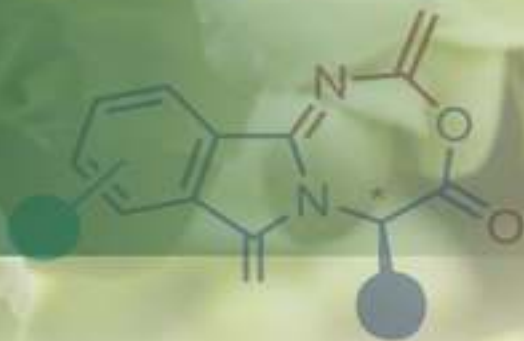


Second Edition



Chemistry of Selected Natural Products and Heterocyclic Compounds

for Pharmacy and Science Students



Anees Ahmad Siddiqui



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Second Edition

Chemistry of Selected Natural Products and Heterocyclic Compounds

for Pharmacy and Science Students

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Second Edition

Chemistry of Selected Natural Products and Heterocyclic Compounds

for Pharmacy and Science Students

Anees Ahmad Siddiqui MPharm PhD

Professor

BM College of Pharmacy
Gurugram, Haryana

Formerly Professor and Head
Department of Pharmaceutical Chemistry
School of Pharmaceutical Education and Research
Jamia Hamdard, New Delhi



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Corporate Office: 204 FIE, Industrial Area, Patparganj, New Delhi-110092

Ph: +91-11-49344934; Fax: +91-11-49344935; Website: www.cbspd.com; www.eduport-global.com;

E-mail: eresources@cbspd.com

Head Office: CBS PLAZA, 4819/XI Prahlad Street, 24 Ansari Road, Daryaganj, New Delhi-110002, India.

Ph: +91-11-23289259, 23266861, 23266867; Fax: 011-23243014; Website: www.cbspd.com;

E-mail: publishing@cbspd.com; eduportglobal@gmail.com.

Branches

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- **Bengaluru:** Seema House 2975, 17th Cross, K.R. Road, Banasankari 2nd Stage, Bengaluru - 560070, Karnataka Ph: +91-80-26771678/79; Fax: +91-80-26771680; E-mail: bangalore@cbspd.com
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Preface to the Second Edition

Natural products are a rich source of bioactive compounds with a wide range of medicinal and clinical applications, which have been used for centuries as prominent medicines. These compounds and their synthetic analogs have shown interesting clinical results and gained substantial importance in recent years. Their importance is also growing with increasing concerns about the adverse effects of synthetic additives on human health and the environment. The increasing demand for natural products makes them especially important to the food, pharmaceutical, and cosmetics industries. Several natural products are routinely used as functional foods, ingredients in formulations, additives (colorants, antioxidants, etc.) or as final products (nutraceuticals and supplements). In several applications, consumers demand the substitution of synthetic compounds with natural ones since there is a widespread belief that natural products are effective and free from toxic effects. Therefore, the importance of natural products has seen a progressive and steady increase in recent decades.

The first edition of the book titled *Precise Chemistry of Natural Products and Heterocyclic Compounds* was compiled to cover the syllabus of the subject 'chemistry of natural products' prescribed for BPharm course in most of the universities. In the present scenario, the subject has been removed from BPharm course prescribed by PCI but topics of this subject have been merged with other subjects in the BPharm curriculum.

The second edition, titled *Chemistry of Selected Natural Products and Heterocyclic Compounds* has been updated with a comprehensive approach to cover the undergraduate and postgraduate courses in science and pharmacy. In pharmacy, the book will be helpful in the subject of chemistry of natural products (MPC 104T) while in science, the chemistry of natural products is taught as an independent subject or a part of organic chemistry.

The book is divided into 14 chapters, covering the properties, biosynthesis, structural elucidation, activity, and uses of drugs. In most of the naturally occurring compounds, spectral data has been provided to increase the utility of this book. The chapter on 'protein' has been completely revised to cover the postgraduate syllabus in science and pharmacy.

I am thankful to my colleagues especially Dr Anupam Pathak (Bhopal); Dr Mukul Tailang (Gwalior); Dr Ashok Khusnoor (Baghpat); Dr Ravinesh Mishra (Baddi); Dr Sarvesh Paliwal (Banasthali); Dr Rajani Chauhan (Banasthali); Dr Mohd Asif (Bareilly); Dr Rajiv Tonk (Delhi); Dr Mamta Kadawla (Gurugram), and Dr Santosh Verma (Roorkee) for their assistance.

I am thankful for the enduring association and help of Sh S K Jain CMD and Sh Varun Jain MD, CBS Publishers & Distributors Pvt. Ltd, New Delhi, in publishing this book.

I have tried my best to avoid typographic errors. However, despite my sincere efforts, some mistakes might have crept in unintentionally. I shall be obliged if these are brought to my notice. I am hopeful that the present edition will be a helpful edition to the students of UG/PG courses in science and pharmacy.

Anees Ahmad Siddiqui

Preface to the First Edition

Precise Chemistry of Natural Products and Heterocyclic Compounds has been compiled primarily for pharmacy undergraduates to provide a modern text to complement lecture courses dealing with natural products chemistry/pharmacognosy and use of natural products in medicine. Nevertheless, it should be of equal value in most of other courses where the study of natural products is included, although the examples chosen are predominantly those possessing biological activity. For example, in India, natural products chemistry is also included in science and biotech courses.

For centuries, drugs were entirely obtained from natural sources and composed of herbs, animal products, and inorganic materials. Earlier, remedies were selected from natural products sources based on certain facts but it is certain that these effective treatments were subsequently recorded and documented, leading to early Herbals. The knowledge of these natural occurring drugs grew from these records to provide a disciplined subject—Natural Products Chemistry. Natural products were present in the bulk of these remedies. With the improvements in chemical technique, the active constituents were isolated from plants, were structurally characterized and in due course, many were synthesized in the laboratory. Sometimes, more active, better drugs were produced by chemical modification or by total synthesis of analogues of the active principles.

As the technique becomes advanced, synthetic compounds superseded many of the old plant drugs, though certain plant-based drugs were never surpassed and remain as important drugs even today. Natural drugs derived from microorganisms have a much shorter history and played a prominent role in introducing the antibiotics during last 6–7 decades. Microbially-derived antibiotics now account for a very large proportion of drugs commonly prescribed.

The pharmacy courses in India include a pharmacognosy or natural products chemistry components, covering a study of plant-derived drugs in their syllabi. It includes the morphological studies, isolation of active constituents, structural elucidation, etc. To cover these, the 1st edition of the present book has been compiled in an easy and understandable language.

The book is divided into 14 chapters, covering the properties, biosynthesis, structural elucidation and activity of drugs. There is extensive use of chemical schemes, chemical structures, mechanism and tables to make the subject easy. The author is quite hopeful that this book will be helpful to the students doing the BPharm/BTech courses.

The author is thankful to his professional friends especially Dr Anupam Pathak, Dr Mukul Tailang, Dr M Shaharyar, Dr Ashok Khusnoor, Dr Ravinesh Mishra, Dr Sarvesh Paliwal, Dr Jaya, Dr (Mrs) Shastri, Dr Rajani (Banasthali), Mohd Ajmal Siddiqui (Dehradun), Mohd Asif (Dehradun), Manni Dutta (Ananpuria), Harish Kumar (Bahadurgarh) for their assistance.

The author is thankful for continuing association and help of Shri Satish K Jain, MD, CBS Publishers & Distributors Pvt Ltd for providing support in publishing this book.

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New Delhi

Dr Anees A Siddiqui

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Natural Products—An Introduction

Natural products are organic and inorganic compounds that are found in various types of natural sources—plants, microbes and animals. These natural products can be organism itself (plant, animal, and microorganism), any part of an organism (leaf or flower of plant; an isolated gland or other organ of animals) and extract or pure compound (alkaloids, coumarins, steroids, etc). However, in practice, the term natural product refers to secondary metabolites, small molecules (molecular weight <1500 amu), produced by an organism, but not strictly necessary for the survival of the organism.

It has been estimated that well over 3,00,000 secondary metabolites exist in nature. These mediate certain functions that help in their survival and reproductive ability. Alkaloids, such as morphine; eicosanoids, such as prostaglandin E₁ and antibiotics, such as erythromycin and penicillins, are good examples of secondary metabolites.

Classification of Natural Products

There is no rigid scheme for classifying natural products—their immense diversity in structure, function, and biosynthesis are too great to allow them to fit neatly into a few simple categories. Initially, the natural products are classified into two broad divisions: (i) Primary metabolites (occur in all organisms) and (ii) Secondary metabolites (occur only in certain organisms). The primary metabolites are organic molecules that have an intrinsic function; essential for the survival of the organism that produces them. Examples of primary metabolites include the core building block molecules (nucleic acids, amino acids, carbohydrates, lipids, etc. In contrast, secondary metabolites are not essential for growth but do increase the competitiveness of the organism within its environment. These are usually produced in response to stress include steroids, terpenes, alkaloids, etc. In practice, however, workers in the natural products field often speak of five main classes of natural products: terpenoids and steroids, fatty acid-derived substances and polyketides, alkaloids, nonribosomal polypeptides, and enzyme cofactors (Fig 1.1).

- **Terpenoids and steroids:** This includes a vast group of substances—more than 35,000 are known—derived biosynthetically from isopentenyl diphosphate. Terpenoids have an immense variety of apparently unrelated structures, while steroids are modified terpenoids, having a common tetracyclic carbon skeleton. These are biosynthesized from the triterpene, lanosterol.



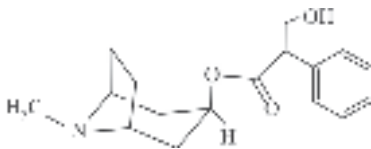
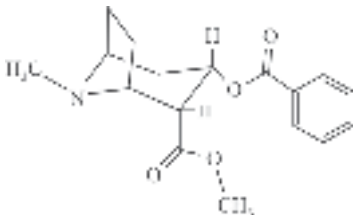
Fig. 1.1: Classification of natural products

- **Alkaloids:** Like terpenoids, there are a large diverse class of compounds, with more than 12,000 examples known at present. Alkaloids are organic substances that contain nitrogen atom, typically in a ring structure and are derived biosynthetically from amino acids.
- **Fatty acid-derived substances and polypeptides:** About more than 10,000 of these are known, which are biosynthesized from simple acyl precursors such as acetyl CoA, propionyl CoA, and methylmalonyl CoA. Natural products derived from fatty acids, such as eicosanoid prostaglandin E_1 , generally have most of the oxygen atoms removed, but polyketides, such as the antibiotic erythromycin A, often have many oxygen substituents.
- **Non-ribosomal polypeptides** are peptide like compounds that are biosynthesized from amino acids by a multifunctional enzyme complex without direct RNA transcription. Non-ribosomal peptide antibiotics (e.g. bacitracin), cytostatics (e.g. bleomycin), and immunosuppressants (e.g. ciclosporin) are in commercial use.
- **Enzyme cofactors** do not fit one of the other general categories of natural products and are usually classed separately.

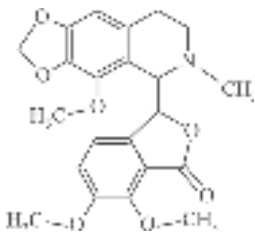
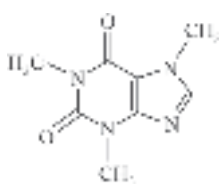
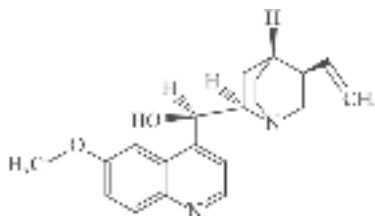
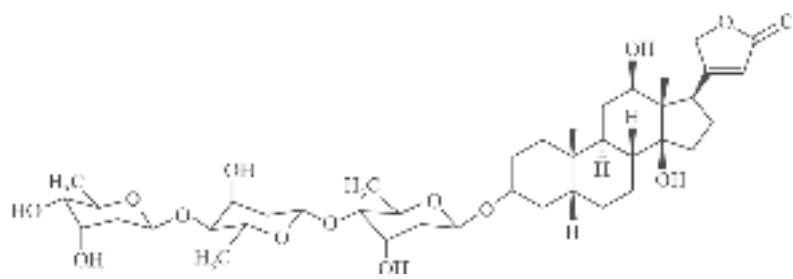
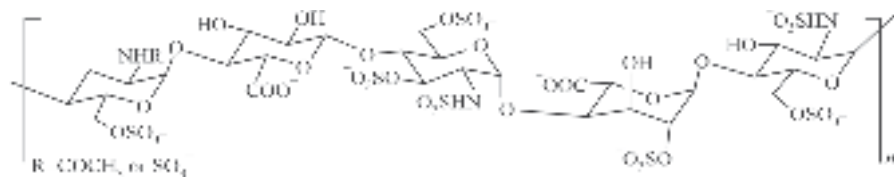
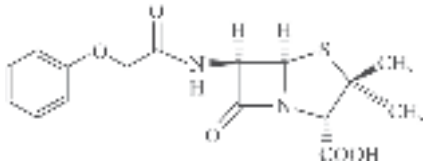
Natural Products in Medicine

Natural products have been a potential source of therapeutic agents for thousands of years. An impressive number of modern drugs have been derived from natural sources. Over the last century, a number of top selling drugs have been developed from natural products. For example, anticancer drug—vincristine from *Vinca rosea* and Taxol from *Taxus brevifolia*, narcotic analgesic—morphine from *Papaver somniferum*, antimalarial drug—artemisinin from *Artemisia annua*, and antibiotic penicillins from *Penicillium* sp. are just a few examples (Table 1.1)

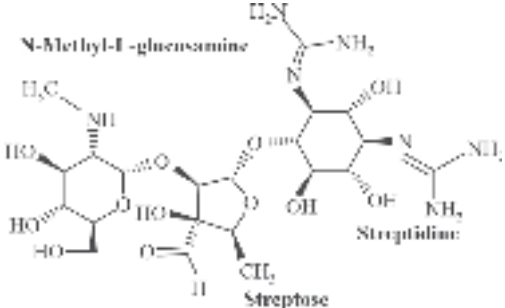
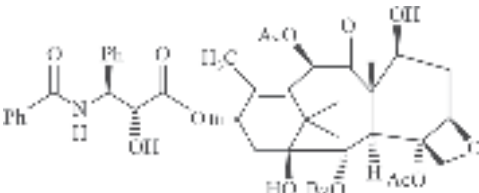
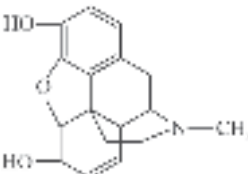
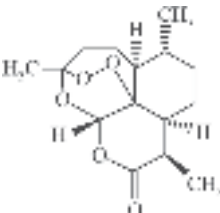
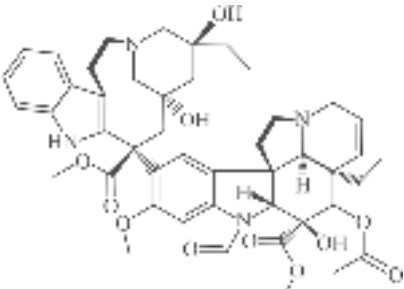
Table 1.1: Uses of natural products as medicine

Name	Chemical Structure	Origin (use)
Atropine (alkaloid)		<i>Atropa belladonna</i> (ophthalmic applications, antispasmodic)
Cocaine (alkaloid)		<i>Erythroxylon coca</i> (local anaesthetic, stimulant)

(Contd.)

Name	Chemical Structure	Origin (use)
Noscapine (alkaloid)		<i>Papaver somniferum</i> (cough remedy)
Caffeine		Tea, coffee (stimulant)
Quinine (alkaloid)		<i>Cinchona ledgeriana</i> (antimalarial)
Digoxin (steroidal glycoside)		<i>Digitalis purpurea</i> (heart medicine)
Heparin (carbohydrate) (mucopolysaccharide)		Liver/lung tissue of cattle (anticoagulant)
Phenoxymethylpenicillin (peptide)		<i>Penicillium</i> sps. (semisynthetic antibiotic)

(Contd.)

Name	Chemical Structure	Origin (use)
Oxytocin (peptide)		Brain (hormone)
Streptomycin (carbohydrate)		<i>Streptomyces griseus</i> (antibiotic)
Taxol (Paclitaxel)		<i>Taxus brevifolia</i> (anticancer)
Morphine		<i>Papaver somniferum</i> (analgesic)
Artemisinin		<i>Artemisia annua</i> (antimalarial)
Vincristine		<i>Catharanthus roseus</i> (anticancer)

Apart from natural-product-derived modern medicine, these products are also used directly in the herbal pharmaceutical industry. The use of these natural products is growing rapidly throughout the world.

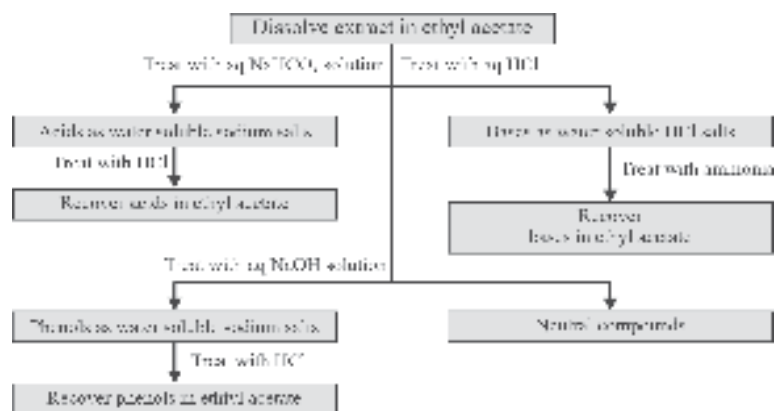
Isolation of Natural Products

Secondary metabolites, with some exceptions, occur very less in amounts, about 0.01% of the dry weight of the plant. Usually extraction of 1 kg of dry plant material is likely to yield less than 100 mg of a natural product. These compounds may be unstable and present as part of a complex mixture. The isolation, separation and purification of these natural products require considerable skill. The source of a secondary metabolite requires proper identification and so a voucher specimen needs to be retained. Within the same species there are sometimes chemotypes, each with a particular composition. Some compounds are found in the roots, some are components of the bark, and others may be found in the leaves, the flowers or the fruits. Some compounds play a seasonal role in the plant, for example as insect antifeedants. Thus, the part of the plant and the place and date on which the plant was collected should all be recorded.

Microorganisms are usually deposited in national culture collections. The production of microbial metabolites often depends upon the medium on which the microorganism is grown and on other fermentation details.

Some fungal metabolites are retained in the fungal mycelium, whilst others are excreted into the broth. Insects, marine organisms and fungi that were collected in the wild may have stored and modified compounds which they had obtained from their food. Natural products may be obtained from the crushed biological material by **extraction** with a solvent such as petroleum ether, chloroform, ethyl acetate or methanol. Several solvents of increasing polarity may be used. Thus, lipid material (waxes, fatty acids, sterols, carotenoids and simple terpenoids) can be extracted with non-polar solvents such as petroleum ether, but more polar substances such as the alkaloids and glycosides are extracted with methanol, aqueous methanol or even hot water. Many alkaloids are present as their salts with naturally occurring acids such as tartaric acid.

Commercial extractions may use tonne quantities of plant material, and a range of different extraction procedures including steam distillation. Most of the isolation procedures still employ basic extraction techniques like infusion, decoction, maceration, percolation, soxhlet, and reflux extraction. Recently, commercial procedures have been developed using supercritical carbon dioxide as a mild solvent, but because of the pressures involved, this requires quite complicated apparatus. The initial extraction is then followed by a **separation** into acidic, basic and neutral fractions. A typical separation procedure is given in Scheme 1.1. A solution of the extract in an organic solvent (such as



Scheme 1.1: Separation of an extract into acidic phenolic, basic and neutral fractions

ethyl acetate) is shaken with an inorganic base (such as aqueous sodium hydrogen carbonate) to remove the carboxylic acids as their water-soluble sodium salts. The more weakly acidic phenols may only be extracted with a sodium hydroxide solution. Extraction of the original solution with an acid such as dilute hydrochloric acid removes the bases such as the alkaloids as their salts. The neutral compounds remain behind in the organic phase. The acid and the phenols may be recovered from the aqueous solution of their sodium salts by treatment with dilute hydrochloric acid and re-extraction with an organic solvent, and the bases may be recovered by treatment of their salts with ammonia and re-extraction.

Although some abundant natural products may be obtained merely by extraction, a simple fractionation or partition and crystallization, the majority are obtained after further careful chromatography. A typical example might involve chromatography on silica or alumina and elution with increasing concentration of ethyl acetate in petroleum ether. The chromatographic separation may be monitored by a bioassay or by thin layer chromatography (TLC). A number of useful spray reagents have been developed, which produce colored TLC spots, indicative of particular classes of compounds.

During the isolation of natural products, various types of reaction such as ester hydrolysis, autoxidation, rearrangement, etc. may occur, leading to the formation of artefacts. Hence, efforts are made to keep the mild condition.

SHORT AND LONG QUESTIONS

1. Classify the natural products? Illustrate the general techniques to isolate the phytoconstituents of various categories.
2. Comment on the role of natural products in the discovery of drugs. Mention the examples of naturally occurring substances used as medicine.

MULTIPLE CHOICE QUESTIONS

1. **Synthesis of most of the complex natural products is a challenging task. Which of the following factors is not a contributing factor to this?**
 - a. Presence of variety of functional groups present
 - b. Presence of multicyclic or unusual ring systems
 - c. Lack of asymmetric centres
 - d. Variety of substituents
2. **Which of the following is known as Ginseng of India?**
 - a. *Penax ginseng*
 - b. *Withania somnifera*
 - c. Tulsi
 - d. Giloe

Answers

1. (c) 2(a)

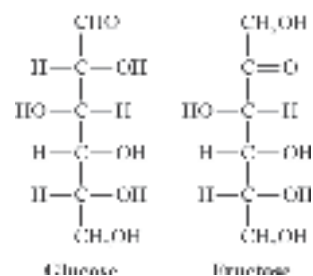
Chapter 2

Carbohydrates—An Overview

The term 'carbohydrates' is derived from the French word: 'hydrate de carbone'— means 'hydrates of carbon'. Carbohydrates are also called **saccharides**. The word 'saccharides' is derived from the Greek word 'sakcharon' means 'sugar'. Thus, carbohydrates are naturally occurring sugar, comprising of carbon, hydrogen and oxygen atoms.

Chemically, carbohydrates can be defined as simple organic compounds that are either aldehydes or ketones with many hydroxyl groups added, usually one on each carbon atom that is not part of the aldehyde or ketone functional group.

Strictly, all carbohydrates have one carbonyl group ($>C=O$) either in the form of aldehyde or ketone. There are many hydroxyl groups ($-C-OH$) attached to the carbon backbone. The structure of most commonly occurring carbohydrates—glucose and fructose are given here.



FUNCTIONS OF CARBOHYDRATES

Carbohydrates are initially synthesized in plants from a complex series of reactions involving photosynthesis which is a complex series of reactions carried out in the leaves in plants, which utilizing the energy from the sun. The simplified version of this chemical reaction is to utilize carbon dioxide molecules from the air and water molecules and the energy from the sun to produce a simple sugar such as glucose and oxygen molecules as a by-product. The simple sugars are then converted into other molecules such as starch, fats, proteins, enzymes, and nucleic acid, required for the survival of plants.



The main functions of carbohydrates are as follows:

- Store energy in the form of starch (**in plants**) or glycogen (**in animals and humans**).
- Provide energy through various metabolic pathways.
- Supply carbon for synthesis of other compounds.

CLASSIFICATION OF CARBOHYDRATES

There are number of inter-related classification systems, based on various facts. These are as follows:

Based on Number of Sugar Units

The most useful classification system divides the carbohydrates into various groups according to the number of individual simple sugar units. The carbohydrates are classified into monosaccharides, disaccharides, oligosaccharides and polysaccharides based on number of sugar units (Table 2.1).

Table 2.1: Classification of carbohydrates with examples

<i>Monosaccharides</i>	<i>Disaccharides</i>	<i>Oligosaccharides</i>	<i>Polysaccharides</i>
Glucose	Sucrose	Raffinose	Starch
Galactose	Maltose		Glycogen
Fructose	Lactose		Cellulose

Monosaccharides contain a single sugar unit. These cannot be further hydrolysed. These are the only sugars that can be absorbed and utilized directly by the body. Disaccharides and polysaccharides must be ultimately broken down into monosaccharides in the digestive process known as *hydrolysis*. After conversion into simple sugars (monosaccharides), carbohydrates are utilized by the body. Glucose, fructose, and galactose are three monosaccharides, particularly important in the study of nutritional science.

Disaccharides contain two sugar units. For example, the disaccharide sucrose contains two monosaccharide units—glucose and fructose. Other disaccharide carbohydrates include lactose (milk sugar), mannose, and maltose.

Oligosaccharides contain 3–6 monosaccharide units and are rare in nature. Many oligosaccharides are prepared by breaking down polysaccharide complex carbohydrates. Most of the naturally occurring oligosaccharides are found in plants. For example, **raffinose** is a trisaccharide consisting of melibiose (galactose and glucose) and fructose. In comparison, **maltotriose**, a trisaccharide of glucose, occurs both in plants and in the bloodstream of certain arthropods.

Polysaccharides contain many monosaccharide sugar units as in polymers. The most of the polysaccharide contains glucose as the monosaccharide unit. Polysaccharides may contain up to 60,000 simple carbohydrate molecules. These carbohydrate molecules are arranged in long chains in either a straight or in a branched chain structure. There are four polysaccharides that are important in the study of nutritional science: starch, dextrin, glycogen and cellulose—most of them contain glucose as the monosaccharide unit. The glycogen—the stored carbohydrate found in the muscles and liver of humans and many animals—consists of a complex chain of glucose molecules. The two most well-known polysaccharides are cellulose and starch. Cellulose, the basic structural material in plants contains over 3,000 glucose molecules.

Based on Number of Carbon Atoms

Monosaccharides can be further classified by the number of carbons present as given in Table 2.2.

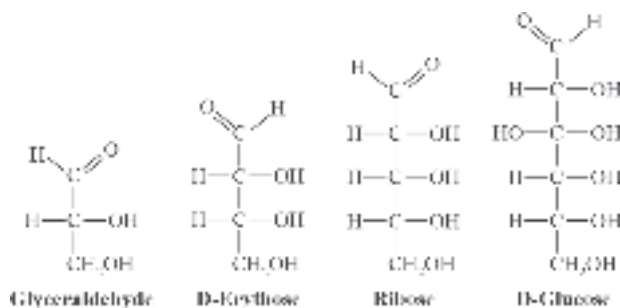
Table 2.2: Classification of monosaccharides based on number of carbon atoms

Number of carbons	Name of sugar	Formula	Examples
4	Tetrose	$C_4H_6O_4$	Erythrose, Threose
5	Pentose	$C_5H_{10}O_5$	Arabinose, Ribose, Ribulose, Xylose, Xylulose, Lyxose
6	Hexose	$C_6H_{12}O_6$	Fructose, Galactose, Glucose, Idose, Mannose, Sorbose, Talose
7	Heptose	$C_7H_{14}O_7$	Sedoheptulose, Mannoheptulose

Based on Functional Group

Carbohydrates are classified into aldoses or ketoses on the basis of functional groups.

Aldoses contain the aldehyde group—Monosaccharides in this group are glucose, galactose, ribose, erythrose and glyceraldehyde (Fig. 2.1).

**Fig. 2.1:** Examples of aldoses

Ketoses contain the ketone group. The major sugar in this group is fructose. Dihydroxyacetone may not be a sugar, but it is included as the ketone analog of glyceraldehyde. The carbonyl group is commonly found at C-2 position in most of the ketoses (Fig. 2.2).

**Fig. 2.2:** Examples of ketoses

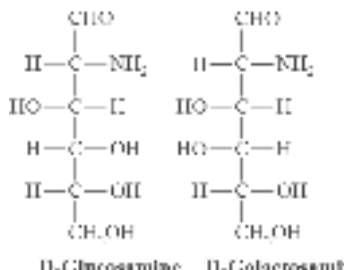
Based on Reactivity

These are classified into reducing and non-reducing sugars.

Reducing sugars: These contain a hemiacetal or hemiketal group. Glucose, galactose, fructose, maltose, lactose are the examples of reducing sugar. These reduce the Fehling's solution, Benedict's reagent, etc.

Non-reducing sugars: These do not contain hemiacetal group. Sucrose and all polysaccharides are the examples of this group. These do not reduce the Fehling's solution. Benedict's reagent, etc.

Amino sugar: These contain an -NH_2 group in place of an -OH group. The amino sugar is constituent of some glycoprotein hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH). D-glucosamine, D-galactosamine are the examples of amino sugars.



CHEMICAL TESTS FOR CARBOHYDRATES

Molisch's Test: The sample is mixed with a small amount of Molisch's reagent (which is α -naphthol dissolved in ethanol) in a test tube. A small amount of concentrated sulphuric acid is gradually added to the mixture along the wall of the test tube without mixing to generate a bottom layer. The appearance of a purple ring at the border between the acid and the test layers indicate a positive reaction. It is a rapid generalised positive test for carbohydrates, monosaccharides while disaccharides and polysaccharides react slower.

The test is named after Austrian botanist, **Hans Molisch**. It is a sensitive chemical test for all carbohydrates, generally based on the dehydration of carbohydrate by sulphuric acid to produce an aldehyde (either furfural in case of pentose sugar or hydroxy methyl furfural in case of hexose sugar), which then condenses with the phenolic structure resulting in a red or purple colored compounds (Fig. 2.3).

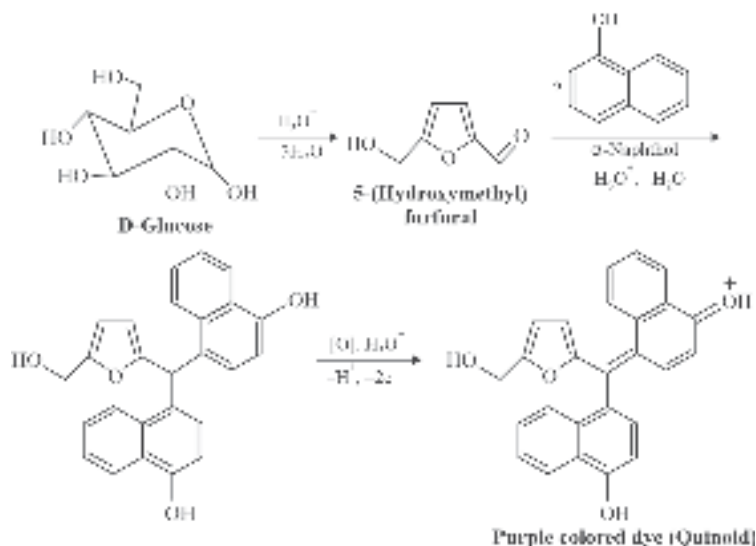


Fig. 2.3: Molisch's test: Dehydration and quinoid formation

Barfoed's Test: It is a chemical test for carbohydrate that detects the presence of monosaccharides. To the 1 ml of a sample solution in a test tube, 3 ml of Barfoed's reagent (acetic acid and cupric acetate solution) is added. The solution is heated on a boiling

water bath for three minutes—formation of a reddish precipitate indicates the presence of monosaccharides.

Barfoed's solution contains cupric ions in an acidic medium. This mild condition allows oxidation of monosaccharides, but does not oxidize disaccharides. If the time of heating is carefully controlled, disaccharides do not react while reducing monosaccharides give the positive result (red colored Cu_2O precipitate). Ketoses do not isomerize with this reagent.

Benedict's Test: 1 ml of the sample solution is placed in a test tube with 2 ml solution of the Benedict's reagent. A reddish precipitate appears within the three minutes, if any reducing sugar is present when the solution is heated in a boiling water bath.

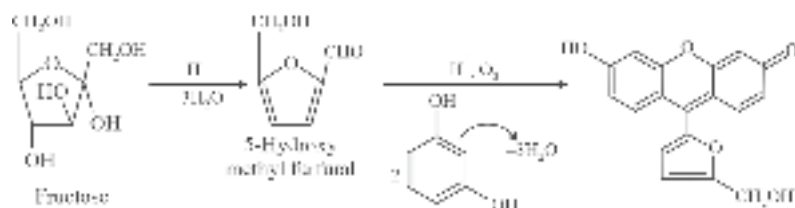
This test is discovered by the American chemist, **Stanley Rossiter Benedict**. The Benedict's test detects the presence of reducing sugars. **Benedict's reagent** contains Cu^{2+} ions in alkaline solution with sodium citrate, which added to keep the cupric ions in solution. The alkaline conditions of this test causes isomeric transformation of ketoses to aldoses, resulting in reduction of the blue colored Cu^{2+} ion to cuprous oxide (Cu_2O), appeared as brick red-orange precipitate.

Fehling's Test: The mixture of 2 ml of Fehling's solution A, 2 ml of Fehling's solution B, and 2 ml of sugar solution is heated in test tube on a water bath. The appearance of yellow or brick red colored precipitate indicates the presence of reducing sugar.

It is test for detecting the presence of reducing sugar. It is composed of two solutions, which are mixed in situ. Fehling's solution A composed of 0.5% w/v of copper sulphate whereas Fehling solution B composed of sodium potassium tartarate.

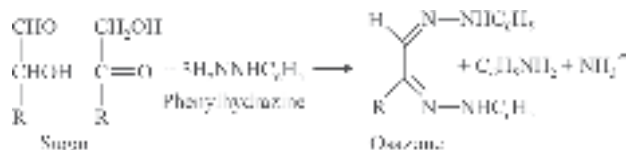
Seliwanoff's Test: The 3 ml of Seliwanoff's reagent and 1 ml of sugar solution are heated to boiling for 2 minutes. On cooling, the formation of red color or red colored precipitate indicates the presence of ketoses like fructose, sucrose.

This test is used for identification of ketohexoses or to distinguish between ketoses and aldoses. This test is based on the fact that ketoses on heating, are more rapidly dehydrated than aldoses. The Seliwanoffs reagent (resorcinol in 6M HCl) gives the formation of cherry red color in presence of ketose (Fructose) due to formation of hydroxy methylfurfural. Sucrose gives a positive ketohexose test because of partial hydrolysis to glucose and fructose.



Rapid Furfural Test: The mixture of 2 ml of sugar solution, 1 ml of 1% alcoholic α -naphthol solution and 5 ml concentrated HCl is heated. The formation of deep purple color indicates the presence of ketoses like fructose, sucrose, etc.

It is a test performed to distinguish between glucose and fructose. It is similar to Molisch test but uses conc. HCl solution instead of conc. sulphuric acid.



Osazone Test: To a solution of the 0.2 g unknown sample in a test tube, 0.4 g of phenylhydrazine hydrochloride, 0.6 g of crystallized sodium acetate, and 4 ml of distilled water are added. The test tube is placed in a boiling water bath. Note the time that the test tube is immersed and the time of the precipitation. After 20 min, the test tube is removed from the hot water bath and set it aside to cool. A small amount of the liquid and solid is poured on a watch glass. The watch glass is tilted from side to side to spread out the crystals, and absorbed some of the mother liquid with a piece of filter paper, taking care not to crush or break up the clumps of crystals. The crystals are examined under a low-power microscope (about 80–100X), and compared with photomicrographs.

- (a) Greenish yellow needle-shaped crystals: Glucosazone, i.e. glucose present.
- (b) Badminton ball, powder puff shaped crystals: Lactosazone, i.e. lactose present.
- (c) Sunflower or its petals shaped crystals: Maltosazone, i.e. maltose present.

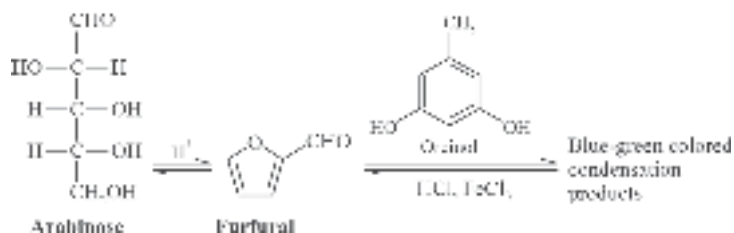
The formation of tarry products due to oxidation of the phenyl hydrazine may be prevented by the addition of 0.5 ml of saturated sodium bisulphite solution. This should be done before heating, if it is desired to isolate the osazone and determine its melting point.

The time required for the formation of the osazones can be a valuable aid in distinguishing among various sugars. The following figures are the times required for the osazone to precipitate from the hot solution: fructose, 2 min. glucose; 4–5 min. xylose; 7 min. arabinose; 10 min. galactose; 15–19 min. raffinose; 60 min. osazone formation involves hydrazone formation at C-1 of an aldose (or C-2 of a ketose) and oxidation of C-2 (or C-1) of an alcohol group to a ketone (or an aldehyde). The new carbonyl group is also converted to a hydrazone.

Iodine Test: To about 2 ml suspension or solution of polysaccharides, 1–2 drops of N/50 iodine solution is added. The formation of blue violet color indicates the presence of starch while brown wine color indicates the presence of glycogen.

Bial's Test: The 2 ml of aqueous solution of sugar is mixed with 4 ml of Bial's reagent and then heated to boiling. The production of blue-green color is indicative of presence of pentose sugar.

It is used to distinguish between pentoses and hexoses. Pentoses reacts with Bial's reagent (Orcinol in conc. HCl and traces of FeCl₃ as catalyst) to form furfural, which condenses with orcinol to produce blue-green product.



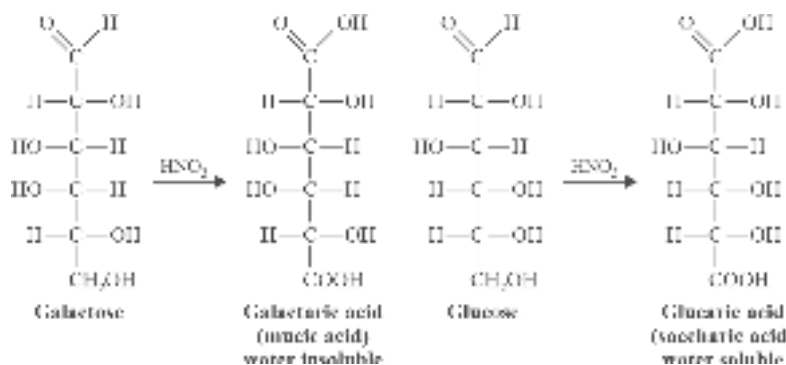
Diphenylamine Test: To the 0.6 ml of a 0.01% carbohydrate solution, add 1 ml of the diphenylamine reagent and heat on a boiling-water bath for 10 minutes. If 2-deoxy sugar is present, a blue-green color is produced.

It is specific test for desoxy sugar (e.g. digitoxose, ribulose). The reagent is prepared by dissolving 1 g of diphenylamine in 10 ml of glacial acetic acid, followed by addition of 0.25 ml of sulphuric acid solution.

Mucic Acid Test: In an evaporating dish, 2 mg of unknown sugar, 6.0 ml of water and 6.0 ml of concentrated nitric acid are placed. The contents are evaporated over a small

free flame with stirring to avoid spattering. When the contents become pasty, cool, dilute with cold water and filter. Wash with cold water. The presence of a white precipitate on the filter paper confirms the presence of galactose. The absence of white precipitates confirms glucose.

This test is specific for galactose. Hot nitric acid oxidizes a sugar to the carboxylic acid. Aldoses are oxidized at both ends of the ring-opened form to provide dicarboxylic acids. Ketoses oxidize to give a mixture of carboxylic acids resulting from chain fragmentation.



Galactaric acid (or mucic acid), formed from the oxidation of galactose is much less soluble in the oxidizing medium (and water) than the saccharic acids obtained from other aldoses. This is partly due to the high molecular symmetry of galactaric acid (it is a meso compound).

REACTIONS OF MONOSACCHARIDES

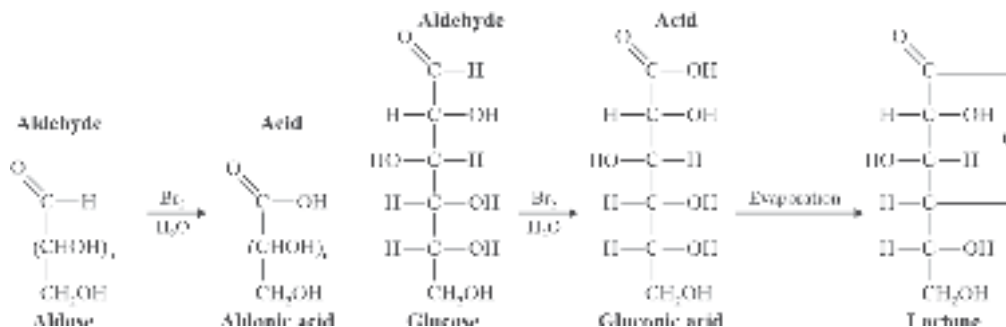
The monosaccharides are polyhydroxy aldehydes or ketones. In general, the order of reactivity of the various hydroxyl groups within D-glucopyranose was observed to be: 01 (hemiacetal) > 06 (primary) > 02 (adjacent to C-1 and therefore, more acidic) > 03 > 04.

Oxidation

There are many ways to oxidize a compound with so many hydroxyl groups.

(a) Oxidation by bromine solution

The bromine solution selectively oxidizes the aldehyde group without affecting the OH groups at all. This produces an acid, an aldonic acid, at one end of the molecule. The resulting aldonic acids usually form cyclic lactone (on evaporation).

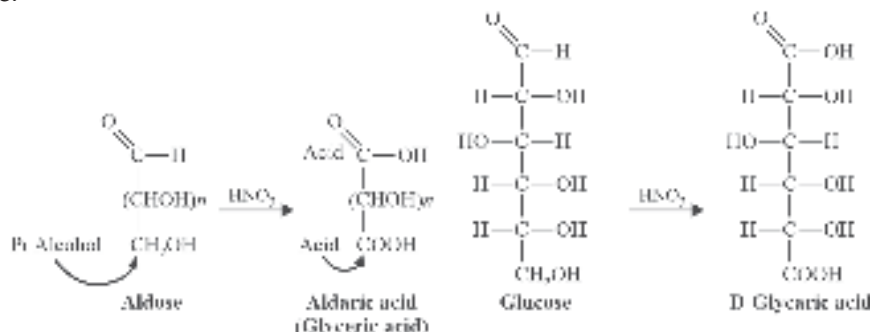


Note: Lactone may be reduced to original aldose by sodium amalgam in presence of traces of acid.

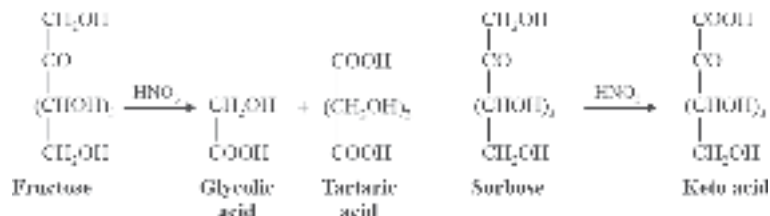
Bromine water do not oxidize ketone. It means, fructose is unaffected due to presence of keto group.

(b) Oxidation by strong oxidant, HNO_3

Nitric acid oxidizes both the aldehyde group and the primary alcohol at the other end of the chain. This produces the dicarboxylic acid, or glycaric acid. This diacid may form a dilactone.

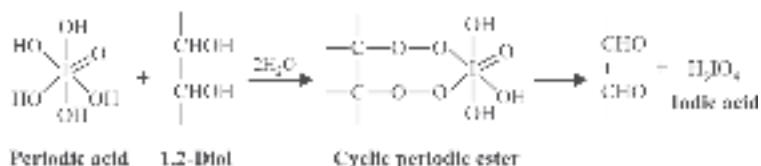


The strong oxidizing agent breaks the ketoses, e.g. fructose to form a mixture of acids having fewer number of carbon atoms but sorbose (keto-hexose) is oxidized with nitric acid to a keto acid.

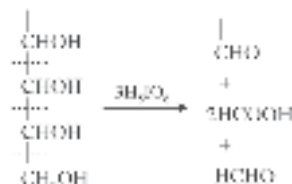


(c) Oxidation by periodic acid

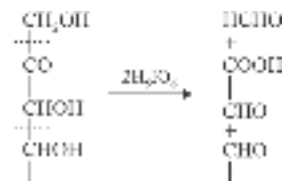
These reagents oxidize the adjacent 1,2-glycolic hydroxyl group to give the dialdehydes (rupturing of C-C bond) (**Malaprade reaction**).



This reagent does not attack the sugar unit devoid of adjacent hydroxyl groups. On the other hand, between every two 1,2-glycolic groups, one molecule of the reagent is consumed. For example, in the given below, three moles of H_5IO_6 are consumed. The reaction with periodic acid is used in the determination of ring size in sugars.

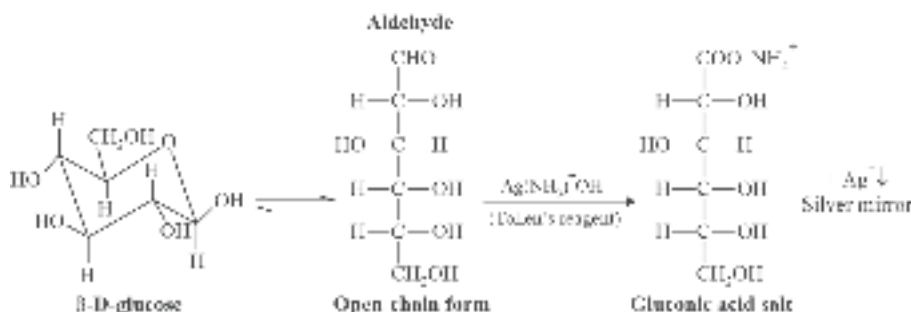


These reagents also attack the α -hydroxyl compound but in this, the carbonyl group is attacked from only one side.

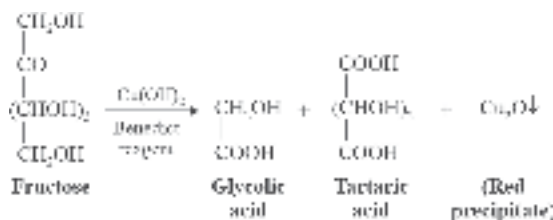


(d) Oxidation by Tollen's reagent

Tollen's reagent (alkaline silver nitrate solution) oxidizes the aldehyde (glucose), to give the corresponding aldonic acid and get themselves reduced. Sugars that give a silver mirror with Tollen's reagent are called reducing sugars.

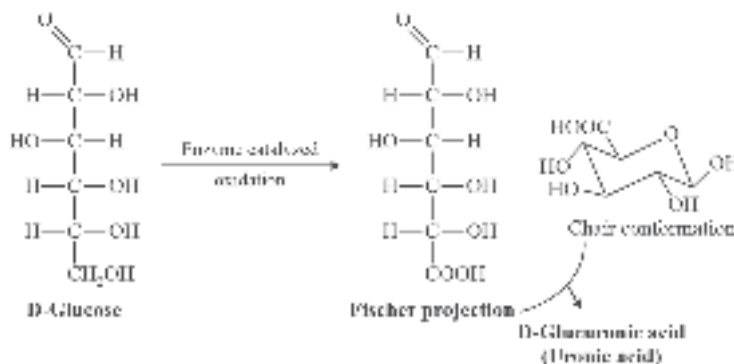


Similarly, the other reagent like Fehling solution, Benedict's reagents (alkaline copper sulphate solution) also oxidize the aldoses and so useful in the determination of reducing sugar.



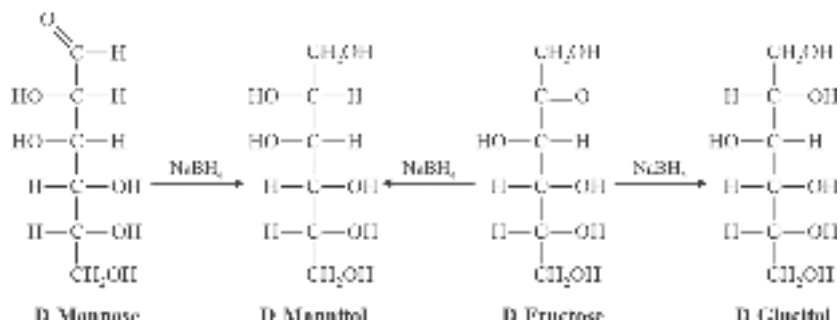
(e) Enzymatic oxidation

Enzyme-catalyzed oxidation of the primary hydroxyl group occurs at C-6 position of a hexose yields a uronic acid. Enzyme-catalyzed oxidation of D-glucose, for example, yields D-glucuronic acid, shown here in both its open-chain and cyclic hemiacetal forms.

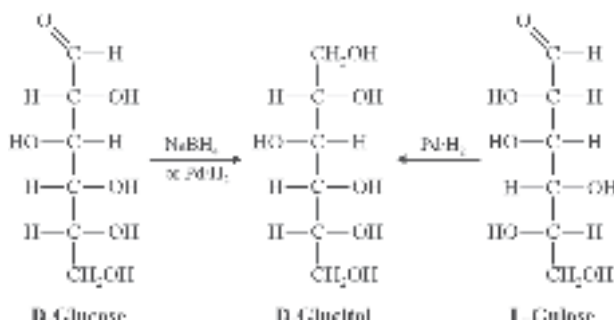


Reduction

The carbonyl group of aldoses and ketoses can be reduced *via* NaBH_4 to yield a polyalcohol referred to as **alditol**. The reduction of aldoses gives a single product, whereas the reduction of ketoses yields two diastereomeric compounds due to the formation of another asymmetric carbon.

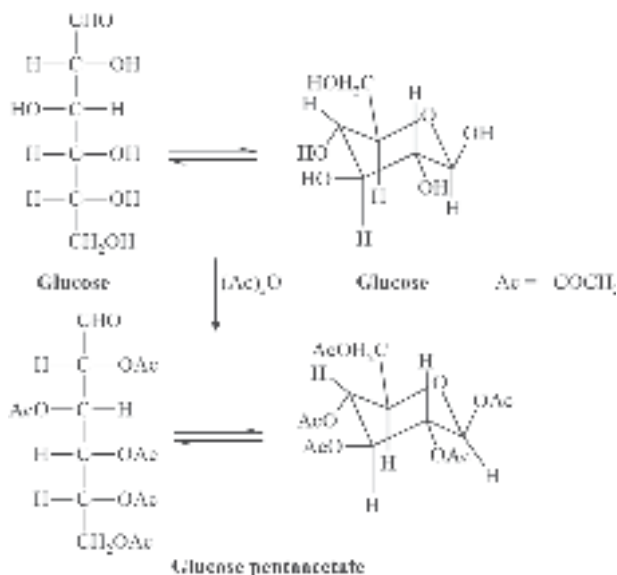


D-Glucitol is obtained from the reduction of either D-glucose or L-gulose. D-Glucitol (also called sorbitol) is about 60 times as sweet as sucrose.



Acetylation

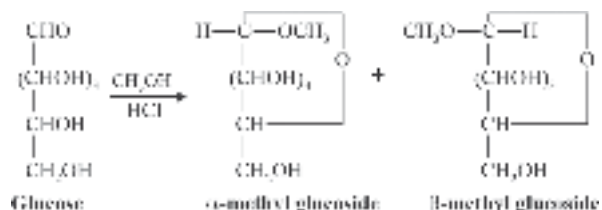
Due to presence of hydroxyl groups, monosaccharides can be acetylated with acid anhydride to form the polyacetate esters. This reaction is used in determining the number of hydroxyl groups.



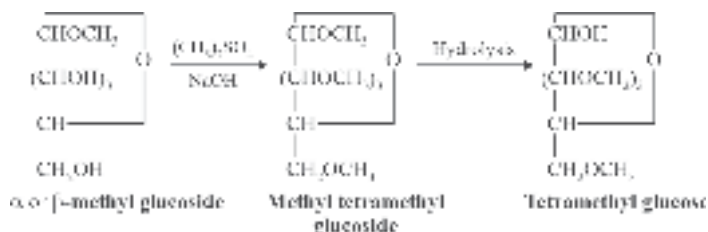
Glucose and fructose forms penta-acetate derivatives due to presence of five hydroxyl groups.

Glycoside Formation

Monosaccharides on treatment with methanolic hydrogen chloride form the two isomeric, α - and β -methyl glycosides. The reaction takes place through the conversion of aldehyde group to an acetal group. Acetals (and thus glycosides) are acid labile but stable in alkaline solution.



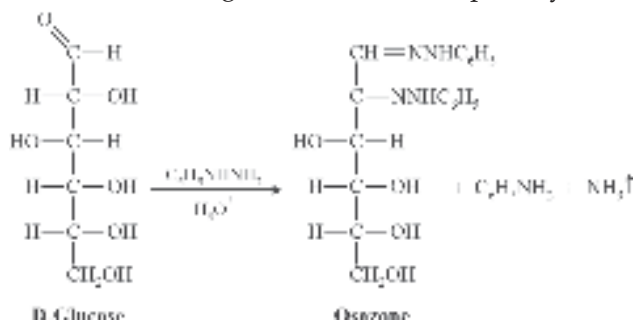
Methylation of other hydroxyl groups of sugar requires strong methylating agents to produce methyl ether. The dimethyl sulphate is used for the formation of methyl ethers. The methyl ethers on acid hydrolysis only at hemiacetal linkage give the tetramethyl glucose.



- This is a Williamson ether synthesis.
- The hemiacetal must be protected as a glycoside prior to methylation.
- Acid-catalyzed hydrolysis of the fully methylated product produces 2,3,4,6 tetramethyl glucose, indicating that the (unmethylated) hydroxyl group at C-5 position is involved in cyclization.
- This reaction is used in establishing the ring structure of the sugar.

Reaction with Phenylhydrazine (Osazone formation)

Carbohydrates that have an aldehyde or ketone carbonyl group, either free or in equilibrium with a hemiacetal, react with phenylhydrazine to form bright yellow, crystalline derivatives called osazones. These derivatives may usually be identified readily both by their melting points and by their crystalline forms. Glucose gives broomstick shaped crystals, with this whereas maltose gives sunflower shaped crystals (Scheme 2.1).



Scheme 2.1: Osazone formation

It involves two reactions, firstly, glucose with phenylhydrazine gives us glucose phenylhydrazone by elimination of a water molecule from the functional group. The next step involves reaction of one mole of glucose phenylhydrazone with two moles of phenylhydrazine (excess) to form osazone. During this oxidation, hydrazine is reduced to aniline and ammonia.

First phenylhydrazine is involved in oxidizing the α -carbon with respect to carbonyl group, and the second phenylhydrazine involves in removal of one water molecule with the formation of carbon nitrogen bond. The α -carbon is attacked here because it is more reactive than the others. They are highly colored and crystalline compounds and can be easily detected. Similarly, fructose reacts with phenylhydrazine through the formation of formyl group (Fig. 2.4). It indicates that osazone formation is characteristic reaction for α -hydroxyaldehyde and α -hydroxyketones. Thus, benzoin $\text{C}_6\text{H}_5\text{CHOHCOC}_6\text{H}_5$ also forms the osazone.

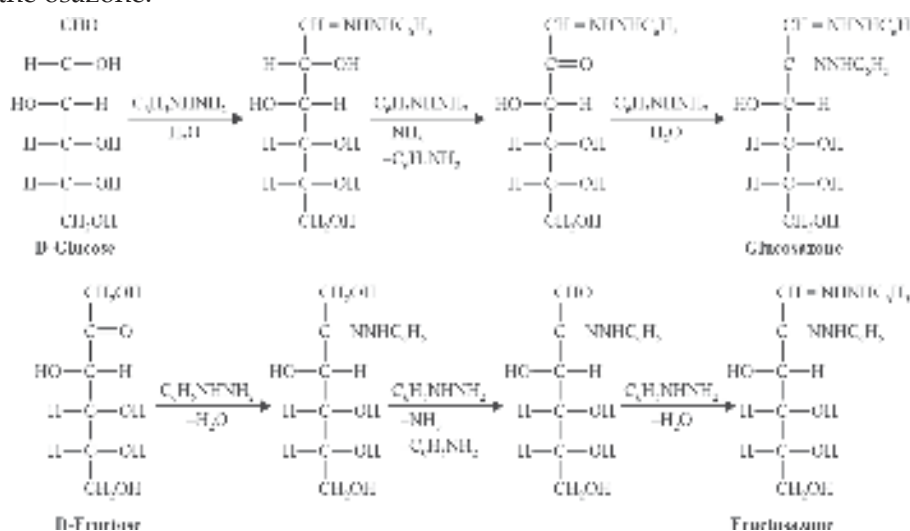
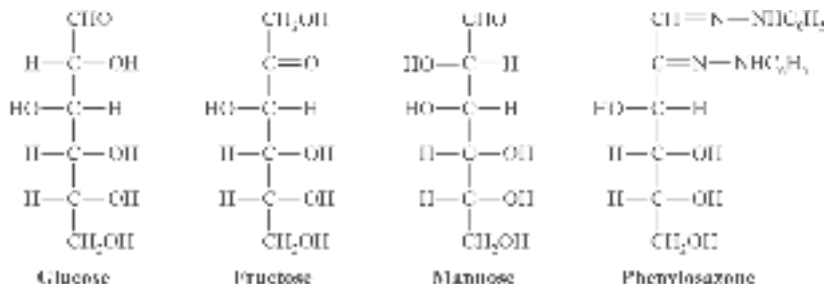


Fig. 2.4: Osazone formation

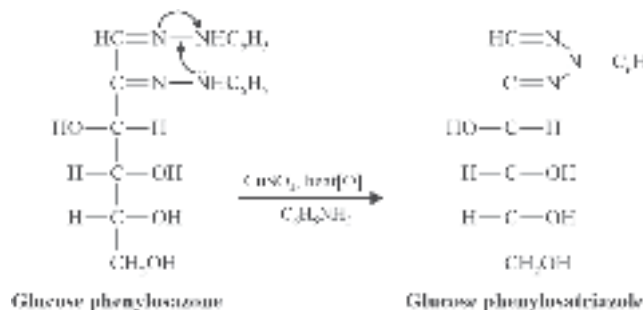
There are two aldoses and one ketose which yield the same osazone. Thus, glucose, mannose and fructose give the same osazone product. This is because osazone formation involves the reaction with C1 and C2 and the asymmetry in both carbon atoms is destroyed. Sugars which give the same osazone are known as **epimers** *aldoses which differ in the configuration of C-2 position*. For example, glucose and mannose give the same osazone, are epimers.



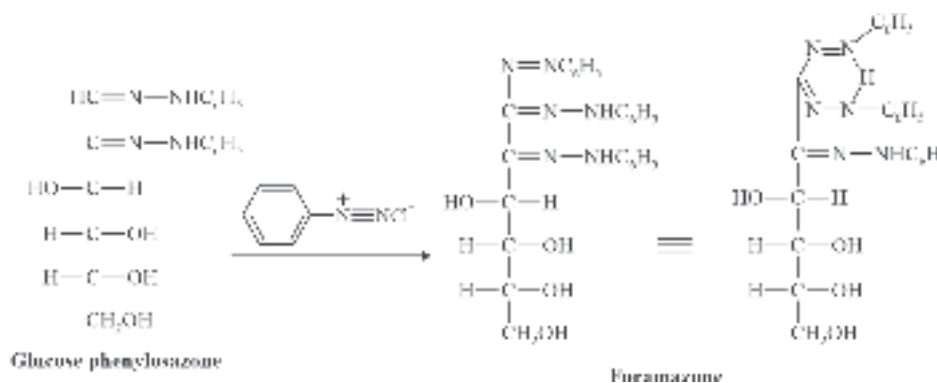
Reactions of Osazones

- Formation of osatriazoles:** The osazones on refluxing with aqueous copper sulphate solution, are converted into the osatriazoles. In this reaction, the osazone

first being oxidized to unstable intermediate, which is then converted into the osatriazoles.



- (ii) **Foramazone:** The reaction of carbohydrate phenyllosazone with aryldiazonium compound yields foramazone.



Epimerisation

The epimers are the optical isomers which differ in configuration at only one asymmetric carbon. The process of converting one epimer into other (generally in a molecule containing several asymmetric carbon atoms) is known as epimerisation. The glucose and mannose are the epimers, differ at C-2 position.

Glucose on treatment with dilute alkali or organic bases such as pyridine or quinoline, epimerised to yield a mixture of mannose, fructose and unreacted glucose. This reaction is useful in preparing of rare sugars from their epimers. The mechanism of this reaction, suggested by Lobrg de Druyn and van Ekenstein, involves the formation of an intermediate enediol. The hydrogen atom attached to the C-2 position may be removed

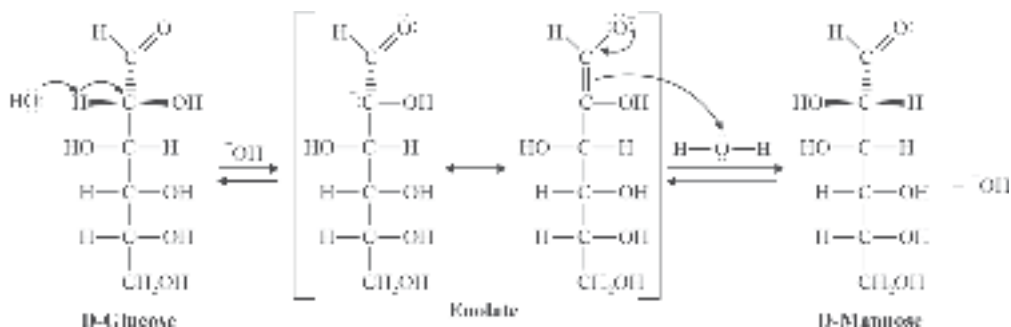
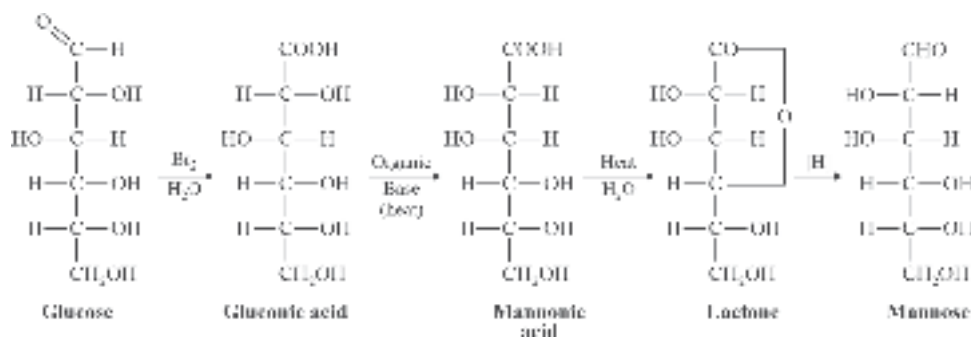


Fig. 2.5: Mechanism of epimerisation

to form enolate ion, thus destroying the asymmetry of C-2. Reprotonation may change the stereochemistry of C-2 position to give D-mannose (Fig. 2.5). If the second hydrogen on C-2 position migrates to C-1 position, a ketose is formed (fructose). Thus, we can convert glucose into mannose and fructose. This process, however, is usually accompanied by a considerable decomposition and it is now no more used for laboratory purposes.

The reaction is best carried out by the epimerisation of aldonic acids (gluconic acid) which are more stable towards alkaline medium. Thus, the aldose is first oxidized to the aldonic acid say gluconic acid, which is then heated with an organic base like pyridine or quinoline and thus it converted to mannonic acid which is then lactonised and reduced to give mannose.



Action of Alkali

The sugar molecule is unstable toward alkalis. It undergoes a series of isomerisation and degradation reactions leading to a number of compounds. Thus, more than 100 different compounds have been isolated from the reaction product of glucose and sodium hydroxide.

SOME INTERCONVERSIONS: ASCENDING AND DESCENDING IN MONOSACCHARIDES

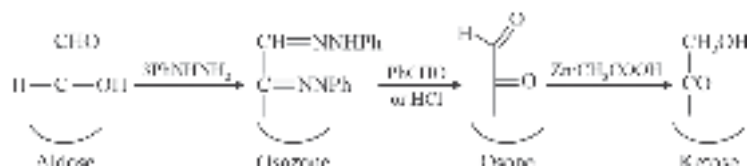
These interconversion reactions have significant role in synthesizing and determining the configuration of aldoses and ketoses.

Interconversions

(i) Conversion of an aldose into the corresponding ketose

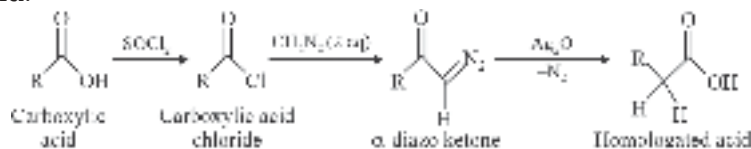
The aldose can be converted into the corresponding ketose, via osazone formation. It involves the following steps:

- The aldose is converted into osazone by reacting with phenylhydrazine.
- The osazone is either treated with conc. acid or benzaldehyde to get osone (elimination of phenylhydrazine residues).
- The osone is reduced with zinc and acetic acid to corresponding ketose.

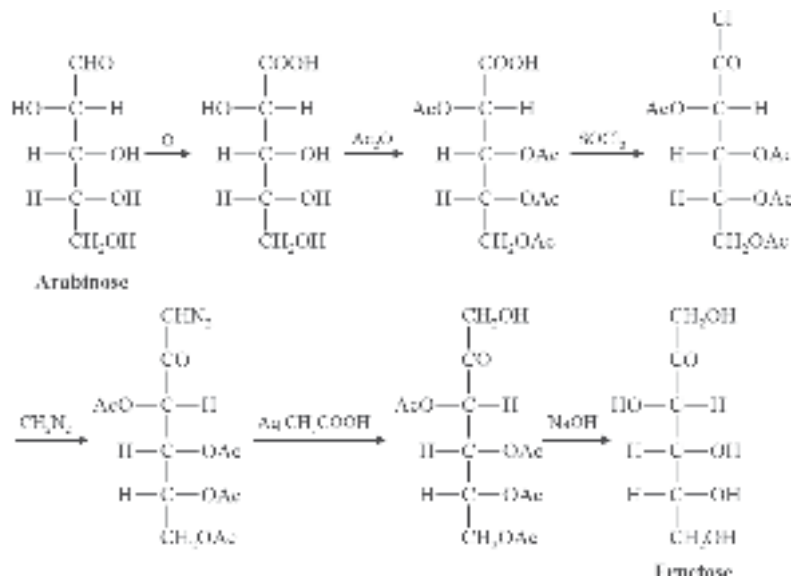


(ii) Conversion of an aldose into the next higher ketose

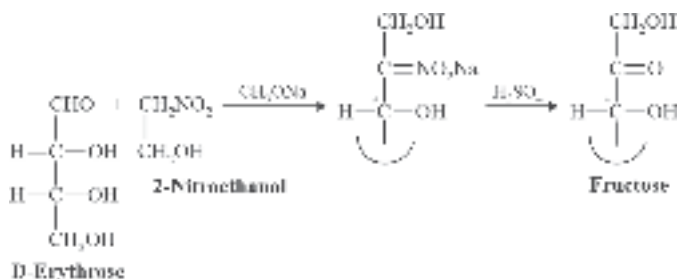
The method follows the **Arndt-Eistert reaction**. The conversion of a carboxylic acid to its homologue (one CH_2 group higher) in three stages is called the Arndt-Eistert synthesis. This homologation is the best preparative method for the chain elongation of carboxylic acids. In the first stage of the process, the acid is converted to the corresponding acid chloride. It is then treated with diazomethane followed by silver oxide to get higher carboxylic acid.



In the same way, D-arabinose (an aldopentose) is converted into D-fructose (α -ketohexose) by using **Arndt-Eistert reaction**. Acetylation is done to avoid methylation of the three alcoholic groups by diazomethane prior to formation of acid chloride. Then, same sequence of reaction is followed to get higher ketose. The example of formation of fructose from arabinose is shown here.

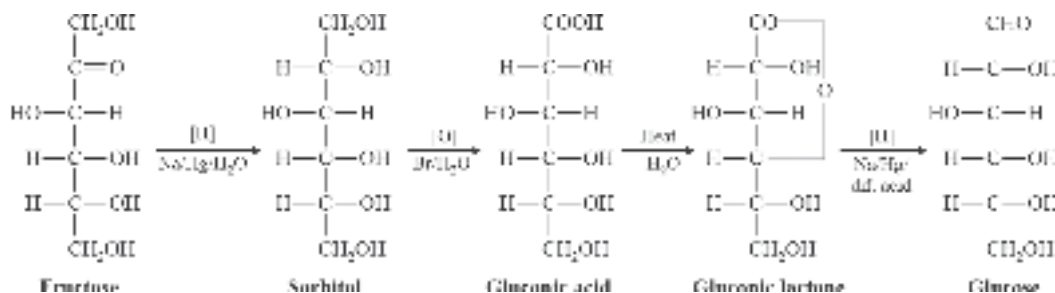
**(iii) Conversion of aldose into the ketose containing two more carbon atoms**

The method is correspondent to **Sowden method** for ascending aldoses by one carbon atom.

**(iv) Conversion of a ketose into the aldose**

The ketose is first reduced to hexahydro alcohol using Na/Hg and water, then the alcohol is oxidized to the corresponding aldonic acid which is then lactonised and reduced to

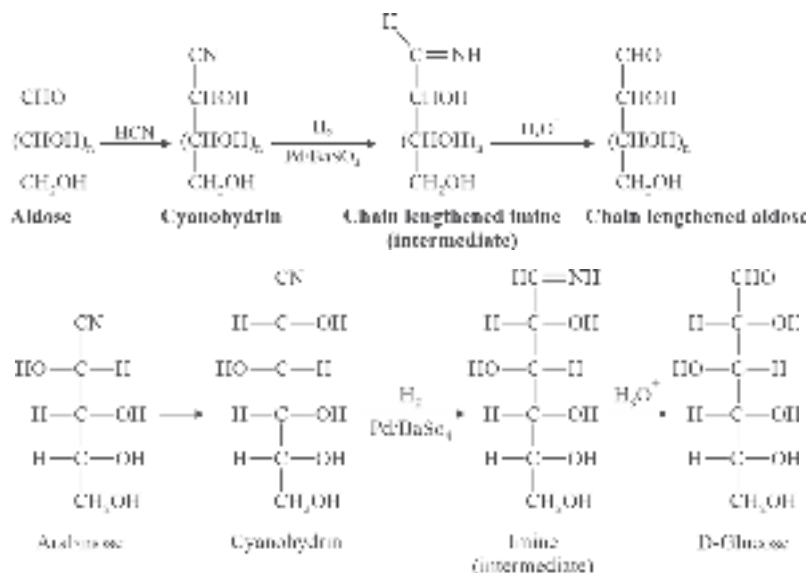
get aldose. The carboxylic group cannot be reduced directly, but firstly it can be reduced via lactone formation.



Ascending aldose series

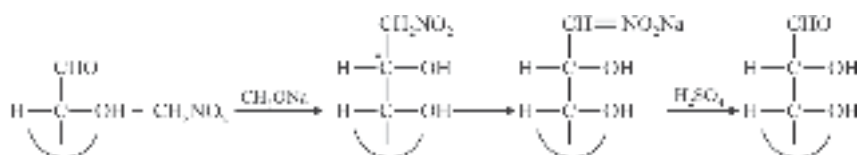
(i) Kiliani-Fischer synthesis

This process lengthens the aldose chain and so mixture of C-2 epimers is formed. This process can be used to prepare aldoses up to ten carbon atoms.

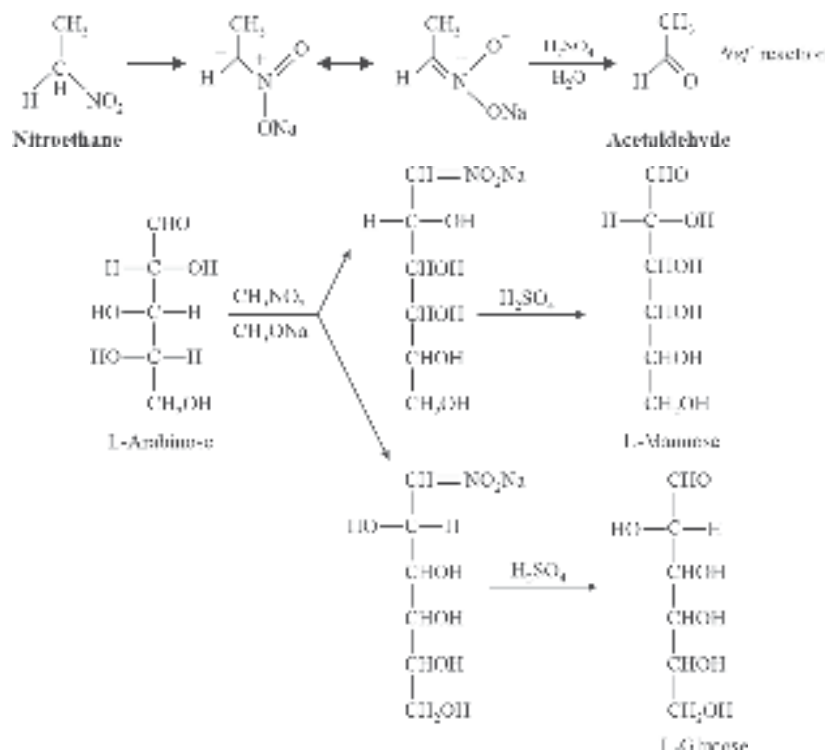


(ii) Sowden or nitromethane synthesis

Sowden observed that the treatment of the nitromethane with methanolic sodium hydroxide led to the formation of 1-nitro-1-deoxy compound which is assorted into corresponding sodium salt. The sodium salt on hydrolysis yields aldose followed by Nef reaction.

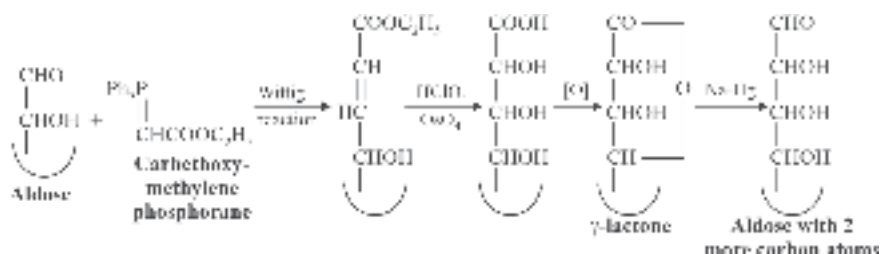


The conversion of nitroalkanes into the corresponding carbonyl compounds is known as the Nef reaction.

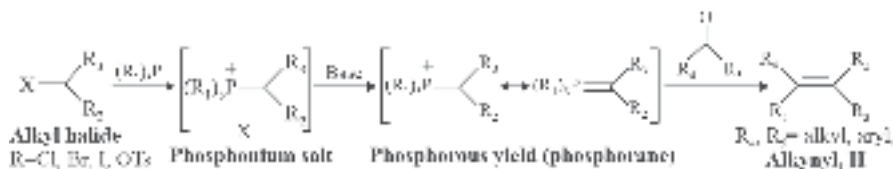


(iii) Kochetkov method

In 1965, Kochetkov found that aldose on treatment with carbethoxymethylene phosphorane have stepped by two carbon atoms (Wittig reaction).



The formation of carbon-carbon double bond (olefins) from carbonyl compounds and phosphoranes (phosphorous yields) is known as the *Wittig reaction*.

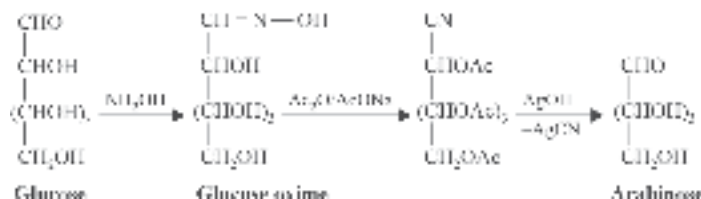


Descending aldose series

The process implies with the elimination of C-1 and the conversion of C-2 position to an aldehyde group. The various methods involved for degrading the aldoses are as follows.

(i) Wohl method

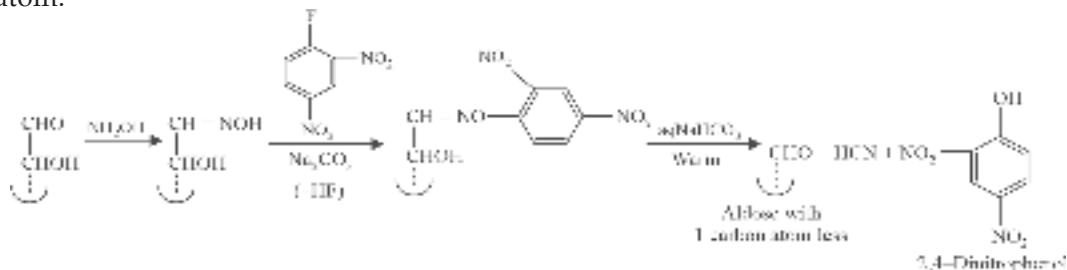
The Wohl degradation in carbohydrate chemistry is a chain contraction method for aldoses. The classic example of conversion of glucose (aldohexose) into arabinose (aldopentose) is shown below:



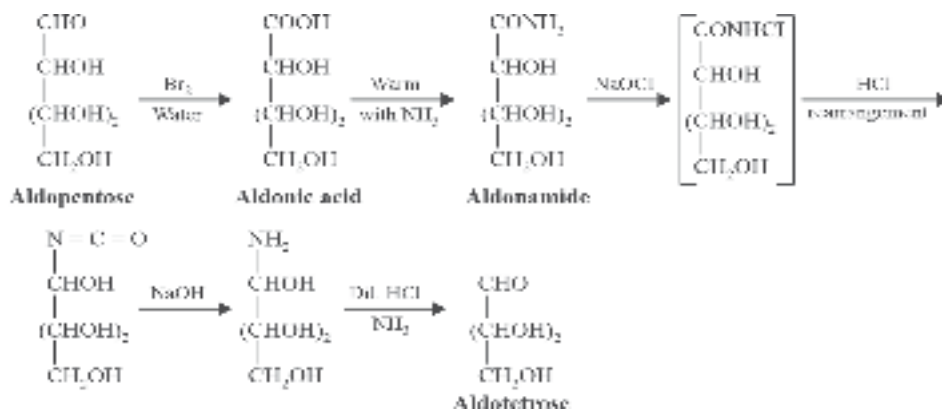
In the above reaction, glucose is converted to the glucose oxime by reaction with hydroxylamine and sodium methoxide. In the second step, the pentaacetyl nitrile derivative is formed by reaction with acetic anhydride in presence of sodium acetate. In this reaction step the oxime is converted into the nitrite with simultaneous conversion of all the alcoholic groups to acetate groups. In the final step, ammonical silver nitrate solution is added by which the nitrite group is split off as sodium cyanide with formation of a new formyl group and acetyl groups are also removed as acetamide.

(ii) Weygand's method

According to Weygand's method, an aldose oxime on reaction with 1-fluoro-2,4-dinitrobenzene followed by sodium bicarbonate gives the aldose with one less carbon atom.

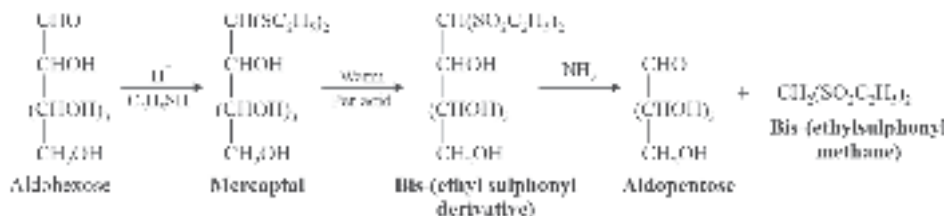
**(iii) Weerman's method**

The Weerman degradation is an organic reaction in carbohydrate chemistry in which an aldonamide (derived from an aldonic acid) is degraded by sodium hypochlorite to get new sugar with one less carbon. The reaction is named after **RA Weerman**. The reaction mechanism is related to that of Hoffmann degradation.



(iv) McDonald method

In 1953, McDonald found that in the presence of conc. HCl, aldose reacted with a mercaptan (thiol) such as ethanethiol to yield mercaptal (thioacetal), which is oxidized with a peracid to form *bis* (ethylsulphonyl) derivative. The ethylsulphonyl derivative on treatment with ammonia gives the lower aldose.

**(v) Ruff method**

In 1898, **Otto Ruff** reported the conversion of D-glucose to D-arabinose now called the Ruff degradation. In this reaction, primarily terminal aldehyde group is converted to carboxylic group, using selective oxidation of aldehyde group using bromine water and then transformed to gluconate ion. Oxidation of resulting product with Fenton's reagent ($\text{H}_2\text{O}_2 + \text{Fe}^{3+}$), followed by decarboxylation gives aldopentose.

**Configuration of Monosaccharides****(i) Aldotrioses**

Glyceraldehyde, $\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CHO}$ is only member of aldotriose series. It exists in two forms which differ in the configurational series as well as in the sign of rotation. Since the D-glyceraldehyde is found to rotate the plane of polarized light to right so-called dextro-(d)-rotatory and the other which rotates the plane of polarized light to left was called levo-(l)-rotatory. Now the configuration of other carbohydrates are derived from D- and L-glyceraldehydes by the fact that the aldoses and ketoses having the configuration of their penultimate carbon atom (highest number of asymmetric carbon atom, C-5 in aldohexose) similar to that of D- or L-glyceraldehyde are said to belong to the D- or L-series respectively of sugars.



Note that the designation D or L merely relates the configuration of a given molecule to that of glyceraldehyde and does not specify the sign of rotation of plane-polarized light. The sign of rotation is indicated only with a + (plus) or – (minus) sign. For example, if two sugars are derived from dextro-glyceraldehyde, it does not mean that they must rotate the plane of polarized light to right, actually they may or may not rotate the beam

of polarized light to right that can only be known by measuring their rotation in a polarimeter. The symbols D and L are used for families while 'd' and 'l' are used to represent the signs of rotation.

The common method for determining the configuration of a monosaccharide consists in ascending the lower homologue of known configuration by one carbon atom (Kiliani synthesis) to give two higher isomeric monosaccharides, the nature of which is determined by their oxidation to acids.

(ii) Aldotetroses

The aldotetroses, $\text{CH}_2\text{OH}-(\text{CHOH})_2-\text{CHO}$ have two asymmetric carbon atoms so four (2^2) isomers are possible. All the four isomers, D- and L-erythrose and D- and L-threose are known. They are obtained by Kiliani synthesis of D(+)-glyceraldehyde. The D-erythrose and D-threose are identified by oxidation reaction. D-erythrose gives the meso-tartaric acid and D-threose gives the L(-)-tartaric acid (Fig. 2.6).

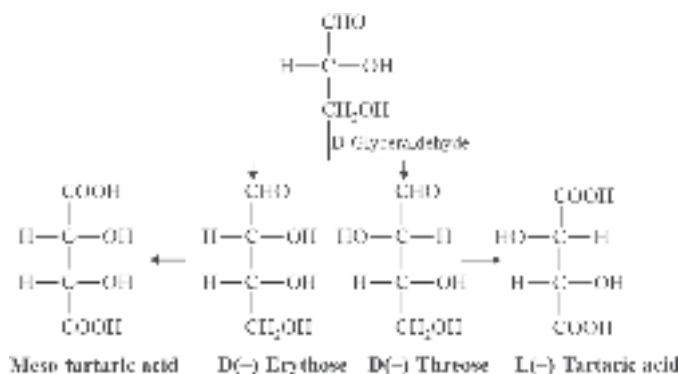


Fig. 2.6: Configuration of aldotetroses

(iii) Aldopentoses

The general structure of aldopentoses is $\text{CHO}-(\text{CHOH})_3-\text{CH}_2\text{OH}$ indicates that they are having three different asymmetric carbon atoms and hence must exist in eight optically active forms, i.e. four pairs of enantiomorphs. All are familiar to D- and L-forms of ribose, arabinose, xylose, lyxose. The configuration of these four enantiomeric pairs may be accomplished by following two methods.

In the first method, they are formed by lengthening the two aldotetroses by one carbon atom (Kiliani synthesis) and the products oxidized to dibasic acids which are optically examined (Fig. 2.7).

In experimental fact, arabinose on oxidation yields an optically active dibasic acid, while ribose on oxidation yields optically inactive acid (due to internal compensation). Similarly, lyxose gives optically active acid while xylose yields inactive acid (due to internal compensation) upon oxidation.

There is alternate method and has no need to know the configuration of parent compounds, i.e. aldotetroses. The D-forms possess four enantiomeric pairs of aldopentoses. On oxidation with nitric acid, compound 2 and 4 give optically active dicarboxylic acid which is arabinose and lyxose, while compound 1 and 3 on similar treatment will yield optically inactive acids due to presence of a plane of symmetry so ribose and xylose will be 1 and 3.

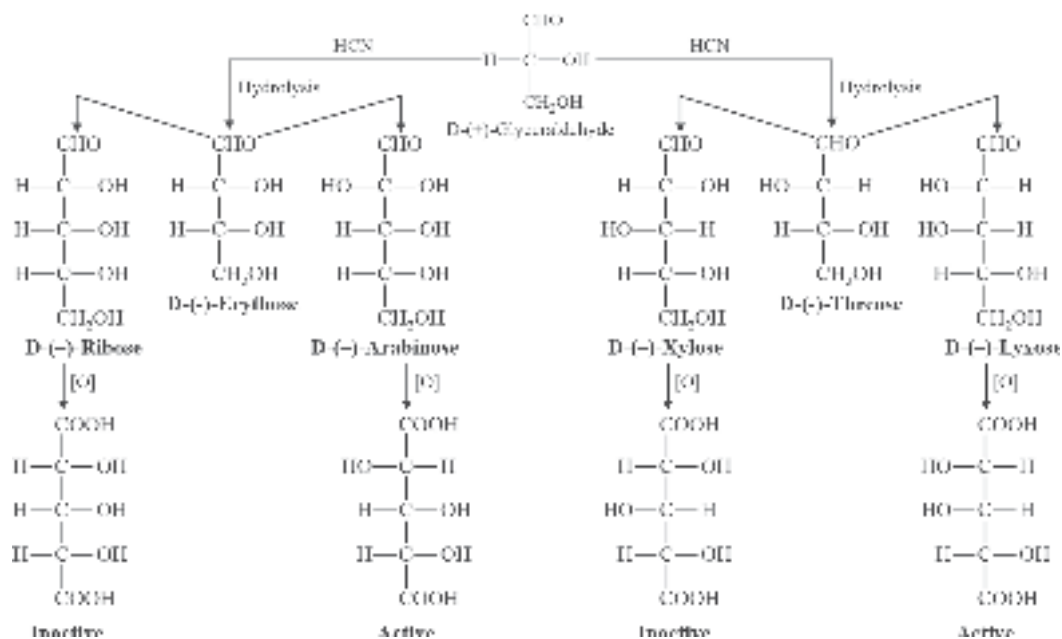
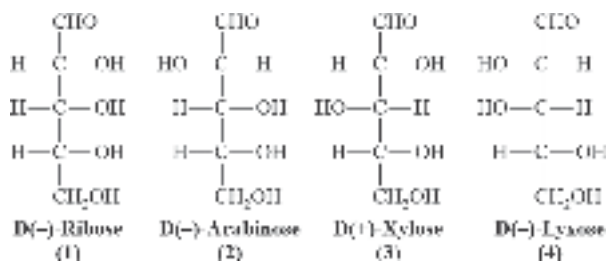


Fig. 2.7: Configuration of aldopentoses



To make it precise, they are stepped by one carbon and then led to oxidation for formation of carboxylic acid. Experimentally it is found that both the ribose and lyxose give one active and one inactive (meso) carboxylic acid while remaining two yields active carboxylic acid.

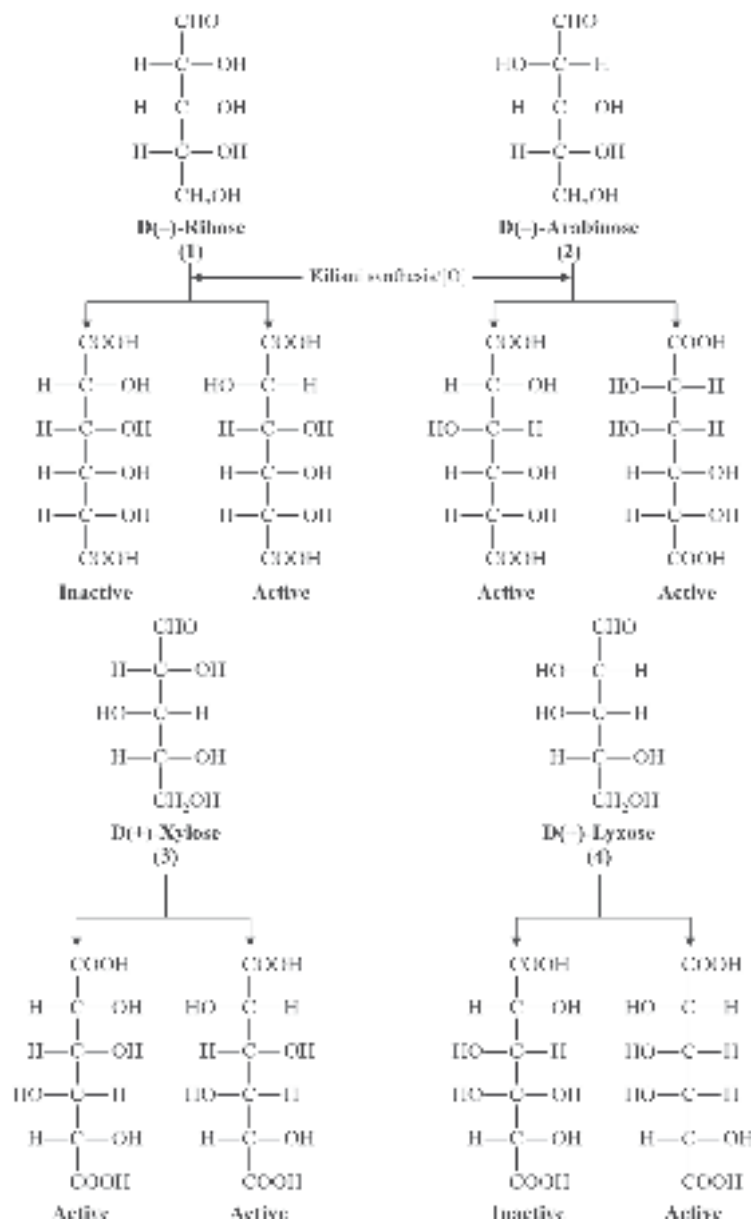
The above accomplished configuration by the experimental fact xylose and lyxose show the same osazone while ribose and arabinose show the same osazone which suggest that in both the cases the 2 sugars only in the configuration of the second carbon atom, i.e. 1 and 2 are epimers as are 3 and 4.

(iv) Aldohexoses

Aldohexose depicts the presence of four asymmetric carbon atoms and hence, they may exist in sixteen ($2^4 = 16$) optically active forms, i.e. eight enantiomeric pairs. The generalized structural formula of the aldohexose and complete D-aldose family are given in Fig. 2.8.

Similar to other aldoses, the oxidation to dicarboxylic acid has the key role to ascertain the configuration.

1. As indicated from oxidation, allose gives an optically inactive acid (allomucic acid), although altrose gives optically active (talmucic) acid.



- Similarly, the configuration of galactose and talose is accomplished on oxidation with nitric acid, the former ascertain an optically inactive (mucic) acid while other gives optically active (talomucic) acid.
- On oxidation both glucose and mannose prevailed from arabinose and gulose and idose from xylose ascertain optically active acids. The configuration is regulated by different method. Experimentally, it has been found that, glucose and gulose give the same dicarboxylic acid (saccharic acid). As we know that, during oxidation only terminal groups CH_2OH and CHO are changed into acidic groups with the rest of the molecule being unchanged.
- D-allose and D-altrose prevailed from D-ribose.

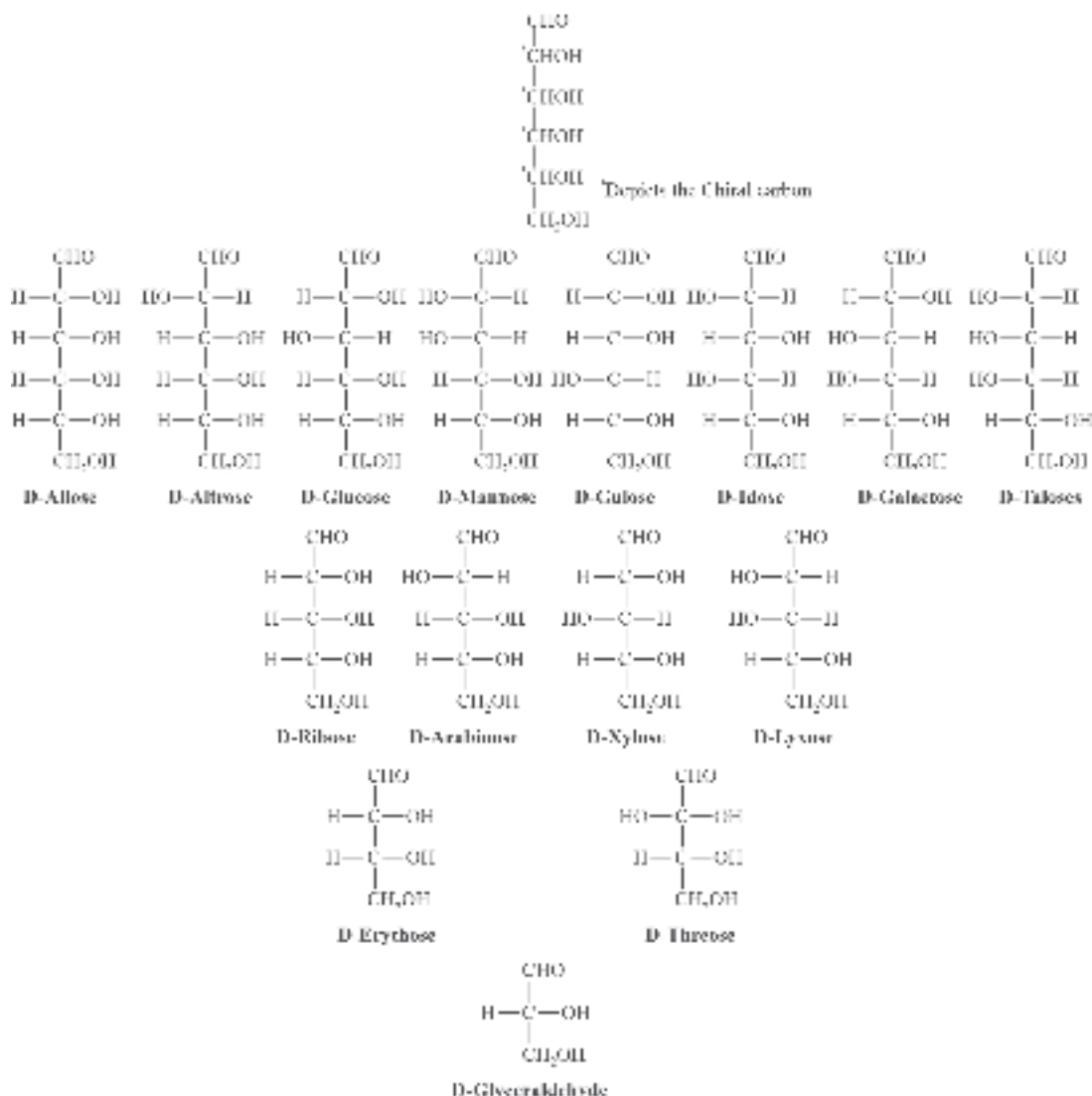


Fig. 2.8: Chart of D-family tree of aldoses

Ketohexoses

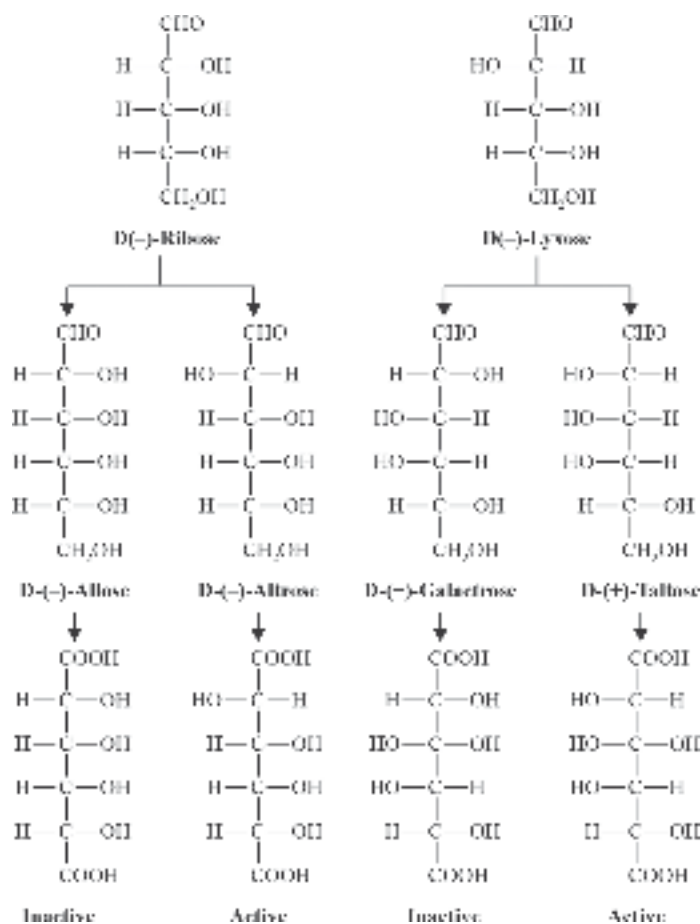
The general formula of ketohexoses:



All the ketohexoses that occur in nature have the keto group adjacent to a terminal CH_2OH group. Thus, there are three chiral carbon atoms present in the structural formula of a ketohexose; hence, eight stereoisomeric forms possible, i.e. four pairs of enantiomers (Fig. 2.9).

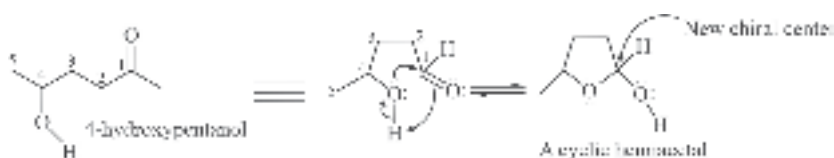
RING STRUCTURE OF ALDOSES

It is a fact that aldehydes and ketones react with alcohols to form hemiacetals. This also implies for the formation of cyclic hemiacetals that form very readily when hydroxyl

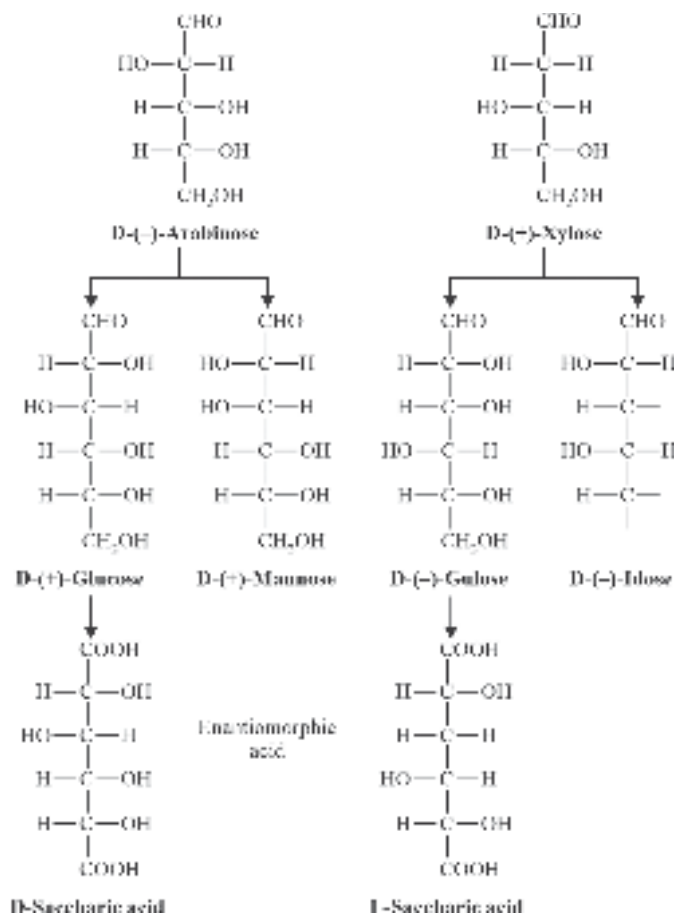


and carbonyl groups are part of the same molecule and their interaction can form a five- or six-membered ring. For example, 4-hydroxypentanal forms a five-membered cyclic hemiacetal.

Note that 4-hydroxypentanal contains one chiral center and that a second chiral center is generated at C-1 position as a result of hemiacetal formation.



Monosaccharides have hydroxyl and carbonyl groups in the same molecule. As a result, they too exist almost exclusively as five/six-membered cyclic hemiacetals. Soon after the determination of open structure formula of glucose, it is apparent that the open chain structure proposed by E. Fischer does not account for following reactions of glucose.



- (i) Glucose does not react with the characteristic reagent of aldehydes such as the color reaction with Schiff's reagent and the formation of a stable addition product with sodium bisulphite.

$$R-CHO \xrightarrow{NaHSO_3} R-\overset{\overset{H}{|}}{\underset{\underset{SO_3Na}{|}}{C}}-OH$$
- (ii) On acetylation glucose yields 2 different pentacetates (designated α and β).
- (iii) Glucose forms of crystalline products when refluxed with methanolic hydrogen chloride: methyl α - and methyl β -D-glucosides, two stereoisomers with: α -isomer (α) D + 158° m.p., 166° β -isomer (α) D -34° m.p., 108°. These glucosides have no reducing properties. In the D-series the sugar isomer with the most positive rotation is the α -isomer, the isomer with the low rotation being called β -isomer.
- (iv) Corresponding with the glucosides two α - and β -modifications of D-glucose itself are isolated α -(α)D + 112°, m.p. 146° and β -(α)D + 18.7° m.p., 156°. α -D-glucose crystallizes from water below 35° or from cold ethanol. β -D-glucose crystallizes from water above 98° or from the pyridine or hot acetic acid, or may be prepared by heating α -D-glucose at 105° for some time.

The two forms are interconvertible in solution when either form is dissolved in water the rotation gradually changes (mutarotation) until an equilibrium value (+52.7°) is reached. Mutarotation occurs through the aldehyde form as intermediates; this form being present normally to the extent of less than 1%. The low concentration of the aldehyde form does not favors reaction with Schiff's reagent.

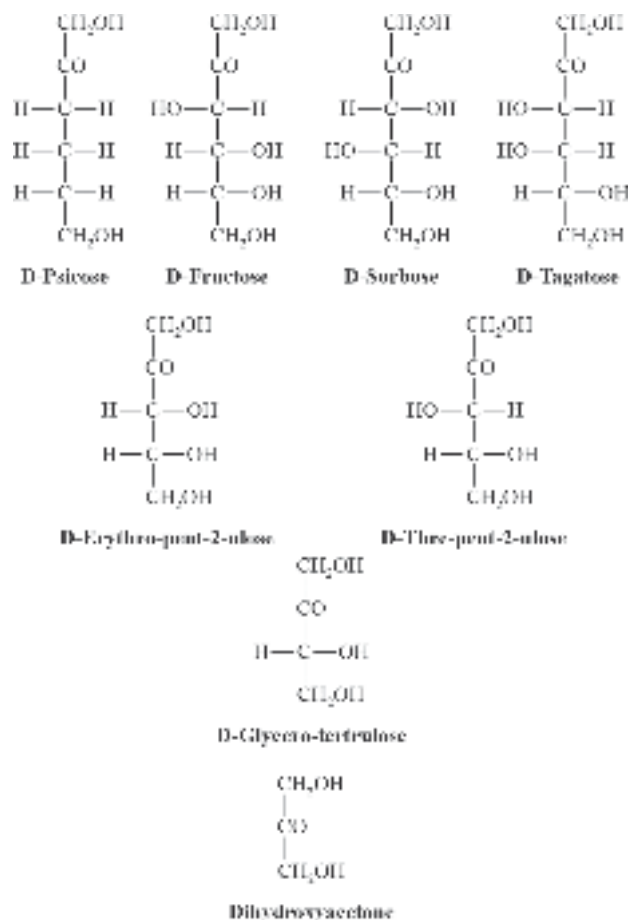
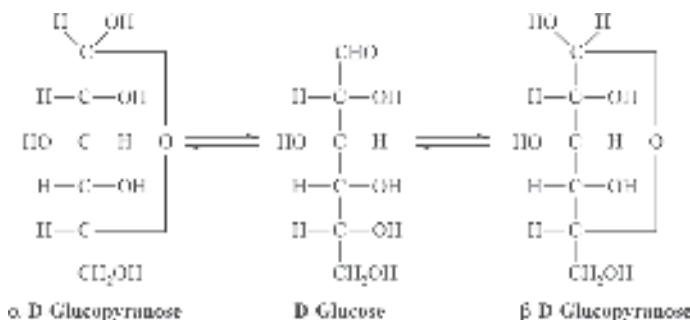


Fig. 2.9: Chart of D-family tree of ketoses

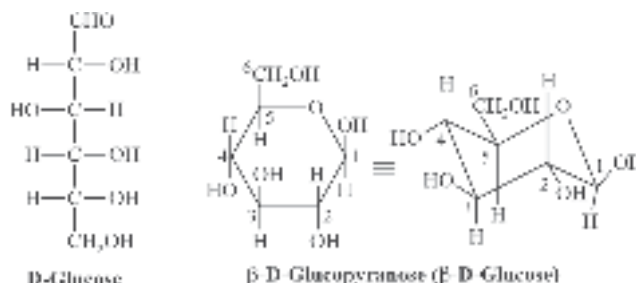
The α - and β -forms which differ in configuration at C-1 position only are known as anomers (ano: upper).



The hydroxyl group on C-1 position is *cis* to the hydroxyl on C-2 position in the α -form and *trans* in the β -form is deduced by Boeseken who found that the α -form of D-glucose increases conductivity of boric acid solution considerably and the increase is great for the *cis*- than the *trans* arrangement of hydroxyl group. The ring structure of glucose (1,5) is a six-membered ring and is described as a pyranose ring having similarity to pyran while the five-membered ring is described as a furanose ring.

The glycosides of the pyranose sugars are described as pyranosides and those of the furanose sugars as furanosides.

A monosaccharide existing as a five-membered ring is a furanose; one existing as a six-membered ring is a pyranose. A pyranose is most commonly drawn as either a Haworth projection or a chair conformation.



Mutarotation

Mutarotation is discovered by French chemist **Dubrunfaut** in 1846, when he noticed that the specific rotation of aqueous sugar solution changes with time. Mutarotation (muta, meaning change) is the change in specific rotation that accompanies the interconversion of α - and β -anomers in aqueous solution. As an example, a solution prepared by dissolving crystalline α -D-glucopyranose in water shows an initial rotation of $+112^\circ$, which gradually decreases to an equilibrium value of $+52.7^\circ$ as α -D-glucopyranose reaches an equilibrium with β -D-glucopyranose (Fig. 2.10).

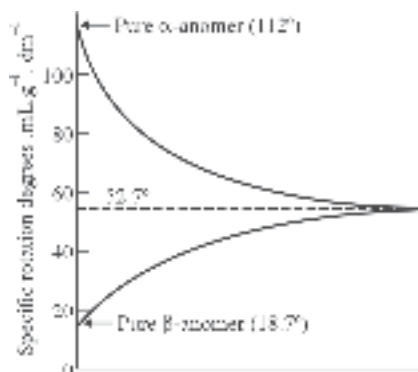
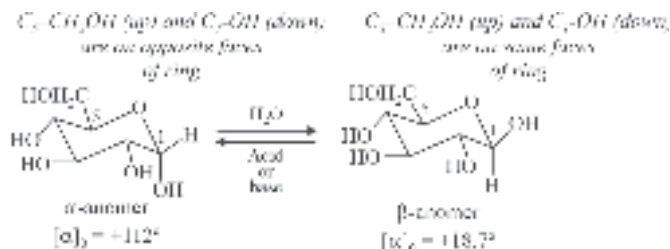


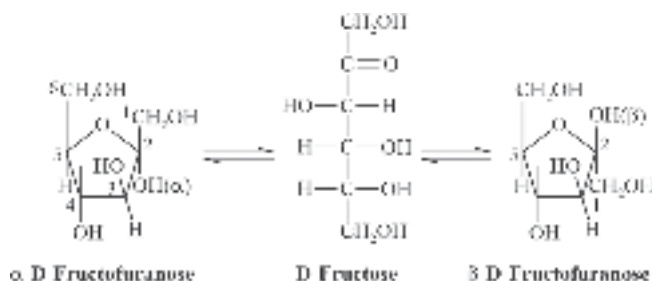
Fig. 2.10: Mutarotation of D-glucose (equimolar aqueous solution of pure α - or β -glucopyranose gradually change their specific optical rotations to the same final value that is characteristic of the equilibrium mixture)

A solution of β -D-glucopyranose also undergoes mutarotation, during which the specific rotation changes from an initial value of $+18.7^\circ$ to the same equilibrium value of $+52.7^\circ$. The equilibrium mixture consists of 64% β -D-glucopyranose and 36% α -D-glucopyranose. It contains only traces (0.003%) of the open-chain aldehydic form. Mutarotation is common to all carbohydrates that exist in hemiacetal forms.

The mutation of glucose is caused by the conversion of the α - and β -glucopyranose anomers into an equilibrium mixture of both. The same equilibrium mixture is formed, as it must be, from either pure α -D-glucopyranose or β -D-glucopyranose. Mutarotation is catalyzed by both acid and base, but also occurs even in pure water.



It is important to notice that mutarotation occurs due to presence of cyclic hemiacetal form of glucose; an aldehyde cannot undergo mutarotation. The ketohexose (fructose) also show the mutarotation.



Note: The β -D-fructofuranose form is found in the disaccharide, sucrose.

Mechanism for Base-catalyzed Mutarotation of Glucose

The phenomenon of mutarotation indicates that the two forms of D(+) glucose are interconvertible and they have a common intermediate. This change presumably takes place via the open change structure of glucose (Fig. 2.11).

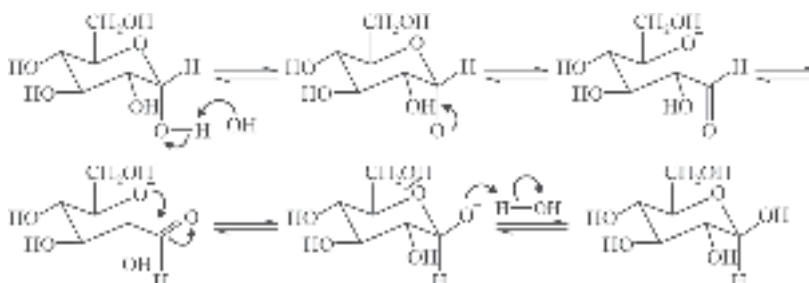


Fig. 2.11: Base catalyzed mutarotation of glucose

When mutarotation takes place, the ring of one form opens up and then recloses in the inverted position.

Mechanism for Acid-catalyzed Mutarotation of Glucose

It is depicted in Fig. 2.12. It also involves the ring opening followed by ring closure.

DETERMINATION OF THE RING SIZE IN ALDOSES

The ring size of sugar molecules is determined by following methods:

1. Haworth methylation method

Methyl glucoside is completely methylated to methyl tetra-*o*-methyl glucoside which, on acidification followed by oxidation with nitric acid, gives 2,3,4-trimethoxy glutaric acid (Scheme 2.2).

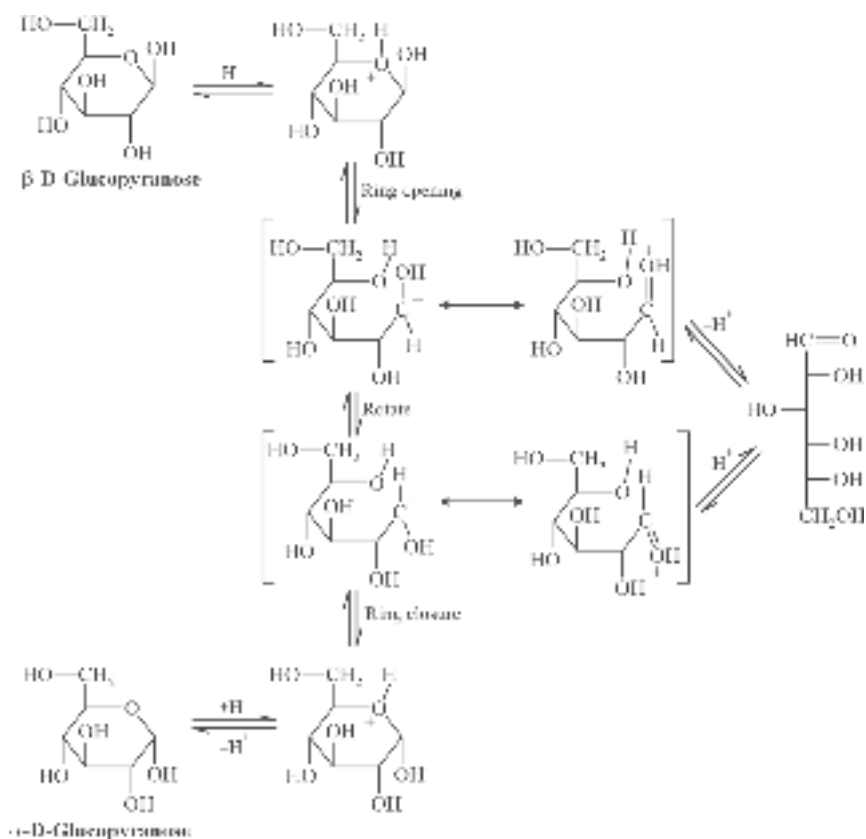
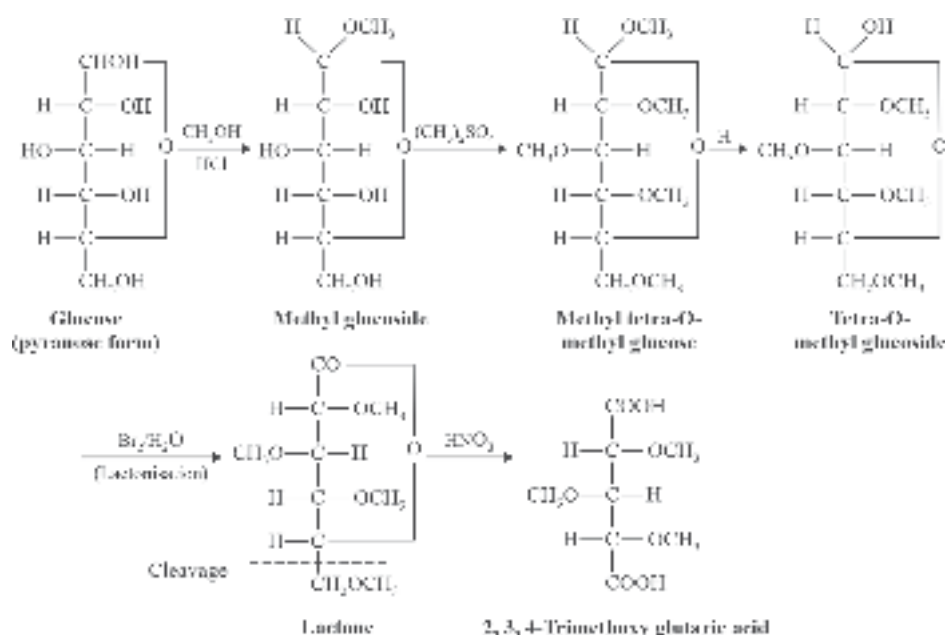
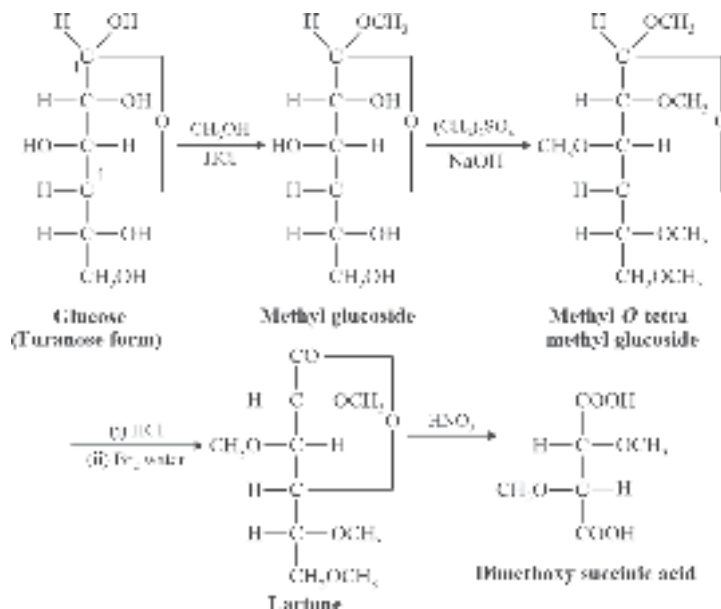


Fig. 2.12: Mechanism of acid catalyzed mutarotation



Scheme 2.2: Determination of pyranose ring by methylation method



Scheme 2.3: Determination of furanose ring by methylation method

Obviously, the carboxylic carbons of the trimethoxy glutaric acid are the one's those originally involved in ring formation.

Hence formation of 2,3,4-trimethoxy glutaric acid indicates the presence of 1,5-linkage (pyranose form). The presence of 1,4-ring (furanose) would give dimethoxysuccinic acid as shown in Scheme 2.3. In both the schemes, it is shown that groups which engage the ring are converted to carboxylic group on oxidation in the final products, xylotrimethoxy glutaric acid and dimethoxysuccinic acid.

2. Jackson and Hudson: Periodic Acid Oxidation Method

The direct method of establishing the ring size involves the oxidation of the glucosides by periodic acid. The reagent cleaves the linkage between adjacent hydroxyl-bearing carbon atoms. A primary alcohol ($-\text{CH}_2\text{OH}$) yields formaldehyde, a secondary alcohol ($-\text{CHOH}$) gives an aldehyde group or, if flanked by two secondary alcohol groups, splits out formic acid.

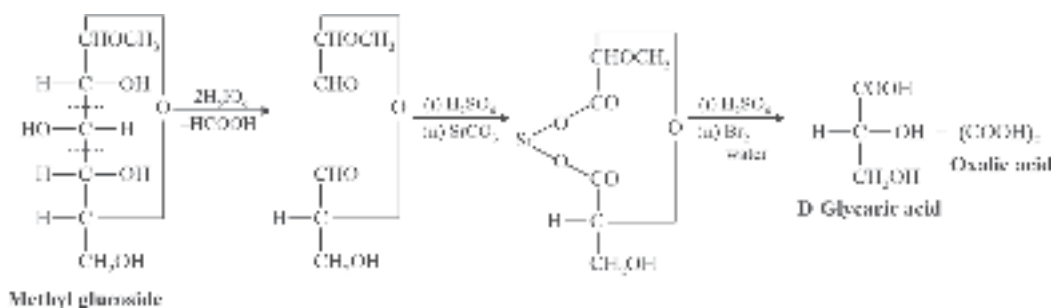
The reaction with periodic acid is quantitative and the consumption of periodic acid or periodate gives a measure of the number of adjacent hydroxyl groups in a compound. The yield of formic acid and formaldehyde is also quantitative and can be estimated after the reaction.

Let us now consider the result of a periodic acid oxidation of the 5 possible ring structures of glucoside. Each structure will give different reaction products. When the reaction is carried out experimentally, we expected results are: 2 moles of periodic acid are consumed, no formaldehyde, one mole of formic acid. These results are in agreement with what is expected with 1,5-ring (Table 2.3).

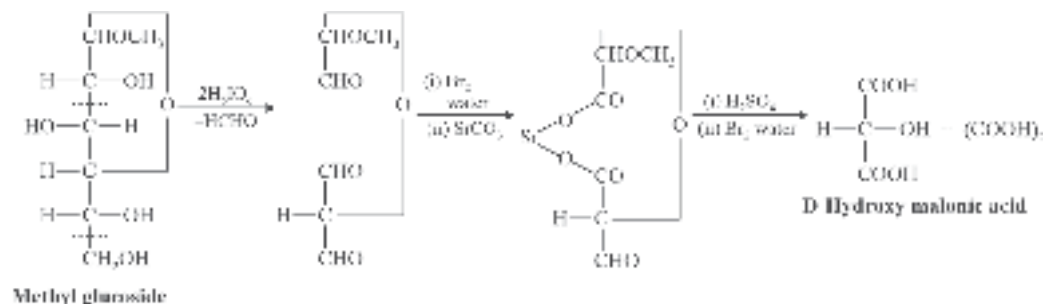
By knowing the oxidation products, the ring size can also be determined. The glucose (as methyl glucoside) in pyranose forms D-glyceric acid and oxalic acid and is shown in the Scheme 2.4.

Table 2.3: Periodic acid oxidation

No. of moles of periodic acid	3	2	2	2	3
No. of moles of HCHO	1	1	1	0	0
No. of moles of HCOOH	2	1	0	1	2

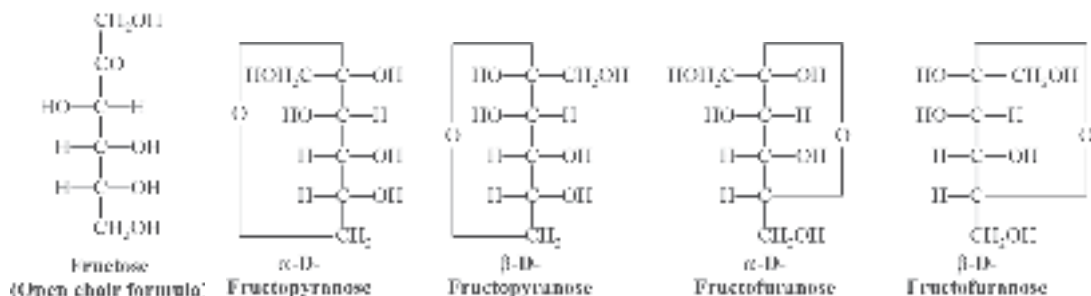
**Scheme 2.4:** Periodic acid oxidation of methyl glucoside (pyranose form)

The glucose in furanose form also consumes the two moles of periodic acid but forms hydroxy malonic acid and oxalic acid (Scheme 2.5). One molecule of HCHO is produced from primary hydroxyl group.

**Scheme 2.5:** Periodic acid oxidation of Methyl glucoside (Furanose form)

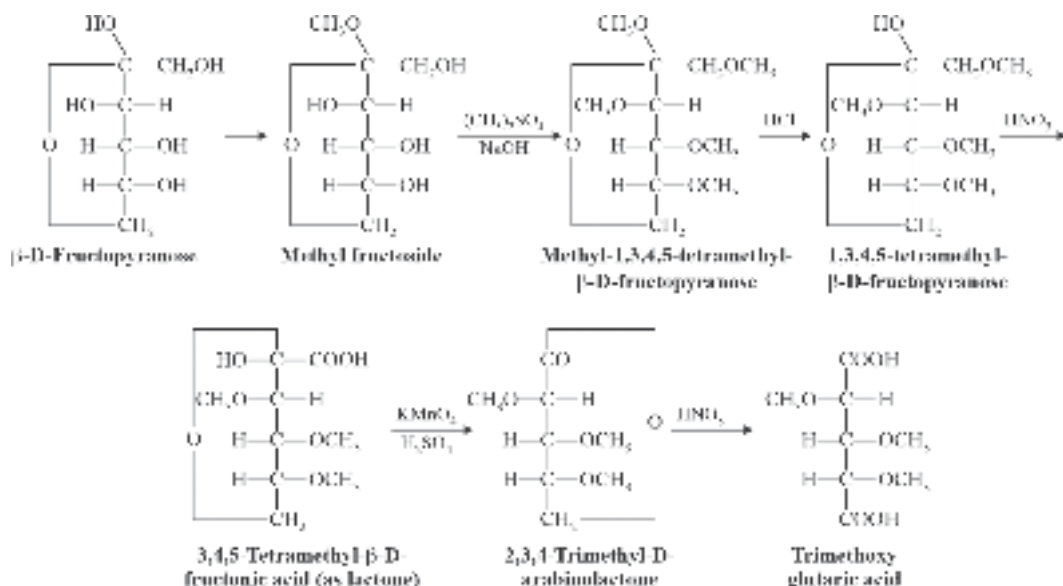
DETERMINATION OF THE RING SIZE IN KETOSES

Like aldoses, ketoses (D-fructose) may also exist in four isomeric forms: α -D-fructopyranose, β -D-fructofuranose, α -D-fructofuranose and β -D-fructofuranose.



Methylation Method

The same sequence of reactions as in aldoses is followed to determine the pyranose or furanose structure by methylation method (Scheme 2.6).



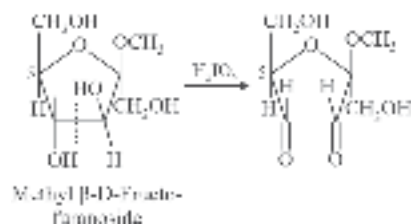
Scheme 2.6: Determination of ring structure in fructose by methylation method

The fructose is converted into methylated tetra-O-methyl fructopyranose or furanoside. The methyl tetra-O-methyl fructoside is hydrolysed with dilute HCl to get tetramethyl-D-fructopyranose which on oxidation with HNO_3 gives trimethyl- β -fructonic acid. On lactonisation, followed by oxidation, this acid in pyranose form gives trimethoxy glutaric acid. In a similar sequence of reaction, fructose in a furanose form (1,4-oxide) gives the dimethyl tartaric acid.

Periodic Acid Method

The periodic acid react only a one site in the molecule. The fructoside, therefore, must be a five membered ring (α fructofuranoside).

Methyl 13- β -fructoside consumes only one mole of periodic acid and produces neither formaldehyde nor formic acid. Thus, oxidizable groups exist on adjacent carbons.



STRUCTURAL REPRESENTATION OF SUGARS

The structure of monosaccharides is presented in three forms:

1. The Fischer projection: Straight chain representation
2. Haworth projection: Simple ring in perspective
3. Conformational representation: Chair and boat configurations.

1. The Fischer Projection

It is devised by **Hermann Emil Fischer** in 1891, is a two-dimensional representation of a three dimensional organic molecule by projection. All bonds are depicted as horizontal or vertical lines.

The carbon chain is depicted vertically, with carbon atoms represented by the center of crossing lines. The orientation of the carbon chain is so that the C-1 carbon is at the top. All the presentations used above are Fischer projections.

2. Haworth Projection

A common way of representing the cyclic structure of monosaccharides is the Haworth projection, named after the English chemist **Sir Walter N. Haworth** (1937 Nobel Prize for chemistry). In a Haworth projection, a furanose (five)-or pyran (six-membered) cyclic hemiacetal is represented as a planar pentagon or hexagon, as the case may be, lying perpendicular to the plane of the paper. The ring is labelled clockwise starting from carbon adjacent to oxygen atom as shown in the Fig. 2.13. Groups bonded to the carbons of the ring then lie either above or below the plane of the ring. If the substituent is to the right in the Fischer projection, it will be drawn down in the Haworth projection (*Down-Right Rule*). If the substituent is to the left in the Fischer projection, it will be drawn up in the Haworth projection. In D-sugars the highest numbered carbon (furthest from the carbonyl) is drawn up. For L-sugars, it is drawn down. The new chiral center created in forming the cyclic structures called an anomeric carbon. Stereoisomers that differ in configuration only at the anomeric carbon are called anomers. The anomeric carbon of an aldose is carbon 1; that of the most common ketoses is carbon 2. Haworth projections are most commonly written with the anomeric carbon to the right and the hemiacetal oxygen to the back. In the terminology of carbohydrate chemistry, the designation 13 means that the OH on the anomeric carbon of the cyclic hemiacetal is on the same side of the ring as the terminal CH₂OH. Conversely, the designation a means that the OH on the anomeric carbon of the cyclic hemiacetal is on the opposite side of the ring as the terminal CH₂OH. Here, in the furanose or pyranose structure, a carbohydrate has one more asymmetric carbon than the open chain form C-1 in the case of the aldoses. Thus, there are two possible diastereomers of D-glucopyranose.

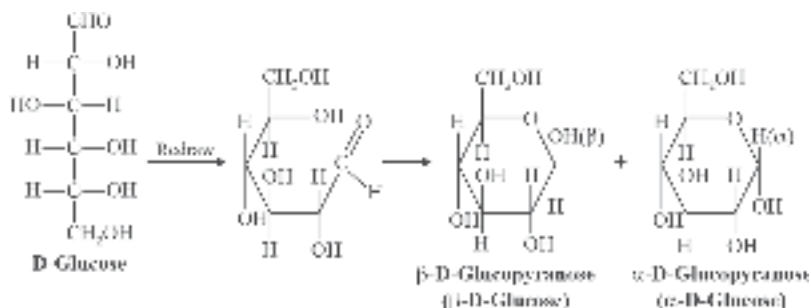


Fig. 2.13: Haworth projection

3. Conformational Representation

The six-membered pyranose ring is not planar because of the tetrahedral geometry of its saturated carbon atoms. Instead, pyranose rings adopt two classes of conformations, termed *chair* and *boat* because of the resemblance to these objects.

Chair form is more stable than the boat form in which the bulky atomic groups (e.g. the hydroxyl and hydroxymethyl groups) are equatorial or within the plane of the ring (see Fig. 2.14).

The hydrogens would then be axial or perpendicular to the plane of the ring. Only in β -D-glucopyranose are all of the bulky groups equatorial; thus, β -D-glucopyranose is more stable than α -D-glucopyranose, in which the C-1 hydroxyl group is axial to the ring.

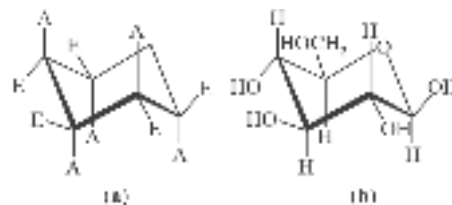


Fig. 2.14: Chair form (a) showing the equatorial and axial position (b) β -D-glucopyranose

CONSTITUTION OF SOME SIGNIFICANT MONOSACCHARIDES

1. Glucose

Glucose, a simple monosaccharide sugar, is one of the most important carbohydrates and is used as a source of energy in animals and plants. The natural form (D-glucose) is also referred to as **dextrose**, especially in the food industry. Glucose is produced commercially via the enzymatic hydrolysis of starch.



Structural Elucidation

- (i) Molecular formula: It is determined to be $C_6H_{12}O_6$.
- (ii) Reduction with hydroiodic acid and red phosphorus yield several products including hexaiodo-*n*-hexane and *n*-hexane itself, denoting that the carbon atoms are linked in an open chain structure in glucose molecule.
- (iii) Glucose gives the characteristic reactions of carbonyl compounds, e.g. it gives an oxime, semicarbazone, and phenylhydrazone, etc.
- (iv) On oxidation, it gives gluconic acid that possess the same number of carbon atoms. This concludes that the carbonyl group is aldehydic in nature.
- (v) On acetylation, it gives a pentacetate denoting that it contains 5-hydroxyl groups (Fig. 2.15).
- (vi) The 6 remaining hydrogen atoms are then added to complete the valency of carbon atoms. It is clear that such a molecule have 4-asymmetric carbon atoms.
- (vii) On the basis of above evidences, it is suggested that glucose is a pentahydroxy aldehyde and has the structure as given in Fig. 2.15 which can explain all the reactions.
- (viii) **Configuration of glucose:** The configuration is determined with the help of Kiliani synthesis as discussed above.

- (ix) **Ring size determination:** It is determined by methylation method or periodic acid oxidation method as discussed above.

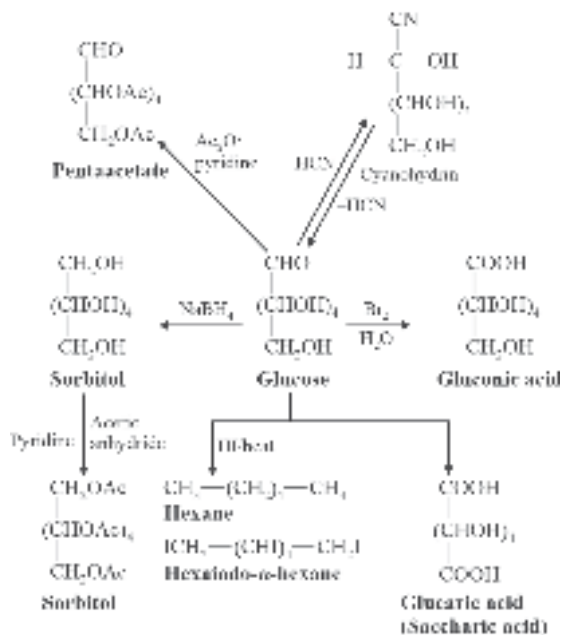


Fig. 2.15: Important reaction of glucose

Spectral data: IR (cm^{-1}): 3402, 3318 (OH stretch), 1140–1070 (C—O stretching), 1150 to 1075 (primary alcohols).

2. Fructose

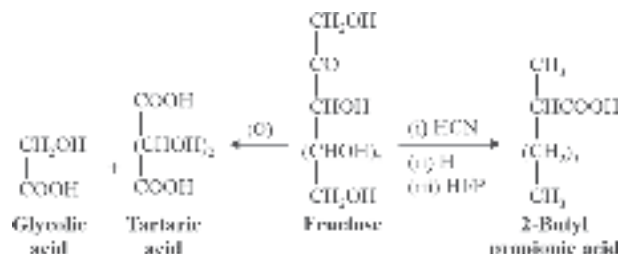
Fructose is more commonly found together with glucose and sucrose in honey and fruit juices. Fructose, along with glucose is the monosaccharide found in disaccharide, sucrose. Fructose is classified as a monosaccharide, the most important **ketose sugar**, a hexose, and is a reducing sugar. Crystalline fructose melts at 104°C . It is the sweetest sugar (about 1.2–1.8 times sweeter than sucrose) but is seldom used in pure form due to multiple health consequences.

An older common name for fructose is levulose, after its levorotatory property of rotating plane polarized light to the left (in contrast to glucose which is dextrorotatory). Glucose and fructose are formed in equal amounts when sucrose is hydrolyzed by the enzyme invertase or by heating with dilute acid; the resulting equimolar mixture of fructose and glucose, called invert sugar, is the major component of honey.

Structural Elucidation

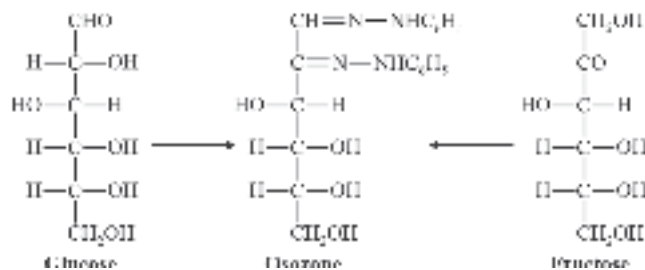
- (i) Fructose has molecular formula, $\text{C}_6\text{H}_{12}\text{O}_6$.
- (ii) The acetylation reaction indicates the presence of five hydroxyl groups.
- (iii) Like glucose, it reacts with HCN and NH_2OH to form cyanohydrins and oxime, respectively. All these reactions show the presence of carbonyl group. Since fructose on oxidation with nitric acid gives a mixture of tartaric acid and glycolic acid, both having less number of carbon atoms, carbonyl group is present as keto group. The formation of glycolic acid and tartaric acid also suggests the presence of keto group at C-2 position. Fructose is not oxidized by bromine water. This rules out the possibility of presence of aldehyde group.

- (iv) On reduction with sodium amalgam, fructose gives hexahydric acid, sorbitol and mannitol. These alcohols on reduction with HI/red P gives the 2-iodohexane and hexane, which indicates that fructose is a straight chain compound.
- (v) Fructose on reaction with HCN gives the cyanohydrins, which on hydrolysis followed by reduction produces the 2-butyl propionic acid. The formation of this acidic compound again confirms the presence of keto group at 2-position.

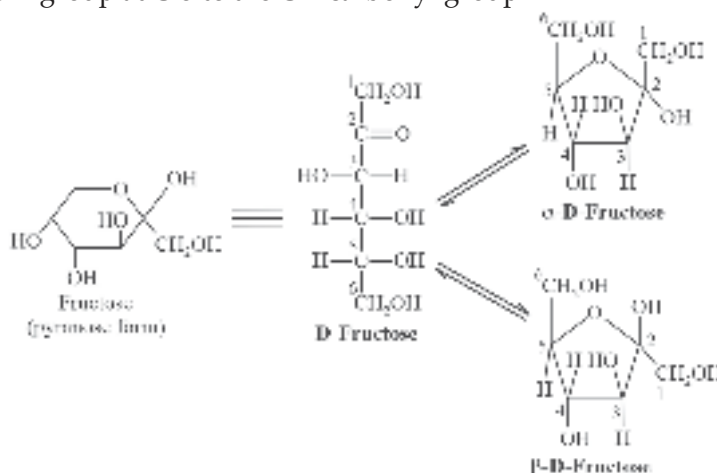


On the basis of above evidences, the structure of fructose may be written as given below.

- (vi) **Configuration of fructose:** The above assign structure contains three asymmetric carbon atoms so eight isomers ($2^3 = 8$) should exist. As it gives the same osazone as produced by glucose, the configuration of C-3 to C-5 should be same as glucose. Hence, the complete open chain formula may be written which can give the same osazone formation.

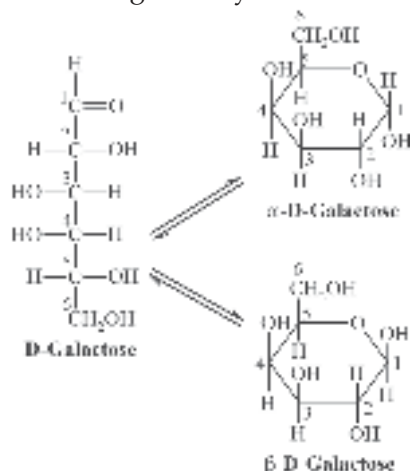


- (vii) **Ring size of fructose:** Fructose exists to the extent of about 80% in the pyranose form and about 20% as the five-membered furanose form resulting from addition of the —OH group at C-5 to the C-2 carbonyl group.



3. D(+)-Galactose (mol formula: $C_6H_{12}O_6$)

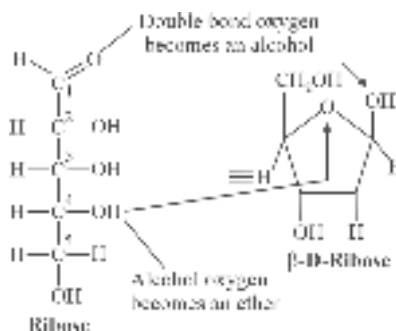
Galactose is found in the biological system as a component of the disaccharide lactose (milk sugar). Most of the galactose ingested by humans is converted to glucose.



The aqueous solution of galactose exhibits mutarotation. The specific rotation of α -D-galactose, β -D-galactose and equilibrium are $+1.4^\circ$, $+52^\circ$ and 83.3° , respectively. Chemically, it behaves like glucose.

4. Ribose

Ribose is an aldo pentose sugar occurring as a component of riboflavin, nucleotides, and nucleic acids. Ribose and its related compound, deoxyribose are the building blocks of the backbone chains in nucleic acid.



CONSTITUTION OF SOME SIGNIFICANT DISACCHARIDES

1. Sucrose (β -D-fructofuranosyl-(2 $\rightarrow$ 1)- α -D-glucopyranoside)

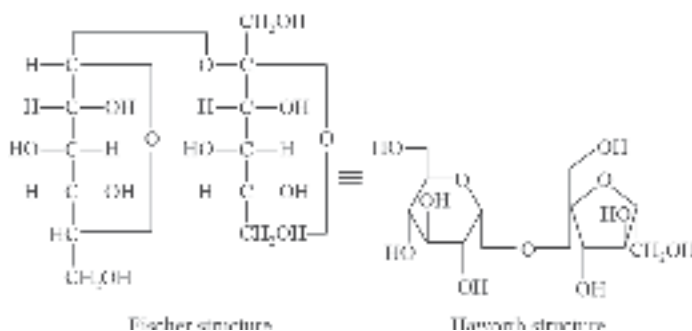
Sucrose is commonly known as table sugar and sometimes called saccharose. It is a white, odourless, crystalline powder with a sweet taste, it is best known for its role in human nutrition. The most important sources are sugarcane and sugar beets; other sources are maple saps, honey and fruits juices.

Structural elucidation

- (i) It has molecular formula, $C_{12}H_{22}O_{11}$.
- (ii) On hydrolysis with acids or enzymes, it gives equal parts of D-glucose and D-fructose, which constitutes the two monosaccharide units of sucrose.

- (iii) It neither reacts with phenylhydrazine nor reduce Fehling's solution indicating that carbonyl group of both the monosaccharides are involved in linkage, i.e. the glucose is linked via its C-1 (CHOH) to the C-2 of fructose unit.
- (iv) It is hydrolyzed by maltase but not by emulsin, thus indicating an α -D-glucose unit. It is also hydrolyzed by an enzyme take invertase that is believed to be specific for β -fructofuranosides, thus indicating a β -D-fructofuranose unit in sucrose.
- (v) Now the only problem in assigning the complete structure to sucrose is to determine the size of glucose and fructose units. It is ascertained by usual methods. Complete methylation of sucrose with dimethyl sulphate in basic solution followed by hydrolysis gives 2,3,4,6-tetra-O-methyl D-glucose and 1,3,4,6-tetra-O-methyl D-fructose (Scheme 2.7). It indicates that glucose is present in pyranose form while fructose residue is in the form of fructofuranose.

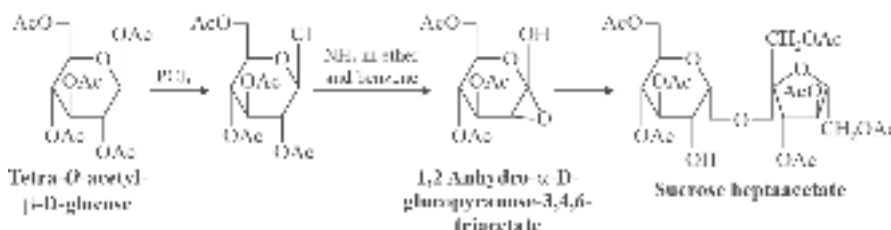
Thus, the sucrose has the following structure which accounts for all the above facts. Both the Fischer and Haworth structures are shown here.



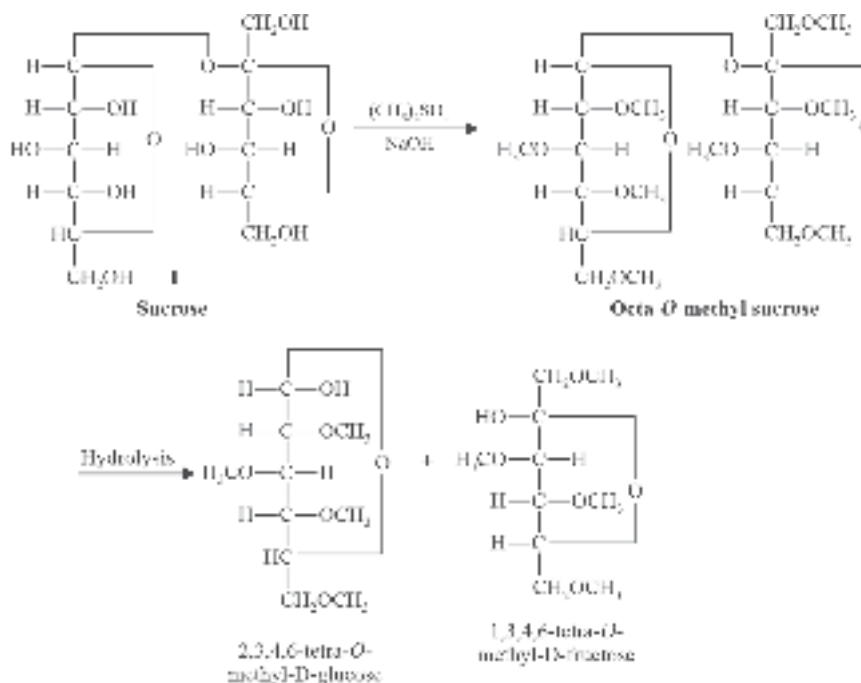
The assigned structure accounts for all of the above facts as shown in Scheme. 2. 7.

This structure for sucrose is confirmed by several physical and chemical evidences.

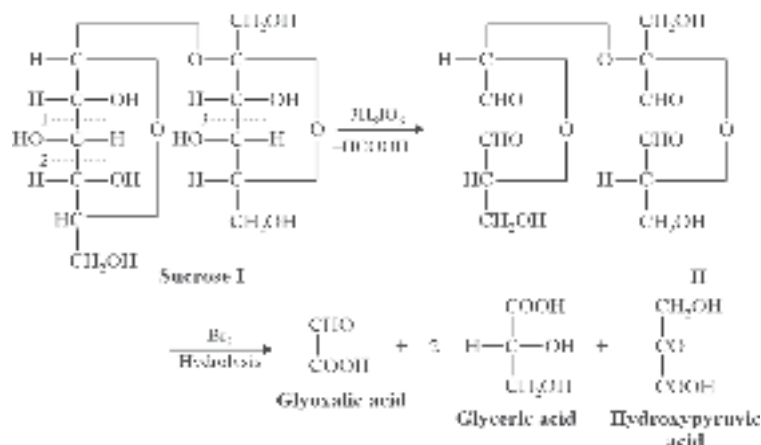
- a. **Periodic acid oxidation:** Periodate oxidation confirms the structure of sucrose (except the nature of the glycosidic link), especially the size of the two monosaccharide units. Sucrose I consumes three moles of periodic acid and forms one mole of formic acid and a tetra-aldehyde, the latter on oxidation with bromine followed by acid hydrolysis gives glyoxalic acid, glyceric acid, and hydroxypyruvic acid, (Scheme 2.8).
- b. **X-ray analysis:** The X-ray of sucrose sodium bromide dihydrate confirmed the stereochemical configuration found chemically, and also the five membered ring of fructose.
- c. **Synthesis of sucrose:** Although sucrose is invariably isolated from natural sources, its chemical synthesis as shown below, first achieved in 1953 by Raymond Lemieux.



Spectral data: IR (cm^{-1}): 3385, 3338 (O—H stretching), 2932 (C—H stretching), 1119 (C—O stretching).

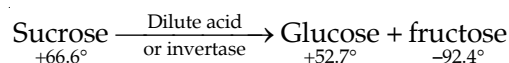


Scheme 2.7: Determination of ring structure in sucrose by methylation method



Scheme 2.8: Determination of ring structure in sucrose by periodate method

Invert sugar: Sucrose (disaccharide) is found to be dextrorotatory, has a specific rotation of $+66.5^\circ$. This on hydrolysis with dilute acid or enzyme, invertase yields equal amount of D(+)-glucose ($+52.7^\circ$) and D(-)-fructose (-92.4°). The resulting mixture shows levorotatory activity and so-called inversion of sucrose and mixture is known as invert sugar.

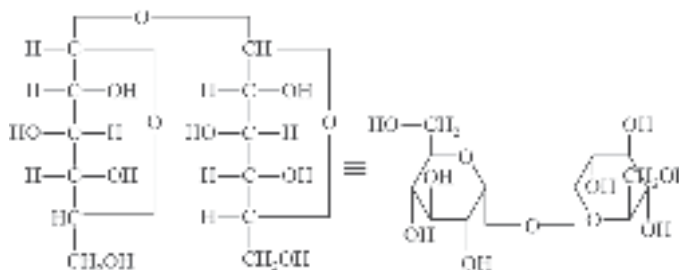


2. Trehalose (α -D-glucopyranosyl-(1 $\rightarrow$ 1)- α -D-glucopyranoside)

It is found in mushrooms, yeasts, and fungi. Trehalose is a disaccharide formed by α -1,1-glucoside bond between two α -glucose units. Because trehalose is formed by the bonding of two reducing aldehyde groups, it has no capacity to participate in the **Maillard reaction**.

Structural elucidation

- (i) Its molecular formula is $C_{12}H_{22}O_{11}$.
- (ii) Although it is relatively stable to acid hydrolysis. It gives two moles of D-glucose.
- (iii) It shows the reactions of non-reducing monosaccharides. For example, it neither reacts with phenyl hydrazine nor reduces Fehling's solution.
- (iv) On complete methylation followed by hydrolysis, trehalose yields two moles of 2,3,4,6-tetra O-methyl-D-glucopyranose, thus indicating that both of the glucose units are present as pyranose rings in trehalose. This is confirmed by periodic acid oxidation: four moles of acid are used to yield two moles of formic acid.
- (v) The higher specific rotation $[\alpha]_D = +197^\circ$ indicates that the two glucose residues are α,α -linkages.
- (vi) Thus, the structure of trehalose may be written as follows:



3. Maltose (4-O- α -D-Glucopyranosyl-D-glucose)

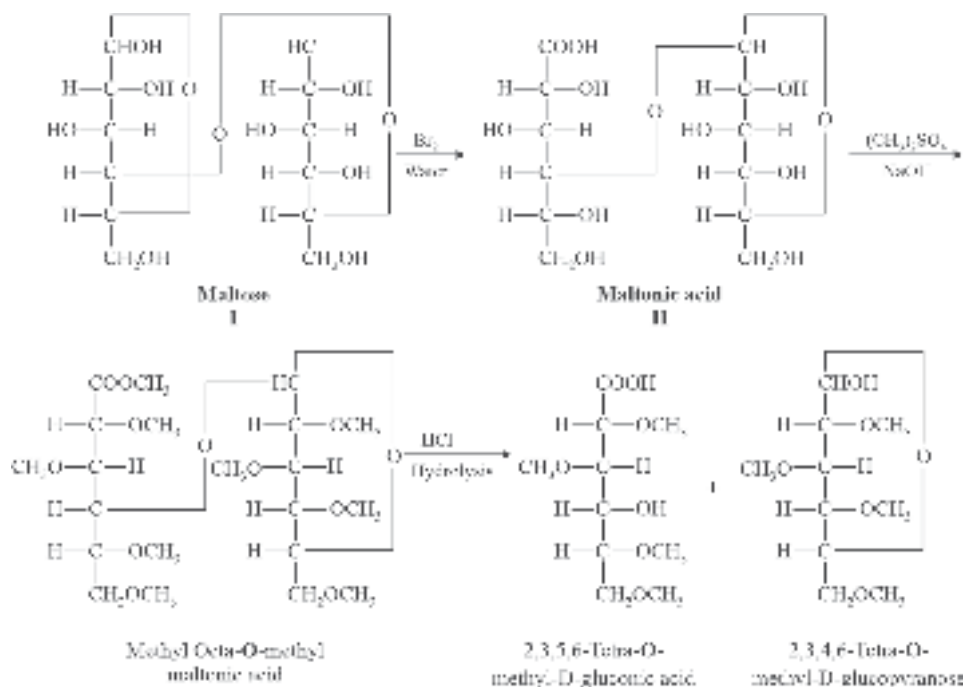
Maltose, or malt sugar, is a disaccharide formed from two units of glucose joined with an α (1 $\rightarrow$ 4) bond, formed from a condensation reaction. The isomer "isomaltose" has two glucose molecules linked through an α (1 $\rightarrow$ 6) bond. Maltose is the second member of an important biochemical series of glucose chains. Maltose is the disaccharide produced when amylase breaks down starch. It is found in germinating seeds such as barley as they break down their starch stores to use for food. It is also produced on caramelization of glucose. Maltose is a white crystalline solid, m.p. 160–165°C.

Structural elucidation

- (i) Its molecular formula is found to be $C_{12}H_{22}O_{11}$.
- (ii) On acidic hydrolysis, maltose yields only D-glucose indicating that glucose is the only monosaccharide unit present.
- (iii) Maltose reduces Fehling's solution, Benedict's solution, and Tollen's reagent. It is oxidized by bromine water to maltonic acid or maltobionic acid, forms osazone, m.p. 206°C with phenylhydrazine and exhibits mutarotation. All of these reactions indicates the presence of free aldehydic group in one of its monosaccharide unit.
- (iv) It is hydrolysed by maltase and by enzyme, emulsin indicating the presence of α -linkage between the reducing half of the maltose and non-reducing half.

(v) The ring sizes of the monosaccharide units are established by the following experiments:

(a) On oxidation with bromine water, maltose is oxidized to maltonic acid (i.e. the terminal aldehyde group is oxidized to carboxyl group). Complete methylation of maltonic acid to methyl octa-O-methyl maltonate followed by hydrolysis gives 2,3,4,6-tetra-O-methyl-D-glucopyranose (from the glucoside component) and 2,3,5,6-tetra-O-methyl gluconic acid (from the alcohol component). The presence of free hydroxyl groups in these products at C-1 and C-4 positions respectively indicates that the glycosidic linkage in maltose involved these carbons atoms, and that the original ring sizes in maltose are pyranose. Thus, maltose may be written as **I** which explains all the above degradations (Scheme 2.9).



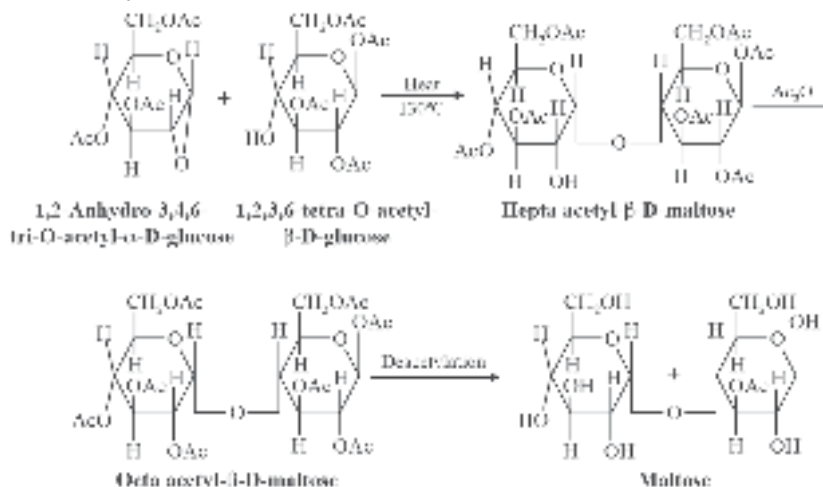
Scheme 2.9: Determination of ring size in maltose by methylation method

(b) On the basis of this, it can be proposed that C-1 position of the first molecule is linked with C-4 position of the 2nd glucose unit. The glucose molecule linked via C-4 position constitutes the reducing half.

(c) Lastly, it must be noted that the presence of free CHO in maltose causes it to exist in two different anomers, α and β .

The existence of two anomeric maltoses explains the phenomenon of mutarotation; the values of specific rotation $[\alpha]_D$ of α -maltose, β -maltose and the equilibrium mixture are 168° , 112° and $+136^\circ$, respectively.

(vi) Finally, the structure of maltose is confirmed by its synthesis from 1,2-Anhydro-3,4,6-tri-O-acetyl- α -D-glucose.



4. Cellobiose (4- β -D-glucopyranosyl-D-glucopyranose)

Cellobiose does not found in nature. It is formed as an intermediate product during the acidic hydrolysis of cellulose and may also be isolated in crystalline form by careful hydrolysis of cellulose. In structure it is similar to that of maltose except that it has the β -configuration at its glycosidic component which is indicated by its hydrolysis by emulsin. Hence, the structure of cellobiose is elucidated exactly in the same manner as maltose.

Now again the C-1 of the reducing half portion of the cellobiose causes the cellobiose to exist in two anomeric forms, *viz.* α and β . The specific rotation $[\alpha]_D$ of α , β and the equilibrium mixture is $+72^\circ$, $+16^\circ$ and $+35^\circ$, respectively.

The structure of cellobiose has been confirmed by several syntheses; the simplest unambiguous one consist in treating 2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl bromide (acetobromoglucose), **I** with 1,2,3,6-tetra-O-acetyl-D-glucopyranoside, **II** to form cellobiose acetate, **III** which on hydrolysis gives cellobiose, **IV**.

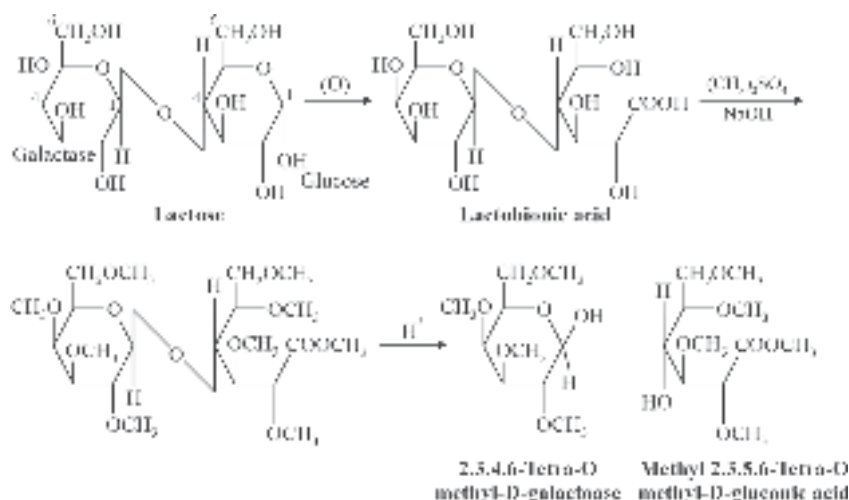


5. Lactose (4- β -D-galactopyranosyl-D-glucopyranose)

Lactose (milk sugar) occurs in mammalian milk, e.g. cow's milk contains 4–6% and human milk contains 5–8% of sugar. Lactose is white crystalline solid, m.p. 203°C (with decomposition), soluble in water and dextrorotatory in nature.

Structural elucidation

- (i) Its molecular formula is found to be $C_{12}H_{22}O_{11}$.
- (ii) Lactose responds the characteristics of reducing sugar, *viz.* it forms osazone, exhibits mutarotation, and reduces Fehling's, Tollen's and Benedict solutions. Therefore, lactose must possess at least one carbonyl group which is not involved in the disaccharide linkage.
- (iii) On acidic or enzymatic (*lactase*) hydrolysis, lactose gives equimolar amounts of glucose and galactose.
- (iv) Since lactose is hydrolysed only by *lactase* (identical with emulsin), the two monosaccharide units are linked through β -glycosidic linkage. This is also indicated by its two specific rotations.
- (v) Lactose, on complete methylation gives methyl heptamethyl lactoside which on vigorous hydrolysis yield 2,3,6-tri-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl galactose indicating that the glucose unit constitutes the reducing half of lactose.
- (vi) Lactose on oxidation with bromine water yields lactobionic acid which on methylation followed by hydrolysis gives 2,3,5,6-tetra-O-methyl-D-gluconic acid and 2,3,4,6-tetra-O-methyl-D-galactose indicating that in lactose C-4 of glucose is linked to C-1 of galactose (Scheme 2.10).
- (vii) The point of linkage (C-4) of glucose unit further confirmed by osazone formation and **Zemplen** degradation.

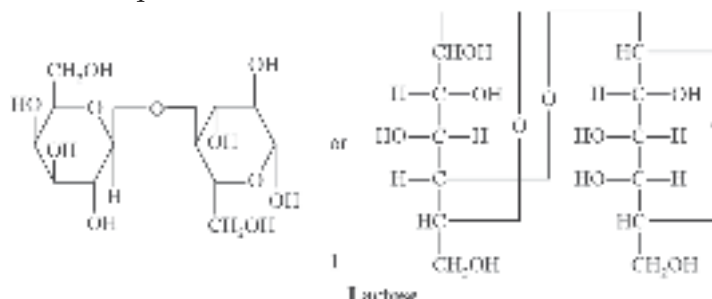


Scheme 2.10: Degradation of lactose

Note that the structure of lactose differs only in the configuration of C-4 position in the nonreducing unit from that of cellobiose. Thus, the below given structure of lactose, I can be assigned.

- (viii) Like cellobiose, lactose has been synthesised by the condensation of acetobromogalactose and 1,2,3,6-tetra-O- β -D-glucopyranoside.

The specific rotation value $[\alpha]_D$ is $+90^\circ$ for α -form (m.p. 223°); $+35^\circ$ for β -form (m.p. 252°) and $+55^\circ$ for equilibrium form.

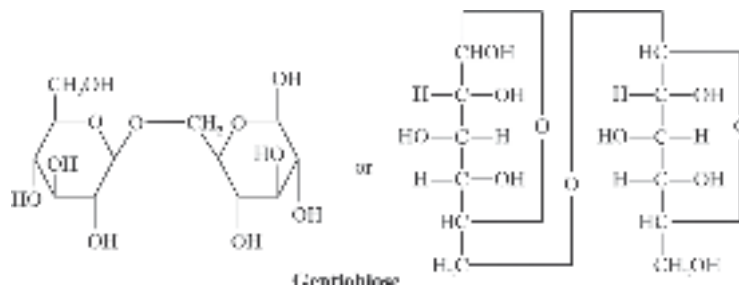


6. Gentiobiose (6-O- β -D-glucopyranosyl-D-glucopyranose)

Initially, gentiobiose was isolated by the careful hydrolysis of the trisaccharide, genti-anose, but later on it is also isolated from the glucoside, amygdalin.

Structural elucidation

- (i) Its molecular formula is found to be $C_{12}H_{22}O_{11}$.
- (ii) It responds the characteristic reaction of reducing sugar indicating the presence of at least one free aldehydic group.
- (iii) Gentiobiose is hydrolysed by acids to two moles of glucose suggesting that it has two glucose units.
- (iv) Gentiobiose is also hydrolysed by emulsin, indicating the presence of β -glycosidic linkage.
- (v) On oxidation with bromine water, gentiobiose gives gentibionic acid which on methylation followed by hydrolysis yields 2,3,4,5-tetramethyl-D-gluconic acid and 2,3,4,6-tetramethyl-D-glucopyranose. The presence of free hydroxyl group in the two products C-6 and C-1 position respectively which indicates that the C-6 of reducing glucose unit (which gives 2,3,4,5-tetramethyl-D-gluconic acid) is linked to the C-1 of non-reducing glucose unit (which gives 2,3,4,6-tetramethyl-D-glucopyranose) in gentiobiose. Hence, the structure of gentiobiose may be drawn as follows:



- (vi) The structure has been confirmed by synthesis from condensation reaction of tetra-O-acetyl-D-glucosyl bromide with 1,2,3,4-tetra-O-acetyl- β -D-glucopyranoside.

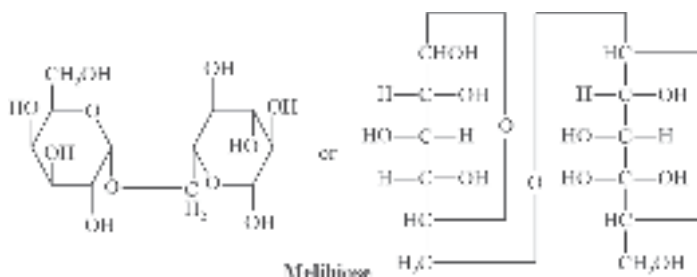
7. Melibiose

Melibiose is obtained along with D-fructose by the incomplete hydrolysis of raffinose, a trisaccharide found in sugar beet.

Structural elucidation

- (i) Its molecular composition is found to be $C_{12}H_{22}O_{11}$.
- (ii) It responds to the characteristic reaction of reducing sugar. For example, it forms osazone and undergoes mutarotation.
- (iii) On hydrolysis with dilute acids, it gives D-glucose and D-galactose.
- (iv) On complete methylation followed by hydrolysis it gives 2,3,4-trimethyl-D-glucose and 2,3,4,6-tetramethyl-D-galactose; indicating that the free aldehydic group is present in the glucose unit.
- (v) The nature of linkage is settled by its specific rotation $[\alpha]_D = +113^\circ$ which suggest the presence of α -glucosidic linkage.
- (vi) On oxidation with bromine water, it gives melibionic acid which on methylation followed by hydrolysis gives 2,3,4,5-O-tetra-O-methyl-D-gluconic acid and 2,3,4,6-tetra-O-methyl-D-galactose. The formation of these two products indicate that C6 of the glucose (reducing part) and C₁ of the galactose (non-reducing portion) are involved in the glycosidic linkage.

Thus, the complete structure of melibiose can be written as below:



OLIGOSACCHARIDE

An **oligosaccharide** (from the Greek **oligos**, *a few*, and **sacchar**, *sugar*) is a saccharide polymer containing a small number (typically two to ten) of component sugars. Oligosaccharides can have many functions; for example, they are commonly found in the plasma membrane of animal cells where they can play a role in cell-to-cell recognition. In general, they are found either *O*- or *N*-linked to compatible amino acid side-chains in proteins or to lipid moieties.

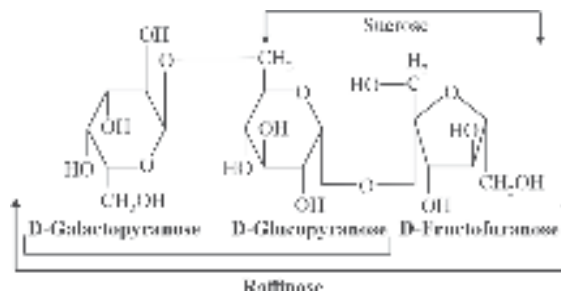
1. Raffinose

It is the most abundant trisaccharide found in nature and is a D-galactosylated version of sucrose. It has only 20% of the sweetening intensity of sucrose. It is found in the seeds, leaves, branches, and roots of many plants.

Structural elucidation

- (i) Its molecular formula is found to be $C_{18}H_{32}O_{16}$.
- (ii) Raffinose corresponds to the characteristics of non-reducing sugars, indicating that all of its anomeric carbon atoms are involved in glycosidic linkage.
- (iii) On vigorous hydrolysis, it gives D-galactose, D-glucose and D-fructose in equal proportion, indicating that the raffinose is composed of D-galactose, D-glucose and D-fructose (one mole each).

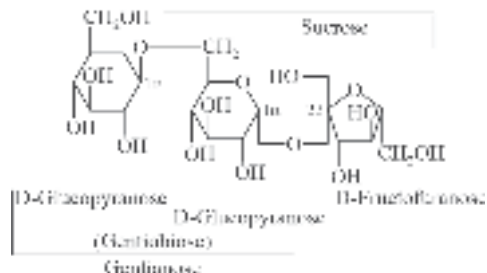
- (iv) Raffinose on hydrolysis, by invertase gives D-fructose and melibiose, while α -galactosidase (specific enzyme for α -galactoside linkage) gives D-galactose and sucrose. This hydrolytic reaction indicates that in raffinose molecule the D-glucose and D-galactose units are linked as in melibiose; and D-fructose is linked to D-glucose as in sucrose, *i.e.* the three monosaccharide molecules are linked in the following order: galactose-glucose-fructose. Thus, the structure of raffinose may be written as below:



- (v) The above structure for raffinose is confirmed by its complete methylation followed by hydrolysis to a mixture of 2,3,4,6-tetra-O-methyl-D-galactopyranose; 2,3,4-tri-O-methyl-D-glucopyranose, and 1,3,4,6-tetra-O-methyl-D-fructofuranose.

2. Gentianose

It occurs in gentian roots. It is also a non-reducing sugar and gives two moles of D-glucose and one mole of D-fructose, on hydrolysis. Its structure is established exactly in the same manner as that of raffinose. For example, on hydrolysis, with invertase it gives D-fructose and gentiobiose, while with emulsin it yields D-glucose and sucrose. Thus, in gentianose 'Structure, the two glucose and one fructose units are combined so as to give gentianose and sucrose, *i.e.* the arrangement of the three monosaccharides molecules is as: glucose-glucose/fructose. Hence, gentianose structure is as below.



3. Cyclodextrins

Cyclodextrins α , β , γ are a group of cyclic oligosaccharides that contain six, seven and eight sugar rings, respectively and are formed by the enzymatic degradation of starch. As such, these molecules consist of a ring of six to eight D-glucopyranose units held together by α -1,4-linkages; the result is a bottomless "tub" with a hydrophilic exterior and a hydrophobic interior, into which "guest" molecules can fit.

POLYSACCHARIDES

Polysaccharides are polymeric carbohydrate structures, consist of repeating units (either mono- or di-saccharides) joined together by glycosidic bonds. These often possess linear structure, but may contain various degrees of branching.

Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water. Polysaccharides are high molecular weight (16,000–14,000,000) carbohydrates which yield ultimately monosaccharides on complete hydrolysis and hence, chemically they are regarded as the condensation product of monosaccharides.

They may be categorized into two groups—**homopolysaccharides** which contain only one kind of monosaccharide units, the **heteropolysaccharides** which contain more than one kind of monosaccharide units.

Polysaccharides occur widely in nature. Examples of homopolysaccharide are cellulose, inulin and starch; while α -heparin, chondroitin sulphate A and hyaluronic acid are the example of heteropolysaccharides.

Polysaccharides may also be classified into two groups according to their function, viz. (i) those which represent reserve carbohydrates stored up by plants or animal (e.g. glycogen, starch and inulin) and (ii) those which serve as skeletal material (e.g. cellulose).

Structural elucidation

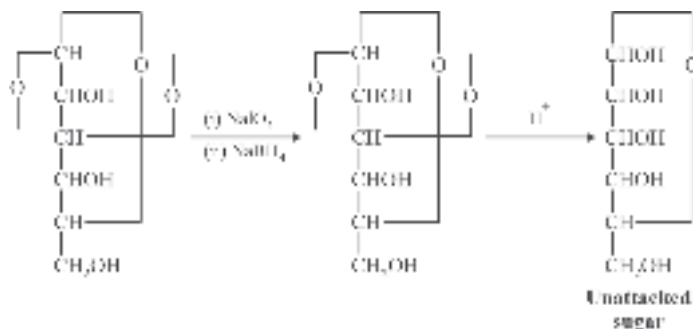
Similar to the structure of disaccharides, the elucidation of polysaccharide structure involves the following points:

- (i) The nature of the monosaccharides constituting the polysaccharide, and their arrangement.
- (ii) Ring size of the monosaccharides (present as pyranose or furanose).
- (iii) Glycosidic linkage (α or β or a mixture of the two).
- (iv) Arrangement of the monosaccharides in polysaccharide (linear or branched structure).
- (v) The determination of molecular weight for knowing the approximate total number of the monosaccharide units.

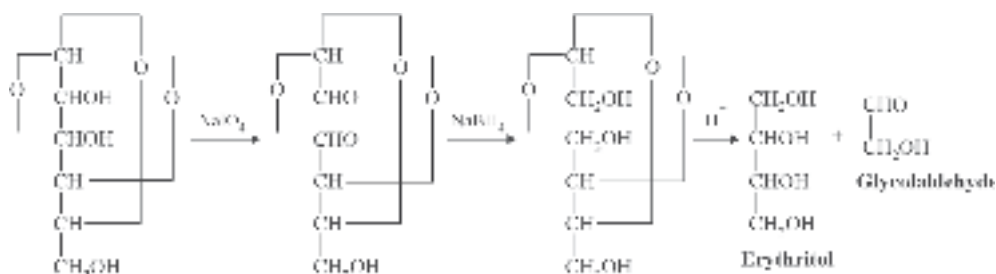
The general methods used for elucidating the structure of a polysaccharide are discussed below:

1. **Acid hydrolysis:** Hydrolysis of a polysaccharide with acid produces its constituent monosaccharide which is identified by the physical characteristics and preparation of derivatives. Quantitative estimation may be done by paper chromatography, GLC, TLC, and zone electrophoresis. However, careful hydrolysis sometimes gives di-, tri- and higher oligosaccharides, which in turn indicate the mode of linkage and also the ring size of the individual monosaccharide. However, it must be noted that 1,6-linkage are the most resistant to acid hydrolysis. The acid hydrolysis also gives direct information about the ring size of the monosaccharide. The easiness of hydrolysis of polysaccharide indicates that monosaccharide units are present in the furanose form; where the difficult hydrolysis of polysaccharide indicates the pyranose form of the monosaccharide. However, this should be confirmed by other methods.

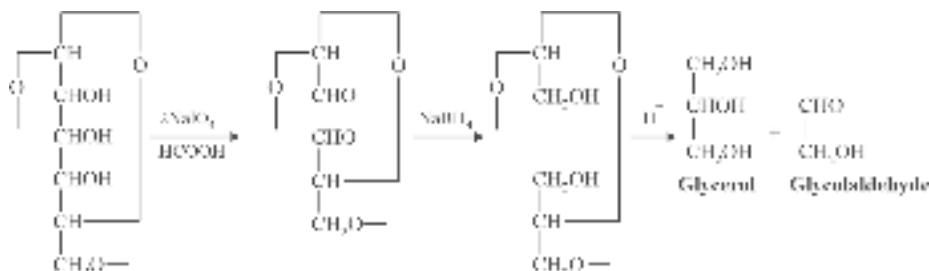
2. *Enzymatic hydrolysis*: This method is especially useful when a series of enzymes capable of removing one or more sugar residues at a time, starting from the reducing end of the polysaccharide.
3. *Acetolysis (simultaneous acetylation and hydrolysis)*: It consists in treating the polysaccharide with a mixture of acetic anhydride and sulphuric acid (3–5%). Acetolysis attacks readily 1, 6-linkage in addition to other linkage also.
4. *Methylation*: Polysaccharides are methylated by the usual methods described earlier, however, Srivastava *et al.* (1963) gave a recent quantitative method for the methylation of lower molecular weight polysaccharide. They carried out methylation by adding barium oxide and methyl iodide to a solution of the polysaccharide in dimethyl sulphoxide at 20°C. Completely methylated polysaccharide are then hydrolysed and the product identified by usual method. The compounds so obtained indicate the point of attachment of sugar units. These are further hydrolysed and/or oxidised to know the ring size and the point of linkage.
5. *Periodic oxidation (Smith degradation)*: This method is of course the most important one for establishing the mode of linkage in the di- and polysaccharides. It gives different products in 1,3-linkage, 1,4-linkage, 1,6-linkage. The method consists in the oxidation of the polysaccharide molecule followed by reduction and the acidic hydrolysis to give fragments that indicate the mode of linkage present in the original polysaccharide. For example:
 - (i) A polysaccharide having 1→3 linkage. Here in the 1→3 linkage, neither oxidation nor reduction by NaBH_4 takes place since no adjacent hydroxyl groups are present in the polysaccharide.



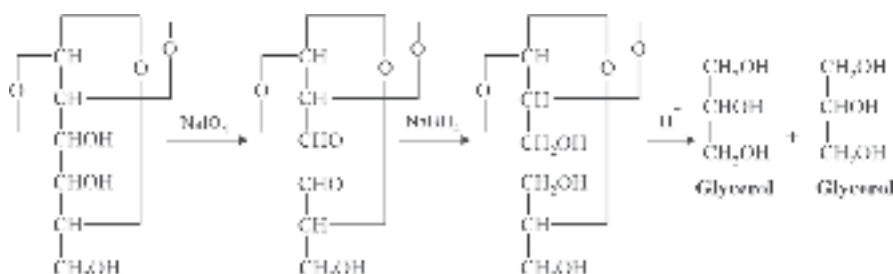
- (ii) A polysaccharide having 1→4 linkage.



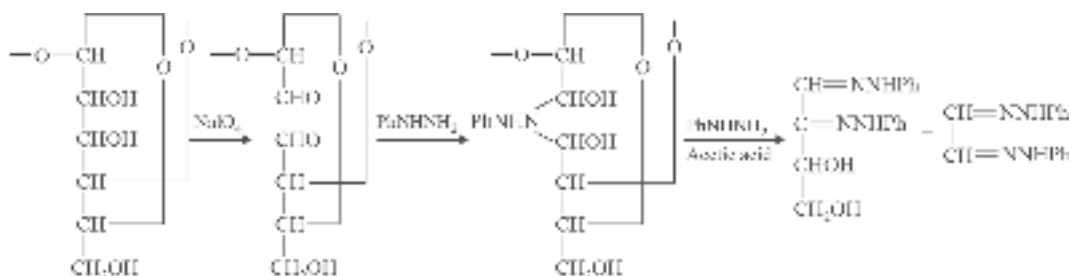
(iii) A polysaccharide having 1→6 linkage.



(iv) A polysaccharide having 1→2 linkage.



Barry et al. (1954) have developed an alternative technique for the periodate-oxidised polysaccharide. He treated the polysaccharide with phenylhydrazine acetate, followed by heating phenylhydrazine in acetic acid to form osazones as shown below.



Linear and branched structure are differentiated by the fact that only the former forms fibers.

CONSTITUTION OF SOME SIGNIFICANT POLYSACCHARIDES

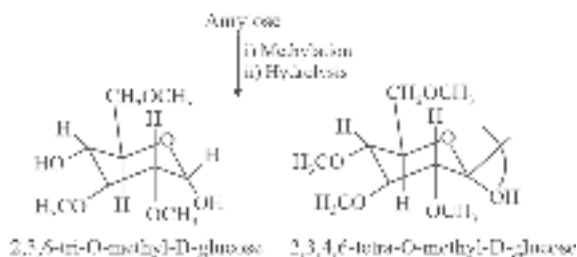
1. Starch

Starch is the major form of stored carbohydrate in plants, mainly potatoes and cereals. Starch is white amorphous substance, composed of microscopic granules. Starches obtained from different sources do not differ much in their appearance but they can be differentiated microscopically from one another on the basis of size and shape of granule. The intact starch is insoluble in cold water, but when the outer membrane of 'S' is broken, the granules swell forming a translucent liquid which contains starch in colloidal solution and which if sufficiently concentrated sets on cooling to a jelly, called starch mucilage.

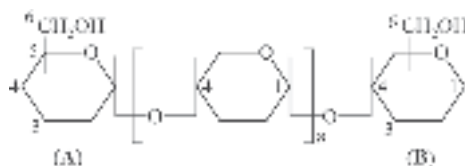
Starch is composed of a mixture of two polymeric substances: *amylose*, an essentially linear polysaccharide, and *amylopectin*, a highly branched polysaccharide. Both forms of starches are polymers of α -D-Glucose. Natural starches contain 0–20% amylose and 80–90% amylopectin. Amylose forms a colloidal dispersion in hot water (which helps to thicken gravies) whereas amylopectin is completely insoluble. Amylose on treatment with iodine solution gives a blue color while amylopectin on a similar treatment gives a violet to reddish violet color.

(a) Structural elucidation of amylose

- (i) Its empirical formula is found to be $C_6H_{10}O_5$.
- (ii) On complete hydrolysis, it gives D-glucose in quantitative yield. This indicates that amylose is composed of only D-glucose unit. Amylose molecules consist typically of 200–20,000 glucose units, which form a helix as a result of the bond angles between the glucose units.
- (iii) On enzymatic hydrolysis with diastase, it yields maltose (100% conversion) as the only disaccharide. The maltose is 4-O-(α -D-glucopyranosyl)-1-D-glucopyranose. This means:
 - (a) Amylase is composed of D(+)-glucose unit.
 - (b) It is a linear molecule.
 - (c) Each glucose unit is joined by α -glycosidic linkage via C-1 of glucose to C-4 of the next one.
- (iv) On complete methylation followed by hydrolysis, 2,3,6-tri-O-methyl-D-glucose along with 2,3,4,6-tetra-O-methyl-D-glucose (0.2–0.4%) is produced.



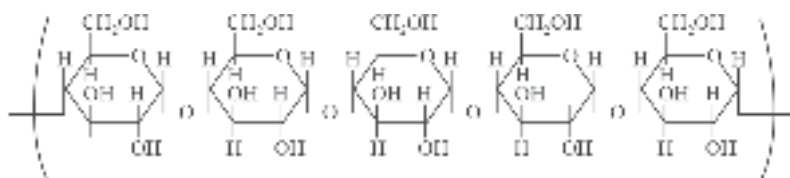
Glucose itself has five free $-OH$ groups for methylation when two glucose units are joined together (maltose), each glucose unit has four $-OH$ groups. Now, if a glucose unit is joined by two glucose units from both side (by glycosidic linkage), the center glucose unit should have three OH groups. This is the case with amylose in which glucose unit is linked on both side by 1,4-linkage to other glucose unit except the end terminal glucose unit. It is anticipated when each D(+) glucose unit in amylose is joined to two others, as it contains only three free $-OH$ groups which are methylated to give the tri-methyl product. However, a small amount of 2,3,4,6-tetra-O-methyl-D-glucose, that is about 0.2–0.4% of the total product, is also formed. In the below given structure, there are two terminal units denoted by A and B. In the center, C-1 of one glucose unit is joined to C-4 unit of another glucose unit.



- (v) Therefore, it has been concluded that each D-glucose unit in amylose is attached to two other D-glucose units, one through C-1 and other through C-4 position. The formation of the above products indicates that amylose is a straight chain polymer with one end having free aldehyde group. The other end has a D-glucose unit that has a free —OH at C-4 position.

This on methylation followed by hydrolysis, the terminal-B glucose unit is converted to open chain (or is degraded) having free aldehydic group.

- (vi) Thus, each molecule of completely methylated amylose on hydrolysis should give one molecule of 2,3,4,6-tetra-O-methyl-D-glucose (from terminal-A glucose unit) along with a number of tri-O-methyl-D-glucose molecules (center glucose units). The formation of 0.2% of tetra-O-methyl derivative indicates the chain length of about 500 units.
- (vii) The hydrolytic reaction does not produce, 2,3-dimethyl derivative which indicates, amylose is a linear polymer of D-glucose units joined together by α -linkages as in maltose.
- (viii) On the basis of above facts, the structure is assigned to amylose.

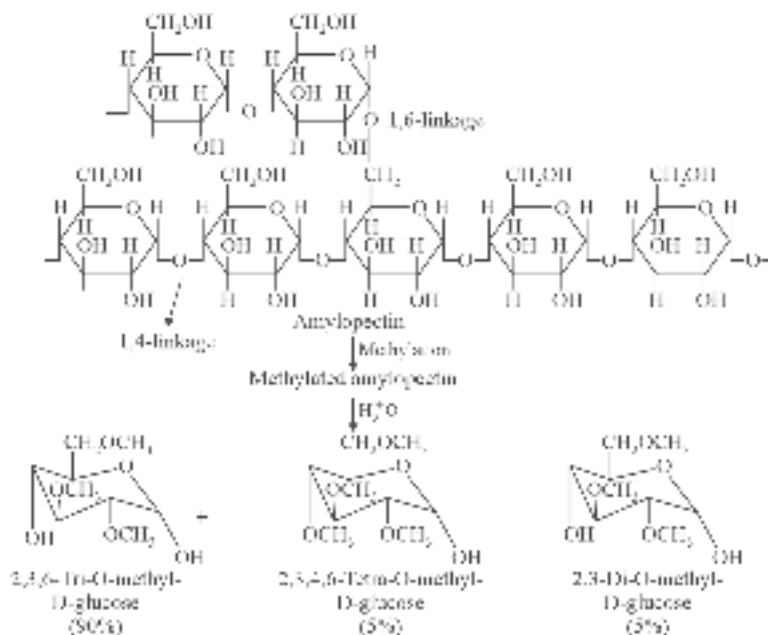


(b) Structural elucidation of amylopectin

- (i) It has empirical formula $C_6H_{10}O_5$.
- (ii) On complete acidic hydrolysis, amylopectin gives D-glucose. It indicates that amylopectin is composed of D-glucose unit.
- (iii) **Amylopectin** differs from amylose in being highly branched. Short side chains of about 30 glucose units are attached with 1 $\rightarrow$ 6 linkages approximately every twenty to thirty glucose units along the chain. Amylopectin molecules may contain up to two million glucose units.
- (iv) On enzymatic hydrolysis with diastase, it gives maltose (55% yield) and a mixture of compounds called as dextrans. It indicates that amylopectin has some other linkages, and it is not a linear molecule.
- (v) On methylation followed by hydrolysis of amylopectin, it produces mainly 2,3,6 tri-O-methyl-D-glucose. Therefore, amylopectin is made-up of chains of D-glucose with each unit joined by α -glycosidic linkage of C-4 of the next unit. Hydrolysis of methylated amylopectin also yields about 5% of 2,3,4,6-tetra-O-methyl-D-glucose along with an equal amount of 2,3 di-O-methyl-D-glucose.
- (vi) Formation of 5% of tetra-O-methyl-glucose indicates that there are 20 end D-glucose units per chain and the formation of di-methyl-glucose suggests that amylopectin has a highly branched structure consisting of several hundred short chains of about 20–25 D-glucose units. One end of each of these chains is joined through C-1 to C-6 on the next chain.

Therefore, amylopectin is a branched α -1,4' polymer of glucose with α -1,6-linkage that provides the attachment point for another chain.

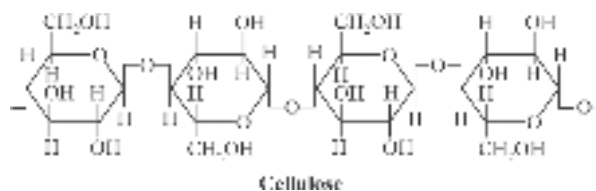
Spectral data: IR (cm^{-1}): 3393 (O—H stretching), 2927 (C—H stretching), 1155 (C—O stretching).



2. Cellulose

Cellulose is a polymer of **β -D-Glucose**, which in contrast to starch, is oriented with **CH₂OH** groups alternating above and below the plane of the cellulose molecule thus producing long, unbranched chains. The absence of side chains allows cellulose molecules to lie close together and form rigid structures. Cellulose is the major structural material of plants. Wood is largely composed of cellulose, and cotton is almost pure cellulose. Cellulose can be hydrolysed to its constituent glucose units by microorganisms that inhabit the digestive tract of termites and ruminants.

Cellulose may be modified in the laboratory by treating it with nitric acid (HNO₃) to replace all the hydroxyl groups with nitrate groups (—ONO₂) to produce cellulose nitrate (nitrocellulose or guncotton) which is an explosive component of smokeless powder. Partially nitrated cellulose, known as pyroxylin, is used in the manufacture of collodion, plastics, lacquers, and nail polish.



Cellulose monomers (β -glucose) are linked together through 1,4-glycosidic bonds. Cellulose is a straight chain (no coiling occurs). In *microfibrils*, the multiple hydroxide groups hydrogen bond with each other, holding the chains firmly together and contributing to their high tensile strength. This strength is important in cell walls, where they are meshed into a carbohydrate *matrix* helping keep plants rigid.

Cellulose is a colorless solid, insoluble in water and organic solvents but readily soluble in ammoniacal cupric hypoxide solution. It is also soluble in strong hydrochloric acid, but undergoes degradation to give products of lower molecular weights.

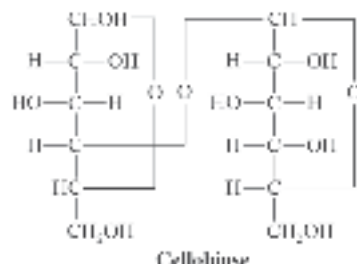
Cellulose from cotton linters: The impurities (nearly 2%) of cotton cellulose consisting mainly of the protective film of wax, oil from the seed, pectic substance, mineral substance such as sand, and some colouring matter are removed carefully to avoid degradation of the cellulose molecule. The heavy impurities like stones and leaves are removed by mechanical cleaning. After removing the heavy impurities the linters are boiled with dilute aqueous sodium hydroxide in an inert atmosphere to remove pectin, wax and oil. The linters are then bleached with dilute sodium hypochlorite and the product thus obtained is nearly 99.7% pure cellulose; the residue is found to consist of mainly silica.

Cellulose from wood (wood pulp): Cellulose obtained from wood is mainly used in paper industry. Some amount is also used in fibre production. The fatty and waxy impurities are removed by organic solvent. Lignin and hemicellulose are removed by treating the wood pulp either with dilute acids or with calcium and sodium sulphite (sulphite process) or with sodium hydroxide or with sodium sulphide (sulphate process) at high temperature and pressure. The fibres are then bleached either with chlorine or with sodium hypochlorite. Finally, the fibres may be purified by the use of 15–20% of sodium hydroxide.

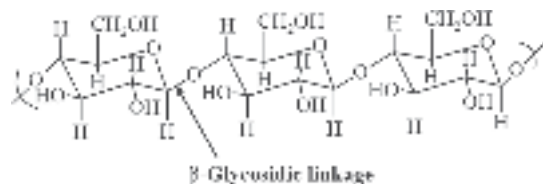
Although, the purification steps in the two processes are relatively mild, some of the cellulose is hydrolysed. By changing time of the various steps in the purification processes, different forms of cellulose may be achieved differing very much in the average size of the molecule. The three important cellulose forms are α , β and γ .

(a) Structural elucidation

- (i) It has empirical formula of $C_6H_{10}O_5$.
- (ii) It is composed of entirely D-glucose units as complete hydrolysis by acid yields D(+)-glucose as only monosaccharide.
- (iii) Hydrolysis of completely methylated cellulose gives a high yield of 2,3,6-tri-O-methyl-D-glucose. It indicates that cellulose is made-up of chains of D-glucose units, each unit via C-1 joined by a glycoside linkage to C-4 of the next. It also suggests that hydroxyl groups are present at C-2, C-3 and C-4 positions.
- (iv) On treatment with acetic anhydride and sulphuric acid, cellulose yields octa-O-acetyl-cellobiose. Therefore, it is considered as a polymer of **cellobiose**.
- (v) Further, cellulose on hydrolysis with enzymes yields cellobiose. It has been found that cellobiose has β -glycosidic linkage, it signifies all glycosidic linkages in cellulose are β -linkages.
- (vi) The structure of cellulose suggests that it is made of a long chain of cellobiose units in which glucose is linked alternately through the carbon atoms at C-1 and C-4 positions. The linkage is β and conformation is completely equatorial. Each glucose unit contains three free hydroxyl groups except at the end of an extra hydroxyl group at C-4 position.

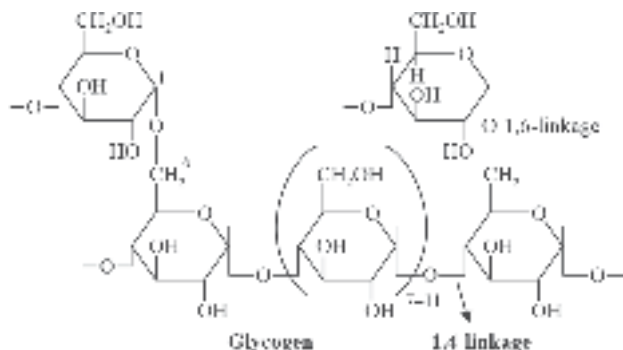


- (vii) The molecular weight of cellulose ranges from 2,50,000 to 1,000,000. It signifies that there are at least 1500 glucose units present in cellulose. These long chains lie side-by-side in bundles, which are held together by hydrogen bonds between the neighbouring $-OH$ groups. These bundles are twisted together to form rope like structures which are grouped to form the fibers. In wood, these cellulose ropes are embedded in lignin to give the compact structure. On this basis, under mentioned structure is assigned to the cellulose.



3. Glycogen

Glycogen is the analogue of starch, a glucose polymer in plants, and is sometimes referred to as *animal starch*, having a similar structure as amylopectin but more extensively branched and compact than starch. Glycogen is a polymer of $\alpha(1\rightarrow4)$ glycosidic bonds linked, with $\alpha(1\rightarrow6)$ -linked branches. Glycogen is found in the form of granules in the cytosol/cytoplasm in many cell types, and plays an important role in the glucose cycle. Glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose, but one that is less compact than the energy reserves of triglycerides (lipids).

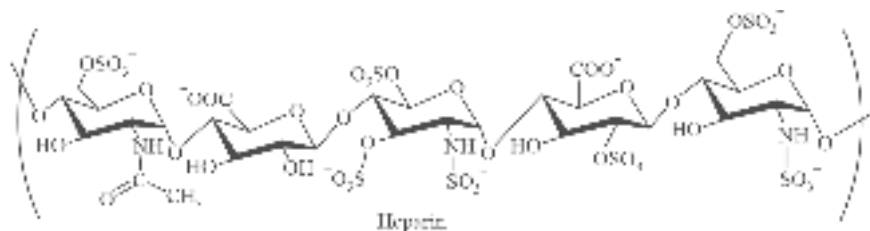


Spectral data: IR (cm^{-1}): 3330 (O—H stretching), 2895 (C—H stretching), 1103 (C—O stretching)

4. Heparin

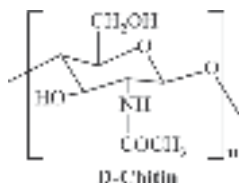
Heparin is a heterogeneous mixture of variably sulphonated polysaccharide chains, ranging in molecular weight from 6000–30,000 g/mol. Heparin has many biological functions, the best known and understood of which is its anticoagulant activity. It binds strongly to antithrombin III, a plasma protein involved in terminating the clotting process.

The repeating monosaccharide units of heparin are N-acetyl-D-glucosamine, D-glucuronic acid, D-glucosamine, and L-ioduronic acid bonded by a combination of α -1,4- and β -1,4-glycosidic bonds.

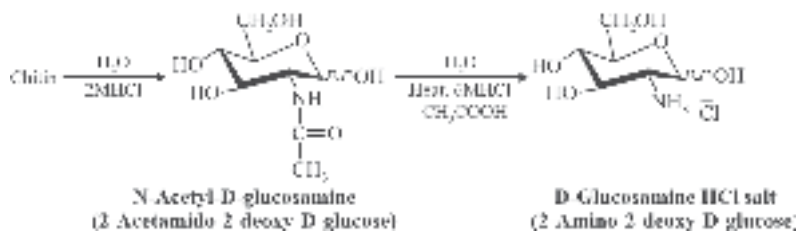


5. D-Chitin

Chitin is a polysaccharide that also occurs widely in nature—notably, in the shells of arthropods (for example, lobsters and crabs). Crab shell is an excellent source of nearly pure chitin.



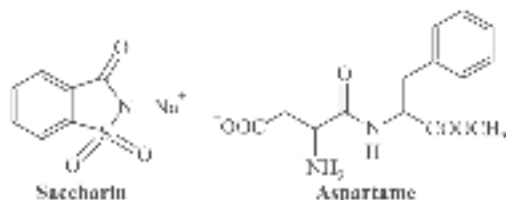
Chitin is a polymer of N-acetyl-D-glucosamine (or, as it is known systematically, 2-acetamido-2-deoxy-D-glucose). Residues of this carbohydrate are connected by β -1,4-glucosidic linkages within the chitin polymer. N-Acetyl-D-glucosamine is liberated when chitin is hydrolyzed in aqueous acid. Stronger acid brings about hydrolysis of the amide bond to give D-glucosamine hydrochloride and acetic acid. Chitosan, a related water soluble polysaccharide in which the vast majority of residues is not acetylated. It can be obtained from chitin by deacetylation in concentrated sodium hydroxide solution.



Glucosamine and N-acetylglucosamine are the best-known examples of the amino sugars. A number of amino sugars occur widely in nature. Amino sugars linked to proteins (glycoproteins) are found at the outer surface of cell membranes, and some of these are responsible for blood-group specificity.

Relative Sweetness of Some Carbohydrate and Artificial Sweeteners

Although, all monosaccharides are sweet to the taste, some are sweeter than others (Table 2.4). D-Fructose tastes the sweetest, even sweeter than sucrose. The sweet taste of honey is attributable largely to D-fructose and D-glucose. Lactose has almost no sweetness. It occurs in many milk products and is sometimes added to foods as a filler. Some people lack an enzyme that allows them to tolerate lactose well; they should avoid these foods.



The sweetening power of sugars is undoubtedly their most important characteristic, in-so-far as the public is concerned. If the sweetness of sucrose is taken as a standard, then other sweet tasting compounds may be ranked accordingly, as shown here. Saccharin and aspartame are synthetic sweeteners, their structures are shown on the right. The other compounds are natural sugars.

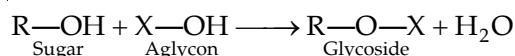
Table 2.4: Relative sweetness of some carbohydrates and artificial sweeteners

<i>Carbohydrate</i>	<i>Sweetness relative to sucrose</i>	<i>Artificial sweetener</i>	<i>Sweetness relative to sucrose</i>
Fructose	1.74	Saccharin	450
Invert sugar	1.25	Aspartame	160
Sucrose (table sugar)	1.00		
Honey	0.97		
Glucose	0.74		
Maltose	0.33		
Galactose	0.32		

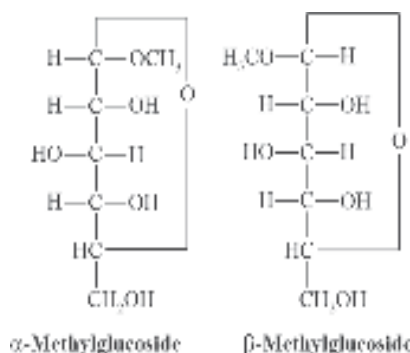
Glycosides

Glycosides are organic compound, isolated from plants and animal source, which on hydrolysis gives one or more sugar moieties along with a nonsugar moiety. Sugar moiety is called glycone while nonsugar moiety is referred to as aglycone or genin. Sugars are hemiacetal and occur as oxide rings. The aglycone moiety may be of diverse nature phenolic, triterpenoidal, steroidal, thiocyanate, etc.

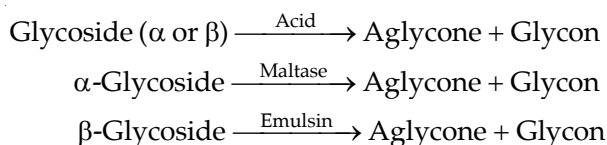
Glycosides can be defined as the condensation product of hydroxyl group of aglycone and hemiacetal hydroxyl group of sugar. The aglycone may be any compound containing at least one hydroxyl group through which glycosidal hydroxyl group of sugar molecule is linked.



Glycosidic hydroxyl group reacts with large number of organic compounds and acid labile, i.e. the organic moiety attached at glycosidic hydroxyl group is hydrolyzed with acids whereas others are not. The sugars present in glycoside are of two isomeric form, i.e. α form and β form, but all the natural glycosides contain β -type of sugar.



The simplest glycosides are α -methyl glycoside and β -methyl glycoside. Both of these glycosides can be hydrolysed using mineral acids whereas these can specifically be hydrolysed using enzyme like maltase specific for α -glycoside and emulsin specific for β -glycoside. Hence, this reaction can be used for determination of type of linkage and nature of aglycone present in glycoside.



Classification of glycosides

Various ways to classify the different glycosides are as follows:

(i) Based on Linkage between Glycon and Aglycone Portion

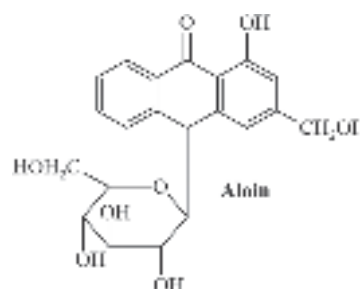
The OH groups may link with any of the following group like OH, CN, SH, NH present in aglycone part.

(a) C-Glycoside

Sugar molecule is directly attached with C-atom of aglycone.



Some of the anthraquinone glycoside like cascarioside in cascara, aloin in aloes shows this particular linkage. They do not hydrolyzed by heating with dilute acid or alkalis but by oxidative hydrolysis with FeCl_3 . Cochineal contains C-glycoside in the form of coloring matter called **carminic acid**.



(b) O-Glycoside

Sugar molecule is combined with phenolic OH group of aglycone, e.g. Amygdalin, Arbutin, Salicin, cardiac glycosides, anthraquinone glycosides like sennosides, etc. They are hydrolyzed by treatment with acid or alkali into glycone and aglycone portion.

(c) S-Glycoside

The occurrence of this glycoside is restricted to isothiocyanate glycoside like sinigrin in black mustard formed by the condensation of sulphahydryl group (aglycone) to OH group of glycon.

(d) N-Glycoside

The most typical representation of N-glycoside is nucleoside where the amino group reacts with OH group of ribose or deoxyribose sugar resulting into N-glycoside.

(ii) On the Basis of Type of (Glycon) Sugar

- Glucoside: Sugar portion is glucose.
- Rhamnoside: Sugar portion is rhamnose.
- Pentoside: Sugar portion is pentose.
- Fructoside: Sugar portion is fructose.
- Arabinoside: Sugar portion is arabinose.

In other words, the terminal -e- of the name of the corresponding cyclic form of the monosaccharide (sugar) is replaced by -ide-. Furthermore, if it is glucuronic acid, then the molecule is a glucuronide, and so forth. In the body, toxic substances are often bonded to glucuronic acid to increase their water solubility; the resulting glucuronides are then excreted.

(iii) On the Basis of Number of Sugar Units Attached to Aglycone Part

One sugar	Monosides, e.g. Salicin
Two sugar	Biosides, e.g. Diosmin
Three sugars	Triosies, e.g. Digoxin

(iv) Nature of the Glycoside

- Primary glycosides: Originally present in the plant, e.g. Purpurea-A
- Secondary glycosides: Resulted from removal of one sugar from the primary glycosides, e.g. Digitoxin (formed from primary glycoside—Purpurea-A).

(v) On the Basis of Type of the Glycosidic Linkage

In α -glycosides, sugar has the α configuration at the hemiacetal carbon; the sugar is usually of the L-series, e.g. L-rhamnose. There are only few medicinal α -glycosides known, especially the rhamnosides. In contrast, in the β -glycosides, sugar involved has the β configuration at the hemiacetal carbon. The majority of plant glycosides isolated are β -glycosides.

(vi) Botanical Source

- Digitalis glycosides.
- Senna glycosides.

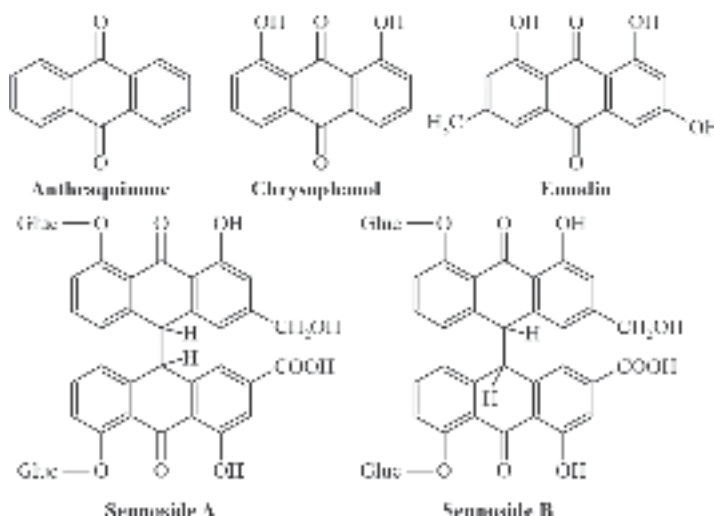
(vii) Therapeutic Use

- Analgesic glycosides, e.g. salicin.
- Purgative glycosides, e.g. aloin.
- Cardiac glycosides, e.g. digitoxin.

(viii) On the Basis of Chemical Nature of Aglycone

Glycosides are also classified according to the chemical nature of the aglycone. For the purposes of biochemistry and pharmacology, this is the most useful classification.

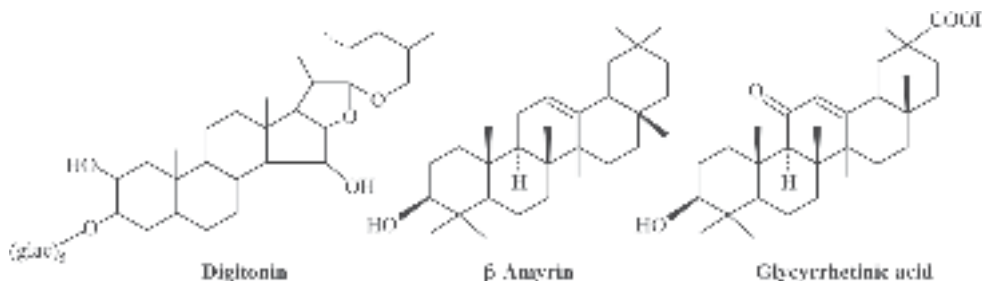
- (a) **Anthraquinone glycosides:** These are derivative of anthraquinones, possess purgative property, may be dihydroxyphenol (chrysophanol), trihydroxyphenol (emodin), or tetra-hydroxyphenols. Anthraquinone derivatives are often orange red colored compounds, soluble in hot water and alcohols, e.g. Sennosides A, B, C and D.



- (b) **Steroidal glycosides or cardiac glycosides:** The aglycone in steroidal glycoside contains the steroidal nucleus. These glycosides are found in the plant genera *Digitalis*, *Seil/a*, and *Strophanthus* (for details please see cardiac glycosides).

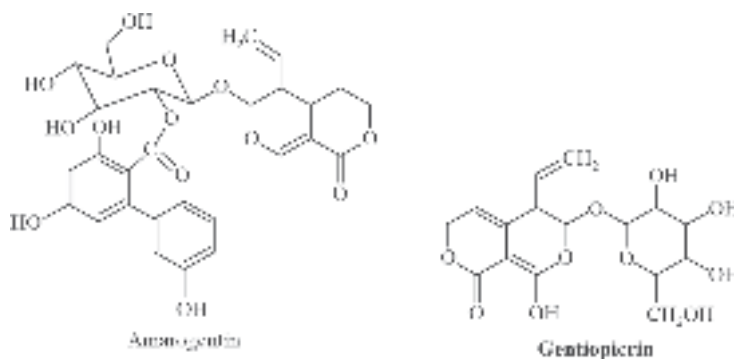
- (c) **Saponin glycosides:** These forms honey comb like foam when shaken with water and causes hemolysis of blood. Saponins are complex organic compounds distributed in higher plants and are toxic to cold-blooded animals and lower organisms like earthworm and fishes. These are soluble in alcohol but insoluble in ether and light petroleum; on hydrolysis it gives aglycone known as sapogenin (generally steroids) and sugars. Depending on the aglycone moiety. Saponins are of two types:

Steroidal saponin (tetracyclic triterpenoids): These contain cyclopentenoperhydro phenanthrene ring. For example, digitonin (isolated from *Digitalis purpurea*) obtained from Dioscorea species is good example of steroidal saponin. It contains steroidal digitogenin (aglycone) combined with 5 molecules of galactose (glycone).

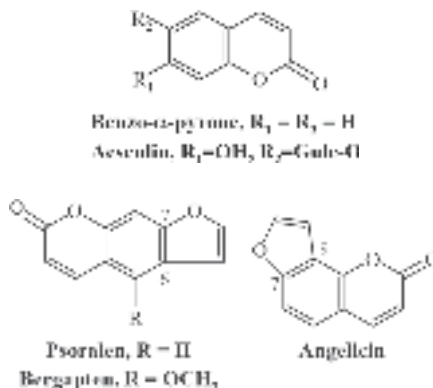


Pentacyclic triterpene saponin: These are polymer of isoprene units and mainly derivatives of β-amyrin. For example, Glycyrrhetic acid, obtained from *Glycyrrhiza glabra*, is example of pentacyclic triterpene saponin.

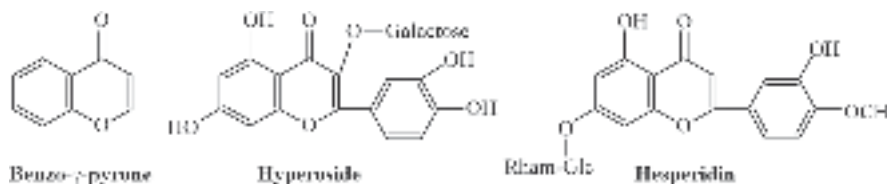
- (d) **Cyanophoric or cyanogenetic glycosides:** The glycosides which on hydrolysis yields hydrocyanic acid (HCN), benzaldehyde and sugars. The medicinal activity of cyanogenetic glycosides is due to presence of hydrocyanic acid and these are the characteristics of family rosaceae. For example, amygdalin (obtained from bitter almond, *Prunus amygdalus*), prunasin (obtained from wild cherry bark) are the common examples of cyanogenetic glycosides.
- (e) **Isothiocyante glycosides:** These are sulphur containing compounds, which on hydrolysis yields isothiocyante (–NCS) group. These glycosides are generally irritant and hence, used externally as counter irritant. For examples, sinigrin from black mustard, sinalbin from white mustard and gluconapin from rape seed.
- (f) **Bitter glycosides:** These are complex organic compounds containing lactone ring, soluble in water and very bitter in taste even in much diluted solutions. These increase the secretions of GIT by reflex action and are used as stomachic, febrifuge and bitter tonic. For example, gentiopicroin and amarogentin from gentian root and chirata.



- (g) **Coumarin glycosides:** These are aromatic compounds containing benzo- α -pyrone ring system. The alcoholic solution of coumarins shows blue-green fluorescence on addition of alkali. These are generally used externally, in skin disorders and in sun protection preparations because they have property to absorb UV radiation of sunlight, e.g. aesculin from plants of family Rosaceae. Some coumarins contain the furan ring, attached at 6-7 or 7-8 position in coumarin ring and so are called as furano coumarin. These are generally present in family rutaceae, umbelliferae and leguminosae like psoralen, bergapten and angelicin.



- (h) **Flavone glycosides:** These are complex organic compounds containing phenyl-benzo- γ -pyrone ring system. Flavones are present in plants in free state or in glycosidal state (O-glycoside or C-glycoside) with its different derivatives like flavone, flavonol, flavonone, isoflavone and chalcones. The examples include rutin, quercetin, hyperoside, hesperidin (lemon and orange peel) and vitexin (Carategus).



Flavonoids have been known for their antioxidant effect and also known to decrease capillary fragility.

- (i) **Alcoholic glycosides:** An example of an alcoholic glycoside is salicin, which is found in the genus *Salix* (willows) discussed later.
- (j) **Phenolic glycosides (simple):** These types of glycosides contain aglycone having simple phenolic structure. For example, arbutin is prominently found in the common Bearberry, *Arctostaphylos uva-ursi*. It has a urinary antiseptic effect. Rutin is found in citrus fruits, black tea, apple peel.

Distribution of Glycosides

Glycosides are widely distributed in nature. Some plant families containing important glycosides are listed below:

1. Scrophulariaceae, e.g. *Digitalis purpurea* and *Digitalis lanata*, *Picrorhiza kurroa*.
2. Apocyanaceae, e.g. *Nerium oliander* and *Thevetia peruviana*.
3. Liliaceae, e.g. *Urgenea indica* and *Umaritima*, *Aloe vera*.

4. Leguminosae, e.g. *Cassia acutefolia* and *C. angustefolia*, *Glycyrrhiza glabra*, *Psoralea corylifolia*.
5. Dioscoreaceae, e.g. *Dioscorea jloribunda*.
6. Rosaceae, e.g. *Prunus amygdalus*, *Carategus oxycantha*.
7. Cruciferae: *Brassica* sp.
8. Gentianaceae: Gentian and Chirata.
9. Acanthaceae: Kalmegh.
10. Simarubaceae: Quassia.
11. Umbelliferae: *Ammi majus*, *Ammi visnaga*.
12. Rutaceae: Citrus sp., *Ruta graveolens*.
13. Polygonaceae: *Fagopyrum* sp.
14. Myrtaceae: *Eucalyptus* sp.

GENERAL CHARACTERISTICS

- Glycoside contains sugar but still the physical, chemical and therapeutic activity is based on aglycone portion. Sugar facilitates the absorption of the glycoside, helping it to reach the site of action.
- Glycosides are crystalline, amorphous substance which are soluble in water, and dilute alcohol but insoluble in the CHCl_3 and ether. The aglycone moiety is insoluble in non polar solvent like C_6H_6 .
- Glycosides are easily hydrolyzed by mineral acids, water and enzyme. They show optical activity normally they are levorotatory.
- Glycoside do not reduce Fehling's solution until they are hydrolysed into aglycone and sugar.
- They are believed to facilitate growth and protection of plant.

Chemical Tests of Glycosides

Generally, aqueous or alcoholic extracts of crude drugs are tested with specific reagents for the presence of various types of glycosides.

1. Chemical Tests for Anthraquinone Glycosides

- (a) **Borntragar's test:** Little quantity of drug is boiled with 5–10 ml of dilute HCl for 10 minutes and then filtered. The filtrate is extracted with equal volume of dichloromethane or chloroform. Separate the lower layer of chloroform and shake it with half volume of dilute ammonia solution. A rose pink to red color is produced in the ammonical layer due to presence of anthraquinone moiety.
- (b) **Modified Borntragar's test:** To 1 g of drug, 5 ml dilute HCl followed by 5 ml ferric chloride (5% w/v) is added. The contents are boiled for 10 minutes, then cooled and filtered. The filtrate is extracted with dichloromethane or chloroform. Separate the lower layer of chloroform and shake it with half volume of dilute ammonia solution. A rose pink to red colour is produced in the ammonical layer due to the presence of anthraquinone moiety. It is used for C-type of anthraquinone glycosides.

2. Chemical Tests for Saponin Glycosides

- (a) **Hemolysis test:** Few drops of blood are mixed on slide with a few drops of aqueous saponin solution. RBCs become ruptured in presence of saponins (haemolysis).
- (b) **Foam test:** To 1 g of drug, shaken for few minutes with 10–20 ml of water. The appearance of frothing which persists for 60–120 seconds indicates the presence of saponins.

3. Chemical Tests for Steroid and Triterpenoid Glycosides

- (a) **Libermann-Bruchard test:** The alcoholic extract of drug is evaporated to dryness and then extracted with CHCl_3 . Few drops of acetic anhydride followed by Conc. H_2SO_4 is added from side wall of test tube to the CHCl_3 extract. Formation of violet to blue colored ring at the junction of two liquid, indicates the presence of steroid moiety.
- (b) **Salkovaski test:** The alcoholic extract of drug is evaporated to dryness and extracted with CHCl_3 . Few drops of Conc. H_2SO_4 are added from sidewall of test tube to the CHCl_3 extract. Formation of yellow colored ring at the junction of two liquid, which turns red after 2 minutes, indicates the presence of steroid moiety.
- (c) **Antimony trichloride test:** The alcoholic extract of drug is evaporated to dryness and extracted with CHCl_3 . The saturated solution of SbCl_3 in CHCl_3 containing 20% acetic anhydride is added. Formation of pink color on heating indicates presence of steroids and triterpenoids.
- (d) **Trichloroacetic acid test:** Triterpenes on addition of saturated solution of trichloroacetic acid forms colored precipitate.
- (e) **Tetranitro methane test:** It forms yellow color with unsaturated steroids and triterpenes.
- (f) **Zimmermann test:** The *m*-dinitro benzene solution is added to the alcoholic solution of drug containing alkali. The appearance of violet color on heating indicates the presence of keto-steroid.

4. Chemical Tests for Cardiac Glycosides

- (a) **Keller Kiliani test:** To the alcoholic extract of drug, equal volume of water and 0.5 ml of strong lead acetate solution are added, shaken and filtered. The filtrate is extracted with equal volume of chloroform. The chloroform extract is evaporated to dryness and then dissolved in 3 ml of glacial acetic acid followed by addition of few drops of FeCl_3 solution. The resultant solution is transferred to a test tube containing 2 ml of cone. H_2SO_4 . Formation of reddish brown layer, which turns bluish green after standing indicates the presence of desoxy sugar (digitoxose).
- (b) **Legal test:** The chloroform extract is dissolved in 2 ml of pyridine. Then 2 ml of sodium nitropruside is added, followed by addition of NaOH solution to make alkaline. Formation of deep red color which faded to brownish yellow indicates the presence of glycosides or aglycone moiety.
- (c) **Baljet test:** The thick section of leaf of digitalis or the part of drug contain ing cardiac glycoside, is dipped in sodium picrate solution. The formation of yellow to orange color indicates the presence of aglycones or glycosides.
- (d) **Kedde test:** To the alcoholic solution of drug, few drops of NaOH fo llowed by 2% solution of 3,5-dinitro benzoic acid is added. Formation of violet color indicates the presence of cardiac glycosides. The test indicates the presence of lactone ring in cardenolide.

5. Chemical Tests for Coumarin Glycosides

- (a) **FeCl₃ test:** To the concentrated alcoholic extract of drug, few drops of alcoholic FeCl₃ solution is added. Formation of deep green color, which turned yellow on addition of cone. HNO₃, indicates presence of coumarins.
- (b) **Fluorescence test:** The alcoholic extract of drug is mixed with 1N NaOH solution (1 ml each). Development of blue-green fluorescence indicates presence of coumarins.

6. Chemical Tests for Cyanophoric Glycoside

- (a) **Sodium picrate test:** The powdered drug is moistened with water in a conical flask and added few drops of cone. sulphuric acid. The filter paper is impregnated with sodium picrate solution followed by sodium carbonate solution and placed the paper on the neck of flask by using cork. Formation of brick red color due to volatile HCN indicates the presence of cynophoric glycosides.

7. Chemical Tests for Flavonoid Glycosides

- (a) **Ammonia test:** The filter paper dipped in alcoholic solution of drug and dried. The filter paper is exposed to ammonia vapor. Formation of yellow spot on filter paper indicates the presence of flavonoid glycosides.
- (b) **Shinoda test:**
 - (i) To the alcoholic extract of drug, magnesium turning and dilute HCl is added. Formation of red color indicates the presence of flavonoids.
 - (ii) To the alcoholic extract of drug, zinc turning and dilute HCl is added. Formation of deep red to magenta color indicates the presence of dihydro flavonoids.
- (c) **Vanillin HCl test:** The Vanillin HCl solution is added to the alcoholic solution of drug, formation of pink color indicates the presence of flavonoids.

Medicinal uses of glycosides: Glycosides are widely used in pharmaceutical industries including production of steroidal hormones, vitamins, active pharmaceutical ingredients and also for therapeutic purposes. Some important applications are listed below:

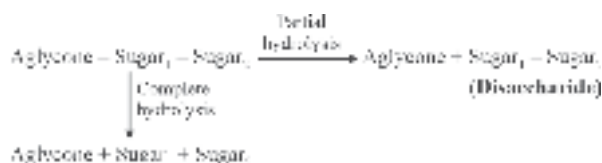
1. Digitalis: Used in the treatment of congestive heart failure.
2. Strophanthus: Used as a cardiac tonic and diuretic.
3. Squill: Used as rodenticide.
4. Oleandrin and adynerin: Used as anti-cancer
5. Senna and rhubarb: Used as laxative and purgative drugs.
6. Aloe: Used as purgative drug.
7. Diosgenin: Used for production of steroidal sex hormone like estrogen and progesterone.
8. Glycyrrhetic acid: Used as anti-inflammatory.
9. Glycosides of quillaic acid: Used as emulsifier.
10. Almond (Bitter): Used as expectorant.
11. Bitter glycosides: Used as bitter and in liver disorders.
12. Rutin: It is used to strengthens capillaries and can help people who bruise or bleed easily. Studies have demonstrated that rutin can help to stop venous edema, that is an early sign of chronic venous disease of the leg.

Generalised Methods of Structural Elucidation for Glycosides

The structure of glycoside is determined in two main steps: (i) Degradation and (ii) Synthesis.

(i) Degradation

The glycoside is hydrolysed with the help of dilute acid followed by separation of aglycone and sugar parts by chromatographic technique. The configuration of glycosidic linkage is determined by enzymatic hydrolysis, in general, the enzyme emulsin hydrolyses the beta glycosides (and maltase hydrolyses the alpha glycosides. If the sugar unit is present as disaccharide or trisaccharide unit, the partial hydrolysis of glycoside is done in order to identify the complete sugar part.



Determination of size of ring: The size of ring (pyranose or furanose) is established by usual method, e.g. complete methylation method.

Formation of xylotrimethoxy glutaric acid indicates the possibility of C-1 and C-6 involvement in bond formation (pyranose). In this condition, the product formed would be tetra-methylsaccharic acid or 2,3,4,5-tetramethyl gluconic acid.

The formation of dimethyl tartaric acid indicates the involvement of C-1 and C-4 positions in the formation of ring (furanose).

(ii) Synthesis

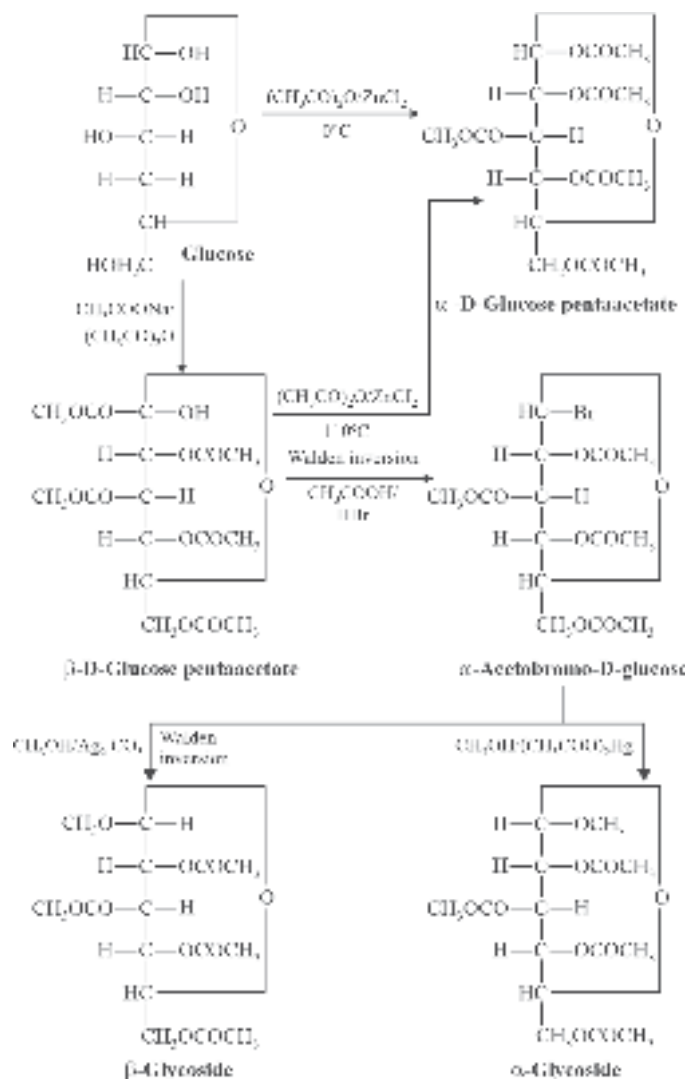
On the basis of degradation the structure of aglycone, sugar and glycosidic linkage is predicted and then structure of glycoside is predicted. The proposed structure is confirmed by synthesis. In general, synthesis of a glycoside involves the use of acetobromohexose (tetra-O-acetyl-D-hexopyranosyl-1-bromide) (Scheme 2.11). The bromine in acetobromo-D-glucose is very active. On treatment with alcohol in presence of mercuric acetate, it gives the α -glycoside while on treatment with alcohol in presence of silver carbonate, it gives the β -glycoside (Walden inversion).

Examples of Glycosides

1. Salicin

It is a salicyl alcohol glucoside, isolated from the leaves and barks of *Salix* species (Willow bark). For isolation of salicin, dried bark is extracted with a mixture of 80% alcohol and 20% water. The hydroalcoholic extract is then subjected to evaporation under reduced pressure to get salicin.

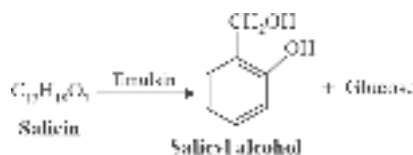
It is available as white crystals or powder. It is used as analgesic, antipyretic and anti-inflammatory drug. Salicin is closely related to aspirin. On absorption in body, it is metabolized to salicylic acid. Salicin shows two absorbance maxima (210 nm and 268 nm) in the UV spectrum.



Scheme 2.11: Synthesis of glycoside

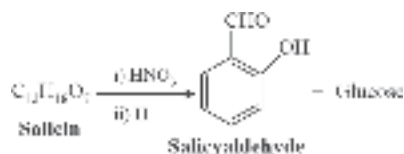
Structural elucidation of salicin

- Molecular formula:** It is found to be $\text{C}_{13}\text{H}_{18}\text{O}_7$.
- Nature of aglycone:** Hydrolysis of salicin by enzyme-emulsin, produces salicyl alcohol and glucose (*in vivo*, salicyl alcohol is metabolically oxidized to salicylic acid, the active component). It indicates that salicyl alcohol and D-glucose units are linked via β -linkage.

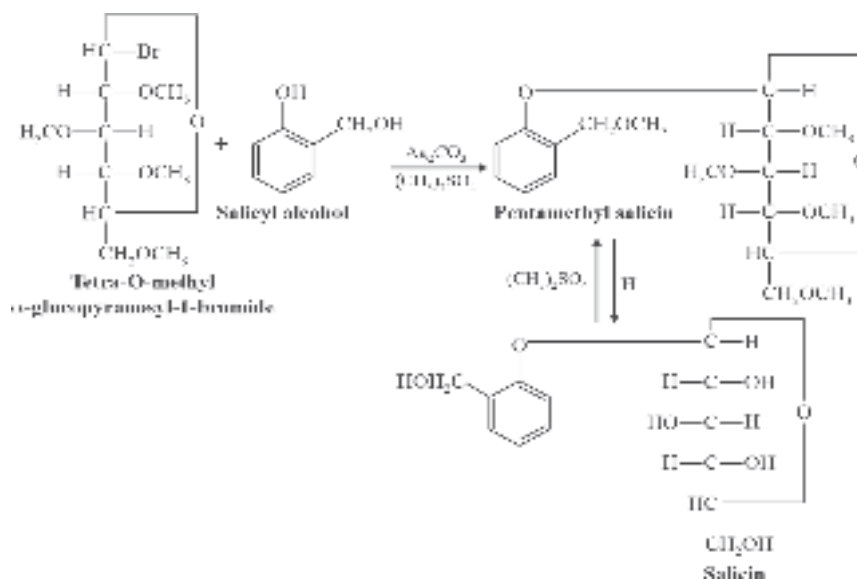


As salicyl alcohol contains phenolic hydroxyl group and alcoholic hydroxyl group, glucose moiety may be linked via phenolic hydroxyl or alcoholic hydroxyl group.

- (iii) Salicin on mild oxidation with nitric acid, followed by hydrolysis gives salicylaldehyde. This reaction indicates that alcoholic group is free in salicin. Hence, glucose moiety is attached through phenolic hydroxyl group.



- (iv) On the basis of above evidences, the structure is assigned to salicin, further confirmed by synthesis.



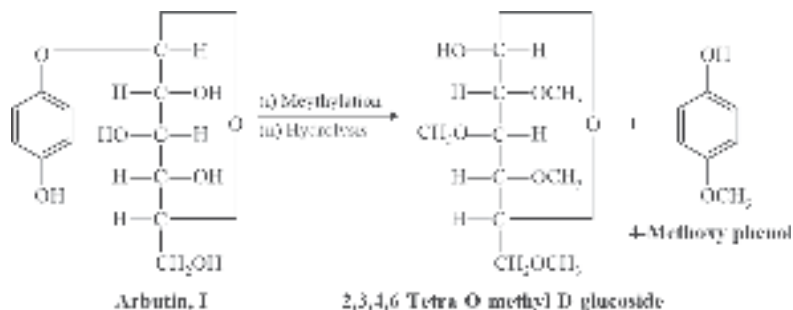
2. Arbutin and Methyl Arbutin

Arbutin is phenolic glycoside, found in the dried leaves of a number of different plant species including bearberry (*Arctostaphylos uva-ursi*). It is generally present in plants in combination with methyl arbutin, especially plants of the Ericaceae family. The leaves of uva-ursi typically contain 5–15% *arbutin* and up to 4% *methyl arbutin*. The leaves and leaf extracts from uva ursi are used in non-prescription medicinal products mainly to treat urinary tract infection, cystitis, kidney stones, and as a diuretic. The active component, arbutin, is converted to hydroquinone (HQ), which has antimicrobial, astringent, and disinfectant properties. Arbutin is also an inhibitor of melanin formation and is used in some skin-lightening products. Arbutin shows two absorbance maxima (285 and 225 nm) in the UV spectrum.

Structural elucidation of arbutin

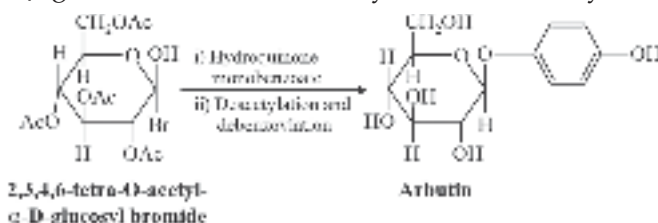
- (i) Arbutin has molecular formula, $\text{C}_{12}\text{H}_{16}\text{O}_7$.
- (ii) The enzyme, emulsin hydrolyses the arbutin into D-glucose and quinol. This reaction indicates the presence of D-glucose and quinol as glycone and aglycone part respectively, linked by β -glycosidic linkage.

- (iii) Arbutin on methylation forms pentamethylarbutin which on hydrolysis with methanolic HCl gives 2,3,4,6-tetra-O-methyl-D-glucoside and methoxy phenol. This reaction can be explained if structure, I is assigned to arbutin.



- (iv) The assigned structure is confirmed by synthesis.

Arbutin can be synthesized from tetra-O-acetyl- α -D-glucosyl bromide and hydroquinone monobenzoate in presence of silver oxide and quinoline. The Walden inversion takes place to give β -glucoside which is deacetylated and benzoylelated to get arbutin.

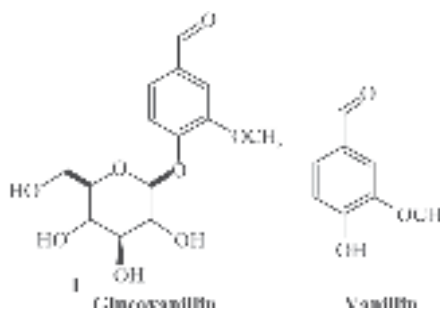


Methyl arbutin: It is hydrolysed by emulsin to one molecule of D-glucose and monomethyl-quinol. This indicates that methyl arbutin is β -glucoside of methylquinol. Methyl arbutin can be synthesized by condensing tetra-O-acetyl- α -D-glucopyranosyl-1-bromide with monomethylquinol in the presence of silver carbonate, followed by deacetylation.



3. Glucovanillin

It is vanillin D-glucoside or 4-(β -D-glucopyranosyloxy)-3-methoxy benzaldehyde. It is isolated from vanilla pods and used as flavoring agent.



On hydrolysis with acids or emulsion, it gives pleasant smelling vanillin and D-glucose, on the basis of this hydrolytic reaction, structure I is assigned to glucovanillin.

4. Sinigrin

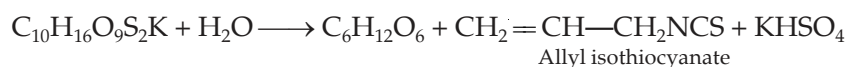
Sinigrin is sulphur containing glycoside, widely distributed in family Cruciferae.

- Sinigrin: In seeds of *Brassica nigra* (black mustard).
- Sinalbin: In seeds of *Brassica alba* (white mustard).

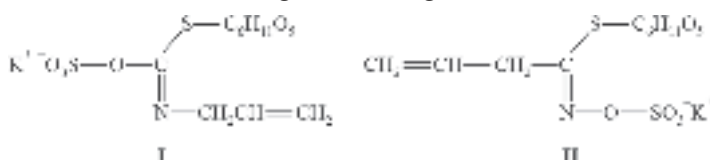
It is used as rubefacients, counter irritants and condiment.

Structural elucidation of sinigrin

- It has molecular formula, $C_{10}H_{16}O_9NS_2K$.
- On hydrolysis with enzyme, myrosin, it produces the D-glucose, allyl isothiocyanate and potassium hydrogen sulphate. This reaction indicates that it is α -glucoside.



- Sodium methoxide degrades sinigrin to give thioglucose as one of the products. On this basis two structures are assigned to sinigrin.



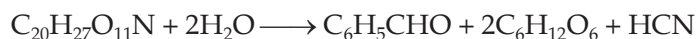
The structure II is confirmed on the basis of X-ray analysis. The rearrangement process in the structure II responsible for production of allyl isothiocyanate. The sinigrin has *syn*-configuration. The sulphate and thioglucose moiety are in *syn* position in the structure II.

5. Amygdalin

It is a cyanogenic glucoside present in bitter almonds and seeds of other plants of the family—Rosaceae. Chemically, it is D-mandelonitrile- β -D-glucoside-6- β -D-glucoside. It has maximum absorption of 268, 262, and 257 nm of wavelength.

Structural elucidation of amygdalin

- It has molecular formula, $C_{20}H_{27}O_{11}N$.
- On acid hydrolysis, it produces one molecule of benzaldehyde, two molecules of D-glucose and one molecule of HCN.



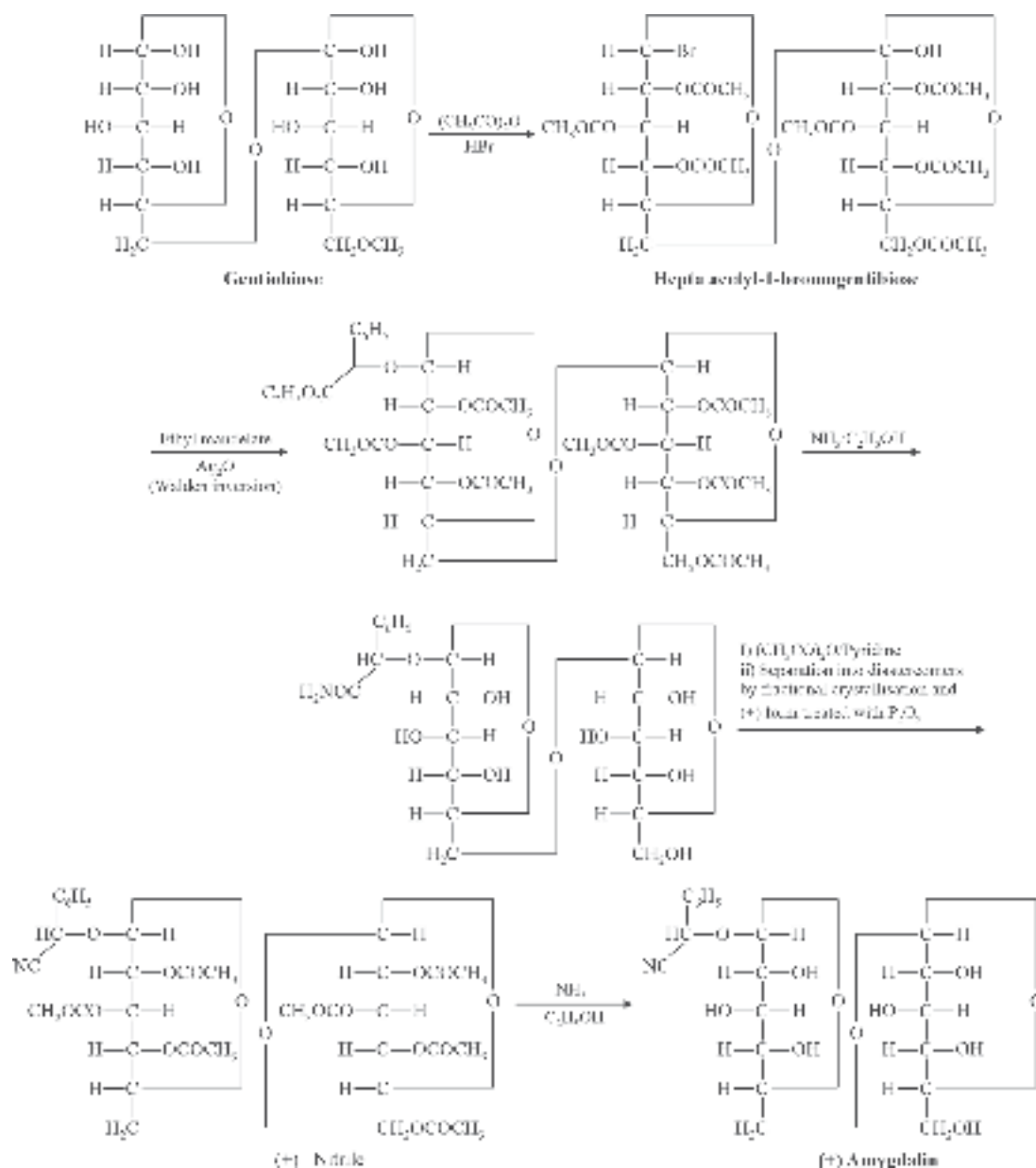
- Hydrolysis of amygdalin by enzyme, emulsin indicates the β -glycosidic linkage.
- The amygdalin on hydrolysis by enzyme, zymase produces one molecule of glucose and a glucoside of (+)-mandelonitrile.



The glucoside of (+)-mandelonitrile is identical to prunasin, naturally occurring glucoside. This reaction also indicates that aglycone and glycone part of amygdalin

are mandelonitrile and disaccharide, gentibiose, respectively. Both the part is linked by β -glycosidic linkage.

- (v) On the basis of above evidences, the structure is assigned to amygdalin which is further confirmed by synthesis from gentiobiose.

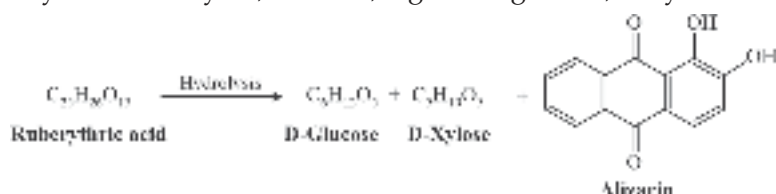


6. Ruberythric acid

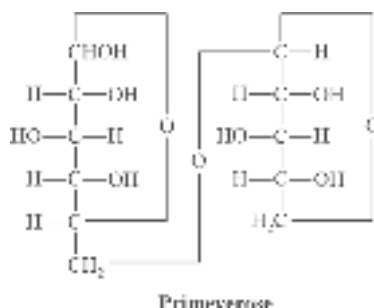
It is a yellow crystalline acidic glycoside occurring in madder root, *Rubia tinctorum* and yielding alizarin and primeverose on hydrolysis. It is soluble in hot water, slightly soluble in cold water, ethanol. It decomposes in acids and alkalis.

Structural elucidation of ruberythric acid

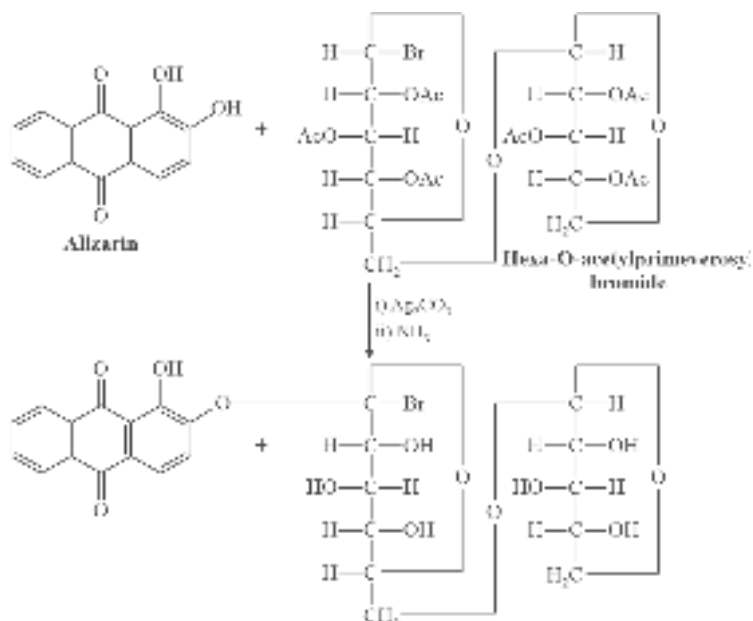
- (i) It has molecular formula, $C_{25}H_{26}O_{13}$.
 (ii) On hydrolysis with enzyme, emulsin, it gives D-glucose, D-xylose and alizarin.



- (iii) Ruberythric acid on controlled enzymatic hydrolysis, it gives disaccharide and alizarin. With usual method, disaccharide is identified as 6-O-β-D-xylopyranosyl-D-glucopyranose (primeverose).



- (iv) Methylation followed by hydrolysis of ruberythric acid gives the alizarin-1-methyl ether. This reaction indicates that the C-1 position of alizarin is free and C-2 position is linked with primeverose molecule.
 (v) Finally, the structure is confirmed on the basis of synthesis.

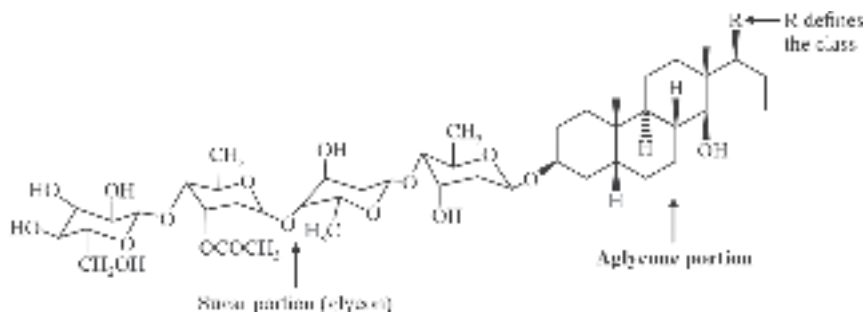
**CARDIAC GLYCOSIDES**

The cardiac glycosides are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart. Plants containing cardiac glycosides

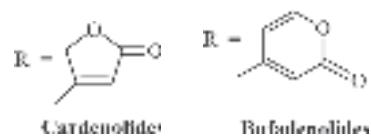
have been used as poisons and heart drugs at least since 1500 BC. Throughout history, these plants or their extracts have been variously used as arrow poisons, emetics, diuretics, and heart tonics. Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation and flutter.

Structural Features

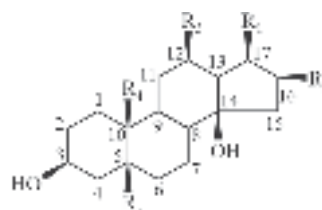
Cardiac glycosides are composed of two structural features: the sugar (glycon) and the non-sugar (aglycone-steroid) moieties as shown below:



The R group at the 17-position defines the class of cardiac glycoside. Two classes have been observed in nature—the cardenolides and the bufadenolides (see structure). The cardenolides have an unsaturated butyrolactone ring while the bufadenolides have an α -pyrone ring.

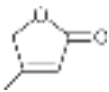
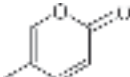
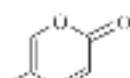


Nomenclature of cardiac glycoside: The cardiac glycosides occur mainly in plants from which the names have been derived. *Digitalis purpurea*, *Digitalis lanata*, *Strophanthus gratus*, and *Strophanthus kombe* are the major sources of the cardiac glycosides. The term 'genin' at the end refers to only the aglycone portion (without the sugar). Thus, the word digitoxin refers to an agent consisting of digitoxigenin (aglycone) and sugar moieties (three). The aglycone portion of cardiac glycosides is more important than the glycone portion.



Name	R_1	R_2	R_3	R_4	R_5
Digitoxigenin		H	H	CH ₃	H
Digoxigenin		H	OH	CH ₃	H
Gitoxigenin		OH	H	CH ₃	H
Quabagenin		H	H	CH ₂ OH	OH

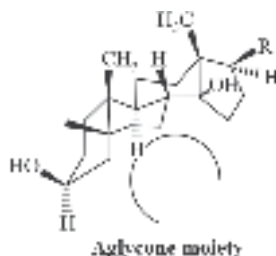
Note: OH group is also present at C-1 and C-11 positions

Strophanthidin		H	H	—CHO	OH
Bufalin		H	H	CH ₃	H
Scillarenin		H	H	CH ₃	H

Note: Double bond is present at C4–5 position

The aglycone moiety: The steroid nucleus has a unique set of fused ring system that makes the aglycone moiety structurally distinct from the other more common steroid ring systems. Rings A/B and C/D are *cis*-fused while rings B/C are *trans* fused. Such ring fusion give the aglycone nucleus of cardiac glycosides the characteristic 'U' shape as shown below.

The steroid nucleus has hydroxyls at 3- and 14-positions of which the HO sugar uses the 3-OH group for attachment. 14-OH is normally unsubstituted. Many genins have —OH groups at 12- and 16-positions. These additional hydroxyl groups influence the partitioning of the cardiac glycosides into the aqueous media and greatly affect the duration of action.



The lactone moiety at C-17 position is an important structural feature for cardiac activity. The size and degree of unsaturation varies with the source of the glycoside. Normally, plant sources have a 5-membered α,β -unsaturated lactone while animal sources give a 6-membered α,β -unsaturated lactone.

Sugar moiety: One to four sugars units are found to be present in most cardiac glycosides attached to the 3 β -OH group. The sugars most commonly used include L-rhamnose, D-glucose, D-digitoxose, D-digitalose, D-diginose, D-sarmentose, L-vallarose, and D-fructose. These sugars predominantly exist in the cardiac glycosides in the β -conformation. The presence of acetyl group on the sugar affects the lipophilic character and the kinetics of the entire glycoside. Because the order of sugars appears to have little to do with biological activity, nature has synthesized a numerous cardiac glycosides with differing sugar skeleton but relatively few aglycone structures.

Structure–Activity Relationships

- The sugar moiety appears to be important only for the partitioning and kinetics of action. It possesses no biological activity. For example, elimination of the aglycone

moiety eliminates the activity of alleviating symptoms associated with cardiac failure.

- The “backbone” U shape of the steroid nucleus appears to be very important. Structures with C/D *trans* fusion are inactive.
- Conversion to A/B *trans* system leads to a marked drop in activity. Thus, although not mandatory A/B *cis* fusion is important.
- The 14 β -OH groups is now believed to be dispensible. A skeleton without 14 β -OH group but retaining the C/D *cis* ring fusion is found to retain activity.
- Lactones alone, when not attached to the steroid skeleton, are not active. Thus, the activity resides in the steroid skeleton.
- α,β -unsaturated lactone ring at C-17 position plays an important role in receptor binding. Saturation of the lactone ring dramatically reduced the biological activity.
- The lactone ring is not absolutely required. For example, using α,β -unsaturated nitrile (C=C-CN group) the lactone could be replaced with little or no loss in biological activity.

Pharmacokinetics of Cardiac Glycosides

The commercially available cardiac steroids differ markedly in their degree of absorption, half-life, and the time to maximal effect (see table below).

<i>Agent</i>	<i>GI absorption</i>	<i>Onset (m)</i>	<i>Peak (h)</i>	<i>Half-life</i>
Quabain	Unreliable	5–10	0.5–2	21 h
Deslanoside	Unreliable	10–30	1–2	33 h
Digoxin	55–75%	15–30	1.5–5	36 h
Digitoxin	90–100%	25–120	4–12	4–6 days

Usually, this is due to the polarity differences caused by the number of sugars at C-3 and the presence of additional hydroxyls on the cardenolide. Although, two cardiac glycosides may differ by only one sugar residue their partition coefficients may be significantly different resulting in different pharmacokinetics. For example, lanatosides C and digoxin differ only by a glucose residue and yet the partition coefficient measured in CHCl₃/16% aqueous MeOH are 16.2 and 81.5, respectively.

<i>Glycoside</i>	<i>Partition coefficient</i>
Lanatoside C (glucose-3-acetyldigitoxose-digitoxose ₂ -digoxigenin)	16.2
Digoxin (digitoxose ₃ -digoxigenin)	81.5
Digitoxin (digitoxose ₃ -digitoxigenin)	96.5
Acetyldigoxin (3-acetyldigitoxose-digitoxose ₂ -digoxigenin)	98.0
G-Strophanthin (rhamnose-quabagenin)	Very low

In general, cardiac glycosides with more lipophilic character are absorbed faster and exhibit longer duration of action as a result of slower urinary excretion rate. Lipophilicity is markedly influenced by the number of sugar residues and the number of hydroxyl groups on the aglycone part of the glycoside. Comparison of digitoxin and digoxin structures reveals that they differ only by an extra-OH group in digoxin at C-12, yet their partition coefficients differ by as much as value of 15%.

Cardiac glycosides show positive inotropic effects due to following reasons:

1. Cardiac glycosides inhibits $\text{Na}^+\text{-K}^+\text{-ATPase}$ pump system on cardiac muscle cell membrane.
2. Due to inhibition, there is increased intracellular Na^+ concentration.
3. There is less expulsion of calcium from the cells by the Na^+ and Ca^+ exchanger.
4. There is facilitation of Ca^+ entry through the voltage gated calcium channel.
5. There is increased release of calcium from intracellular storage site (Sarcoplasmic reticulum).
6. Net result is increased cardiac contractility.

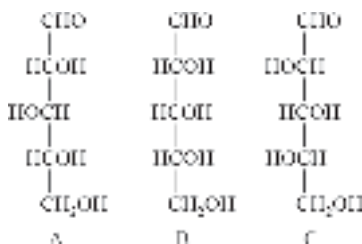
SHORT AND LONG QUESTIONS

1. What are carbohydrates? How these are classified.
2. Discuss the structure and stereochemistry of glucose.
3. How will you distinguish between glucose and fructose?
4. Write the hydrolytic products of lactose and starch.
5. How will you distinguish between glucose and sucrose (cane sugar)?
6. Treatment of glucose with HIO_4 gives results that confirm its aldohexose structure. What product should be formed, and how much HIO_4 should be consumed
7. Why aldoses react with Fehling's solution and phenyl hydrazine, but not with NaHSO_3 ?
8. Glucose on oxidation with conc. HNO_3 gives glucaric acid. Glucaric acid on dehydration gives two six-membered lactones. Explain the reactions involved with the formation of lactones.
9. Discuss the methylation method to determine the ring size in glucose molecule.
10. Distinguish between the oxidation and reduction of monosaccharides.
11. Define what is meant by anomers.
12. a. Identify the product formed when a given monosaccharide is reduced with sodium borohydride.
b. Identify the monosaccharide which should be reduced in order to form a given polyalcohol (alditol)
13. Determine whether a given monosaccharide will exist as a pyranose or furanose.
14. Discuss the structural elucidation of sucrose.
15. What is mutarotation? Discuss the mechanism of mutarotation.
16. Define what is meant by anomers and describe how they are formed
17. Discuss the chemistry of Lactose.
18. Classify the carbohydrates and their properties
19. Define the term 'Carbohydrate'. Elucidate the structure of fructose with appropriate reactions.
20. a. How will you convert aldose to ketose and vice versa
b. Write two methods of synthesis for increasing carbon number in the carbohydrate.
21. Glucose, fructose and mannose form the same osazone on reaction with phenylhydrazine HCl and sodium acetate. Why? Write only the structure of osazone (indicating the configuration at all asymmetric carbon atoms).
22. a. What is mutarotation?
b. Discuss the chemistry of Lactose.

23. Give the following reactions:
 - a. Glucose + Br₂ Water
 - b. Glucose + Dil HNO₃
 - c. Glucose + Na-Hg/ H₂O
 - d. Glucose + HCN
 - e. Glucose + NH₂ OH
 - f. Glucose + CH₃-CO-Cl
24. Draw the Fischer projection formula of the following:
 - a. α-D-glucopyranose
 - b. β-D-glucopyranose
 - c. α-D-fructopyranose
 - d. β-D-fructopyranose
25. Draw the Haworth projection formula of following:
 - a. α-D-glucopyranose
 - b. β-D-glucopyranose
 - c. α-D-fructopyranose
 - d. β-D-fructopyranose
26. Explain the following:
 - a. Glucose has pyranose ring.
 - b. Osazone formation
 - c. Oligosaccharides with suitable example
27. Write notes on:
 - a. Mutarotation
 - b. Kiliani-Fischer synthesis
 - c. Reducing properties of monosaccharides
28. What are cardiac glycosides? Discuss its important structural features.
29. What are epimers? Give the mechanism of epimerization.
30. Give the essential features of chitin.
31. Classify the glycosides. Discuss the chemistry of amygdalin.
32. Classify the glycosides with examples.
33. Enlist the phenolic glycosides? Give the structural elucidation of salicin.

MULTIPLE CHOICE QUESTIONS

1. Consider the sugars shown below:



Answer the following three questions about these carbohydrate structures.
What kind of isomers are A and B?

What kind of isomers are A and C?

What kind of isomers are B and C?

- a. Anomers; epimers; enantiomers
 - b. Enantiomers; anomers; diastereomers
 - c. Diastereomers; enantiomers; epimers
 - d. Epimers; enantiomers; diastereomers
 - e. Enantiomers; epimers; anomers
2. β -D-glucopyranose in the chair form is the most widely occurring form of glucose in nature and it has the following characteristics except:
- a. Forms a six-membered ring
 - b. Carbon 6 is above the plane of the chair
 - c. All of the -OH groups are equatorial
 - d. The anomeric carbon has a hydroxyl that is below the plane of the chair
 - e. All axial positions are occupied by -H
3. In plant cells, starch is hydrolyzed by to release and a starch molecule with
- a. Starch phosphorylase; glucose-1-phosphate; one less glucose unit
 - b. Salivary α -amylase; maltose 1-phosphate; two less glucose units
 - c. Starch phosphorylase; maltose 1-phosphate; two less glucose units
 - d. A (1 $\rightarrow$ 6) glucosidase; glucose-6-phosphate; one less glucose unit
 - e. Starch hydrolase; glucose; one less glucose unit
4. Cooked starch is more digestible because:
- a. It has been partially hydrolyzed during cooking.
 - b. The amylopectin is converted to amylose during cooking.
 - c. The enzymes that hydrolyze the starch are only active when the starch is hot.
 - d. The starch granules take up water, swell and are more accessible to the enzymes.
 - e. All are true
5. Which of the following statements about cellulose fiber is true?
- a. It is a linear polymer of β (1 $\rightarrow$ 4) glycosidic linked glucose and galactose residues.
 - b. It is extremely susceptible to hydrolysis
 - c. It is water-soluble
 - d. It is made up of very weak microfibrils
 - e. Intermolecular hydrogen bonds play a major role in stabilization of the microfibrils
6. Cellulose is extremely resistant to hydrolysis, but cellulase catalyzes the hydrolysis and comes from:
- a. The pancreas
 - b. Bacteria that live in the lumen
 - c. The liver
 - d. H^+ secreted into the lumen
 - e. The intestinal wall

7. and are found in tendons, cartilage and other connective tissue.
 - a. Heparin; chondroitins
 - b. Chondroitin's; keratin sulfate
 - c. Keratin sulphate, dermatan sulfate
 - d. Heparin, keratin sulphate
 - e. All are true.
8. Maltose is a disaccharide of which simple sugars?
 - a. Fructose and lactose
 - b. Glucose and glucose
 - c. Glucose and galactose
 - d. Glucose and lactose
9. Which one of these sweeteners is used in sugarless gums and candies?
 - a. Ribitol
 - b. Xylitol
 - c. Inositol
 - d. Mannitol
10. Starch consists of
 - a. Branched amylose and branched amylopectin
 - b. Unbranched amylose and branched amylopectin
 - c. Unbranched amylose and unbranched amylopectin
 - d. None of the above
11. Which of the following is also known as inverted sugar?
 - a. Sucrose
 - b. Fructose
 - c. Dextrose
 - d. Glucose
12. Which of the following is also known as table sugar?
 - a. Sucrose
 - b. Lactose
 - c. Dextrose
 - d. Glucose

Answers

1. (d) 2. (d) 3. (e) 4. (e) 5. (e) 6. (b) 7. (b) 8. (b) 9. (b) 10. (b) 11. (a) 12. (a)

Alkaloids—An Overview

Alkaloids are basic nitrogenous compounds, obtained from plants, animals and microorganisms having a marked physiological action. The term 'alkaloid' was first introduced by **W Meissner** in 1918. According to him, alkaloids (alkali-like) are basic nitrogenous compounds isolated from plants. According to **Ladenburg** "alkaloids are defined as naturally occurring compounds that have basic character and contains at least one nitrogen atom in a heterocyclic ring and have marked physiological action." The name 'proto-alkaloid' or 'amino-alkaloid' is sometimes applied to compounds such as ephedrine and colchicine, which lack one or more of the properties of typical alkaloids.

Characteristics of Alkaloids

Most of the alkaloids are crystalline substances, generally occurring as solids. A few alkaloids are amorphous in nature, e.g. emetine. Some alkaloids are liquid, for example, nicotine and pilocarpine, which are a volatile and non-volatile liquid, respectively. Majority of alkaloids are colorless except berberine and colchicine, which are yellow colored alkaloid. The alkaloidal bases are soluble in organic solvent and insoluble in water. The alkaloidal salts are soluble in water but insoluble in organic solvents. The alkaloids are mostly found in plants as free base, or as salt with organic acid (maleate, oxalate, citrate) or inorganic acid (hydrochloride, sulphate) or with special acid (meconic acid, quinic acid), as N-oxides and glycosidal form (solanine in solanum sp.).

Most of the alkaloids are optically active except papaverine, which is optically inactive. The optical isomers may show different physiological activities. For example, atropine (racemic form) is physiologically active. The levo isomer of quinine is antimalarial while its dextro isomer is antiarrhythmic.

Qualitative Tests for Alkaloids

There are several general reagents, which may be used to detect the presence of alkaloids or to help in their identification. These include the alkaloidal precipitating reagent and the alkaloidal coloring reagents. In addition, there are some special reagent that can be used for recognizing and confirming the identity of particular alkaloid. The various alkaloidal reagents are classified as follows:

A. Alkaloidal Precipitating Reagent

1. *Reagent that form double salt*

- (i) **Mayer's reagent (potassium mercuric iodide solution):** Alkaloids give cream or pale yellow colored precipitate with Mayer's reagent.

- (ii) **Dragendroff's reagent (potassium bismuth iodide solution):** Alkaloids give orange colored precipitate with this reagent. Caffeine is a pseudo alkaloid, which does not react with this reagent.
- (iii) **Gold chloride:** It gives yellow colored precipitate with alkaloids.

II. Reagents containing halogens

Wagner's reagent (Iodine–potassium iodide solution): Alkaloids give brown or reddish brown colored precipitate with Wagner's reagent.

III. Organic acids

- (i) **Hager's reagent (saturated solution of picric acid):** Alkaloids give yellow colored precipitate with Hager's reagent.
- (ii) **Tannic acid:** Alkaloids give buff colored precipitate.

IV. Oxygenated acids

- (i) **Phosphomolybdic acid (Sheibler's reagent):** It precipitates the alkaloids and similar nitrogenous compounds in the form of yellowish or brownish-yellow colored solids.
- (ii) **Phosphotungstic acid:** It behaves like phosphomolybdic acid.
- (iii) Silicotungstic acid also gives precipitate with alkaloids.

B. Alkaloidal Coloring Reagents

These develop the color with alkaloids.

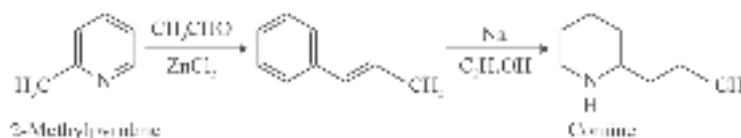
1. Marqui's reagent (Formaldehyde–sulphuric acid)
2. Mandalin's reagent (Sulphovanadic acid)
3. Erdmann's reagent (Nitric acid–sulphuric acid)

History of Alkaloids

Alkaloids containing plants were used by humans since ancient times for therapeutic and recreational purposes. For example, medical plants have been known in the Mesopotamia at least around 2000 BC. A Chinese book on indoor plants written in 1st–3rd centuries BC mentioned a medical use of ephedra and opium poppies. Also, coca leaves have been used by South American Indians since ancient times. Extracts from plants containing toxic alkaloids, such as aconitine and tubocurarine, were used since antiquity for poisoning arrows. Ancient people used plant extracts, containing alkaloids for treating a large number of ailments including snakebite, fever and insanity.

Studies of alkaloids began in the 19th century. The French chemist, **Derosne** in 1803, isolated narcotine. **Freidrich Serturmer**, the German chemist who first isolated morphine from opium in 1804 and called it morphium. **Pelletier** and **Caventou** from the School of Pharmacy, Paris, isolated emetine in 1817 and colchicine in 1819. This was followed by isolation of alkaloids from vegetable drugs, like strychnine (1817); brucine, piperine and caffeine (1819); quinine and cinchonine (1820); papaverine (1821), coniine (1826) and thebaine (1835). **Caventous** was pioneer in the use of mild solvent to isolate a number of active ingredients from plants, making a study of alkaloids from vegetables. But in 1870, a landmark in the domain of alkaloids is achieved by determining the structure of coniine which also becomes the first synthesized alkaloid in 1886.

The first complete synthesis of an alkaloid was achieved in 1886 by the German chemist, **Albert Ladenberg**. He synthesized coniine by reacting 2-methylpyridine with acetaldehyde and reducing the resulting 2-propenyl pyridine with sodium. The development of chemistry of alkaloids was accelerated by the emergence of spectroscopical and chromatographical methods in the 20th century and by 2008 more than 12000 alkaloids were identified. Day by day, numbers of documented alkaloids are increasing.



Occurrence

True alkaloids are of rare occurrence in lower plants. In the fungi, the lysergic acid derivatives and the sulphur-containing alkaloids, e.g. the gliotoxins, are the best known examples. Among the pteridophytes and gymnosperms, the lycopodium, ephedra and taxus alkaloids are of medicinal interest. Alkaloid distribution in the angiosperm is uneven. They are found in abundance in the families—apocynaceae, papaveraceae, papilionaceae, ranunculaceae, rubiaceae, rutaceae, and solanaceae. Depending on the type of plants, the maximum concentration is observed in the leaves (e.g. black henbane, *hyoscyamus niger*), fruits or seeds (strychnine tree), root (e.g. *Rauwolfia serpentina*) or bark (e.g. cinchona).

Nearly 300 alkaloids belonging to more than 24 classes are known to occur in the skins of amphibians along with other toxins. They include the potent neurotoxic alkaloid (e.g. steroidal alkaloids, batrachotoxins) of frogs of the genus *Phylllobates*, which are among some of the most poisonous substances known. Other reptilian alkaloids are strongly antimicrobial. Alkaloids derived from mammals belong to indole and isoquinoline classes, a few of them are found in both plants and animals.

Classification of Alkaloids

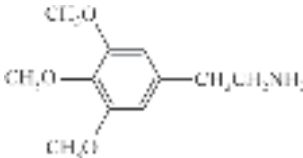
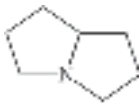
Alkaloids can be classified on the basis of:

- **Chemical structure** (heterocyclic nucleus containing nitrogen).
- **Biosynthetic pathway** (the way they are produced in the plant).
- **Pharmacological activity**.
- **Taxonomical**: This is done according to the source of compound without reference to the chemical types.

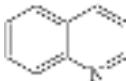
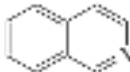
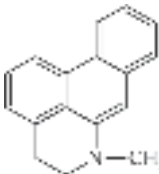
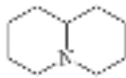
Out of these, classification based on chemical structure is universally acceptable as other ways of classification are confusing. There are two broad divisions:

- I. Non-heterocyclic or atypical alkaloids, sometimes called 'protoalkaloids' or biological amines.
- II. Heterocyclic or typical alkaloids, divided into 14 groups according to their ring structure (Table 3.1).

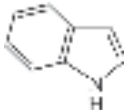
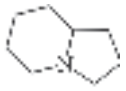
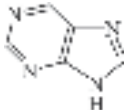
Table 3.1: Types of alkaloids and their occurrence

Example	Source
I. Non-heterocyclic alkaloids	
Mescaline	<i>Lophophora williamsii</i> (Cactaceae)
	
Ephedrine	<i>Ephedra</i> sp. (Ephedraceae)
Colchicine (tropolone nucleus with nitrogen inside-chain)	<i>Colchicum</i> sp. and related genera (Liliaceae)
Taxol (a modified diterpene pseudoalkaloid)	<i>Taxus brevifolia</i> (Taxaceae)
II. Heterocyclic alkaloids	
1. Pyrrole and pyrolidine-based alkaloid	
	
Hygrine	<i>Coca</i> sp. (Erythroxylaceae); often associated with tropane alkaloid, cocaine
2. Pyrolizidine based alkaloid	
	
Symphitine, echimidine	<i>Symphytum</i> sp.
Senecionine, seneciphylline, etc.	<i>Senecio</i> sp.
3. Pyridine and piperidine based alkaloid	
	
Trigonelline	Fenugreek (Leguminosae)
Coniine (piperidine)	<i>Conium maculatum</i> (Umbelliferae)
Arecoline (piperidine)	<i>Areca catechu</i> (Palmae)
Lobeline (piperidine)	<i>Lobelia</i> sp. (Lobeliaceae)
Pelletierine (piperidine)	<i>Punica granatum</i> , the pomegranate (Punicaceae)
Nicotine (pyridine + pyrolidine)	<i>Nicotiana tabacum</i> and other sp. (Solanaceae)
Anabasine	<i>Nicotiana glauca</i> ; <i>Anabasis aphylla</i> (Chenopodiaceae)
Piperine (piperidine)	<i>Piper</i> sp. (Piperaceae)
Ricinine	

(Contd.)

Example	Source
4. <i>Tropane-based alkaloid</i>	
 Tropane Hyoscyamine, atropine, hyoscyne, meteloidine, etc. Cocaine Pseudo-pelletierine	Species of <i>Atropa</i>, <i>Datura</i>, <i>Hyoscyamus</i>, <i>Duboisia</i>, <i>Madragora</i> and <i>Scopolia</i> (Solanaceae) <i>Coca</i> sp. (Erythroxylaceae) <i>Punica granatum</i> (Punicaceae)
5. <i>Quinoline-based alkaloid</i>	
 Quinoline Quinine, quinidine, cinchonine, cinchonidine Cusparine	<i>Cinchora</i> sp. (Rubiaceae), <i>Remijia</i> sp. (Rubiaceae) <i>Angostura</i> or <i>cusparia</i> bark, <i>Galipea officinalis</i> (Rutaceae)
6. <i>Isoquinoline-based alkaloid</i>	
 Isoquinoline Papaverine, narceine, narcotine Hydrastine, berberine Emetine, cephaeline Tubocurarine	<i>Papaver somniferum</i> (Papaveraceae) Numerous genera of the Berberidaceae, Ranunculaceae and Papaveraceae family <i>Cephaelis</i> sp. (Rubiaceae) Curare obtained from plants of family—Menispermaceae
7. <i>Aporphine (reduced isoquinoline/naphthalene)-based alkaloid</i>	
 Aporphine Boldine	<i>Peumus boldus</i> (Monimiaceae)
8. <i>Quinolizidine based alkaloid</i>	
 Quinolizidine Sparteine, cytisine, lupanine, laburnine	Sometimes called 'the lupin alkaloids'. Occur particularly in the Leguminosae, subfamily Papilionaceae, e.g. broom (<i>Cytisus scoparius</i>); dyer's broom (<i>Genista tinctoria</i>); <i>Laburnum</i> and <i>Lupinus</i> sp.

(Contd.)

Example	Source
9. Indole or benzopyrrole-based alkaloid	
	
Indole	
Ergometrine, ergotamine	<i>Claviceps</i> sp. (Hypocreaceae)
Physostigmine	<i>Physostigma venenosum</i> (Leguminosae)
Ajmaline, serpentine, reserpine	<i>Rauwolfia</i> sp. (Apocynaceae)
Yohimbine, aspidospermine	<i>Aspidosperma</i> sp. (Apocynaceae)
Vinblastine, vincristine	<i>Catharanthus rosea</i> (Apocynaceae)
Curare alkaloids	<i>Chondrodendron tomentosum</i> , certain <i>Strychnos</i> sp. (Loganiaceae)
Strychnine, brucine	<i>Strychnos</i> sp. (Loganiaceae)
10. Indolizidine-based alkaloid	
	
Indolizidine	
Castanospermine	<i>Castanospermum australe</i> (Leguminosae), <i>Alexa</i> sp. (Leguminosae)
Swainsonine	<i>Swainsona</i> sp. (Leguminosae)
11. Imidazole-based alkaloid	
	
Imidazole	
Pilocarpine	<i>Pilocarpus</i> sp. (Rutaceae)
12. Purine-based alkaloid	
	
Purine	
Caffeine	Tea (Ternstroemiaceae), coffee (Rubiaceae)
Theobromine	Coca (Sterculiaceae)
13. Steroidal-based alkaloid (some combined as glycosides)	
Solanine (glycoside of solanidine)	Shoots of potato (Solanaceae), etc.
Veratrum alkaline esters and their glycosides	<i>Veratrum</i> spp. and <i>Schoenocaulon</i> sp. (Liliaceae)
Conessine	<i>Holarrhena antidysenterica</i> (Apocynaceae)
14. Terpenoid-based alkaloid	
Aconitine, atisine, lyctonine, etc.	<i>Aconitum</i> and <i>Delphinium</i> sp. (Ranunculaceae)

Nature of nitrogen atom in alkaloids: Alkaloids, taken in their broadest sense, have a nitrogen atom which is primary (mescaline norephedrine), secondary (ephedrine), tertiary (atropine) or quaternary (one of the N atoms of tubcurarine), and this factor affects the derivatives of the alkaloid which can be prepared and the isolation procedure.

Biosynthetic Pathway-based Classification

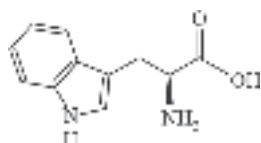
The amino acids are the precursor of the alkaloid (Table 3.2). Hence, alkaloids are classified on the basis of amino acid, responsible for biosynthesis of particular alkaloid.

Table 3.2: Major types of alkaloids, their amino acid precursors and well known examples of each type

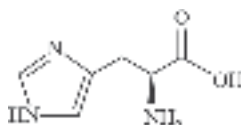
<i>Alkaloid type</i>	<i>Biosynthetic precursor (amino acid)</i>	<i>Examples</i>
Pyrolidine	Ornithine	Nicotine
Tropane	Ornithine	Atropine, cocaine
Piperidine	Lysine (or acetate)	Coniine
Pyrolizidine	Ornithine	Retrorsine
Quinolizidine	Lysine	Lupinine
Isoquinoline	Tyrosine	Morphine, codeine
Phenylethylamines	Tyrosine	Epinephrine
Indole	Tryptophan	Reserpine, Strychnine, Psilocybin
Imidazole	Histidine	Pilocarpine

The most common amino acids that act as precursor in biosynthesis of alkaloids are:

- (i) Ornithine derived alkaloid, $\text{H}_2\text{N}(\text{CH}_2)_3\text{CHNH}_2\text{COOH}$.
Examples: Hygrine, cocaine, atropine, scopolamine.
- (ii) Lysine derived alkaloid, $\text{H}_2\text{N}(\text{CH}_2)_4\text{CHNH}_2\text{COOH}$.
Examples: Anabasine, lupinine.
- (iii) Phenylalanine derived alkaloid, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$.
Examples: Ephedrine, mescaline.
- (iv) Tyrosine-derived alkaloid, $p\text{-OHC}_6\text{H}_4\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$.
Examples: Papaverine, thebaine, codeine, morphine, emetine, colchicine.
- (v) Methionine derived alkaloid, $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$.
Examples: Nicotine, ergonine.
- (vi) Tryptophan derived alkaloid.



- Examples:* Ergot alkaloids, quinine, strychnine, reserpine, etc.
- (vii) Histidine derived alkaloid.



Example: Pilocarpine.

Pharmacological-based Clasasification

It is based on their pharmacological activity or response. For example:

1. Analgesic alkaloids, e.g. morphine
2. Cardiac active alkaloids, e.g. ajmaline, reserpine, quinidine

3. CNS stimulants, e.g. caffeine
4. Depressants, e.g. opium alkaloids
5. Antitussive, e.g. codeine
6. Anticancer, e.g. vincristine

Taxonomy-based Classification

This classification is based on the distribution of alkaloids in various plant families like solanaceous, papilionaceous alkaloids. Sometimes, they are grouped as per the name of grouped genus in which they occur, e.g. ephedra, cinchona.

Biological Functions of Alkaloids

Although biological functions of alkaloids are not clearly understood but they may act as:

- Reserve substances to supply nitrogen, but very little evidence is found for this type of activity.
- End products of detoxification mechanism.
- Act as poisonous substances to protect the plant from insects and animals. For example, nicotine shows insecticidal properties.
- As plant stimulants and regulators. For example, colchicine which inhibits cell division.
- Function as food reservoir for protein synthesis.
- Excretory products of plant.
- Inhibitor of enzymatic activity.

Medical Importance of Alkaloids

A number of alkaloids are used as drug (Table 3.3). For example:

- **Quinine** derived from the bark of the tropical cinchona tree, has been used as antipyretic and antimalarial drug since the ancient times.
- **Quinidine** is primarily used for treatment of cardiac arrhythmia.
- **Vincalukoblastine and vincristine (indole alkaloids)**, two alkaloids derived from the periwinkle plant are used effectively for the treatment of white blood cell cancers. Vincalukoblastine is especially useful against lymphoma (cancer of the lymph glands), while vincristine (oncovin) is used against the most common form of childhood leukemia.
- **Atropine** is an alkaloid produced by several plants, including deadly nightshade (*Atropa belladonna*), Jimson weed (*Datura stramonium*), and henbane (*Hyoscyamus niger*) has ability to relax smooth muscle by blocking action of the neurotransmitter, acetylcholine. Atropine is most commonly used to dilate the pupil during eye examinations. Atropine also relieves nasal congestion and serves as an antidote to nerve gas and insecticide poisoning.
- **Pilocarpine**, derived from several Brazilian shrubs, is another alkaloid used in ophthalmology.
- **Reserpine** found in root of *Rauwolfia serpentina* (Indian snakeroot), is also used in the treatment of high blood pressure. Unfortunately, reserpine also causes drowsiness and sometimes severe depression. Medications without these side effects have been developed in recent decades, and so reserpine is rarely used.

- **Ephedrine** is largely used as substitute for epinephrine against bronchial asthma. It is also used orally and locally in patients suffering from hay fever, urticaria and other allergic reactions.
- **Cocaine** from the leaves of the coca plant has central nervous system stimulatory activity and is used as local anesthetics agent.
- **Quinolizidine and pyrolizidine** type alkaloids are used in modern medicine for treatment of pain, cough and diarrhoea.
- **Purine alkaloids** derived from tea and coffee are widely consumed in human diet.

Extraction Procedure for Alkaloids

There are several methods that can be used for the extraction of the alkaloids from plant materials. However, the common procedures are largely based on: (1) the basic nature of most of the alkaloids; (2) the subsequent ability to form salts with acids; (3) the ease by which the free bases can be liberated from their salts by alkalinization; and finally (4) the relative solubility of the alkaloids and their salts in water and various organic solvents.

The isolation of alkaloid from their plant sources is usually not difficult. First of all presence of an alkaloid is ascertained in the experimental plants; for which the plants extracts is treated with alkaloidal reagent such as picric acid, tannic acid. Because of the structural diversity of alkaloids, there is no single method of their extraction from naturally available raw materials. Most of the methods exploit the solubility property of the alkaloids. The alkaloids as base are soluble in organic solvent but insoluble in water. In contrast, alkaloids as a salt has opposite tendency means insoluble in organic solvents but soluble in water. However, if an appreciable quantity of alkaloid is present, one of the following general methods will usually serve the purpose.

Process A: The powdered material is moistened with water and mixed with lime which combines with acids, tannins and other phenolic substances and sets free the alkaloids (if they exist in the plant as salts). Extraction is then carried out with organic solvents such as ether or petroleum spirit. The concentrated organic liquid is then shaken with aqueous acid and allowed to separate. Alkaloid salts are now in the aqueous liquid, while many impurities remain behind in the organic liquid. The basification of the aqueous liquid gives the precipitate of alkaloids.

Table 3.3: Medicinal importance of alkaloids

<i>Alkaloid</i>	<i>Medicinal use</i>	<i>Alkaloid</i>	<i>Medicinal use</i>
Ajmaline	Antiarrhythmic	Morphine	Analgesic
Atropine, scopolamine, hyoscyamine	Anticholinergic	Reserpine	Antihypertensive
Vinblastine, vincristine	Antitumor	Tubocurarine	Muscle relaxant
Vincamine	Vasodilator, antihypertensive	Physostigmine	Inhibitor of acetylcholinesterase
Codeine	Cough medicine	Quinidine	Antiarrhythmic
Cocaine	Anesthetic	Quinine	Antipyretics, antimalarial
Colchicine	Remedy for gout	Emetine	Antiprotozoal agent
		Ergot alkaloids	Sympathomimetic, vasodilator, antihypertensive

Process B: The powdered material is extracted with petroleum ether to remove the fatty material. The residue obtained is extracted with aqueous alcohol. The alcoholic



Fig. 3.1: Extraction procedure for alkaloids

extract is acidified and the extracted with organic solvent like chloroform or ether. The aqueous extract containing alkaloidal salt is basified with ammonia solution or sodium bicarbonate solution or dilute alkaline solution and then extracted with organic solvents. The solvent is evaporated off to get the crude alkaloids (Fig. 3.1).

The resulting crude alkaloid mixture is separated into individual alkaloids by means of fractional crystallisation, fractional precipitation, column chromatography, gas chromatography, or counter current extraction.

In rare cases, steam distillation can be employed; for example, volatile liquid alkaloids such as nicotine, sparteine and coniine are most conveniently isolated by steam distillation. An aqueous extract of plant is made alkaline with caustic soda or sodium carbonate and alkaloid is distilled off with steam. The distillate is extracted with solvent to isolate the desired alkaloid.

General Methods for Structure Determination of Alkaloids

In structure determination of alkaloids, a variety of general chemical method and more recently physical methods are employed. The generalized procedure, used to determine the structure of alkaloids illustrated here:

- (i) **Determination of molecular formula:** The first step is determination of empirical formula/molecular formula and molecular weight by elemental or combustion

analysis. Molecular formula can be determined with the help of mass spectrometry.

- (ii) **Number of double bond:** The presence of unsaturation in an alkaloid may be ascertained by the addition of hydrogen or bromine or halogen acids or by hydroxylation with dilute alkaline permanganate solution.

Number of double bond present in an alkaloids with the molecular formula, $C_aH_bN_cO_d$ can be determined by following formula:

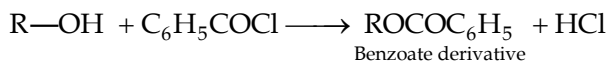
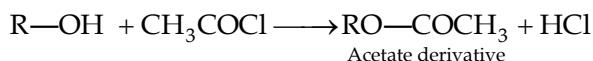
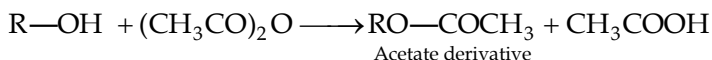
$$\text{Number of double bond} = \frac{a - b}{2} + \frac{c}{2} + 1$$

where a, b and c are the number of carbon, hydrogen and nitrogen atoms, respectively.

- (iii) **Functional group analysis:**

- (a) **Functional nature of oxygen:** Most of the alkaloids contains one or more oxygen atoms, which may be present as hydroxyl, methoxy, methylenedioxy, carbonyl, ester, lactone, amide, lactam, epoxide groups or ether linkage. The nature of oxygen atom is ascertained using the following usual chemical reactions. It can also be determined with the help of infrared spectrum.

Hydroxyl group: Formation of acetate derivative on treatment with acetic anhydride/ acetyl chloride or benzoate on treatment of benzoyl chloride indicates the presence of hydroxyl group.



The phenolic and alcoholic hydroxyl groups give the same reaction. The phenolic group can be differentiated on the basis of following test:

- Alkaloid is soluble in NaOH.
- Reprecipitated by passing CO_2 gas.
- Gives color reaction with $FeCl_3$ solution.

The primary amines present in an alkaloids also form acetate or benzoate derivative.

The **number of hydroxyl group** is determined by acetylation method. The alkaloid is acetylated with acetic anhydride. The acetylated product is hydrolysed with accurately measured excess of standard NaOH solution. The excess of standard NaOH solution is estimated by titration with standard HCl solution. Similarly, blank titration is performed. Number of $-OH$ group can be calculated from the volume of standard NaOH solution used for hydrolysis.



$$\text{Number of hydroxyl groups} = \frac{(b - a) \times \text{mol wt of alkaloid}}{\text{Weight of alkaloidal compound}}$$

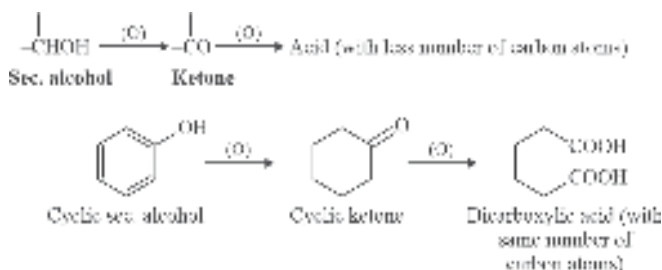
where 'b' and 'a' are volume of standard HCl solution consumed in blank and sample titration, respectively.

The nature of alcoholic hydroxyl group (primary, secondary, and tertiary) is determined by oxidation or dehydration reaction.

The primary alcohol on oxidation gives an aldehyde which on further oxidation yields the acid having same number of carbon atoms.



The secondary alcoholic group gives the acid via ketone formation having fewer number of carbon atoms. However, if secondary alcoholic group is directly attached to cyclic ring then dicarboxylic acid having same number of carbon atom is obtained.



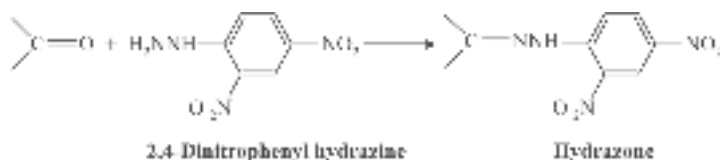
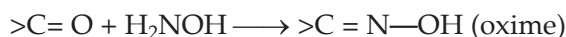
Tertiary alcohol gives first ketone followed by acid. Both ketone and acid have fewer number of carbon atom.

Carboxylic group: The solubility of an alkaloid in bicarbonate or ammonia and reprecipitation with carbon dioxide indicates the presence of carboxyl group. The number of carboxyl groups may be determined volumetrically by titration against a standard solution of sodium hydroxide using phenolphthalein as an indicator.

The formation of ester on treatment with alcohol in the presence of dehydrating agent also indicates the presence of carboxyl group.

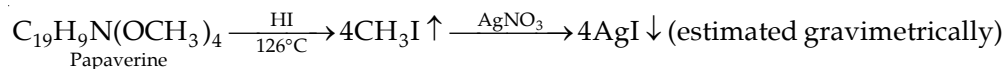
Carbonyl group: The presence of carbonyl group is ascertained by usual reactions with hydroxylamine or semicarbazide or 2,4-dinitrophenylhydrazine when the corresponding oxime or semicarbazone or 2,4-dinitrophenylhydrazone respectively is formed. The weight of corresponding semicarbazone or oxime, or hydrazone is co-related with number of carbonyl groups. The carbonyl group may be present as an aldehyde or a ketone.

This distinction can be made from Tollen's reagent. Aldehydes give silver mirror with Tollen's reagent whereas ketones are unreactive.

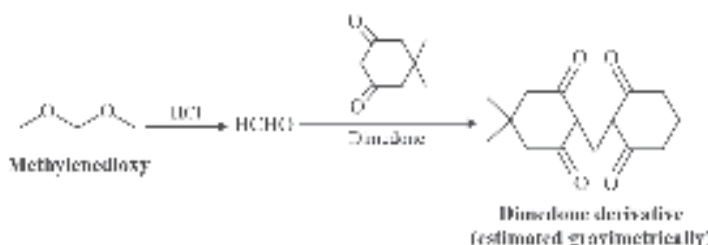


The distinction between aldehyde and ketone can also be done by oxidation or reduction, also by NMR, IR, and UV techniques.

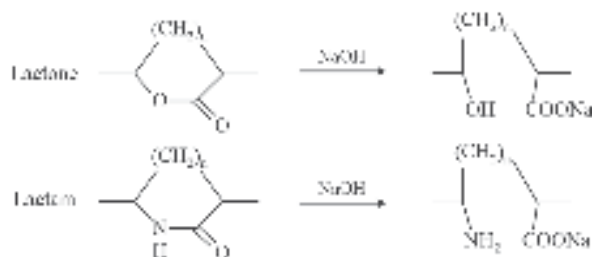
Methoxyl group: It is determined by **Zeisel** method. The methoxy group present in an alkaloid, forms methyl iodide on heating with HI at 126°C. The methyl iodide is treated with silver nitrate to get insoluble silver iodide. The weight of silver iodide is determined gravimetrically and co-related with number of methoxyl groups. For example:



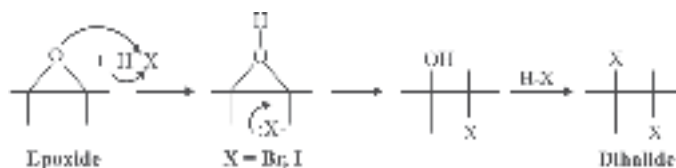
Methylenedioxy group: On heating with concentrated with HCl or H₂SO₄, the methylenedioxy group forms formaldehyde. Formaldehyde thus obtained can be estimated gravimetrically after conversion to dimedone derivative. 5,5-Dimethyl cyclohexane-1,3-dione (also known as dimedone) reacts quantitatively with formaldehyde to form colorless crystalline precipitate of dimedone derivative. The weight of this derivative is co-related with number of methylenedioxy groups.



Amide, lactam, ester, lactone groups: These groups can be detected and estimated through acid or alkaline hydrolysis.



Epoxide and ether linkage: Epoxide or ether linkage is cleaved by the addition of hydrogen bromide or hydroiodic acid to form dihalide.

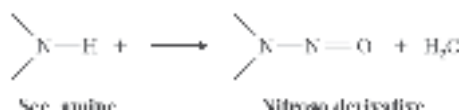


(b) **Functional nature of nitrogen:** In the majority of alkaloids, nitrogen atom(s) is present in heterocyclic ring; therefore, it can be secondary or tertiary in nature.

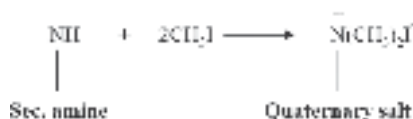
The acetylation or benzylation reaction can distinguish tertiary amine from secondary amine. The former being inert whereas the latter gives acetate or benzoate derivative. This distinction can also be done by treatment with HNO_2 or methyl iodide or oxidation with 30% hydrogen peroxide.

Secondary Amine

Alkaloid having secondary amino group gives nitroso derivative on reaction with nitrous acid.

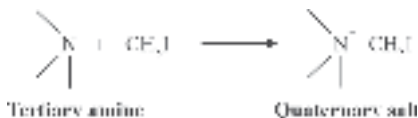


If alkaloids react with two molecule of methyl iodide to form quaternary salt, it means, it contains secondary amino group. For example:

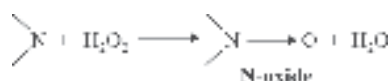


Tertiary Amine

If it consumes one molecule of methyl iodide to form crystalline quaternary salt, nitrogen is tertiary in nature.

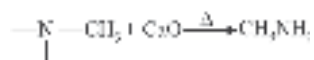


If nitrogen is tertiary, when treated with H_2O_2 (50%) forms N-oxide.

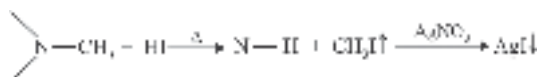


N-Methyl group: The presence of N-methyl group is often detected by distillation of amine with soda lime or estimated by Herzig-Meyer method.

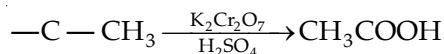
- (i) On treatment with soda lime alkaloid containing N-CH_3 group forms methyl amine. The formation of methyl amine confirms the presence of N-methyl group.



- (ii) **Herzig-Meyer method:** It involves the treatment of alkaloid with hydroiodic acid at $150\text{--}300^\circ\text{C}$ to form the volatile methyl iodide. This is converted into insoluble silver iodide by passing volatile methyl iodide gas into silver nitrate solution. The silver iodide is estimated by gravimetric method and weight of AgI is correlated with number of N-CH_3 group as mentioned for estimation of methoxy groups.



- (iii) **Tertiary C-methyl group:** The presence of C-Me group is quantitatively estimated by **Kuhn-Roth oxidation** ($\text{K}_2\text{Cr}_2\text{O}_7/\text{H}_2\text{SO}_4$) method in which C-methyl groups are converted to acetic acid, which is distilled off and assayed volumetrically by titration against standard base.

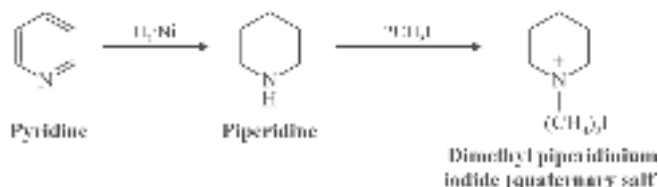


Degradation of Alkaloids

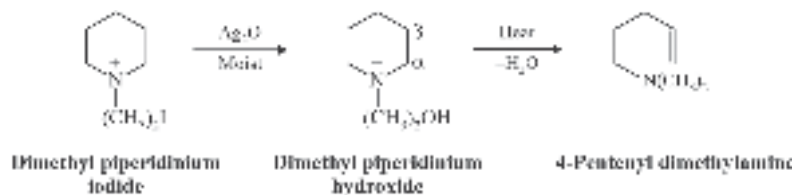
The following methods are used to find out basic skeleton of alkaloid molecules. In this, depending on the nature of functional group present in the alkaloid, the molecule is broken down into fragments of which structures are known. From the structure of simple fragment, the original structure of molecule is predicted.

1. **Hoffmann's exhaustive methylation method:** This is a specific method for alkaloid (Heterocyclic amines) to know the nature of the C-skeleton in the heterocyclic system. It involves following steps:

- (a) The alkaloid (e.g. piperidine/pyridine containing) is treated with excess of CH_3I to form quaternary ammonium iodide.

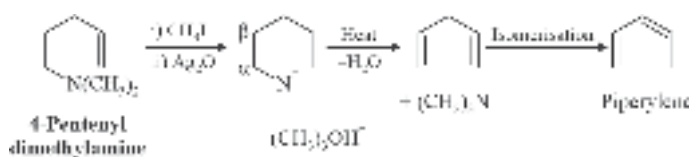


- (b) The resulting salt is then converted into the more basic hydroxide by reacting with silver oxide. Subsequently, the quaternary hydroxide salt is heated at 200°C to give an olefin with the elimination of water molecule.



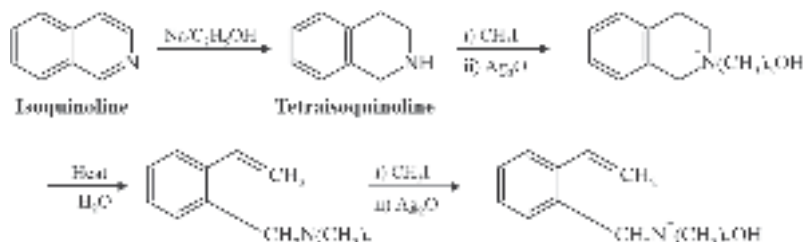
The $-\text{OH}$ of hydroxide extracts hydrogen atom from β -position, causing elimination of a water molecule and also the ring is cleaved at the N-atom to give an open chain tertiary-amine.

- (c) The step 'a' and 'b' are repeated when a second cleavage at the N-atom gives an unsaturated hydrocarbon which on isomerisation may give the conjugated derivative, piperylene.

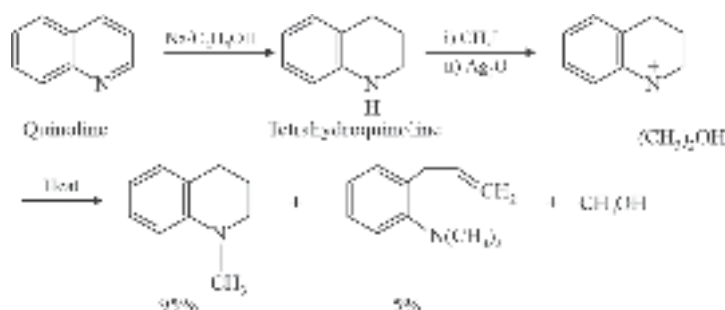


Drawbacks of Hoffmann degradation method: Hoffmann degradation is only depends on the availability of β -hydrogen atom. This method fails if β -hydrogen is not

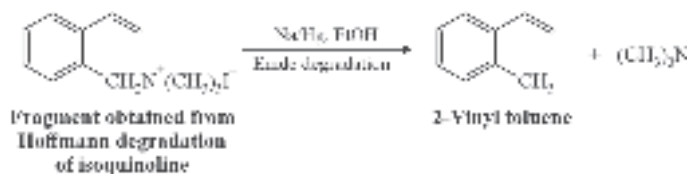
available. For example, isoquinoline is degraded by only one time and so nitrogen is not removed.



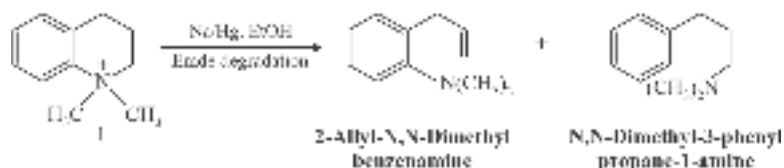
Secondly, sometimes this method is not applicable even β -hydrogen is available. For example, tetrahydroquinoline cannot be degraded with this method although β -hydrogen is available.



2. **Emde degradation method:** If alkaloids do not contain β -hydrogen atom then Hoffmann exhaustive degradation method is failed. In such cases, Emde degradation is applied which involves the reduction of an aqueous or alcoholic solution of the quaternary ammonium halide with sodium amalgam in aqueous ethanol or sodium in liquid ammonia or catalytically. For example, the fragment obtained from Hoffmann degradation of isoquinoline can be subjected to Emde method to get 2-vinyl toluene and triethyl amine.

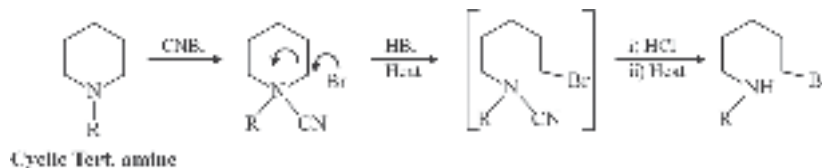


The Emde method can be applied to tetrahydroquinoline where Hoffmann exhaustive methylation fails. The tetrahydroquinoline after converting into quaternary halide salt gives 2-allyl-N,N-dimethyl benzenamine and N,N-dimethyl-3-phenylpropan-1-amine when subjected to Emde method.

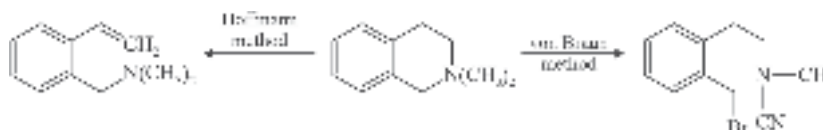


3. von Braun's method: It is of two types:

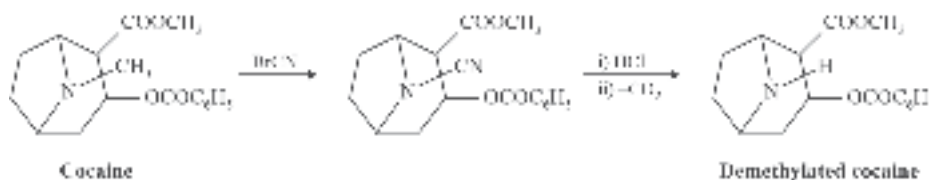
- A. Cyclic tertiary amine, which contains at least one-alkyl substituent, is treated with cyanogen bromide. This results in cleavage of an alkyl nitrogen bond to give an alkyl halide and a substituted cyanamide.



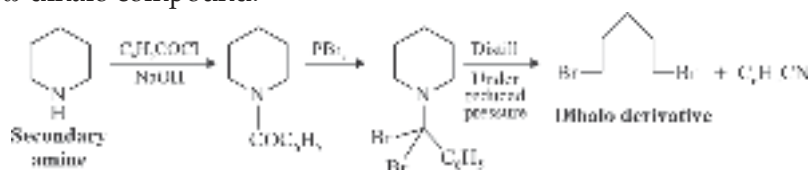
This method is often applicable to such compounds that do not respond to Hoffmann's method. Furthermore, in cases where both the methods are applicable, ring opening takes place at different points.



However, a second possibility in the same von Braun's method is the de-alkylation of the N-alkyl group without ring cleavage. For example, cocaine is simply de-alkylated on treatment with cyanogen bromide.



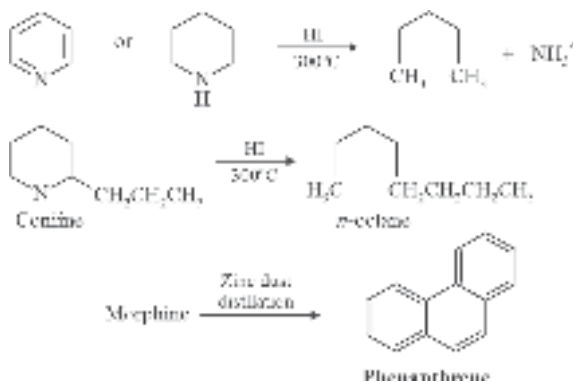
- B. The second type von Braun's method is meant for secondary cyclic amines and involves the treatment of its benzoyl derivative with phosphorus halide to cause the removal of nitrogen atom eliminated as benzonitrile with the formation of α,ω -dihalo compound.



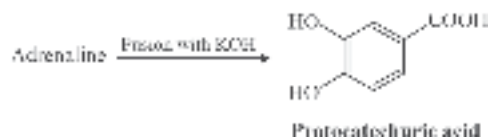
4. Reductive degradation and zinc dust distillation:

In some cases, ring may open by heating up to 300°C with hydroiodic acid, e.g. coniine. Morphine on zinc dust distillation gives phenanthrene, indicating the presence of phenanthrene moiety in its structure.

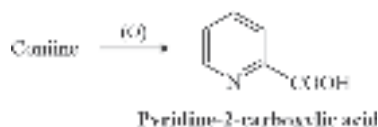
5. **Alkali fusion:** This is very drastic method, which is often employed to break down the complex alkaloidal molecule to simpler



fragment. The nature of fragment can give the information about basic structure present in alkaloid molecule. For example, adrenaline on fusion with KOH gives protocatechuric acid. Formation of protocatechuric acid indicates the presence of 3,4-dihydroxy phenyl moiety in the adrenaline.

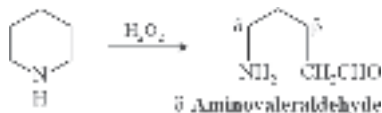


6. **Oxidation:** This method can give quite useful information about the structure of alkaloids. For example, coniine on oxidation gives pyridine-2-carboxylic acid, indicating the presence of basic pyridine nucleus.

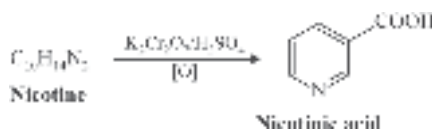


Oxidation can be categorized into mild, moderate and vigorous oxidation depending on the reagent

- A. *Mild oxidation:* H_2O_2 , alcoholic HI solution and alkaline potassium ferricyanide. For example, piperidine ring is opened by reacting with hydrogen peroxide solution.



- B. *Moderate oxidation:* KMnO_4 (Alkali), Cr_2O_3 (acetic acid), etc. are used to carry out the moderate oxidation.



7. **Dehydrogenation:** When Alkaloid is distilled with catalyst such as sulphur, selenium or palladium, dehydrogenation takes place to form relatively simple & easily recognizable product which can provide the clue regarding the basic skeleton.

Synthesis: On the basis of above chemical methods, the structure of the alkaloid is predicted. The final confirmation of the predicted structure is done by the unambiguous synthesis.

Physical Method

Recently, physical methods are used, in conjunction with chemical reaction to elucidate structure of alkaloids. With the use of sophisticated instruments, it is possible to determine a structure in a matter of days given with few milligrams (or less) of a pure alkaloidal compound.

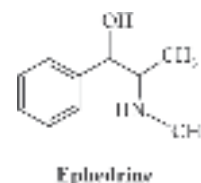
Infrared spectrum gives information about many functional groups. Ultraviolet spectra are used to indicate the nature of unsaturation or aromatic rings. NMR spectroscopy is more versatile for detecting many functional groups, the nature of protons, carbon skeleton, heterocyclic rings etc. Mass spectral fragmentation gives the information about molecular weight/molecular formula and structure.

Single crystal X-ray analysis has offered means for determining or confirming stereochemistry as well as distinguishing between alternate structures that appear to fit well for a particular alkaloid. Further support for the stereochemistry can be obtained by using optical rotatory dispersion or circular dichroism studies.

I. NON-HETEROCYCLIC ALKALOIDS

1. Ephedrine

Ephedrine is an important drug which occurs along with five other alkaloids, namely pseudoephedrine, methylephedrine, methyl pseudoephedrine; norephedrine and norpseudoephedrine in the genus *Ephedra*. The ephedrine base was first isolated from the Ephedra in 1887 by Nagai and much later, Gulland and Virden obtained a very small amount from the Yew (*Taxus baccata* L.). The Ma Huang is a Chinese drug which contains ephedrine, has been used successfully in the treatment of bronchial asthma, hay fever and other allergic conditions. It also increases blood pressure. It is a much stronger stimulant than caffeine.



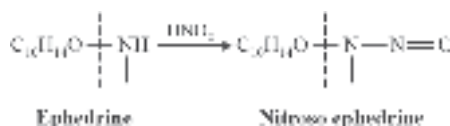
Ephedrine, m.p. 43°C is very soluble in water, alcohol, and chloroform and nearly insoluble in petroleum ether upon cooling. Being a strong base, ephedrine displaces ammonia from its salts. A Ephedrine hydrochloride crystallized out from alcohol in prismatic needles, m.p. 216°C, $[\alpha]_D^{22} - 32.5^\circ$.

Isolation of ephedrine

- (i) The powdered herb is extracted with cold benzene in the presence of dilute Na_2CO_3 solution.
- (ii) The benzene extract is shaken up with a sufficient quantity of dilute HCl to remove the basic substances.
- (iii) The acid solution is made alkaline with solid K_2CO_3 ; the liberated base then extracted with chloroform.
- (iv) The chloroform solution, after drying over anhydrous Na_2SO_4 is evaporated/distilled off to get crude ephedrine.

Structural elucidation of ephedrine

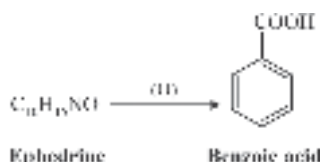
- (i) **Determination of molecular formula:** It is found to be $\text{C}_{10}\text{H}_{15}\text{NO}$.
- (ii) On treatment with nitrous acid, ephedrine gives nitroso compound indicating the presence of secondary amino group.



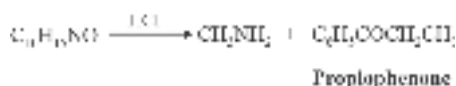
- (iii) On benzylation, it forms dibenzoyl derivative inferring that oxygen atom is present as hydroxyl group since one benzoyl group is introduced at $-NH$ and other at $-OH$.



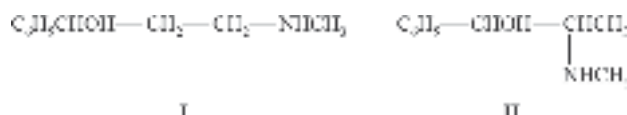
- (iv) On oxidation, it gives benzoic acid inferring that it is monosubstituted benzene.



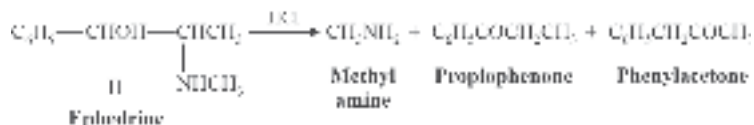
- (v) Ephedrine, when heated with hydrochloric acid, forms methylamine and propiophenone.



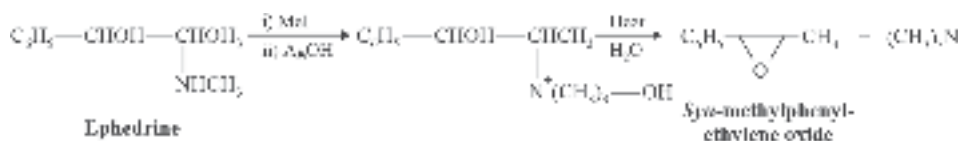
This reaction along with above evidences suggests either of the following two structures to ephedrine.



- (vi) Ephedrine hydrochloride on heating undergo *hydramine fission* to form propiophenone phenylacetone and methylamine. Now since hydramine fission is characteristic of compound having $C_6H_5CH(OH)CH-NHR$ group. Therefore, ephedrine must be structure, II.



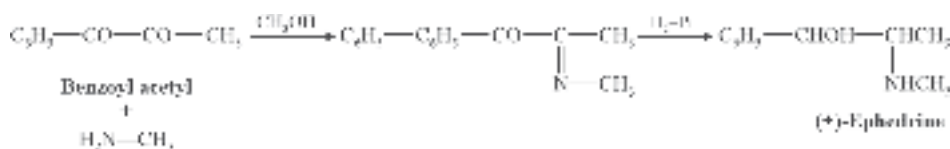
- (vii) The above structure of ephedrine is supported by Hoffmann exhaustive methylation to form *syn*-methylphenylethylene oxide.



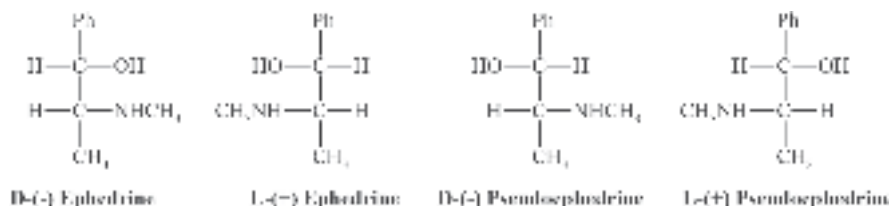
- (viii) The structure proposed by degradative means is further confirmed by its synthesis and optical isomerism.

Synthesis: Manske et al. synthesized racemic ephedrine by catalytic reduction of benzoylacetyl (1-phenylpropane-1,2-dione) in the presence of methylamine in

methanol solution. The racemic ephedrine resolved into optically active ephedrine by means of mandelic acid.



Optical isomerism in ephedrine: Ephedrine has 2 asymmetric carbon atoms; therefore, it has 4 (2^2) optically active isomers, i.e. two enantiomeric pairs, ephedrine (erythro configuration) and pseudoephedrine (threo configuration) are possible. But it is very important to note that only (-) ephedrine and (+) pseudoephedrine are found in nature.



Spectral data

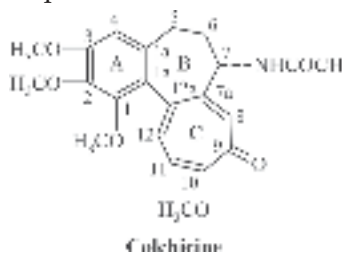
UV (Methanol): λ_{max} 250, 256 and 262 nm (log e 170, 20, 165 respectively).

IR (KBr) (cm^{-1}): 3330 (–OH stretching) 2480 (NH-stretching), 1490, 1450 (Ar–H) 750 and 699 (C–H out of plane deformation).

$^1\text{H-NMR}$ (D_2O) (δ): 7.53 (5H, s, Ar–H), 5.28 (1H, s, OH), 3.2 (1H, s, CH–OH), 2.9 (3H, s, NH–CH₃), and 1.20 (3H, d, CH–CH₃).

2. Colchicine

Colchicine is a type of tropolone alkaloids, first isolated by Pelletier and Caventou in 1820. It is found in the seeds and corns of *Colchicum autumnale* L (known as “meadow saffron”). It has been used for the last many thousand years in the treatment and diagnosis of gout. Later on in 1932, it is observed to arrest cell division at a particular stage. Therefore, this was used from the year 1934 to 1950 for the artificial production of polyploid varieties of numerous plants.



Isolation of colchicine

- (i) For isolation of colchicine, colchicum seeds are extracted with alcohol repeatedly. The alcoholic extract is concentrated, and then diluted with water. This results in the precipitation of fat and resinous mass which are removed by filtration.
- (ii) The filtrate is extracted with chloroform repeatedly and the resultant extract is evaporated to a syrupy consistency.

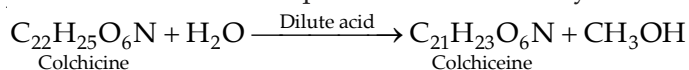
- (iii) To the warm syrupy residue, small quantities of alcohol is added up to such an extent that white precipitate first formed is redissolved in chloroform solution.
- (iv) Then, the resulting solution is allowed to cool at 3°C for such a time period until crystals of colchicine separate out.
- (v) The crystals obtained as above are made to suspend in a small amount of water and then steam is passed through this solution to remove the chloroform. Steam carries away the chloroform vapors along it whereas colchicine goes into water, forming a suspension. The latter on evaporation in vacuum yields yellow colored colchicine powder.
- (vi) Colchicine is crystallized from ethyl acetate as pale yellow needles. It may also be purified through water as trihydrate.

Colchicine is a constituent of colchicum alkaloids. It is believed to be formed in the process of extraction. It readily formed by heating colchicine with dilute sulphuric acid. Colchicine gives intense green color with ferric chloride solution.

Properties: Colchicine is found as yellow colored needle-shaped crystal, m.p. 155–157°C. Colchicine base is soluble in sulphuric acid.

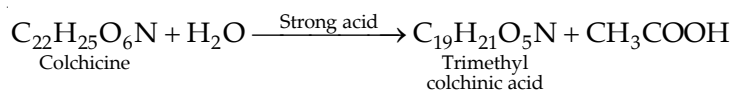
Structural elucidation of colchicine

- (i) Molecular formula is found to be $C_{22}H_{25}O_6N$.
- (ii) **Presence of enol methyl ether group:** On hydrolysis with dilute acids, colchicine yields a mixture of an acidic substance, called colchicine $C_{21}H_{23}O_6N$ and methyl alcohol. Although, it does not contain carboxylic group but still colchicine is acidic in nature. This reaction reveals the presence of enol methyl ether in colchicine.



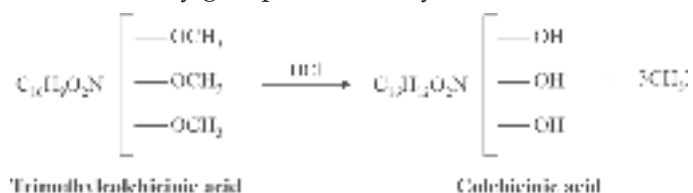
Colchicine shown no color reaction with $FeCl_3$, whereas colchicine gave a positive color test.

- (iii) **Presence of acetamido group:** Colchicine, on hydrolysis with strong acids, yields acetic acid and a new base called trimethyl colchicine acid, $C_{19}H_{21}O_5N$. The reaction could be regenerated by action of $(CH_3CO)_2O$. The latter compound behaves as a primary amine because it is converted into quaternary compound on reaction with CH_3I . This reaction clearly reveals the presence of an acetamido group, $-NHCOCH_3$.

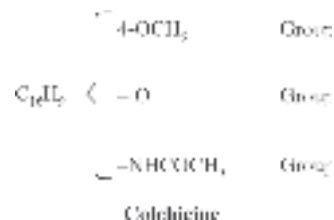


As nitrogen atom in colchicine is not basic in nature, it means that this is not an alkaloid in the true sense.

On further hydrolysis under vigorous conditions, trimethylcolchicine acid yields one mole of colchicine acid and three moles of methyl halide. This indicates the presence of three methoxy groups in trimethylcolchicine acid.

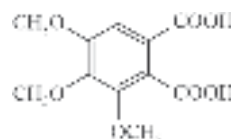


With the above evidences, five oxygen atoms present in colchicine have been accounted in the structure. The remaining sixth oxygen atom, which remains unaccounted so far, has been directly found to be present as an unreactive carbonyl group. Thus, colchicine may now be represented as follows:



- (iv) **Tricyclic nature of colchicine:** From the above tentative formula of colchicine, the expected formula for parent hydrocarbon of colchicine would be $C_{16}H_{16}$. Further, colchicine when hydrogenated catalytically yields hexahydrocolchicine (two double bonds and the ketonic group are reduced) which forms epoxide indicating that hexahydrocolchicine still contains an isolated double bond. Thus, it follows that colchicine must contain 3 double bonds. Further, colchicine and its derivatives when oxidized yield 3,4,5-trimethoxyphthalic acid, indicating the presence of a benzenoid nucleus in colchicine, would possess the composition $C_{16}H_{28}$ ($C_{16}H_{16} + 6H$ for 3 double bonds and $6H$ for the benzenoid nucleus) and therefore, colchicine must be *tricyclic* because its completely saturated hydrocarbon follows the general formula C_nH_{2n-4} for tricyclic compounds. These three rings in colchicine can be designated as A, B and C. Now, we shall establish the nature of these three different rings (A, B and C) one by one.

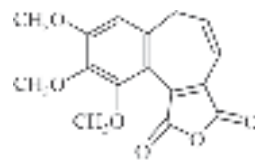
- (v) **Nature or size of one of the ring A:** It is stated earlier that colchicine and many of its derivatives, when oxidized, yield 3,4,5 trimethoxy phthalic acid. This degradation product establishes the structure of ring A as well as the position of the three methoxy groups.



Structure of ring A

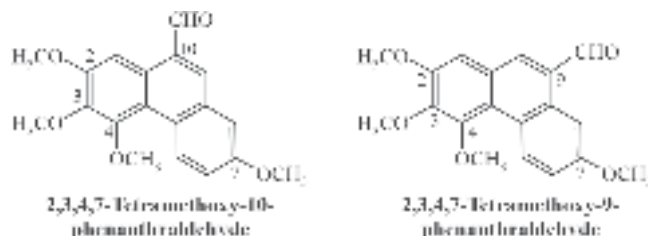
- (vi) **Nature or size of second ring B:** Colchicine on reaction with KI, gives N-acetyliodocolchinel, which on *o*-methylation followed by reduction yields N-acetylcolchinel methyl ether. The latter compound when heated with P_2O_5 yields two nitrogen free compounds called deaminocolchinel methyl ether and isodeaminocolchinel methyl ether. The structure to ring B is assigned on the basis of reactions of N-acyl colchinel methyl ether and above formed nitrogenous substances.

- (a) N-benzoyl trimethylcolchinelic acid when oxidized yields N-benzoyl colchicinic anhydride. The latter compound on heating with P_2O_5 yields a nitrogen free anhydride which does not resemble to naphthalene in chemical behavior but gives a dihydro derivative on reduction. Its structure as given here has been verified by synthesis.

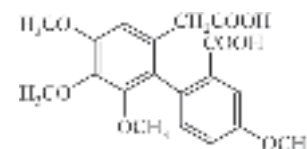


Nitrogen free anhydride

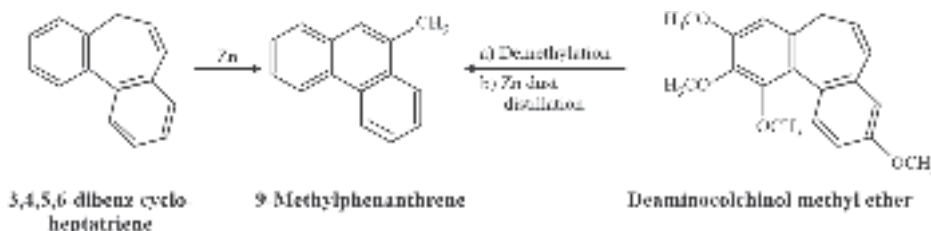
- (b) The two above isomers, deaminocolchinel methyl ether and isodeaminocolchinel methyl ether, give the same dihydro derivative on reduction, revealing that the two isomers differ only in the position of the double bond.
- (c) The two isomers when oxidized with osmium tetroxide yield the two isomeric diols. These are further oxidized to two dialdehydes with lead tetraacetate. On treatment with trace of alkali, these two isomeric dialdehydes are converted into 2,3,4,7-tetramethoxy-10-phenanthraldehyde (obtained from deaminocolchinel methyl ether) and 2,3,4,7-tetramethoxy-9-phenanthraldehyde (obtained from isodeaminocolchinel methyl ether). These results reveal that the **ring B of colchicine must be seven membered**.



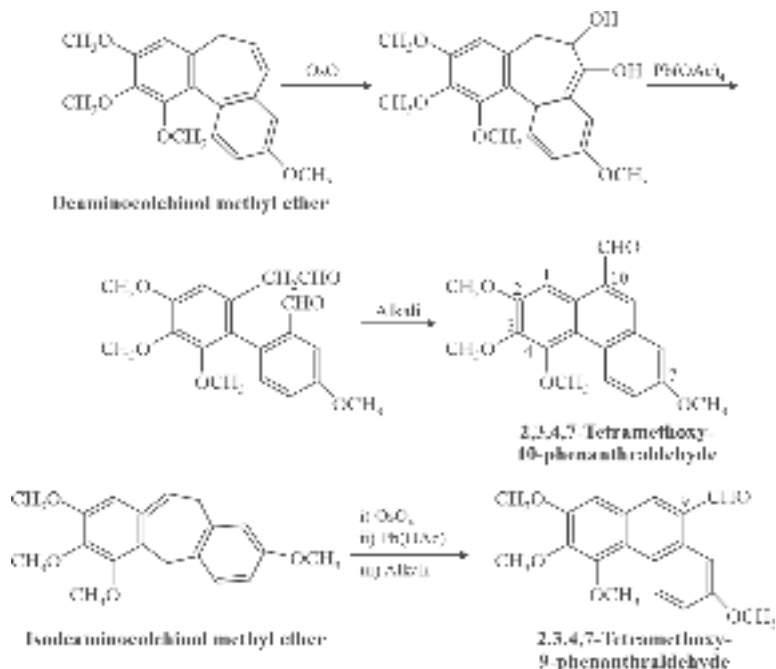
- (d) The seven membered nature of the ring B is further confirmed by the fact that the oxidation of deaminocolchinol methyl ether yields an iodo acid. The iodo acid on reduction yields iodine free acid.



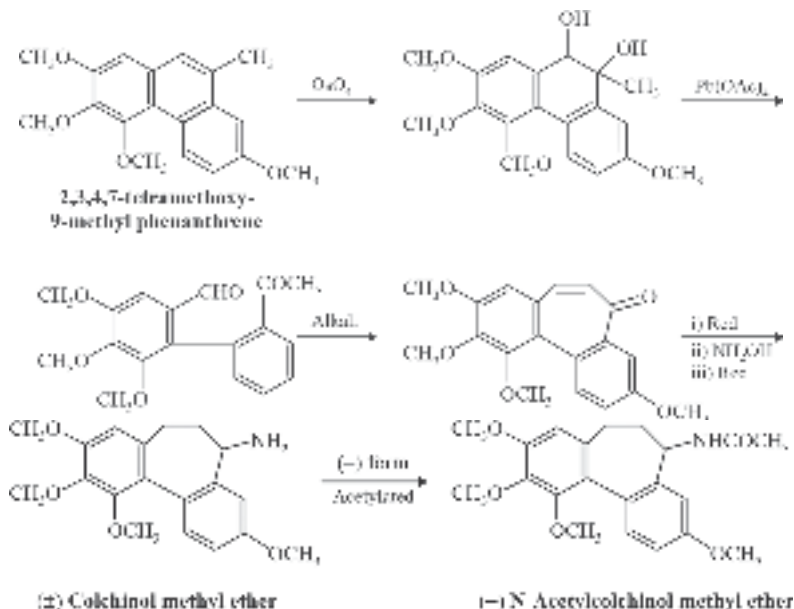
- (e) According to Windaus' reaction, there is the formation of 9-methylphenanthrene from deaminocolchinol methyl ether by demethylation followed by zinc dust distillation. These reactions may be explained by the fact that same compound is also obtained by the zinc dust distillation of 3,4,5,6-dibenz-cycloheptatriene, a compound which is having seven membered B ring.



Now, the above reaction may be explained by considering the following structure of the isomeric deaminocolchinol methyl ether.

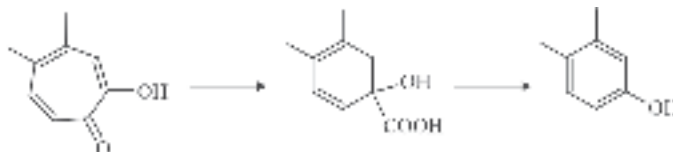


- (f) Finally, the presence of seven membered ring B structure in colchicine is confirmed by the synthesis of its derivative, N-acetylcolchicol methyl ether.

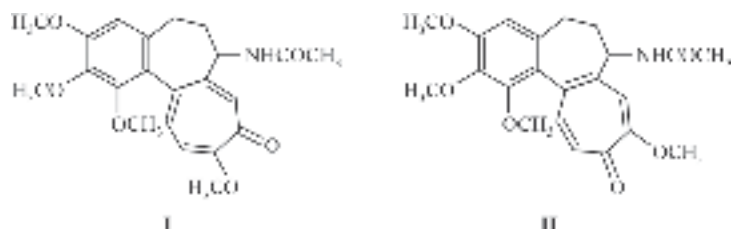


- (vii) **Nature or size of third ring, C:** In the above reaction, ring C has been shown to be six membered. However, Dewar in 1945 cited enough evidences to demonstrate that ring C is seven membered tropolone ring like structure not six membered ring. The nature of ring C as a seven membered ring has been supported by following observations:

- Colchicine on hydrogenation gives hexahydrocolchicine possessing an ethylenic double bond, confirmed by the formation of epoxide and thus, 3 double bond and a ketonic group in the same ring indicates that it is not 6-membered but 7-membered ring structure.
- Hexahydrocolchicine on oxidation with $Pb(OAc)_4$ or periodic acid yields a cyclic product monoaldehyde via an acyclic dialdehyde. Thus, ring must be 7-membered.
- Colchicine does not possess any ketonic property but resembles to tropolone and γ -pyrones.
- The conversion of colchicine into N-acetyliodocolchicol by means of cold alkaline hypiodite can be explained by the fact that the tropolone ring (C) undergoes benzilic acid rearrangement as shown below:



- (viii) **Structure of colchicine:** The structure of colchicine can be written as either **I** or **II**. The correct structure cannot be assigned on the basis of degradation studies. The structure, I is assigned on the basis of X-ray crystallographic and spectral studies.



SYNTHESIS OF COLCHICINE

MG Banwell synthesis (*Pure and Applied Chemistry*, 68, 539–542, 1996).

Banwell synthesis (Fig. 3.2) begins with initial **Claisen-Schmidt condensation** reaction between benzaldehyde (2) and acetophenone (3) derivatives to produce 1, 3-diarylpropanol (4). Subjection of this compound to an oxidative coupling protocol developed by Umezawa ultimately gives the dibenzocyclo-heptenone (5). In anticipation of the enantioselective introduction of the C-7 acetamido group associated with colchicines, it is necessary, for various reasons, to form alcohol (6) with the R configuration at C-7 (colchicine numbering). For this reason, the ketone (5) is subjected to enantioselective reduction with stoichiometric quantities of the CBS-reagent and, after debenzylation, the target compound (6) is obtained in 94%. Taylor-McKillop oxidation of this phenol followed by nucleophilic cyclopropanation of the derived cyclohexadienone (7) with dimethylsulphoxonium methylide then provides the key mono-acetal (8) (98% yield), the structure (including absolute configuration) of which was established by single-crystal X-ray analysis.

Reaction of compound (8) with trifluoroacetic acid in dichloromethane resulted in the desired (biomimetic) ring-expansion and formation of troponoid (10). Most likely this key conversion proceeds via the oxonium ion (9). Then, there is $\text{S}_{\text{N}}2$ displacement of the hydroxy group in compound (10) by azide ion. The resulting azido-compound (11) is subjected to reduction under Staudinger conditions and the amine (12) so-formed is immediately acetylated to get (–)-colchicine, 1 (>81% yield).

Spectral data

UV: λ_{max} 350 nm.

IR (cm^{-1}): 3446, 3300, 2995, 1640, 1618, 1590, 1560, 1490, 1250, 100, 1020.

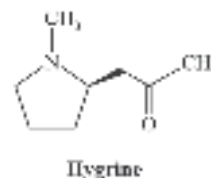
$^1\text{H-NMR}(\delta)$: 7.6 (1H, s, H-8), 7.31 (1H, d, $J = 10.9$, H-12), 6.87 (1H, d, $J = 10.9$, H-11), 6.50 (1H, d, $J = 6.1$ Hz, NH), 6.49 (1H, s, H-4), 4.60 (1H, m, H-7), 3.97 (3H, s, OCH_3 -10), 3.91 (3H, s, OCH_3 -2), 3.89 (3H, s, OCH_3 -3), 3.60 (3H, s, OCH_3 -1), 1.90–2.50 (4H, m, CH_2 -5 and CH_2 -6), 1.92 (3H, s, COCH_3).

II. HETEROCYCLIC ALKALOIDS

PYRROLIDINE-BASED ALKALOIDS

Hygrine

Hygrine is present in the leaves of Peruvian coca shrub (*Erythroxylone truxillense* Rubsy) along with, hygroline, and euskygrine. It is first isolated in 1889 by **Carl Libermann**.



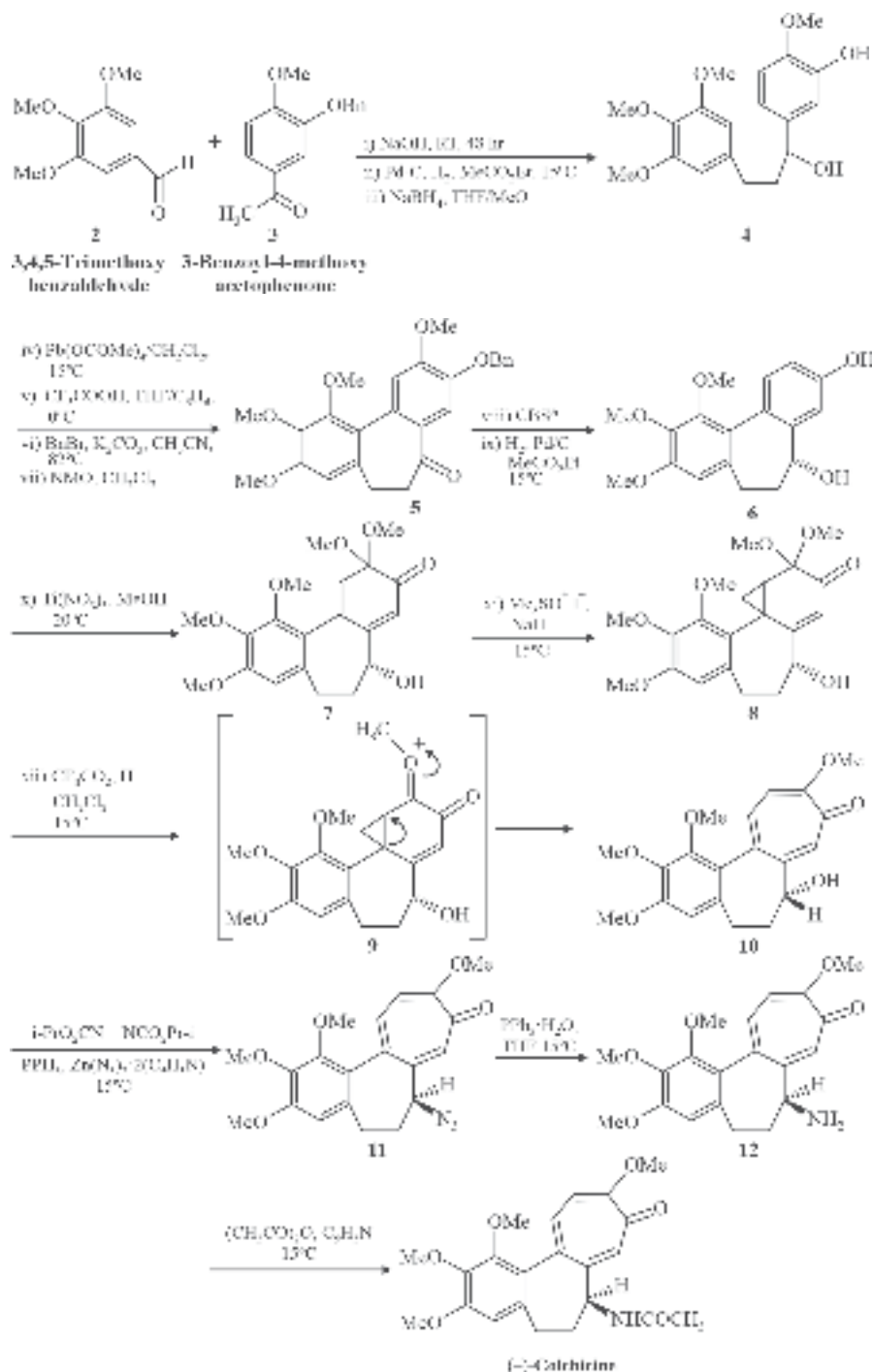


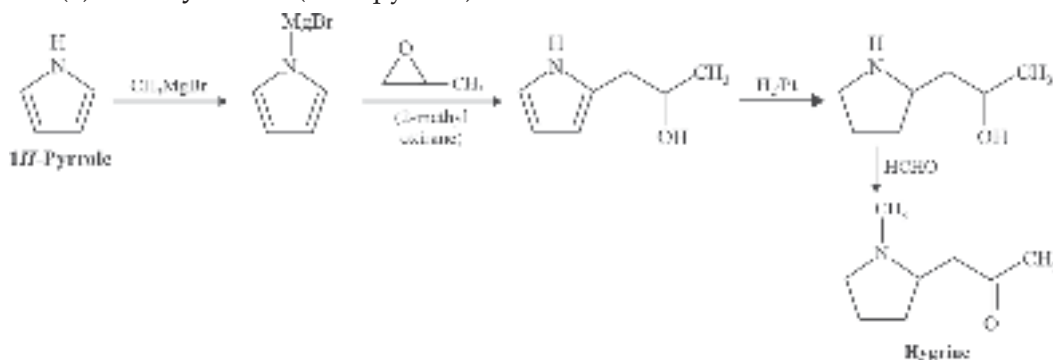
Fig. 3.2: Synthesis of colchicine

*Corey-Bakshi-Shibata catalyst derived from proline

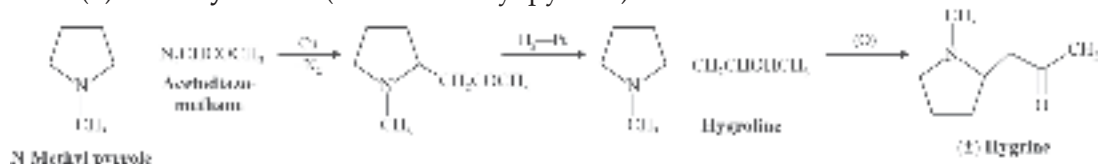
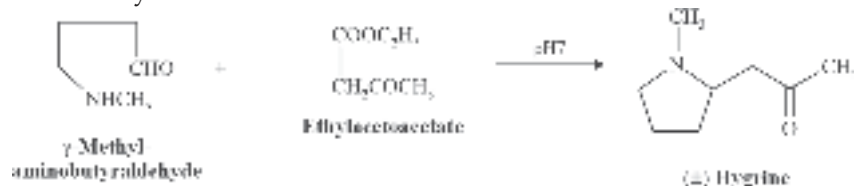
Properties: It is colorless liquid, most volatile (b.p. 193–195°C), and have piperidine like odor. It is a strong base.

Uses: Hygrine is used as sedative, hypnotic, laxative and diuretic agents.

Out of these two structures, the structure, II is assigned on the basis of its synthesis.

(a) **Hess synthesis** (from pyrrole).

In the Hess synthesis, last step is known as the **Eschweilwer-Clarke methylation** in which methylation take place along with oxidation of the secondary alcoholic ($-\text{CHOH}$) group.

(b) **Sorm synthesis** (from N-methyl pyrrole).(c) **Anet *et al* synthesis.****Spectral data**

IR (cm^{-1}): 1720 ($\text{C}=\text{O}$).

$^1\text{H-NMR}$ (δ): 1.2–3.2 (9H, CH_2 and CH), 2.13 (s, 3H, COCH_3), 2.27 (s, 3H, N-CH_3).

MS (m/e): 141 (M^+), 126, 98, 84, 70, 42.

PYROLIZIDINE-BASED ALKALOIDS

Compounds with the characteristic structure depicted in Fig. 3.3 are classified as pyrrolizidine alkaloids. These compounds are widely present in plants with as many as 6000 species containing them. However, about half of the identified pyrrolizidine alkaloids are toxic.

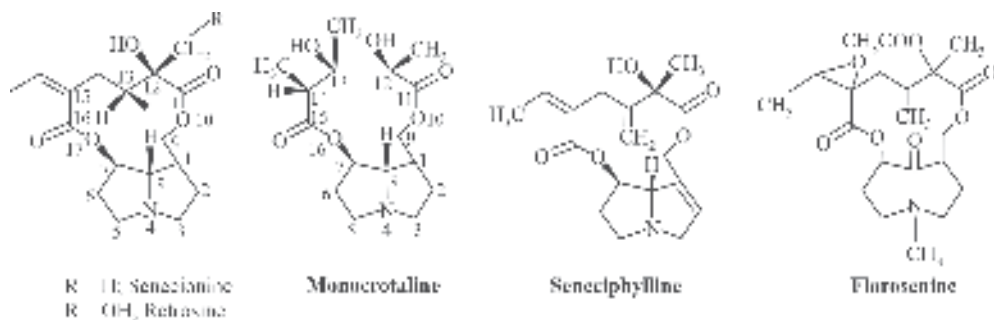
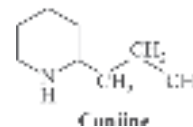


Fig. 3.3. Examples of pyrrolizidine alkaloids

PYRIDINE OR PIPERIDINE-BASED ALKALOIDS

A. Coniine

Coniine is the principal alkaloid of hemlock (*Conium maculatum* L.). The toxic properties of the common hemlock plant are known to Greeks who employed Coniine plant extracts to execute Socrates in 399 B.C. These properties are due to the presence of group of alkaloids, the most important one is coniine. The other alkaloids present in hemlock plants are *y-coniceine*, *A-conhydrine*, and *N-methyl. coniine*. But out of all five alkaloids, coniine is the most significant due to following reason:

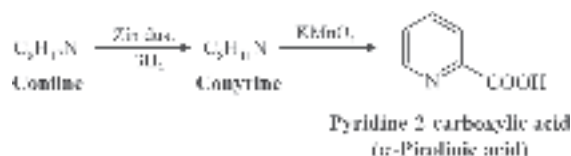


- (a) Due to poisonous nature, hemlock plant causes gradual paralysis, creeping from the feet, followed by convulsions and death. Coniine destroys all forms of animal life.
- (b) It is the first alkaloid to be synthesized due to its simple structure.

Properties: It is optically active, colorless and poisonous oily liquid, b.p. 167°C with burning taste and unpleasant odour.

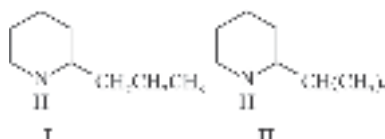
Structural Elucidation of Coniine

- (i) The molecular formula of coniine is found to be $C_8H_{17}N$.
- (ii) **Presence of secondary amine:** Coniine forms nitroso derivative with nitrous acid and quaternary ammonium iodide with two moles of methyl iodide. This indicates that coniine is a secondary amine derivative.
- (iii) When coniine ($C_8H_{17}N$) is distilled with zinc dust, it yield conyryne ($C_8H_{11}N$) which on further oxidation with potassium permanganate yields pyridine-2-carboxylic acid (α -picolinic acid).

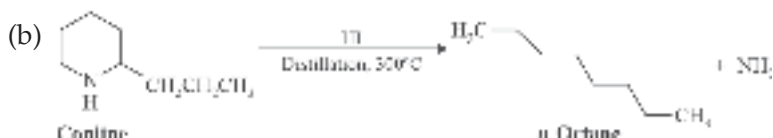
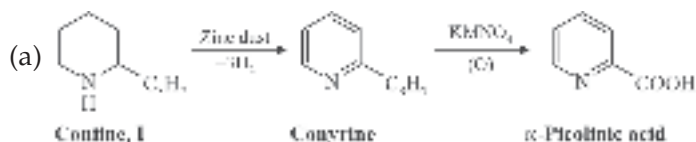


This reaction concludes:

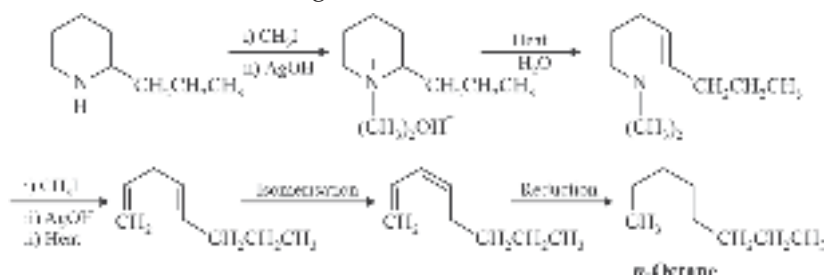
- (a) Conyryne must be pyridine derivative with a side chain $-C_3H_7$ at α -position.
- (b) Coniine is a secondary amine which loses six hydrogen atoms to form conyryne.
- (c) Therefore, it is obviously the corresponding piperidine derivative.
- (iv) Now the only question remaining is the side chain, which may be of *n*-propyl or isopropyl moiety. On the basis of the side chain, coniine may be either I or II.
- (v) Out of the above two possible structures, the structure I is assigned to coniine on the basis of the following observations:
 - (a) Coniine when heated with hydriodic acid at about 300°C under pressure yield *n*-octane, suggesting the structure (I). The structure (II) should give iso-octane. On this basis, it can be stated that coniine is 2-*n*-propyl piperidine, I



- (b) Complete exhaustive methylation of coniine followed by reduction yields *n*-octane.
 (c) von Braun degradation of coniine yields 1,5-dichlorooctane.
 (vi) All the above reaction can be explained by assigning the structure, **I** to coniine.



(c) Hoffmann's exhaustive degradation:

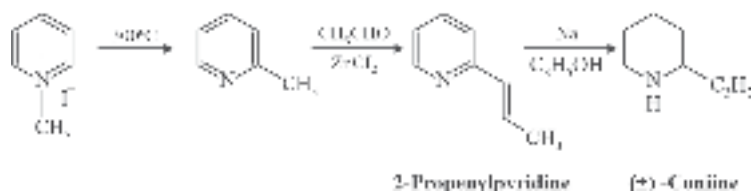


(d) **von Braun's degradation:** 1,5-Dichlorooctane is obtained on von Braun's degradation.



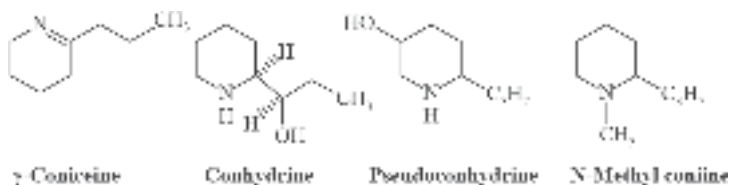
(vii) **Synthesis:** Finally, the structure proposed on the basis of degradation reactions, is confirmed by its synthesis.

Ladenburg's synthesis:



The racemic base may be resolved by the means of (+) tartaric acid, when (+)-coniine (+) tartarate crystals will be crystallize out first, which is removed and decomposed with alkali.

Structure of other hemlock alkaloids are:



Spectral Data

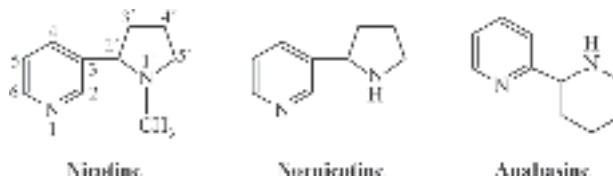
IR (cm^{-1}): 3350 (NH).

$^1\text{H-NMR}$ (δ): 4.2 (1H, NH), 3.1 (1H, CH), 2.3–2.8 (8H, 4XCH_2), 0.9–1.1 (4H, 2XCH_2), 0.8 (3H, CH_3).

MS (m/e): 127, 112, 98, 84 (base peak).

B. Nicotine (Tobacco alkaloid)

Nicotine is a pyridine-pyrrolidine type of alkaloid. It is the most important alkaloid, usually occurs as malate and citrate salt in the tobacco plant namely *Nicotiana tabacum* (family—Solanaceae). It is biosynthesized in roots but stored in leaves. Although, it is distributed throughout but its highest concentration is found in the leaves in varying amounts from 0.6 to 8%. It is one of most toxic alkaloids known, a fatal dose for man being about 40 mg. It causes paralysis of the nervous system, including respiratory control centre. Nicotine in large amount is used as an insecticide and in the preparation of nicotinic acid and niacin. Nornicotine and anabasine are other two alkaloids present in tobacco plant.



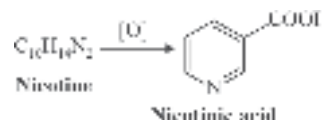
Isolation of Nicotine

- (i) The leaves and the stems of tobacco plants are dried and powdered.
- (ii) To it, milk of lime water is added to make alkaline for liberation of nicotine base. The nicotine is separated out by steam distillation process.
- (iii) The distillate is extracted with ether. When the solvent is evaporated, nicotine is left as an oily liquid which is further purified by fractional crystallization of its salt like oxalate.

Properties: It is colorless, hygroscopic liquid (b.p. 247°C) which becomes discolored in air. It is a levo-rotatory liquid but its salt are dextrorotatory. It is miscible with water in all proportions at temperature below 60°C .

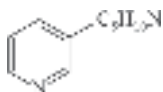
Structural elucidation of nicotine

- (i) **Molecular formula:** It is found to be $\text{C}_{10}\text{H}_{14}\text{N}_2$.
- (ii) **Nature of nitrogen:** Both the nitrogen are shown to be in tertiary state because nicotine take up two moles of methyl iodide to form a dimethiodide. Under suitable conditions, it also forms isomeric monomethiodides, one of the nitrogen atoms is found to be N-methyl group.
- (iii) **Presence of N-CH_3 group:** By Herzig-Meyer method, it is found to contain one N-CH_3 group.
- (iv) It contains three double bonds, indicated by the consumption 3 hydrogen molecules to form saturated compound.
- (v) When nicotine is oxidized with chromic acid or potassium permanganate, it forms nicotinic acid (pyridine-3-carboxylic acid).



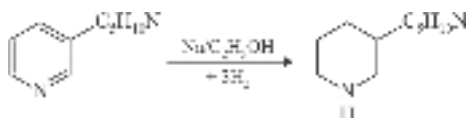
The above reaction concludes:

- (a) Nicotine must have a pyridine nucleus.



- (b) The side chain ($C_5H_{10}N$) is present at C-3 position in the pyridine nucleus.

- (c) The presence of the pyridine nucleus is further confirmed by the formation of a hexahydro derivative when reduced with sodium and alcohol. It means that one of the two nitrogen atoms *is* present as pyridine nucleus of the nicotine.

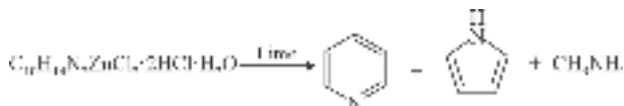


- (d) The pyridine contains three double bonds. It means that side chain is saturated.

- (e) The $N-CH_3$ group is present in the side chain.

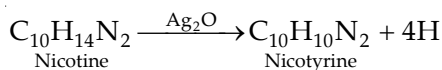
(vi) **Nature of side chain:**

- (a) Nicotine forms an addition product zinc chloride. The zinc chloride product on heating with lime gives pyridine, pyrrole and methylamine.



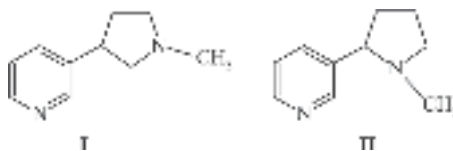
This step clearly suggests that the side chain is pyrrole derivative. But we have already stated that the side-chain is reduced (saturated) and is having one $N-CH_3$, it is N-methyl pyrrolidine.

- (b) Nicotine when oxidized mildly with silver oxide yields nicotyrine with the loss of four hydrogen atoms.



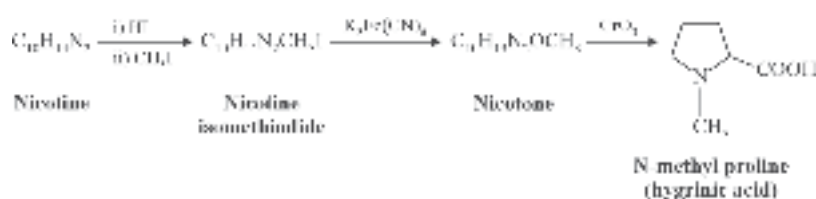
From the above facts, it can be concluded that nicotine contain a pyrrolidine nucleus and therefore, the side-chain in nicotine must be N-methyl pyrrolidine.

- (vii) **Point of attachment of N-methylpyrrolidine to the pyridine nucleus:** The pyrrolidine side chain may be attached to the pyridine ring only in two possible ways, either through C-2 or C-3 position. Thus two structures, I and II for nicotine are possible.

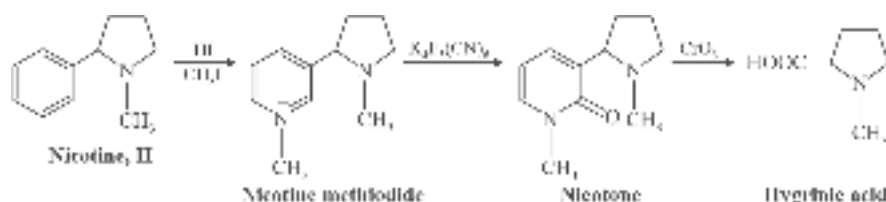


Nicotine hydroiodide on reaction with methyl iodide gives nicotine isomethiodide. This on oxidation with $K_3Fe(CN)_6$ followed oxidation with CrO_3 gives N-methyl proline.

N-methyl proline has carboxylic group at C-2 position. It reveals that it is coming by oxidation of side chain attached at C-2 position in pyrrolidine nucleus.

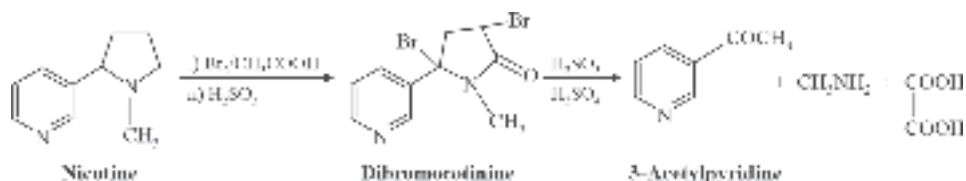


(viii) On the basis of the above points, structure, **II** is assigned to nicotine which can explain all the reactions.

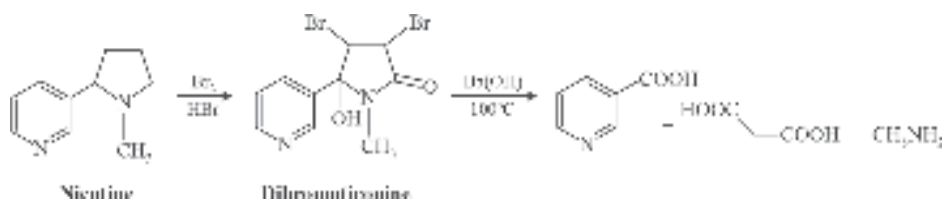


The above structure also explains the reactions proposed by **Pinner**. According to him:

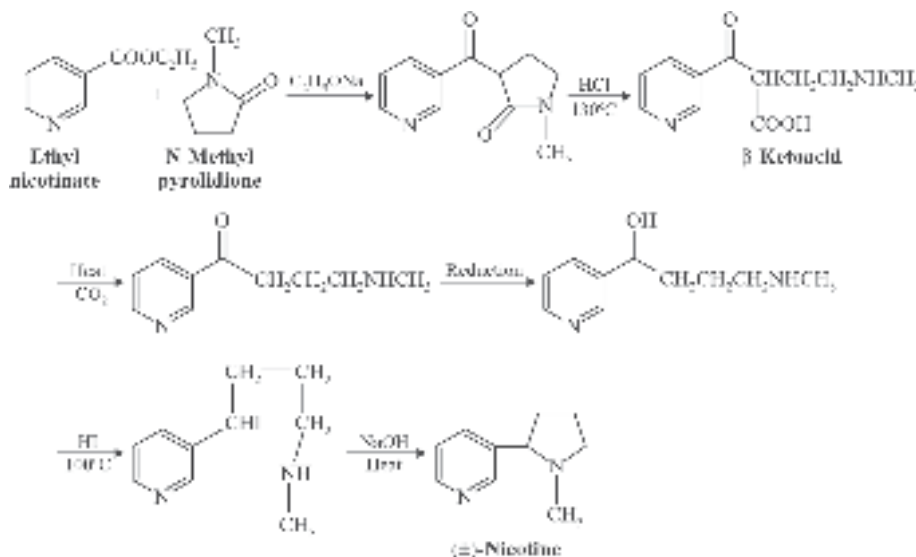
(a) When nicotine is treated with bromine, in acetic acid, it yields many products including the hydrobromide perbromide $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O} \cdot \text{HBr} \cdot \text{Br}_2$ which on treatment with aqueous sulphurous acid is converted into dibromocotinine $\text{C}_{10}\text{H}_{10}\text{Br}_2\text{N}_2\text{O}$. Dibromocotinine when heated with mixture of sulphurous and sulphuric acid at $130\text{--}140^\circ\text{C}$ yields 3-acetylpyridine, oxalic acid and methyl amine. This fragmentation can be explained on the basis of structure, II.



(b) Nicotine on treatment with bromine in presence of hydrobromic acid gives dibromoticonine which on heating with $\text{Ba}(\text{OH})_2$ solution at 100°C affords nicotinic acid, malonic acid and methyl amine.



(ix) Lastly, the structure of nicotine is proved by its synthesis:



Spectral Data

UV (ethanol): λ_{max} 261 (molecular extinction coefficient, $\epsilon = 5.5 \times 10^3$).

IR (KBr) (cm^{-1}): 2970 and 2780 (C–H stretching), 1677 (aromatic C = N double bond stretching), 1691 (aromatic C = C double bond stretching).

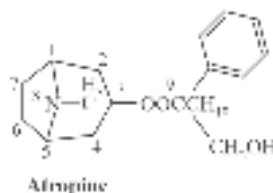
^1H -NMR (δ): 8.60 (1H, m, H-2), 8.55 (1H, m, H-6), 7.77 (1H, m, H-4), 7.3 (1H, m, H-5), 3.16 (1H, m, H-2'), 2.16 (3H, s, N–CH₃), 1.8–2.1 (6H, m, H-3',4' and 5').

MS (m/e): 162(M⁺), 84.

TROPANE-BASED ALKALOID

1. Atropine

This is the most important alkaloid of tropane group and occurs in the roots of deadly nightshade (*Atropa belladonna*), Jimson weed (*Datura stramonium*) and many other members of solanaceous family together with 1-hyoscyamine which is optically active.



Atropine is the racemic mixture of d- and l-hyoscyamine. The levo hyoscyamine readily racemises to atropine on warming in an ethanolic alkaline solution.

Isolation of atropine

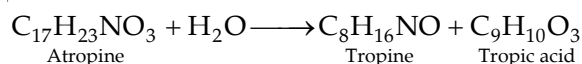
- Atropine is extracted either from belladonna root or from juice of Datura plant. In practice, the juice which contains hyoscyamine is heated with potassium carbonate solution to bring out the racemisation of hyoscyamine to produce atropine.

- (ii) Then, this atropine is extracted with chloroform. The chloroform is recovered by evaporation and the residue is then extracted with dilute sulphuric acid.
- (iii) The acidic extract is made alkaline with potassium carbonate to liberate the atropine base.
- (iv) The precipitated atropine is extracted with ether and purified by converting it into an oxalate or a sulphate salt.

Properties: Atropine is a crystalline compound, m.p. 118°C with bitter taste. Atropine is a tertiary base of pK_a 10.0, which is quite high for an alkaloid. It is optically inactive and is a strong poison. It dilates the pupil of the eyes. Therefore, it finds extensive use in ophthalmology. A single drop of solution containing 1 part of atropine in 40,000 parts of water is sufficient to dilate the pupil of the eye. It has also been used to relieve the night sweats which are a distressing feature of tuberculosis and to diminish the activity of the salivary and gastric glands.

Structural Elucidation of Atropine

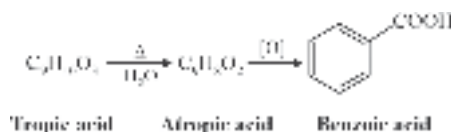
1. Molecular formula is found to be $C_{17}H_{23}NO_3$.
2. On hydrolysis, it gives an alcohol, tropine $C_8H_{15}NO$, and ($\pm$)-tropic acid $C_9H_{10}O_3$. Hydrolytic reaction indicates that atropine is an ester compound (tropine tropate).



Hence, the constitution of atropine is resolved into two parts: the structure of tropine and tropic acid.

A. Constitution of tropic acid

- (i) Its molecular formula is found to be $C_9H_{10}O_3$.
- (ii) As tropic acid consumes one equivalent of alkali and does not add on bromine, it is a saturated monobasic acid.
- (iii) Tropic acid has one hydroxyl group, proved by the formation of monoacetyl derivative on acetylation.
- (iv) On strong heating, it loses a molecule of water to form optically inactive unsaturated acid known as atropic acid, $C_9H_8O_2$, which yields benzoic acid on oxidation. Therefore, the hydroxyl group must be an alcoholic group in nature.



The formation of benzoic acid suggests that atropic acid and hence, tropic acid both contain a benzene ring with one side chain containing a carboxylic group in their structure. In atropic acid and benzoic acid, there is a difference of double bond ($CH=CH$ moiety).

Thus, molecular formula of atropic acid $C_9H_8O_2$, having a benzene nucleus, one carboxyl group and a double bond may be represented by following two structures, I or II. But since structure I is known as cinnamic acid; therefore, structure II must be atropic acid. Lastly, since atropic acid is formed by the dehydration of tropic acid, addition of a molecule of water to the former would therefore give tropic acid. Again there are two

Chemical structures of the four compounds are shown below:

- I**: CC(=O)O/C=C/c1ccccc1 (trans-2-phenylacrylate)
- II**: CC(=O)C(=O)c1ccccc1 (2-phenylacrylate)
- III**: CC(O)(C(=O)O)c1ccccc1 (2-phenyl-2-hydroxypropanoate)
- IV**: OC(O)(C(=O)O)c1ccccc1 (2-phenyl-2-hydroxypropanoic acid)

(i) **MacKenzie and Wood synthesis (from acetophenone):** The synthesis of tropic acid is devised by Wood and MacKenzie. Treating acetophenone with HCN and acidifying produces 2-hydroxy-2-phenylpropanoic acid (atrolactic acid). Further controlled heating, addition of hydrochloric acid followed by treatment with sodium carbonate gives tropic acid.

$$\begin{array}{ccccc}
 \text{H}_3\text{C}_2=\text{C}(\text{CN})_2 & \xrightarrow{\text{HCN}} & \text{H}_3\text{C}_2-\text{C}(\text{OH})(\text{CN})_2 & \xrightarrow{\text{HCl}} & \text{H}_3\text{C}_2-\text{C}(\text{OH})(\text{COOH})_2 & \xrightarrow[\text{(H}_2\text{O)}]{\text{Heat}} & \text{H}_3\text{C}_2=\text{C}(\text{CH}_3)(\text{COOH}) \\
 \text{Acetophenone} & & & & \text{Atrolactic acid} & & \text{Atropic acid}
 \end{array}$$

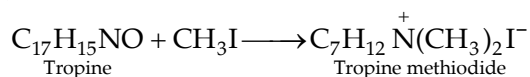
$$\begin{array}{ccc}
 \xrightarrow[\text{ether}]{\text{HCl}} & \text{H}_3\text{C}_2 & \begin{cases} \text{CH}_2\text{Cl} \\ \text{COOH} \end{cases} \\
 & & \xrightarrow[\text{(H}_2\text{O)}]{\text{Na}_2\text{CO}_3} \text{H}_3\text{C}_2 & \begin{cases} \text{CH}_2\text{OH} \\ \text{COOH} \end{cases} \\
 & & \text{(E)-Tropic acid}
 \end{array}$$

Phenylacetic acid

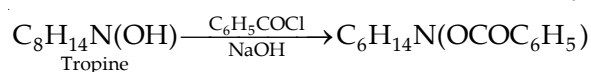
Tropic acid

B. Constitution of alcoholic compound, tropine

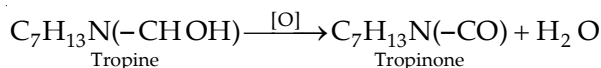
- (a) Its molecular formula is found to be $C_8H_{15}NO$.
 (b) Tropine is found to have tertiary nitrogen atom as it consumes one mole of methyl halide to form quaternary ammonium salt.



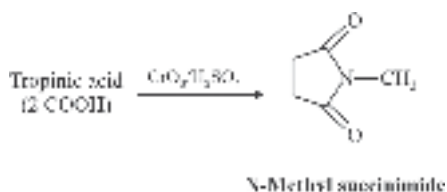
- (c) On fusion with alkali, tropine yields methyl amine, indicating that it must contain N-methyl group. It is also confirmed by the fact that tropine when heated with hydrogen iodide at $150^\circ C$ (Herzig-Meyer method) yields one mole of methyl iodide.
 (d) Tropine contains a hydroxyl group, confirmed by acetylation and benzooylation reaction to form monoacetate and monobenzoate respectively.



- (e) Tropine on oxidation with chromic acid, it yields a tropinone, $C_8H_{13}NO$, which gives characteristic reaction of a ketone. Therefore, the hydroxyl group must be secondary alcoholic ($-CHOH-$) group.

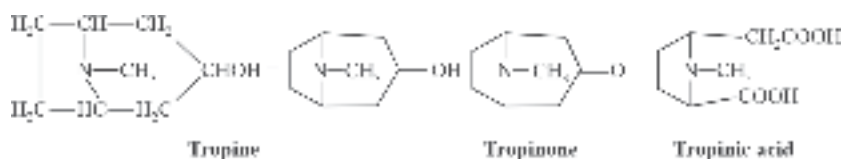


- (f) Oxidation of tropinone yields a dicarboxylic acid, tropinic acid ($C_8H_{13}NO_4$) without loss of carbon. It indicates the $-CH_2CO-CH_2-$ group in tropinone must be present in the ring. Hence, like the corresponding secondary alcoholic group in tropine must be in a ring. It is further confirmed by dibenzylidene formation. In another way, it also indicates the presence of two methylene groups adjacent to keto group.
 (g) Tropinic acid on further oxidation with CrO_3/H_2SO_4 , yields N-methylsuccinimide. This reaction confirms the presence of 5-membered nitrogen containing ring having N- CH_3 group.



On the basis of the above reactions, it can be concluded:

- Presence of $C=O$ group in a ring, in tropinone and $CHOH$ group in ring in tropine.
- Presence of $-CH_2COCH_2-$ in tropinone.
- Five membered ring possessing N- CH_3 group in tropinic acid.
- On the basis of these findings, the structure is assigned to tropinone, tropine and tropinic acid as given below:

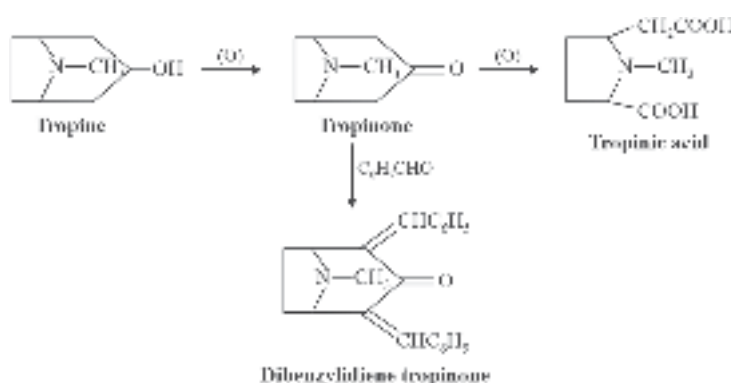


(h) The above proposed structure for tropine is confirmed by its degradation reaction.

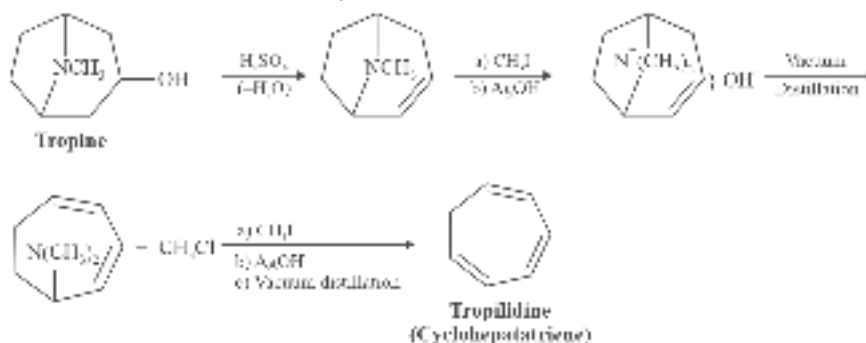
(i) Formation of 2-ethylpyridine from tropine.



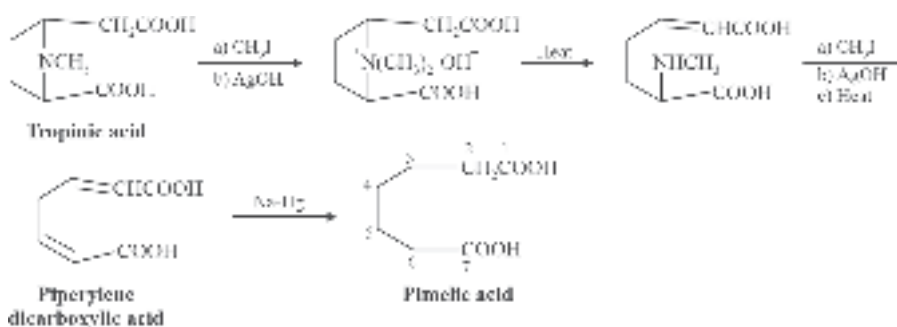
(ii) Formation of tropinone and tropinic acid from tropine.



(iii) Formation of tropilidene (cycloheptatriene) from tropine.



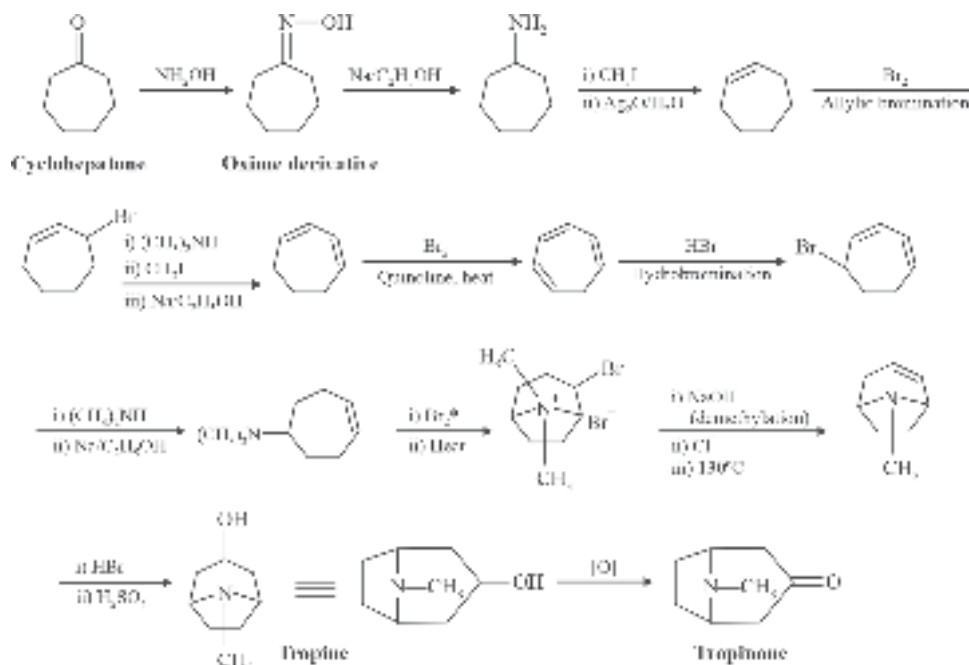
(iv) Formation of pimelic acid from tropinic acid through Hoffmann exhaustive methylation.



(i) Finally, the structure of tropine has been confirmed by its synthesis.

Willstatter' Synthesis

The first synthesis of atropine was achieved by **Richard Willstätter in 1901**. Richard Willstätter was a giant in the field of plant natural products and this was recognised, when he was awarded the Nobel Prize for Chemistry in 1915. He was interested in many alkaloids including atropine and the structurally similar cocaine molecule. Both of these compounds yielded tropinone as a degradation product and so his synthesis targeted this molecule initially. The synthesis begins from cycloheptanone.

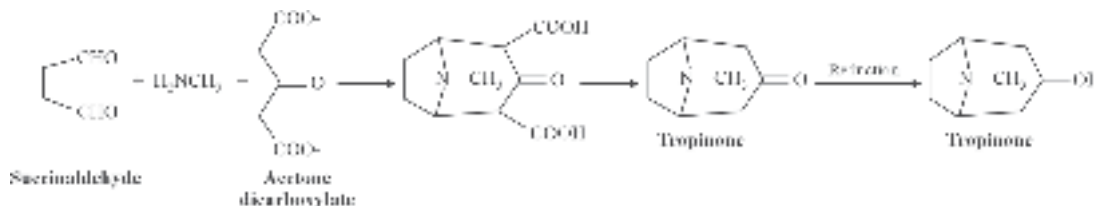


* Bromination followed by intramolecular nucleophilic substitution to form nitrogen bridge

His preparation of tropinone is a competent but long synthesis which demonstrates one of the fundamental difficulties involved in the preparation of complex organic molecules.

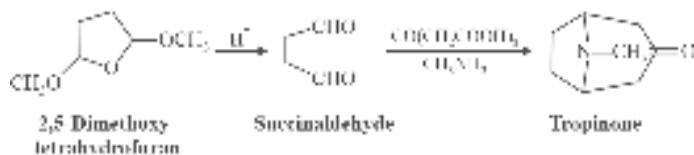
Robinson Synthesis

Tropinone is synthesized by reaction (Mannich reaction) of three components, namely succinaldehyde, methylamine and acetone dicarboxylate. The tropinone on reduction gives tropine.



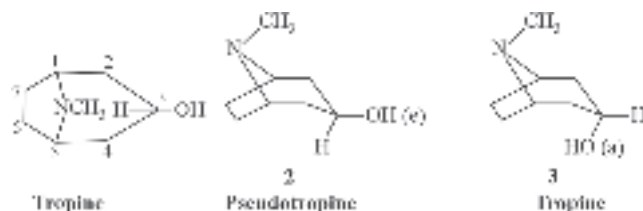
Elming Synthesis

According to Elming synthesis, tropinone is prepared by condensing methylamine, acetonecarboxylic acid and succinaldehyde, which is produced by the action of acid on 2,5-dimethoxytetrahydrofuran.



Conformation of Tropines

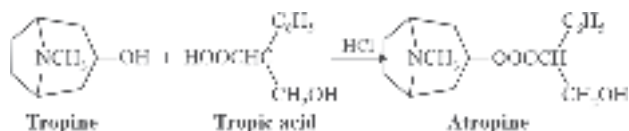
Tropinone on reduction yields a mixture of two alcohols, tropine and ψ -tropine (pseudotropine). Both are epimers. They differ in configuration at C3 position only. One of the isomer has H-atom on C3 on the same side of nitrogen methyl bridge while the other has H-atom on the opposite side of N-methyl bridge.



Although in both of these, there are two chiral centres C-1 and C-5 but none of these is optically active since the molecule has plane of symmetry. The molecule is inactive due to internal compensation and so each isomer is meso form. The C-3 position is pseudo asymmetric due to rigidity provided by ethane chain present at 1,5-position. In these, two forms, one is tropine and other is pseudotropine. Bose et al. (1953) have assured the chair form (lowest energy state) for piperidine nucleus; although boat form also exist as energy barrier in two forms is not so great, hence concluded that in ψ -tropine(2), -OH group is equatorial (e) and in tropine(3) -OH is axial (a). In ψ -tropine, nitrogen bridge and -OH are in *cis* position and has *syn* configuration whereas the tropine is anti-compound (nitrogen bridge and -OH are in *trans* configuration).

Synthesis of Atropine

As it is already proved that atropine is an tropine tropate ester. Thus, atropine can be prepared by heating tropine and tropic acid in the presence of hydrogen chloride (Fischer-Speier esterification).



If (+) or (-) tropic acid is used, the product is (+) or (-) hyoscyamine, respectively. Atropine is the racemic mixture of hyoscyamine.

Spectral Data

UV (Ethanol): $\lambda_{\max}$ 246, 251.6, 257, 263.5, 271 nm (ϵ 147.6, 175.1, 209.8, 143.3 and 24.6, respectively).

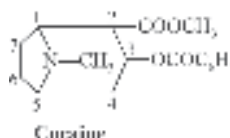
IR (KBr) (cm^{-1}): 3070 (OH-Hydrogen bonded), 2930 (CH stretch), 2810 (N-CH₃ stretch), 1725 (O-C=O), 1595, 1580 (C=C, Ar), 1155, 1030 (C-C-O), 770, 725 and 690 (mono subs Ar).

$^1\text{H-NMR}$ (CDCl_3) (δ): 7.23 (5H, s, Ar-H), 3.9 (1H, m, H-10), 2.93 (2H, bs, H-1, and H-5), 2.56 (3H, s, N-CH₃) 1.66 (8H, m, H-2,4,6,7).

2. Cocaine

Cocaine (benzoylecgonine) is a crystalline tropane alkaloid that is obtained from the leaves of the coca plant. The name comes from “coca” in addition to the alkaloid suffixine, forming cocaine.

Cocaine is sparingly soluble in water. It is a colorless crystalline solid m.p. 98°C. It is used as a local anaesthetic in eye surgery and dentistry but cannot be used for producing prolonged anaesthesia because of its poisonous properties. Deep in the brain, cocaine interferes with chemical messengers—neurotransmitters—that nerve use to communicate with other. Cocaine blocks nor epinephrine, serotonin, dopamine and other neurotransmitters from being absorbed.

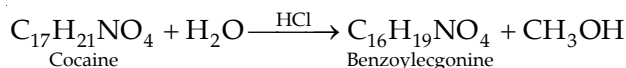


Isolation of Cocaine

- (i) The wet leaves are macerated with dilute sulphuric acid to form the water-soluble sulphate salts of the alkaloids.
- (ii) The mixture is then extracted with petroleum ether.
- (iii) After phase separation, the aqueous layer is basified with ammonia, lime or sodium carbonate to precipitate the alkaloid. The precipitate of cocaine is then recovered by filtration.

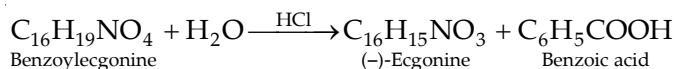
Structural elucidation of cocaine

- (i) Its molecular composition is $\text{C}_{17}\text{H}_{21}\text{NO}_4$.
- (ii) Cocaine on hydrolysis with boiling water yields methanol and benzoylecgonine.



Cocaine is the methyl ester of benzoylecgonine as the usual tests of benzoylecgonine shows the presence of carboxylic group. The above said is proven by the fact that benzoylecgonine in presence of hydrochloric acid gives cocaine on treatment with methyl alcohol.

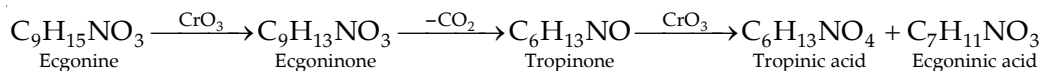
- (iii) Mineral acids, baryta water, or caustic alkali further hydrolyses benzoylecgonine to (–) ecgonine and benzoic acid. The structure of ecgonine must be determined to know the structure of cocaine.



Structure of Ecgonine

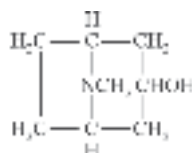
- (i) Its molecular formula is found to be $\text{C}_9\text{H}_{15}\text{NO}_3$.
- (ii) The ecgonine possess:
 - (a) a tertiary nitrogen atom—as it forms a quaternary salt with one mole of alkyl halides,

- (b) a carboxyl group—as it form monoester with methyl alcohol, and
 (c) a secondary alcoholic group—as it gives ketone on oxidation.
 (iii) Ecgonine on oxidation gives a ketone, ecgoninone which soon loses a molecule of carbon dioxide to form tropinone which, on further oxidation, gives tropinic and ecgoninic acids.

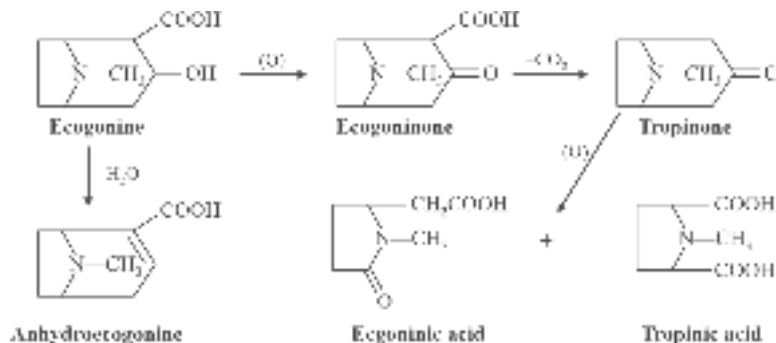


The aforesaid reactions reveal two facts:

- Ecgonine contains the tropane skeleton as it gives the tropinone and tropinic acid on oxidation. Even the position of secondary alcoholic group is similar to tropine. Dehydration of ecgonine to anhydroecgonine followed by decarboxylation to tropidine further strengthens the above fact.

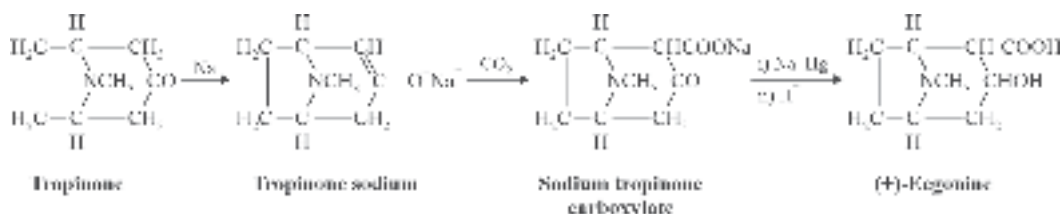


- The easy decarboxylation of the ecgonine suggests that it is a β -keto acid.
- (iv) On the basis of above facts, the structure is assigned to ecgonine which can explain the formation of ecgoninone, tropinic acid, tropinone and ecgoninic acid.

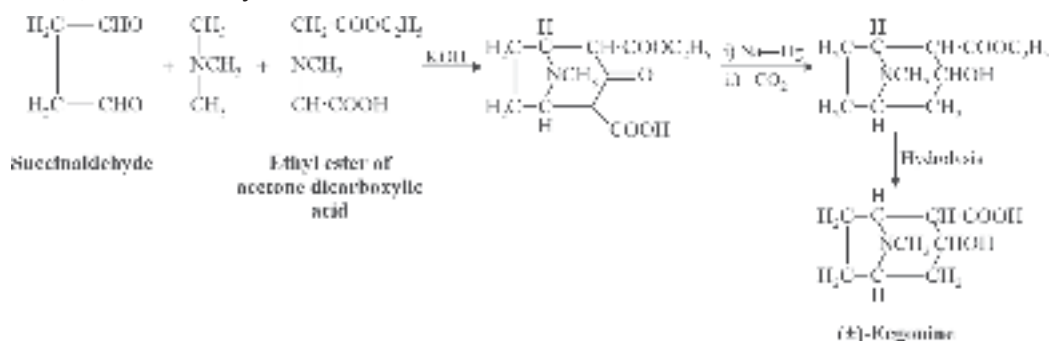


- (v) **Synthesis.** The above structure of ecgonine is confirmed by synthesis (Willstätter and Robinson synthesis).

(a) Willstätter's synthesis:

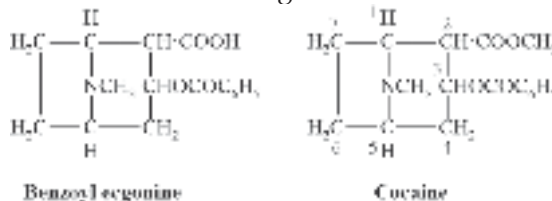


(b) Robinson synthesis:

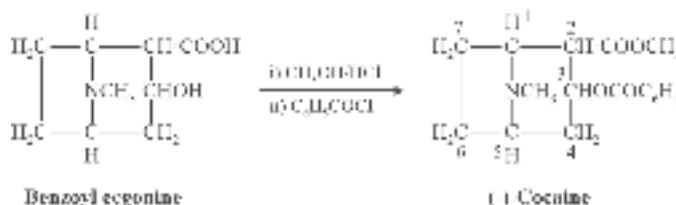


(vi) Structure of Cocaine

- Since cocaine is the methyl ester of benzoyl ecgonine, cocaine and benzoyl ecgonine should have the following structures:



- The above structure of cocaine has been proved by its synthesis which consists in the resolution of the racemic ecgonine. The esterification of (–)-ecgonine with methanol, followed by benzoylation gives cocaine identical to natural (–)-form.



Spectral data

UV: 233 (major peak), 275 (in acidic solution); 230 (major peak), 273 (basic solution).

IR (cm^{-1}): 3000, 1760, 1260.

$^1\text{H-NMR}(\delta)$: 7.95 (2H, m, H-1' and H-5'), 7.64 (3H, m, H-2', 3' and H-4'), 5.59 (1H, m, H-3), 4.23 (1H, m, H-2), 3.66 (OH, s, COOCH_3), 2.89 (3H, s, N-CH_3), 2.74 (1H, m, H-1 and H-5), 2.22 (6H, m, H-4, H-6 and H-7).

MS (m/e): 303 (M^+), 272 ($\text{M}^+ - \text{CH}_3\text{O}$), 198 ($\text{M}^+ - \text{C}_6\text{H}_5\text{CO}$), 182 ($\text{M}^+ - \text{C}_6\text{H}_5\text{COO}$, base peak), 105, 94, 82.

Stereochemistry of Cocaine

Cocaine has four asymmetric carbons so $2^4 = 16$ optically active forms (8 enantiomeric forms) are possible.

Practically, only *cis* fusion of the nitrogen bridge is possible, C_1 and C_5 have only one configuration and hence, actually four enantiomeric forms are there. In structure of cocaine, there is *cis* relationship of the COOH and OH groups, while in ψ -cocaine these groups appear to be *trans* placed. ($\pm$) cocaine and ($\pm$) ψ (pseudo) are two enantiomeric pairs.

Synthetic Substitutes for Cocaine

Some synthetic substitutes of cocaine are discovered having similar anaesthetic properties as good as cocaine but with less toxic effects. Fig. 3.4 illustrates the examples of synthetic cocaine substitutes.

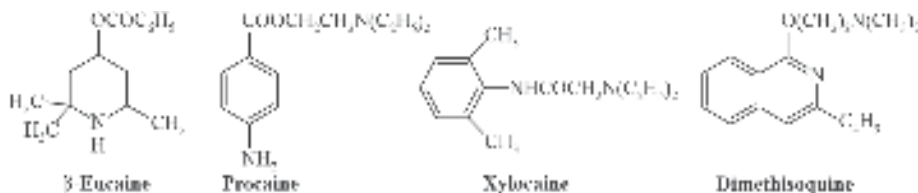


Fig. 3.4: Synthetic substitutes for cocaine

1. **̢-Eucaine:** Equivalent to cocaine in anesthetic properties. It is synthesized by the interaction of diacetoneamine with diethyl acetal.
2. **Novocaine or Procaine:** It is the most important local anesthetic. It is non-irritant, less toxic and more powerful local anesthetic.
3. **Xylocaine:** It is considered as one the most satisfactory local anaesthetic due to its less side effects. It is used for producing derma endure face anaesthetic. Its synthesis is as followed.
4. **Dimethisoquine:** It is used in painful and irritating skin conditions. It is 1000 times more active than cocaine, 100 times more active than procaine and 10 times as active as dibucaine.

QUINOLINE-BASED ALKALOIDS

Cinchona Alkaloids

Cinchona alkaloids are the most important compound class, being isolated from the Cinchona and Rubiaceae genera tree. They have been used from the early seventeenth century, when they were first introduced in Europe after the discovery of the antimalarial properties of Cinchona tree bark extract and the isolation of active principles by **PJ Pelletier** and **JB Caventou** in 1820. About 30 compounds are extracted from the bark of trees. Alkaloid content of ranges from 5 to 16%. The main components of the extract are quinine (60–85%), quinidine, cinchonine and cinchonidine (Fig. 3.5). The alkaloids possess the basic skeleton of 9'-rubanol (d) that is derived from the compound known as ruban (c). This ruban is obtained from the two distinct heterocyclic nuclei: (i) 4-methyl quinoline nucleus, (ii) quinuclidine nucleus. For over 300 years, cinchona alkaloids have played an important role in medicine and more recently in organic synthesis.

Quinine

Quinine is the most important alkaloid obtained from cinchona bark. It is a well known bitter antimalarial drug occurring among the alkaloids of cinchona bark. Quinine and cinchonine are the first cinchona alkaloids to be isolated in pure form by Pelletier and Caventou in 1820. In ~100 years, Rabe elucidated the structure of quinine and cinchonine. Most of the commercial quinine comes nowadays from cinchona bark grown in Java. Although the bark of the native trees contain about 8% quinine, several of the grafted varieties have produced more than 15% quinine (as the sulphate).

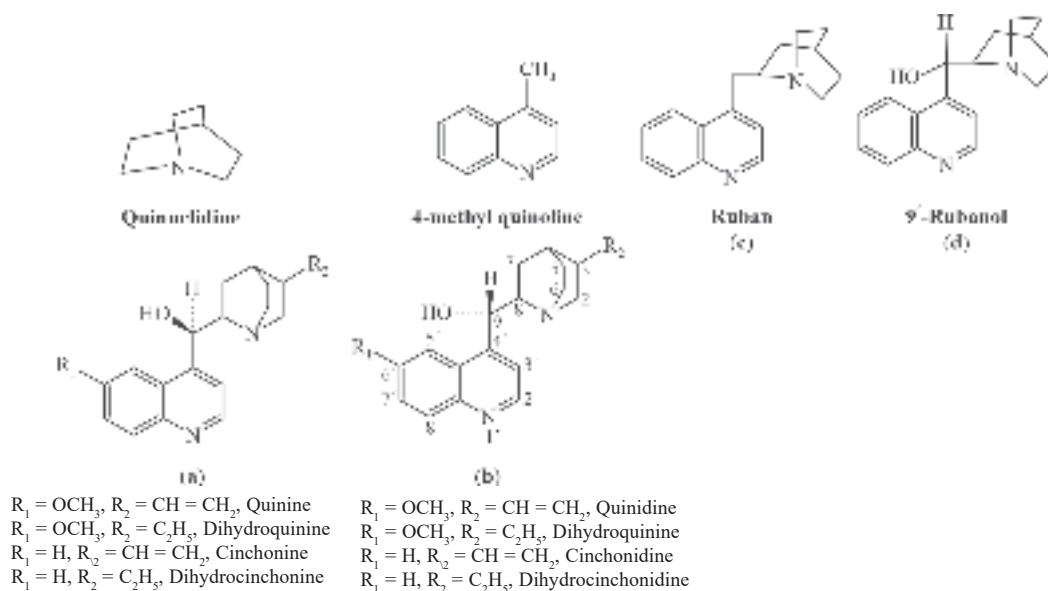


Fig. 3.5: Structures of eight major cinchona alkaloids

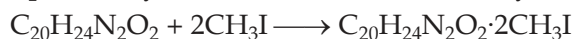
Isolation of Quinine

- The bark is stripped and dried in the Sun, crushed to a fine powder and mixed with lime and caustic soda solution for several hours and finally refluxed with benzene. It is filtered while hot.
- The benzene extract (having alkaloidal bases) is washed with dilute H_2SO_4 solution in a lead lined vessel provided with a powerful stirrer. The acidic aqueous liquid, while still hot is neutralized with Na_2CO_3 solution to pH 6.5 and allowed to stand when the quinine monosulphate is crystallized out. It is separated by filtration.
- The crude quinine (as monosulphate) is further treated with hot sodium carbonate solution to get quinine as free base.
- The other alkaloids (cinchonine, cinchonidine and quinidine) are separated out as per scheme given in Fig. 3.6.

Properties: It is a levorotatory base, m.p. 177°C and has a bitter taste. It is insoluble in water, so usually administered as the sulphate. Quinine crystallizes having three moles of water. Quinine and cinchonine find use in resolution of racemic mixtures via the formation of diastereomeric salts.

Structural elucidation of quinine

- The molecular formula is found to be $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2$.
- Presence of two tertiary 'N' atoms:** As quinine consumes two moles of methyl iodide to form a quaternary salt, it is therefore a ditertiary base.



- Presence of methoxy group:** Quinine on treatment with HCl , eliminates one mole of methyl chloride indicating the presence of a methoxy group.



- Presence of secondary alcoholic group:** Quinine can be monoacetylated and monobenzoylated and converted into a mono-chloro derivative. It indicates the presence

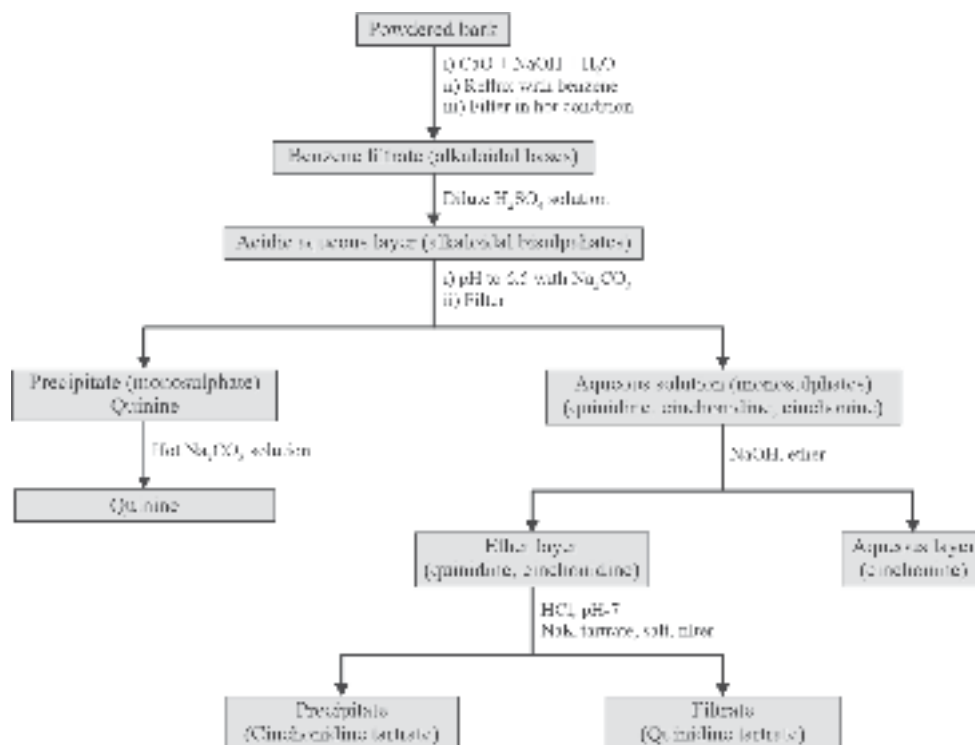
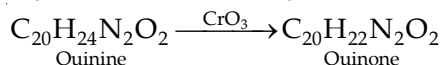
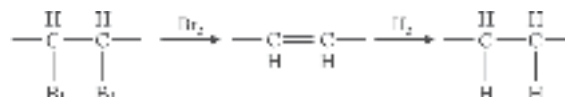


Fig. 3.6: Separation of cinchona alkaloids

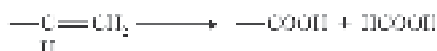
of hydroxyl group. Furthermore, quinine on oxidation gives a ketone (quinone) suggesting that hydroxyl group is secondary in nature.



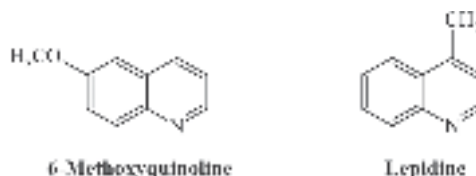
- (v) **Presence of olefinic linkage:** Quinine contains one ethylenic double bond, as it adds one molecule of bromine. The same is proved by the fact that quinine absorbs a molecule of hydrogen in the presence of catalyst.



- (vi) **Presence of vinyl group:** On mild oxidation with KMnO_4 , quinine gives a mono-carboxylic acid along with formic acid. Formation of formic acid indicates the presence of vinyl group in quinine structure.

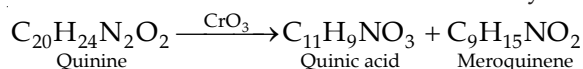


- (vii) **Presence of quinoline group:** On fusion with cone KOH, quinine gives 6-methoxy quinoline and lepidine (γ -methyl quinoline) along with other products. These products prove that quinoline nucleus is present in quinine.



Cinchonine gives quinoline and lepidine under the same conditions indicating that quinine is methoxy cinchonine.

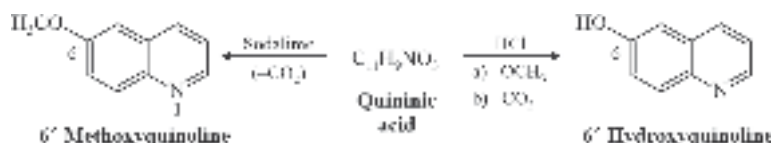
- (viii) Chromic acid oxidation of quinine yields quinic acid and other components known at that time under the name of 'second half' now commonly known as meroquinene.



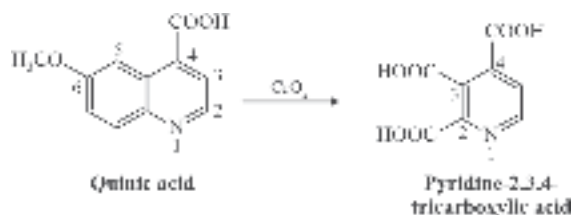
In order to establish the structure of quinine, the structure of these two oxidation products—quinic acid and meroquinene has to be established.

A. Structural Elucidation of Quinic Acid, $\text{C}_{11}\text{H}_9\text{NO}_3$

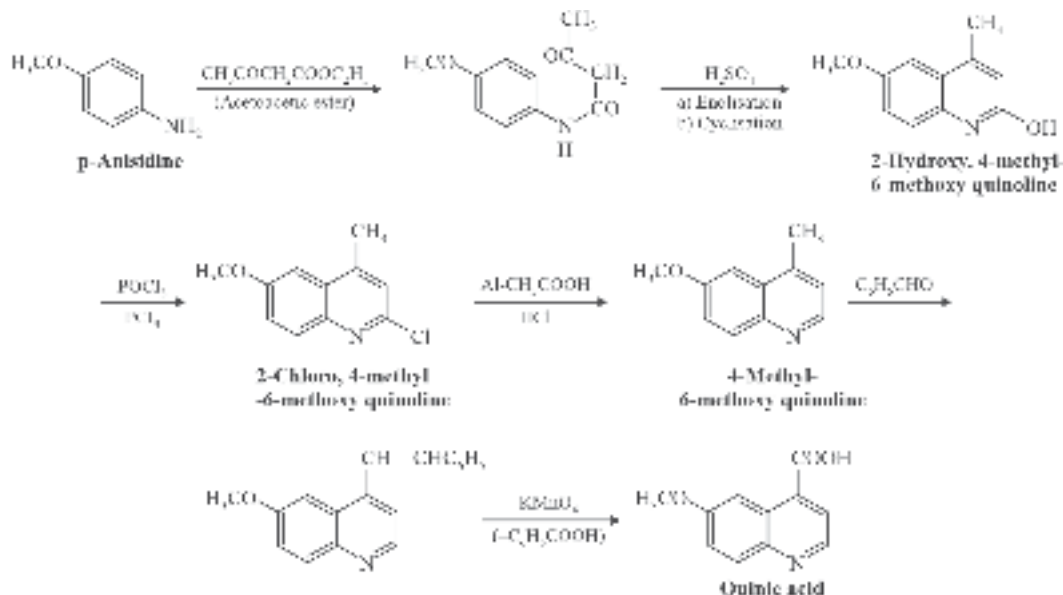
- (i) Quinic acid gives 6-methoxy quinoline on heating with sodalime indicating that quinic acid is 6-methoxyquinoline derivative. The C-6 position for the methoxyl group has been confirmed by the conversion of quinic acid to 6-hydroxyquinoline by heating with HCl.



- (ii) Quinic acid on oxidation with chromic acid yields pyridine-2,3,4-tricarboxylic acid. The formation of latter from quinic acid indicates that the benzene ring of the latter having the methoxy group is oxidized. Furthermore, -COOH group at C-4 indicates that in quinic acid also the COOH group is present at C-4 position. Therefore, quinic acid can be written as:

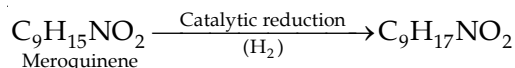


- (iii) Finally, the above proposed structure of quinic acid is proved by its synthesis.

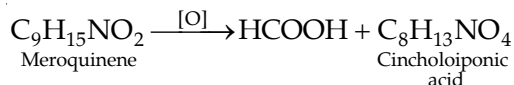


B. Structural Elucidation of Meroquinene

- (i) Its molecular formula is been found to be $C_9H_{15}NO_2$.
- (ii) Formation of monosodium salt as well as monoester reveals the presence of one carboxylic group.
- (iii) When meroquinene is reduced with hydrogen gas, it takes up one molecule of hydrogen, suggesting that a ethylenic double bond is present in it. The presence of ethylenic double bond indicates that $-CH=CH_2$, side chain is still present in meroquinene.

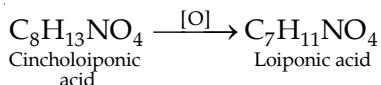


- (iv) Presence of secondary amino group, meroquinene can be benzoylated, acetylated and nitrosated (forms nitroso derivative with HNO_2), it means that a secondary amino group is present in meroquinene.
- (v) When meroquinene is oxidized with cold acidified $KMnO_4$, it yields a cincholoiponic acid (a dicarboxylic acid) and formic acid.

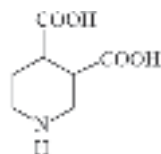


The formation of formic acid reveals the presence of $-CH=CH_2$ (vinyl group) side chain in meroquinene.

- (vi) When cincholoiponic acid is oxidized with acid permanganate, it yields loiponic acid $C_7H_{11}NO_4$. As loiponic acid is a dicarboxylic acid and contains one less methylene group than its precursor cincholoiponic acid. This means that the latter contains at least a side chain $-CH_2COOH$.

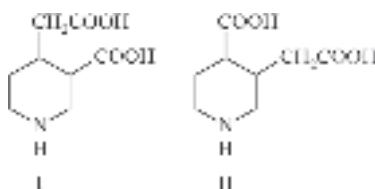


- (vii) Loiponic acid is somewhat less stable and isomerizes to more stable hexahydrocinchomeronic acid, $C_7H_{11}NO_4$ (piperidine-3,4-dicarboxylic acid) on treatment with KOH at about $200^\circ C$; hence loiponic acid should also be piperidine-3,4-dicarboxylic acid.

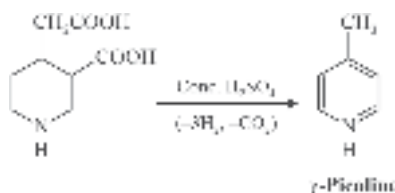


Loiponic acid
(Piperidine 3,4 dicarboxylic acid)

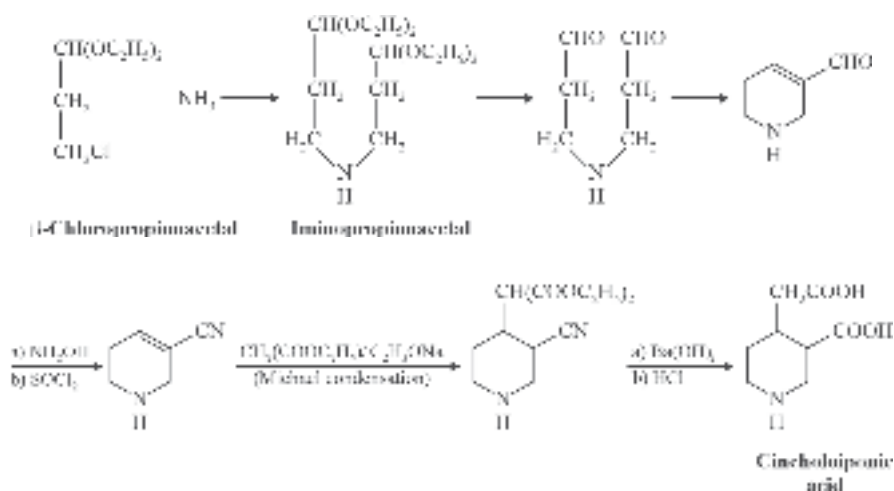
- (viii) Now as cincholoiponic acid is having one more $-CH_2-$, than the loiponic acid, it must be either I or II.



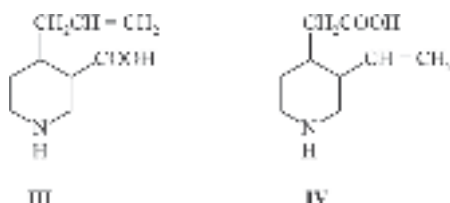
Cincholoiponic acid has been found to be I because on heating with cone. H_2SO_4 , it gives γ -picoline.



The structure, I of the cincholoiponic acid has been further proved by its synthesis.

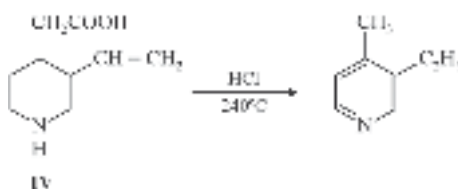


- (ix) Now, since cincholoiponic acid is obtained along with HCOOH by the oxidation of meroquinene. Hence, meroquinene contains $-\text{CH}=\text{CH}_2$ which is responsible for generation of COOH group. Hence, meroquinene may be either **III** or **IV**.



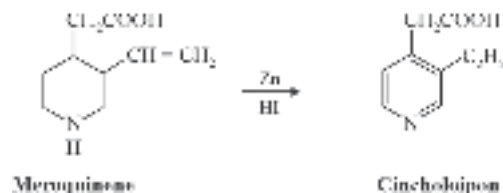
But the structure is found to be IV, confined by following observation:

- (a) Meroquinene on heating with HCl at about 240°C gives 3-ethyl 4-methylpyridine.



In case of structure, III, 4-propylpyridine would have been obtained.

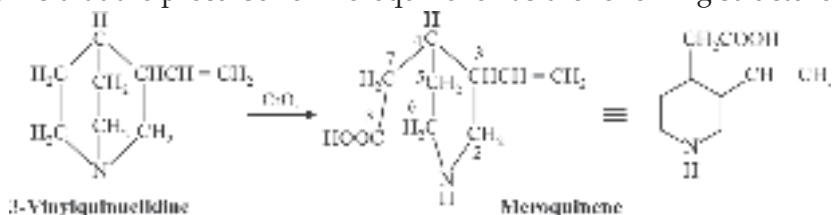
- (b) Meroquinene on reduction with zinc and hydroiodic acid gives cincholoipon, $C_9H_{11}NO_2$, which is found to have a carboxyl group and a ethyl group.



In case of structure, III, propyl group would have been introduced at C-4 position.

Point of linkage between quinic acid and meroquinene in quinine

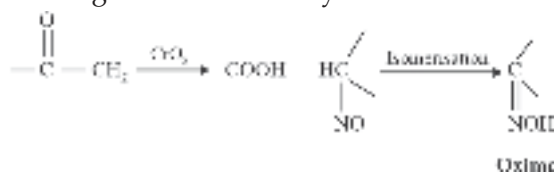
Since quinine has no carboxylic acid, but its oxidation product, viz. quinic acid and meroquinene, have free carboxylic group, indicating that two moieties are linked with each other by means of the carboxylic carbon atom. Further, as the quinine is a ditertiary base, while meroquinene has a secondary nitrogen atom indicating that during oxidation of quinine the second half moiety is oxidized in such a way as to convert its tertiary nitrogen atom into secondary with the introduction of the one carboxylic group. A possible explanation is that the precursor of meroquinene has the following structure.



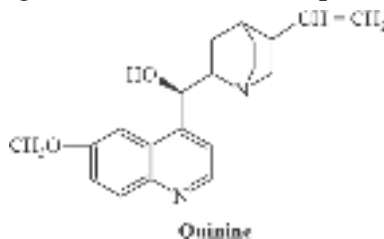
Based on the above facts, it can be stated that in quinine molecule, C-4 position in the quinoline fraction is joined to the quinuclidine via C-8 position.

Position of the secondary alcoholic group

Rabe oxidized quinine to quinonone by gentle oxidation (CrO_3). Quinonone on treatment with amyl nitrite and hydrogen chloride gives quinic acid and oxime a characteristic reaction of compound having $-\text{CO}-\text{CH}-$ moiety.



On the basis of the forgoing facts, the structure of quinine can be represented as:



Synthesis

RB Woodward and WE Doering synthesis: In this, synthesis begins from *m*-hydroxybenzaldehyde to get 4-hydroxy quinoline. From this, quinotoxine is formed via sequence of reactions (Fig. 3.7). The dextro isomer of quinotoxine is subjected to Rabe protocol to produce quinine.

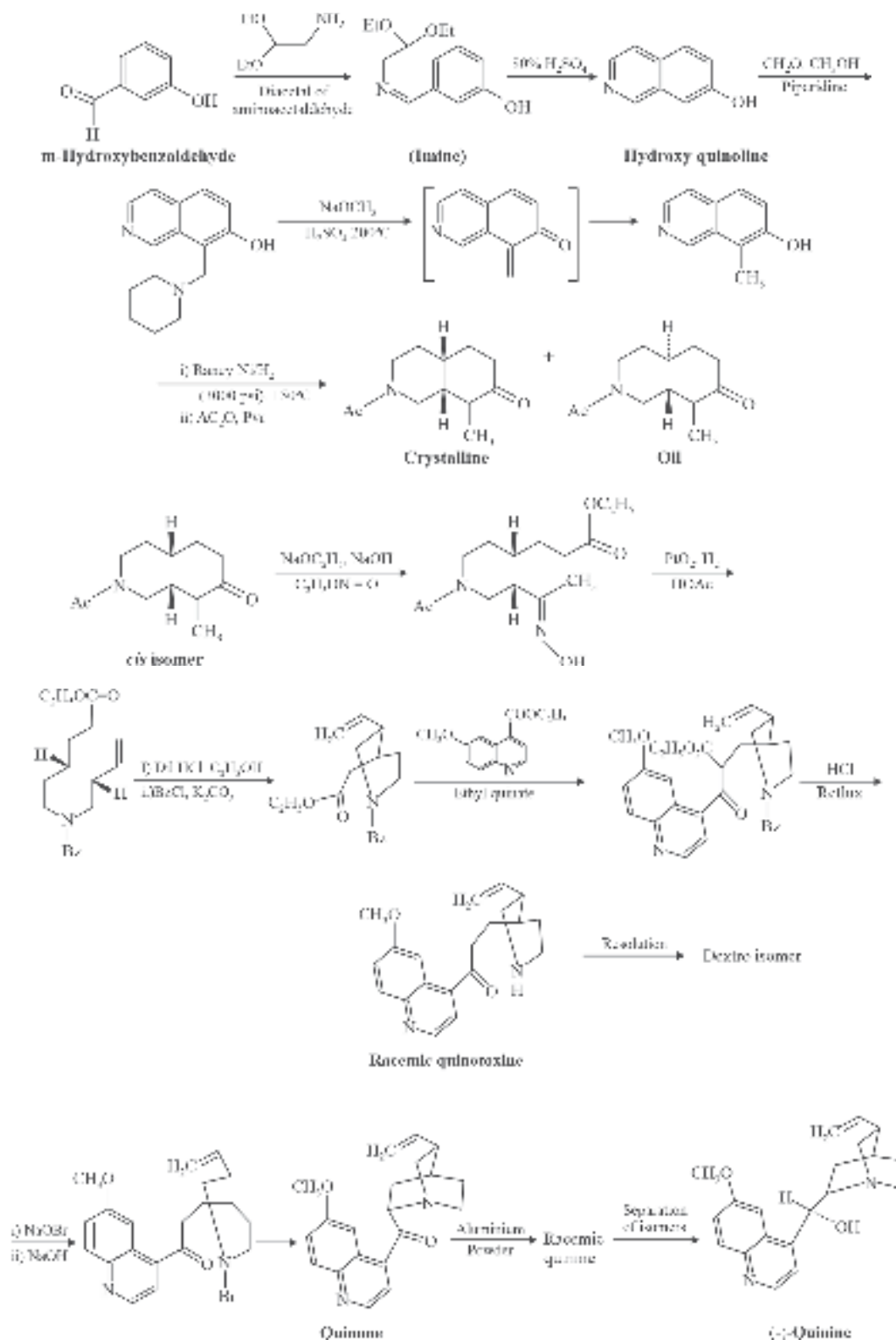


Fig. 3.7: Synthesis of quinine

Stereochemistry: There are four chiral centers (C-3, C-4, C-8, C-9) in the quinine structure. The stereoisomers differ from each other in configuration at C-8 and C-9. For example, although structurally similar, quinine and quinidine form a diastereomeric pair as shown in Fig. 3.8.

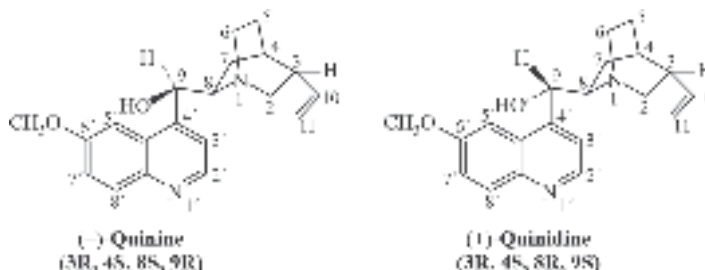


Fig. 3.8: The structure of quinine and quinidine (stereoisomers)

Quinine is levo isomer while quinidine is a dextro isomer. The primary indication of dextro-isomer, quinidine is cardiac arrhythmias. It is a good example of the importance of stereochemistry because it provides a significantly different pharmacological spectrum.

Spectral Data

UV: $\lambda_{\max}$ 277, 321, 332.

IR (KBr) (cm^{-1}), 3300 (OH), 2858(NH), 1615 (C=N, C=C), 1600, 1512, 1480 (C = C, Ar), 1248, 1230, 1100, 1030 (ether linkage), 860, 838, 808, 725 (trisubstituted benzene).

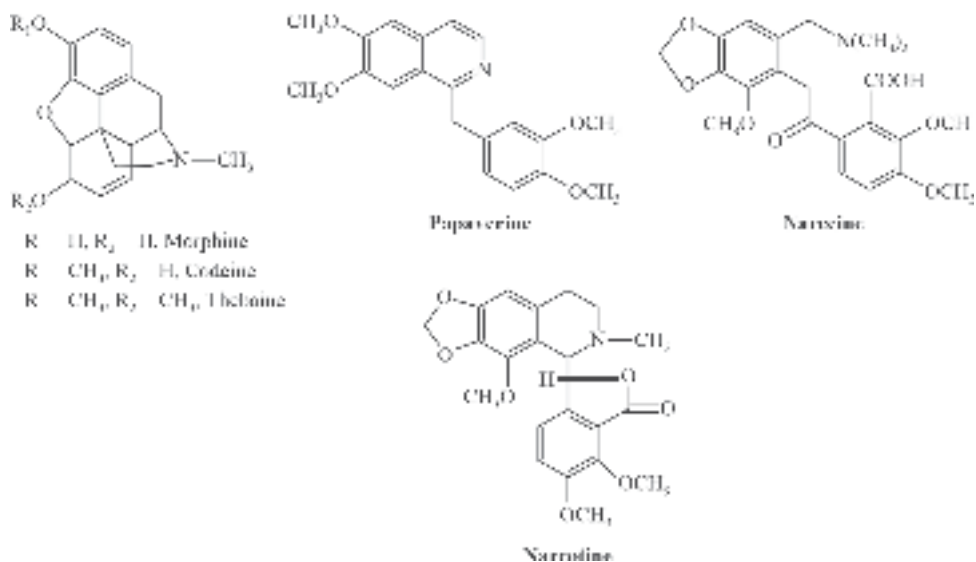
$^1\text{H-NMR}$ (CD_3OD) (δ): 8.68 (1H, d, H-2); 7.78 (1H, d, H-3), 7.93 (1H, d, H-8), 7.83 (1H, d, H-5), 7.40 (1H, dd, H-7), 6.23 (1H, brs, H-9), 5.08 (1H, dd, H-11b), 5.79 (1H, ddd, H-10), 5.02 (1H, dd, H-11a), 4.17 (1H, m, H-6b), 3.72 (1H, dd, H-2b), 3.94 (3H, brs, 6-OCH₃), 3.34 (1H, m, H-6a), 3.46 (1H, dd, H-8), 3.21 (1H, ddd, H-2a), 2.96 (1H, m, H-3), 2.43 (1H, m, H-7b), 2.39 (1H, m, H-5b), 2.03 (1H, m, H-5a), 1.95 (1H, m, H-4), 1.71 (1H, m, H-7a); **$^{13}\text{CNMR}$** . (CD_3OD) (δ): 20.90 (C-7), 27.64 (C-5), 28.77 (C-4), 42.38 (C-3), 56.54 (6-OCH₃), 59.98 (C-6), 64.14 (C-9), 71.08 (C-2), 74.95 (C-8), 102.92 (C-5'), 116.85 (C-11), 120.09 (C-3'), 123.57 (C-7'), 127.56 (C-4'a), 131.23 (C-8'), 139.54 (C-10), 144.55 (C-8'a), 148.09 (C-2'), 149.31 (C-4'), 159.88 (C-6').

MS (m/e), 324 (M^+), 309, 295, 269, 222, 202, 136 (base peak), 95, 81.

6. ISOQUINOLINE-BASED ALKALOIDS

Opium alkaloids

The six opium alkaloids which occur naturally in the largest amounts are morphine, narcotine, codeine, thebaine, papaverine and narceine. These are of two types: phenanthrene alkaloids and isoquinoline alkaloids. The phenanthrene alkaloids (morphine, codeine, and thebaine) are under international control and also used in clinical practice. For example, morphine and codeine are used as analgesic and antitussive drugs respectively while thebaine itself is not used in therapy, but it is an important starting material for the manufacture of a number of opioids. In contrast, isoquinoline type alkaloids (narcotine, narceine and papaverine) are not under international control. Among these, only papaverine is used, due to its vasodilating property but narcotine and narceine have scarcely any medical or other uses.

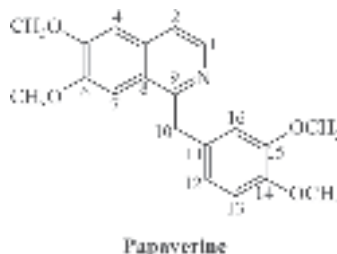


Isolation of opium alkaloids

- (i) The powdered drug is shaken with calcium chloride solution and filtered to remove the meconate and sulphate of calcium.
- (ii) To the filtrate, add 10% NaOH solution. It is filtered. The marc consists of narcotine, papaverine and thebaine and filtrate consists of morphine and codeine.
- (iii) The filtrate is extracted with chloroform. The chloroform layer is separated. The chloroform layer consists of codeine and aqueous layer consists of morphine and narceine. The aqueous layer is first acidified and later on slightly alkalinized with ammonia solution. Morphine is precipitated out by addition of ammonia solution. It is separated out and purified by crystallization.
- (iv) The aqueous layer consists of narceine.
- (v) The marc consisting of narcotine, thebaine and papaverine, is dissolved in alcohol and then acidified with acetic acid.
- (vi) To the acidified solution, add three volumes of boiling water. Precipitates of narcotine and papaverine are formed and thebaine still remains in solution. Papaverine is separated by addition of 0.3% oxalic acid solution. The solution after adding oxalic acid is allowed to cool. On cooling papaverine crystals separate out. It is filtered out and the filtrate is made alkaline for the precipitation of narcotine.

Papaverine

Papaverine together with nearly twenty four alkaloids such as laudanose, laudanine, narcotine, narcine, morphine etc. occurs in opium poppy, *Papaver somniferum* to the extent of 0.5–8% and hence, all of them are usually known as opium alkaloids.



Properties: It is a colorless solid, m.p. 147°C. It is an optically inactive tertiary base. It is insoluble in water but soluble in hot alcohol and chloroform.

It was isolated from opium by **Merch in 1848**. Like morphine, it is a pain killer but has weaker physiological action. However, it finds use as antispasmodic for the relaxation of cardiac muscles and in remedy of coughs. In small doses, it causes light sleep (narcosis) while in larger doses, it causes tetanus and respiratory paralysis.

Structural elucidation of papaverine

The constitution of papaverine is established by **Goldschmidt et al.** and their work is considered to be a very good example for illustrating the application of oxidative degradation to determine the structure of the alkaloids.

(i) **Molecular formula:** The molecular formula of papaverine is found to be $C_{20}H_{21}NO_4$.

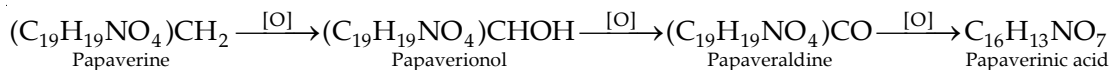
(ii) **Presence of a tertiary nitrogen:** It adds on one molecule of methyl iodide to yield a quaternary methiodide derivative, indicating the presence of tertiary nitrogen present in papaverine.



(iii) **Number of methoxyl groups:** On heating with hydroiodic acid (Zeisel's method), papaverine yields four equivalent moles of methyl iodide. This reaction reveals that papaverine contains four methoxyl ($-OCH_3$) groups. It means that all four oxygen atoms of papaverine are adjusted as $-OCH_3$ groups. The demethylated product is known as papaveroline.



(iv) **Presence of a methylene ($-CH_2$) group:** On oxidation with cold dilute permanganate solution, it yields papaverinol, $C_{20}H_{21}NO_5$. This on more vigorous oxidation with hot dilute permanganate solution yields ketone, papaveraldine $C_{20}H_{19}NO_5$; formation of this ketone reveals that papaverinol is a secondary alcohol. Finally, the prolonged action of hot permanganate solution causes the oxidation of a papaveraldine to papaverinic acid $C_{16}H_{13}NO_7$. The latter compound, papaverinic acid is a dibasic acid which still contains the keto group in its precursor because it forms an oxime derivative. The papaverinic acid also contains two methoxyl group. On the basis of this sequence of reactions, presence of $-CH_2$ group is suspected.



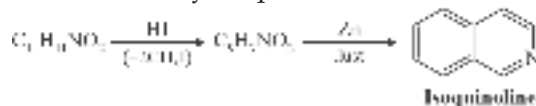
(v) **Degradation method:** Papaverine when fused with potassium hydroxide mainly yields two compounds, A($C_{11}H_{11}NO_2$) and B($C_9H_{12}O_2$) along with some veratric acid. Now the molecular formula of the compounds A and B account for twenty carbon atoms. Therefore, the two compounds must constitute the molecule of papaverine, so it becomes essential to know the structure of these two compounds.

I. Structure of the compound A ($C_{11}H_{11}NO_2$):

(a) Nitrogen atom in the compound has been found to be tertiary one as in the parent compound papaverine.

(b) It has been found to contain two methoxy groups determined by Zeisel's method.

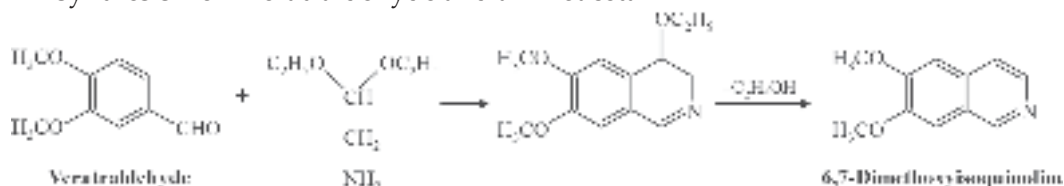
- (c) On demethylation followed by zinc dust distillation, it yields isoquinoline revealing that it is dimethoxy isoquinoline.



- (d) It is possible to know the position of two methoxy group by the oxidation of the compound, **A** to *m*-hemipinic acid. Hence, the compound, **A** is 6, 7-dimethoxy isoquinoline.

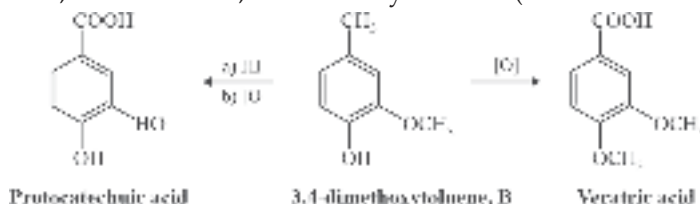


The structure of compound, **A** as 6, 7-dimethoxyisoquinoline can be proved by its synthesis from veratraldehyde and aminoacetal.



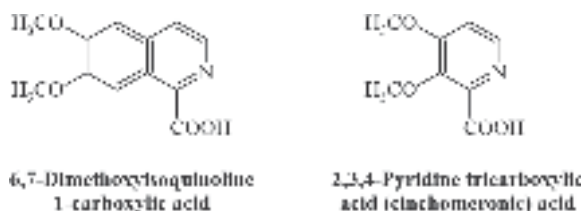
II. Structure of compound, **B** ($C_9H_{12}O_2$):

- (a) It contains two methoxy groups, determined by Zeisel method. On oxidation it yields veratric acid, identified as 3,4-dimethoxybenzoic acid. Secondly, on demethylation followed by oxidation, **B** yields protocatechuic acid. Hence, the compound, **B** should be 3,4-dimethoxy-toluene (homoveratrole).



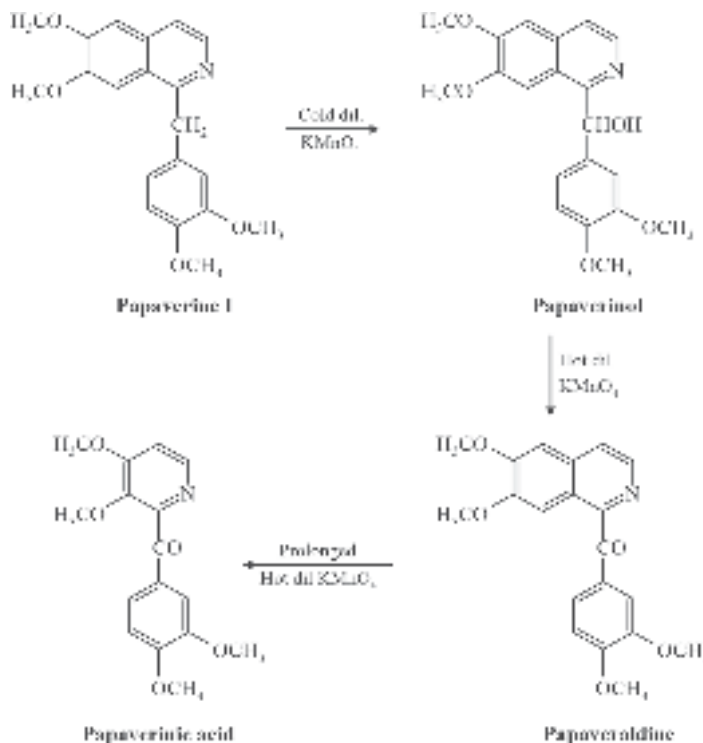
It is also able to explain the formation of some veratric acid during the potassium fusion of papaverine.

- (vi) **Point of linkage between compound A and B:** The papaverine itself contains four methoxy groups. It means that the two components cannot be linked through the methoxy groups. Thus, the component, **B** should be linked to **A** either via the carbon atom of the benzene nucleus or the carbon atom of the methylene (CH_2) group. But the formation of veratric acid during fusion or oxidation reveals that the unit **B** is linked via the carbon atom of the methylene group. Therefore, the point of linkage of isoquinoline nucleus can be decided by the oxidation of papaverine to 6,7-dimethoxyisoquinoline-1-carboxylic acid and also 2,3,4-pyridine tricarboxylic acid (cinchomeronic acid).

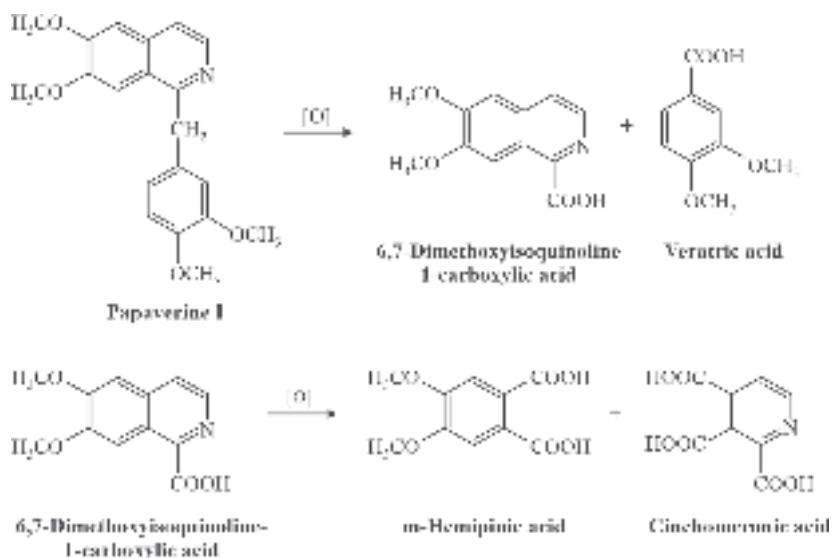


(vii) On the basis of above evidences, the structure of papaverine may be written as **I** which explains all the reactions.

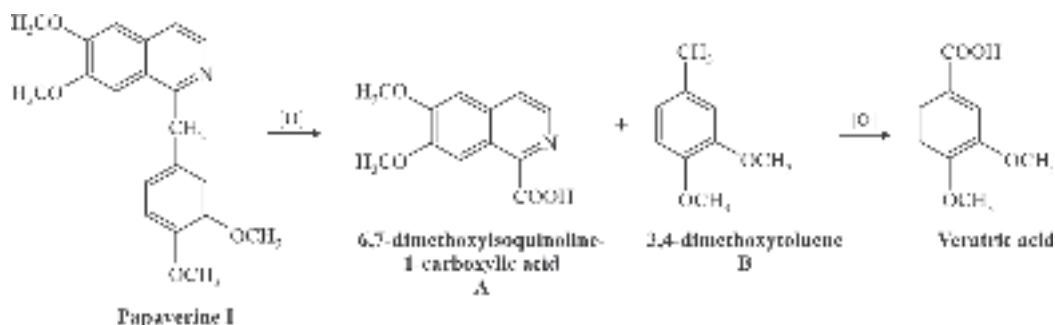
(a) Oxidation of papaverine with cold dilute potassium permanganate solution:



(b) Oxidation of papaverine with hot conc KMnO_4 solution:

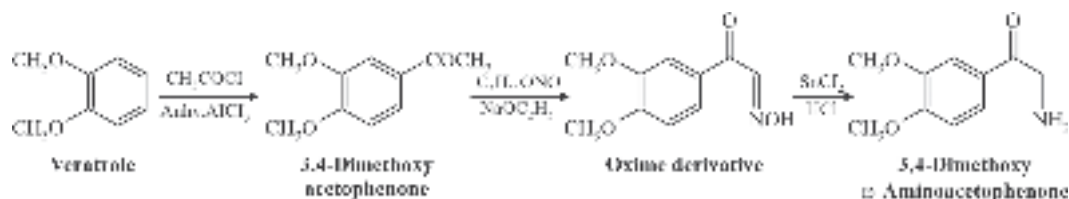


(c) Fusion with potash may be explained as follows:

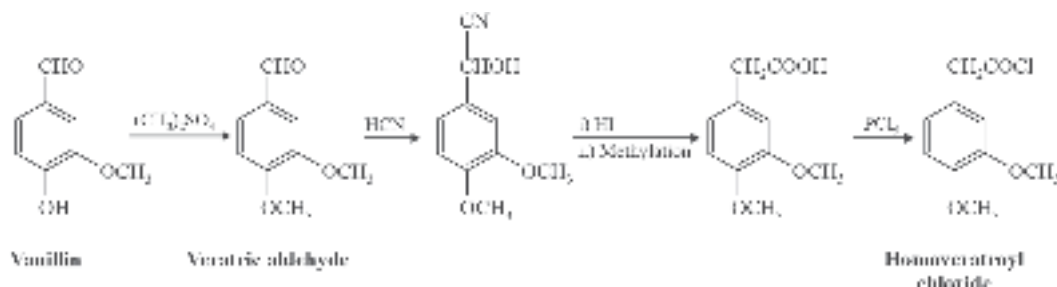


(viii) **Synthesis:** Finally, the structure is confirmed from synthesis. The first synthesis of papaverine is accomplished by **Pictet and Garns**. The synthesis is accomplished through synthesis of following two intermediates:

(a) **3,4-dimethoxy- ω -aminoacetophenone:** 3,4-dimethoxyacetophenone is obtained through a Friedel-Crafts reaction of veratrole with acetyl chloride. This ketone derivative is converted through the oxime-ketone to the corresponding ω -aminoacetophenone by reduction with Stannous chloride.

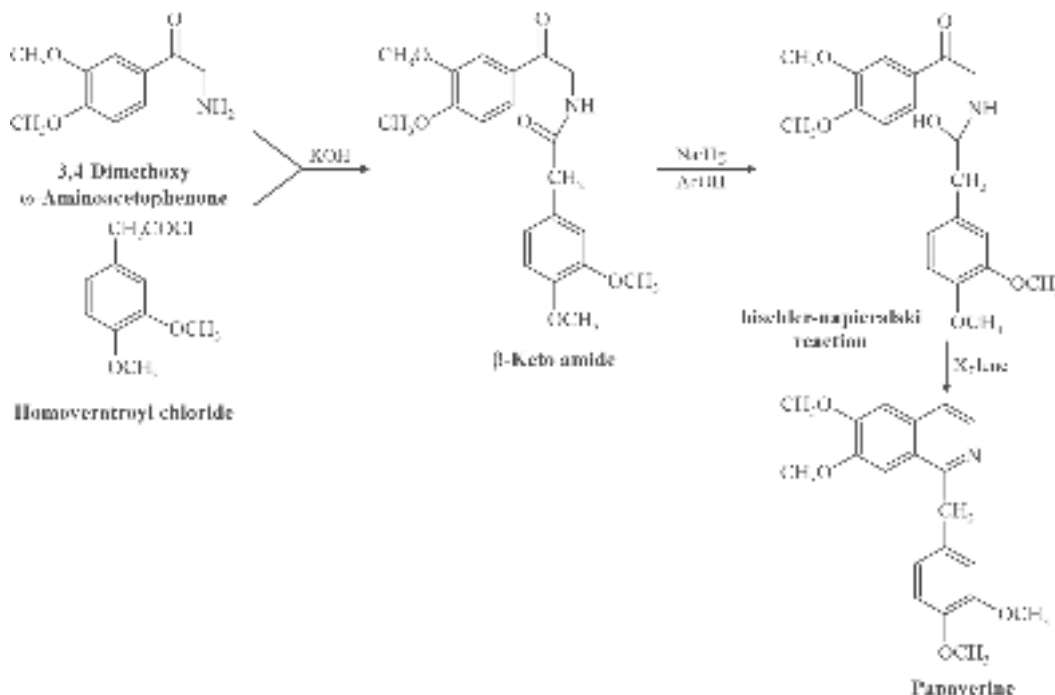


(b) **Homoveratroyl chloride:** Veratric aldehyde is obtained by methylation of vanillin. The cyanohydrin is prepared and upon treatment with hydriodic acid, several transformations take place: demethylation of the phenolic ether groups, saponification of the nitrile group and reduction of the secondary hydroxyl group. Remethylation of the product gave homoveratric acid which is converted to homoveratroyl chloride by means of phosphorus pentachloride.

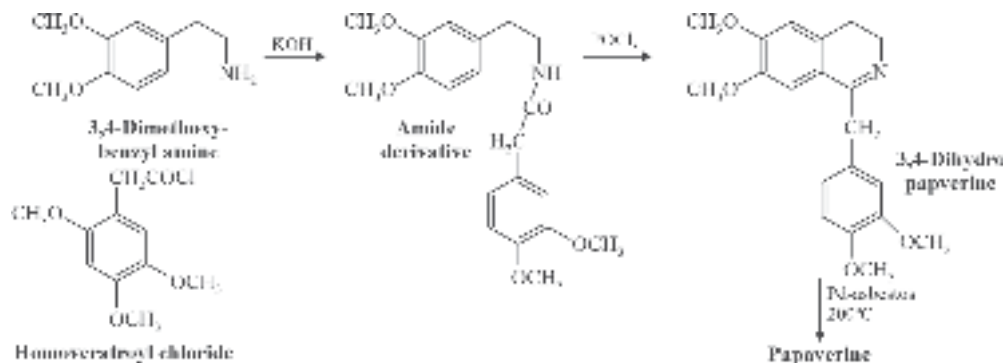


(c) **Synthesis of papaverine:** Condensation of the 3,4-dimethoxy- ω -aminoacetophenone with homoveratroyl chloride yields the 3-keto amide. Reduction with sodium amalgam gives the corresponding 3-hydroxy-amide. Dehydration

of 3,4-Dimethoxy α -aminoacetophenone with phosphorus pentaoxide at the reflux temperature of xylene yields papaverine.



Pictet and **Finkelstein** improved the synthesis by substituting 3,4-dimethoxybenzyl amine for the above α -amino-acetophenone. Condensation of this amine with homoveratryl chloride yields the amide derivative. Cyclization of the amide by the Bischler-Napieralski procedure (phosphorus oxychloride), yields 3,4-dihydropapaverine. Synthesis of papaverine is accomplished with the dehydrogenation of this substance, with palladized-asbestos at 200° in the presence of air.



Spectral Data

UV (HCl, CH₃OH): $\lambda_{\max}$ 325, 312, 283 and 238.

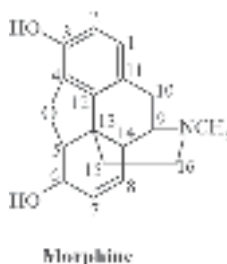
IR (cm⁻¹): 3020, 2965, 2940 (CH), 1635 (C = N), 1520, 1510, 1485 (C = N, conjugated cyclic system), 1610, 1595 (C = C, Ar).

$^1\text{H-NMR}$ (δ): 8.31 (1H, s, H-7) 8.24 (1H, d, H-2), 7.54 (2H, d, H-1, H-4), 7.03 (1H, s, H-16). 6.82 (1H, s, H-12, H-13), 4.48 (2H, s, H-10), 3.90 (6H, s, $2 \times \text{OCH}_3$ -5,6), 3.68, 3.87 (6H, s, $2 \times \text{OCH}_3$, 14, 15).

MS (m/e): 340 (M^+), 339, 338, 324 (100), 322, 305, 294.

B. Morphine

Morphine is usually considered as phenanthrene type of alkaloid. It is the first alkaloid, isolated by **Serturmer** (1806) from opium plant. In opium, it is present in a quantity of 10–23% along with other substances like fats, resins, proteins, carbohydrates, mineral salts, meconic acid about 20 or more alkaloids.



Codeine and thebaine are the other closely related alkaloids to morphine. These three are commonly known as *morphine alkaloids* and form a sub-group of the *opium alkaloids*. In all morphine alkaloids, phenanthrene nucleus present. Due to this, these are also known as *phenanthrene alkaloids*.

The morphine alkaloids have been studied comparatively more due to the following reasons:

- (i) These are widely used as analgesic agents, and
- (ii) These undergo a wide variety of molecular rearrangements.

Isolation of morphine

Morphine, m.p. 254–256°C is extracted from opium by involving the following steps:

- (i) First of all, the raw opium is extracted with cold dichloromethane repeatedly. Papaverine, narcotine and gummy substances go into the dichloromethane layer whereas morphine and other substances remain in the *insoluble residue*. Dichloromethane layer is separated.
- (ii) The residue obtained from the dichloromethane extraction is agitated with lime water at temperature below 20°C. Morphine, codeine and thebaine are solubilized in lime water. The aqueous extract is extracted several times with benzene to remove codeine and thebaine in a benzene layer. Then, the pH of aqueous extract is raised to 8 when the crude morphine is precipitated out. The filtrate still contains morphine. The filtrate after evaporation in vacuum is extracted with amyl alcohol to get further amount of crude morphine. The two samples of crude morphine are mixed.
- (iii) The crude morphine obtained from step (ii) is dissolved in dilute HCl and filtered. Then, the filtrate is neutralized with ammonia followed by the addition of alcohol. This results in precipitation of morphine. The precipitated morphine is dissolved in minimum quantity of dilute HCl and the resulting solution so obtained on concentration and cooling yields crystals of morphine hydrochloride.

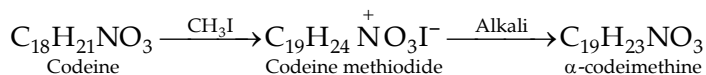
- (iv) The benzene extract obtained from step (ii) is evaporated to yield a residue. This is then treated with hot alcohol, followed by cooling and filtration. The filtrate when treated with sulphuric acid yields a precipitate of codeine sulphate which is filtered out. To the resultant filtrate, tartaric acid is added to precipitate out thebaine as thebaine acid tartrate.

Structural elucidation of morphine

- (i) **Molecular formula:** It is found to be $C_{17}H_{19}NO_3$.
- (ii) **Nature of nitrogen:** It takes up one mole of CH_3I to form quaternary ammonium salt, showing that the nitrogen is tertiary in nature. The tertiary nature of nitrogen is confirmed by Hoffmann degradation of codeine derivative which further indicates nitrogen is present in ring (see point vi).
- (iii) **Nature of oxygen:** Morphine on acetylation form diacetyl derivative, indicating the presence of two hydroxyl groups:
- It exhibits characteristic of the phenolic group, viz. coloration with $FeCl_3$ and solubility in aq. sodium hydroxide to form monosodium salt, which is reconverted into morphine by passing CO_2 gas, hence, one of the hydroxyl groups must be phenolic one.
 - On treatment with halogen acids, morphine forms monohalogen product indicating that an alcoholic hydroxyl group is present in morphine. It is further confirmed by following fact.

Morphine on methylation with methyl iodide gives monomethyl product which does not give any color reaction with $FeCl_3$ indicating that it is the phenolic hydroxyl group that is methylated. The methylated morphine on chromic acid oxidation gives a ketone codeinone showing that the monomethylated morphine or codeine has a secondary alcoholic group.

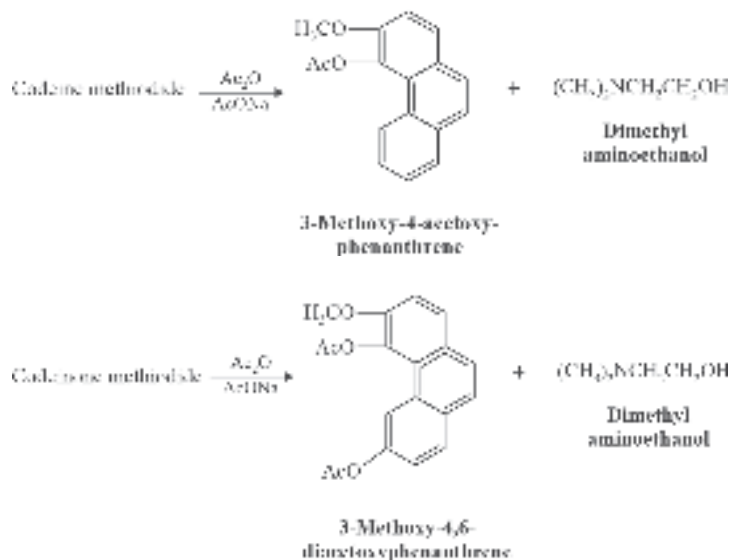
- (iv) From the unreactivity of 3rd oxygen atom and degradation products of morphine, it is concluded that the third oxygen atom is present as ether linkage.
- (v) On catalytic reduction, codeine gives isolated dihydro product $C_{18}H_{23}O_3N$ suggesting the presence of an ethylenic bond.
- (vi) Morphine is brominated to a bromo derivative along with the evolution of a mole of hydrogen bromide, which suggests that morphine possesses a benzene nucleus.
- (vii) Codeine is treated with methyl iodide to form codeine methiodide which on heating with alkali gives α -codeimethine.



Now as these changes correspond to Hoffmann degradation of N-methylpiperidine, the nitrogen atom must be present in a ring.

- (viii) α -Codeimethine on heating with alkali suffers a double bond shift to give isomeric β -codeimethine. When either of these isomers is treated with methyl iodide followed by alkali, methylmorphenol is formed as the main product along with trimethylamine and ethylene. The methyl morphenol when heated with hydrobromic acid gives morphenol, which on reduction with sodium and alcohol

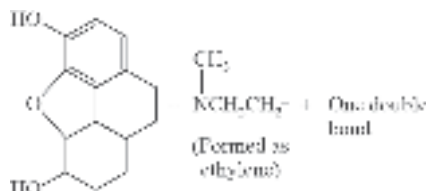
4,6-diacetoxyphenanthrene, respectively. The presence of an additional acetoxy group at C-6 position in the latter indicates that in the former the secondary alcoholic group is lost as water molecule during dehydrogenation to aromatic product while in the latter case the ketonic group enolizes during the route to aromatic product and hence it appears as acetoxy group in the final product.



Thus, the position of all three oxygen function in morphine has been established, one (phenolic) at C-3 position, the other (ether) linkage between C-4 and C-5 and third (secondary alcoholic) at C-6 position in the phenanthrene nucleus.

- (x) As indicated by the fact that morphine forms mono-bromo-derivative with bromine and monosodium salt with sodium hydroxide, it possesses only one benzenoid nucleus. Further, as ethylene is formed as one of the products during the exhaustive methylation of codeimethine and dimethylamino-ethanol is formed, a $\text{CH}_2\text{CH}_2\text{NCH}_3$ chain must be present in morphine.

Further as it contains a double bond and a tertiary nitrogen atom the partial structure for morphine may be written as follow:

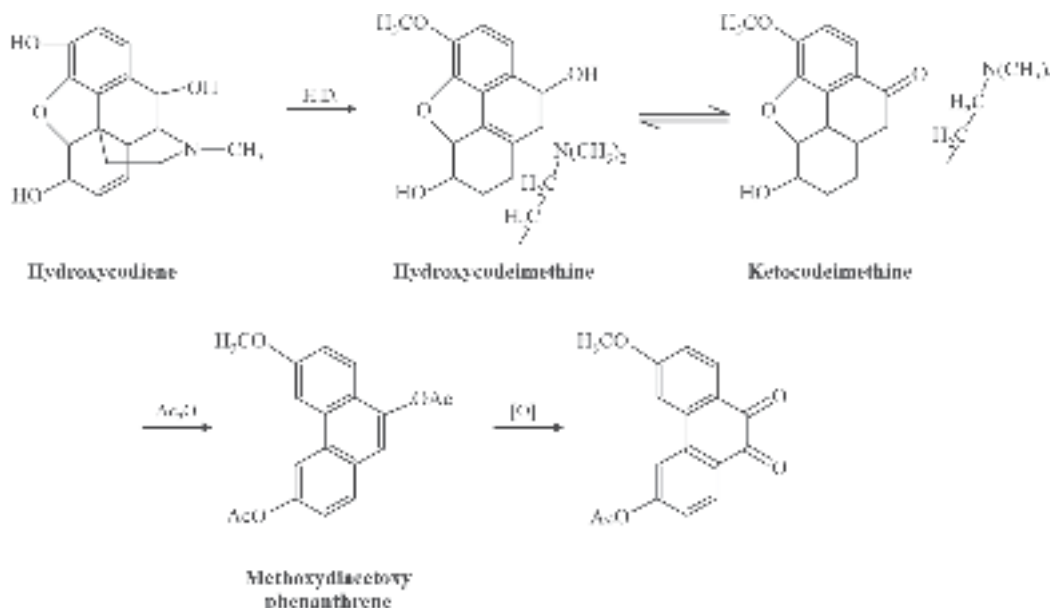


So, now the only problem is to assign the positions of the double bond and the chain $-\text{CH}_2\text{CH}_2\text{NMe}$ in such a manner as to explain all reactions of morphine.

- (xi) **Point of attachment of $\text{CH}_2\text{CH}_2\text{NMe}$ chain:** Codeine on oxidation with chromic acid gives some hydroxycodine along with codeinone. The hydroxycodine on Hoffmann degradation gives a ketocodimethine which on heating with Ac_2O

affords a methoxydiacetoxyphenanthrene. The later on further oxidation gives a quinone with the loss of an acetoxy group.

Codeine $\longrightarrow$ hydroxycodine



As we have observed that the side chain having nitrogen atom is always eliminated with the aromatization of nucleus. **Gulland** and **Robinson** in 1923 stated that "the formation of phenanthrene derivative can also take place for structural reasons unless the ethamine chain is displaced." And now the nitrogen end of the side chain had been previously be shown to be attached at C-9 the carbon end of side chain must be located at an angular position, so that its extrusion from that position becomes essential for aromatization. Out of two such possible positions viz. C-13 and C-14 the former is selected on the basis that such structure explains the rearrangement of thebaine to thebenine and thus the partial structure for morphine may be written as shown in the reaction.

- (xii) **Position of double bond:** Codeine on treatment with PCl_5 yields α -chlorocodide which on further treatment with aqueous acetic acid solution affords a mixture of codeine, isocodeine, pseudocodine and allospseudocodine. The first two gives the same ketone on oxidation indicating that they differ only in the position of the hydroxyl group at C-6 position. While the latter two give same ketone on oxidation again suggesting that these two differ in position of $-OH$ group which is at C-8. These changes can be explained if the double bond is present between C-7 and C-8.
- (xiii) Finally, the structure is confirmed by spectroscopical data and synthesis.

Synthesis

Among various methods, Gates synthesis is one of the pioneer method for morphine synthesis discovered in 1953 and the Parker synthesis is one of the latest methods discovered in 1996. However, one of the highest yields of morphine would be through the Kenner C Rice synthesis with a good 12% yield.

Kenner C Rice Synthesis

The carboxylation of isovanillin (1) gives 3-hydroxy-4-methoxybenzoic acid, (2) which reacts with 3-methoxyphenyl ethylamine to produce compound, (3) (Fig. 3.9). Reduction and formylation occurs and after ketalization and regioselective bromination, compound (5) formed. The bromine acts as a blocking group to prevent para-coupling as cyclization occurs forming compound (6). The compound then has the bromine cleaved and methylation of the amine occurs forming dihydrocodeinone (7). From this, through demethylation and hydrogenation, morphine is finally formed.

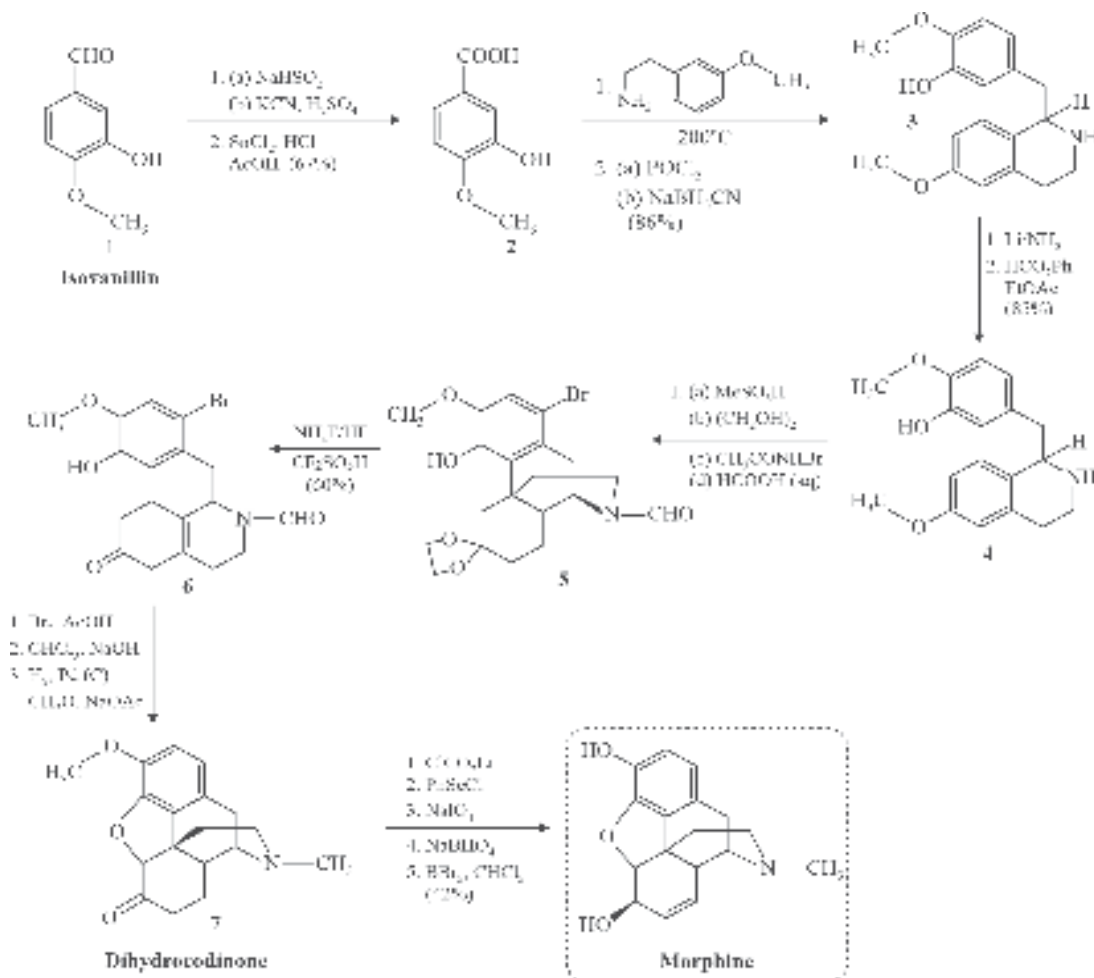


Fig. 3.9: Synthesis of morphine

Spectral Data

UV (Ethanol): λ_{max} 286, 250 and 298 nm (log ϵ 3.256, 3.275, 3.360 respectively).

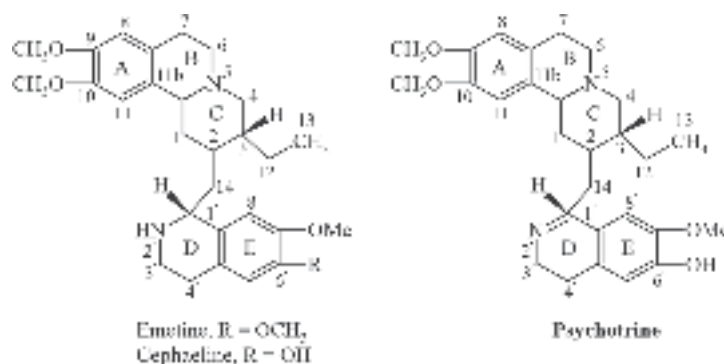
IR (KBr) (cm^{-1}): 3480, 3350 (free OH), 3210 (OH bonded), 2940, 2920 (CH stretch), 2840 (N-CH₃ stretch), 1640 (C = C alkene), 1605 (C = C, Ar), 1250, 1090 (C₄-O-C₅ stretch), 760 (monosubs Ar).

$^1\text{H-NMR}$ (HCl salt in D_2O , δ): 6.76 (d, H-1), 6.68 (d, H-2), 5.75 (d, H-7), 5.40 (d, H-8), 5.06 (d, H-5), 4.37 (m, H-9), 4.20 (m, H-14), 4.18 (1H, H-6), 3.0 (s, N- CH_3), 2.9 (2H, H-10), 2.51 (2H, H-16), 1.88 (2H, H-15).

MS (m/e): 286($\text{M}^+ + 1$), 268, 229, 201, 181, 165, 128, 91.

C. Ipecacuanha Alkaloids

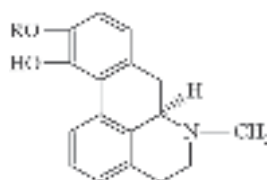
Ipecacuanha alkaloids are obtained from *Cephaelis ipecacuanha* (family—Rubiaceae) consist of mixture of alkaloids derived from tyrosine, via a dopamine intermediate, that have powerful emetic properties. Emetine, the best known of this group of alkaloids, is used as a treatment for amoebic dysentery, but has many side effects. The root and rhizomes of ipecac contain a number of closely related isoquinoline alkaloids in a total concentration of up to 2.5% by weight of the root, primarily emetine, cephaeline, and psychotrine. Cephaeline is demethylated derivative of emetine (Fig. 3.10). Emetine may be manufactured commercially by the chemical modification of either cephaeline or psychotrine.



Emetine also has been used to treat schistosomiasis, leishmaniasis and malaria. Its side effects are cardiotoxicity, muscle weakness and gastrointestinal problems. The alkaloid has a profound reversible effect on DNA synthesis.

7. APORPHINE (REDUCED ISOQUINOLINE/NAPHTHALENE) ALKALOIDS

Aporphine alkaloids are composed of an aporphine nucleus, which is 4H-dibenzo [de, g] quinoline or 3-methyl derivative. Apomorphine and apocodeine are the example of architectural aporphine type alkaloids.



Apomorphine, R = H
Apocodeine, R = CH_3

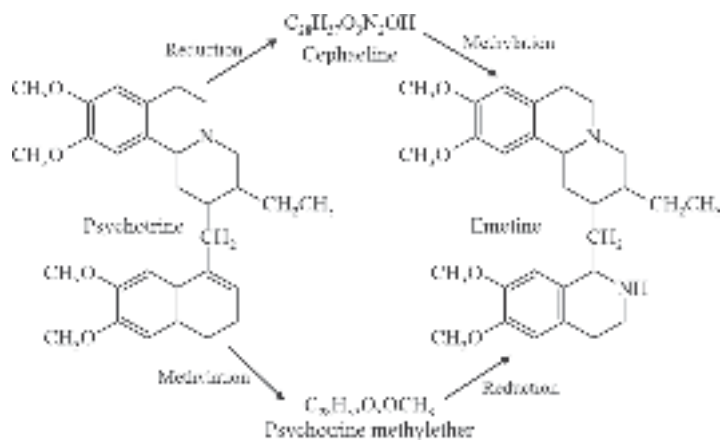


Fig. 3.10: Relationship between the principal alkaloids of Ipecacuanha

8. QUINOLIZIDINE ALKALOIDS

Quinolizidine alkaloids are found in *Lupinus* and other genera of the family—Fabaceae. These are responsible for neurological effects in mammals. These can be divided in more than 6 structural groups (Fig. 3.11).

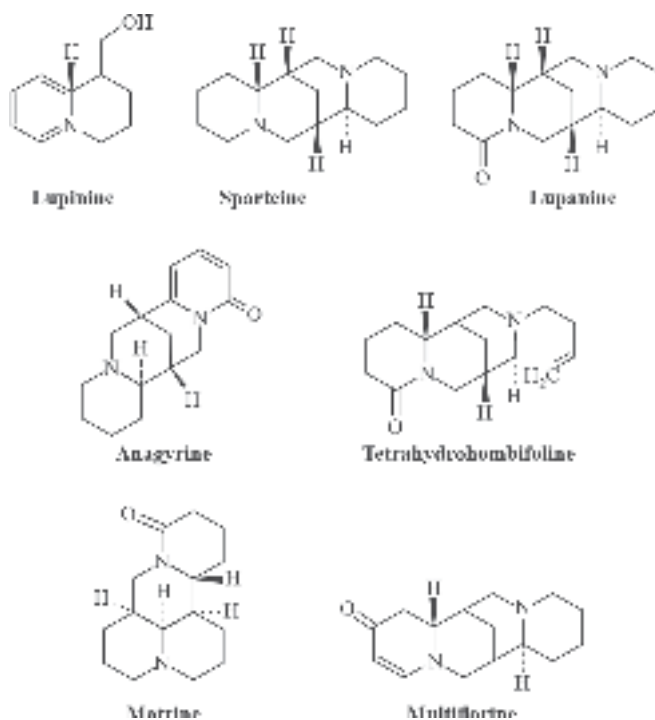


Fig. 3.11: Structural types of Quinolizidine alkaloids

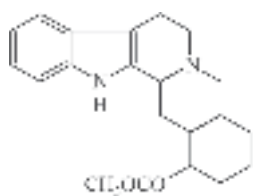
- Lupinine and its ester.
- Tetracyclic quinolizidine alkaloids, such as sparteine and lupanine which can be characterized by additional keto group and upto two hydroxyl groups.
- The α -pyridones such as anagrine.

- D. The tricyclic quinolizidine alkaloids, such as tetrahydrohombifoline.
- E. Marine alkaloids like multiflorine
- F. Other types, e.g. matrine.

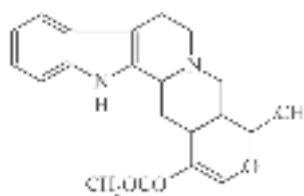
9. INDOLE OR BENZOPYRROLE BASED ALKALOIDS

1. Reserpine

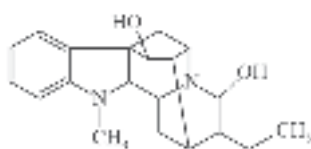
Reserpine is the main constituent of *Rauwolfia species*, particularly *R. serpentina* and *R. vomitoria*. Other important alkaloids presents in these species are yohimbine, ajmalicine and ajmaline. All these alkaloids are antihypertensive and sedative agents.



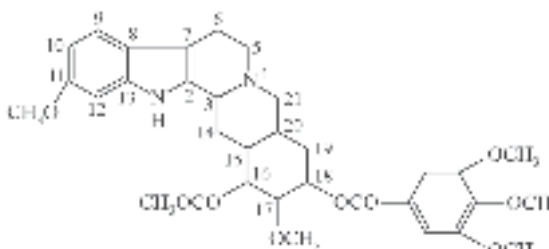
Yohimbine



Ajmalicine



Ajmaline



Reserpine

Reserpine is mainly used for the treatment of hypertension, headache, tension states, asthma and dermatological disorders.

Properties: Reserpine, m.p. 262°C, exists at room temperature as a white or pale-buff to yellow odorless powder. It is practically insoluble in water; freely soluble in chloroform, methylene chloride, and glacial acetic acid; soluble in benzene and ethyl acetate; and slightly soluble in methanol, ethanol, acetone, ether, and weak solutions of acetic and citric acids. It is stable under normal storage conditions but when subjected to oxidation and hydrolysis, reserpine acquires a yellow color with pronounced fluorescence, especially after the addition of acid or exposure to light. When heated to decomposition, it emits toxic fumes of nitrogen oxides. In solution, it unstable in the presence of alkali.

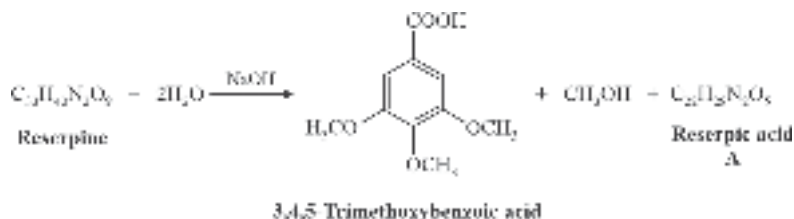
Isolation of Reserpine

- (i) Mature root bark is extracted with methanol in a Soxhlet apparatus for 30 hours.
- (ii) After concentrating the methanolic extract, the residue is treated with NaHCO_3 solution and then with ether. Dissolve the resinous material if separated out in little methanol and retreated in the same way, this process being repeated thrice or until more resin formation is closed.
- (iii) All the aqueous solutions are combined, extracted four times with ether and the combined ether extracts washed with 2% acetic acid (3~100 c.c.) then 2% HCl solution.

- (iv) The combined acid extracts are then basified with NH_3 solution to get a resin which would not dissolve in ether.
- (v) A solution of this material in a little methanol gradually deposited a crystalline precipitate which after several recrystallizations from methanol yields reserpine. A further quantity can be obtained on longer standing.

Structural Elucidation of Reserpine

- (i) **Molecular formula:** It is found to be $\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_9$.
- (ii) **Presence of methoxy groups:** Reserpine, when heated with hydrogen iodide at a temperature of 126°C , yields 5 moles of methyl iodide (Ziesel's method) indicating the presence of 5 methoxyl groups in reserpine.
- (iii) **Nature of the nitrogen atom:** As reserpine is a weak base, it indicates that both the nitrogen atoms should be present in its ring systems. Further, reserpine does not have any hydroxyl group but it forms a monoacetyl derivative, indicating that one of the nitrogen atoms is present as a secondary amino ($>\text{NH}$) group. This has been further confirmed by the I.R. spectral studies which reveal the presence of an iodole nucleus. Reserpine also forms a methiodide with methyl iodide, indicating that the second nitrogen atom must be in tertiary state.
- (iv) **Hydrolysis of reserpine:** When reserpine is subjected to hydrolysis with alkali solution, it yields a mixture of methyl alcohol, 3,4,5-trimethoxybenzoic acid and another acid, **A** corresponding to the composition $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_5$.



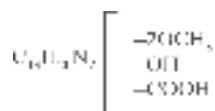
As reserpine does not contain $-\text{COOH}$ or $-\text{OH}$ groups, the introduction of the two carboxyl acidic groups and two alcoholic groups in its hydrolysis products reveal that reserpine is a diester compound. The ester linkage in reserpine has been further confirmed by its reduction with LiAlH_4 to reserpil alcohol, $\text{C}_{22}\text{H}_{30}\text{N}_2\text{O}_4$ and 3,4,5-trimethoxy benzyl alcohol.

To know the reserpine structure, it is must to know structure of reserpil acid.

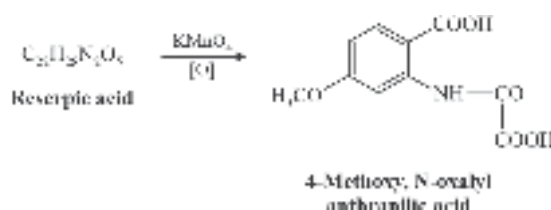
Structural Elucidation of Reserpil Acid

- (i) Molecular formula is found to be $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_5$.
- (ii) **Presence of carboxyl group:** By usual tests, reserpil acid is shown to possess one carboxyl group.
- (iii) **Presence of one $-\text{OH}$ group:** The usual tests reveal that reserpil acid contains one $-\text{OH}$ group. Further, it is shown to be a secondary alcoholic group because reserpil acid on oxidation yields a ketone derivative.
- (iv) **Presence of methoxy groups:** The Ziesel's method concludes that reserpil acid contains two methoxy groups.
- (v) **Nature of two N-atoms:** It is shown that in reserpil add two nitrogen atoms are present in heterocyclic rings, one in the form of a secondary amino group ($>\text{NH}$) while the other in the form of tertiary amino groups.

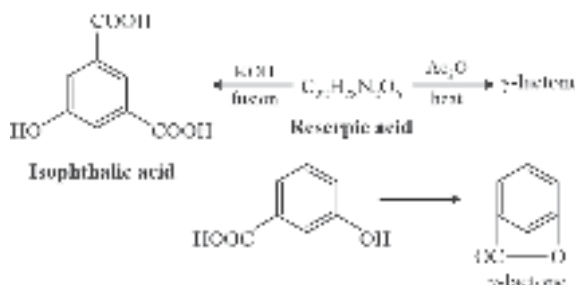
On the basis of the above facts, (a) to (e) reserpic acid may be written as:



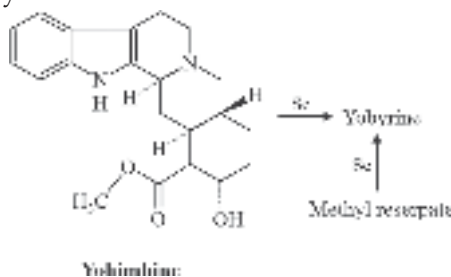
- (vi) **Reduction of reserpic acid:** The acid when reacted with $LiAlH_4$ yields reserpic alcohol which has two $-OCH_3$, one $-OH$, and one $-CH_2OH$ groups.
- (vii) **Oxidation of reserpic acid:** When reserpic acid is oxidized with potassium permanganate, it yields 4-methoxy N-oxalyl anthranilic acid as one of the oxidation products, thus confirming the presence of one indole nucleus in reserpic acid. Moreover, it also reveals that one of the methoxyl groups is present at '*m*' position to $>NH$ group of indole ring.



- (viii) **Fusion with KOH:** When reserpic acid is fused with potash, it yields 5-hydroxy-phthalic acid. Now, since one of the acidic groups of isophthalic acid must be carboxyl groups of reserpic acid itself, it means that the hydroxyl and carboxylic groups in reserpic acid must be present at '*m*' position to each other. This conclusion has been further confirmed by the fact that reserpic acid when heated with acetic anhydride yields a λ -lactone.

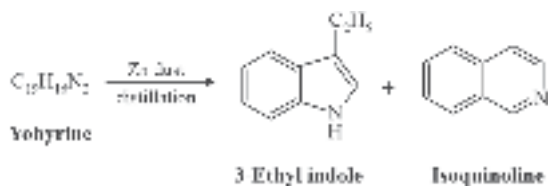


- (ix) **Dehydrogenation:** When methyl reserpate is dehydrogenated with selenium, it yields a hydrocarbon, $C_{19}H_{16}N_2$ as one of the principal product. In order to know the carbon framework of reserpic acid and hence of reserpine, it becomes essential to know the structure of this hydrocarbon. This hydrocarbon is also obtained by dehydrogenation of yohimbine with selenium and is therefore named as yobyryne.

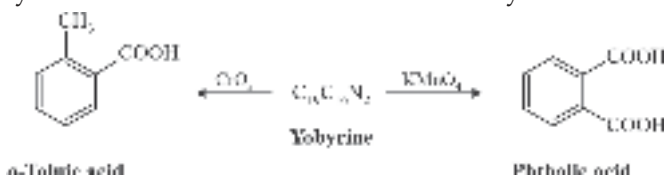


Structural Elucidation of Hydrocarbon, Yobyryne

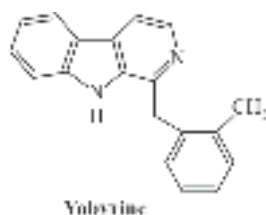
- (i) The yobyryne hydrocarbon on distillation with zinc dust, yields 3-ethyl indole and isoquinoline.



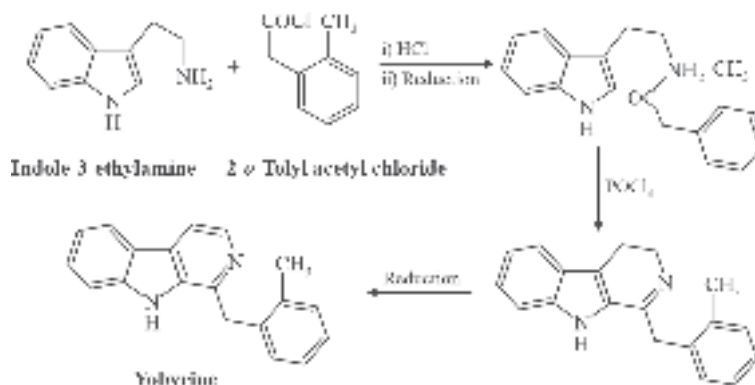
- (ii) On oxidation with permanganate solution, yobyryne yields phthalic acid. In contrast, yobyryne when oxidized with chromic acid yields *o*-toluic acid.



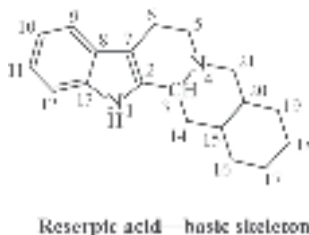
- (iii) Yobyryne gives condensation products with aldehydes, suggesting the presence of a pyridine ring with a $-\text{CH}_2-$ substituent adjacent to the nitrogen. On the above facts and its formation from yohimbine, the following structure has been postulated for yobyryne:



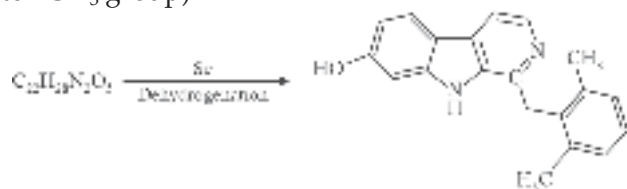
The proposed structure of yobyryne has been further confirmed by synthesis from indole-3-ethylamine.



- (iv) On the basis of yobyryne formation from reserpine acid, basic skeleton is assigned to reserpine.

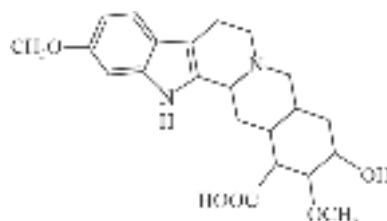


- (v) From the fact (point vii), it follows that one of the methoxyl groups is present in the *m*-position to the >NH group of indole, i.e. on C-11. Reserpine acid when dehydrogenated with selenium yields 11-hydroxy-16-methyl yohbyrine. This may be only formed if a -COOH group is present on C16 (during reaction, -COOH is converted into -CH_3 group).



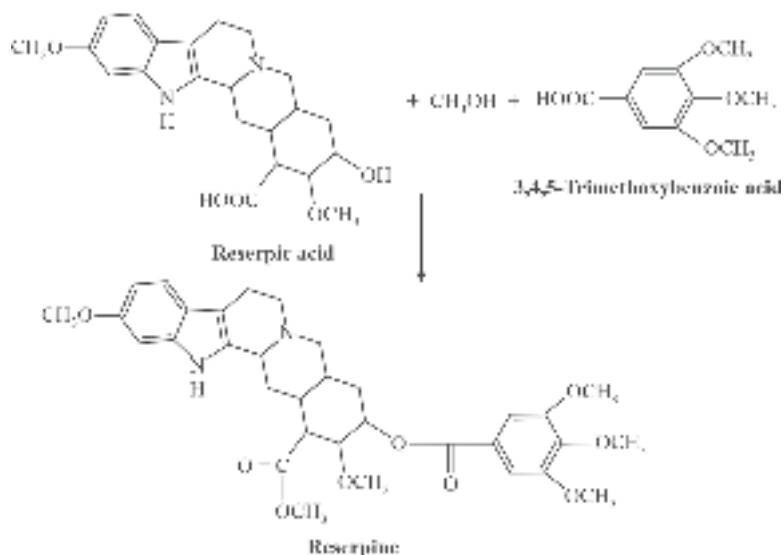
11-hydroxy-16-methyl yohbyrine

In step viii of reserpine acid, it is already inferred that -COOH group and -OH group are in *m*-position to each other so -OH group must be situated at C₁₈ (to form lactone). The C-17 position is assigned to the second methoxyl group on the basis of purely biogenetic reasons. On the basis of the above mentioned facts, the structure of reserpine acid may be written as:



Reserpine acid

- (x) **Structure of reserpine:** As reserpine is a diester of reserpine acid, it means that the structure of reserpine may be written as follows:



- (xi) **Synthesis of reserpine:** The above structure of reserpine has been confirmed by its synthesis, given by Woodward et al. (Fig. 3.12).

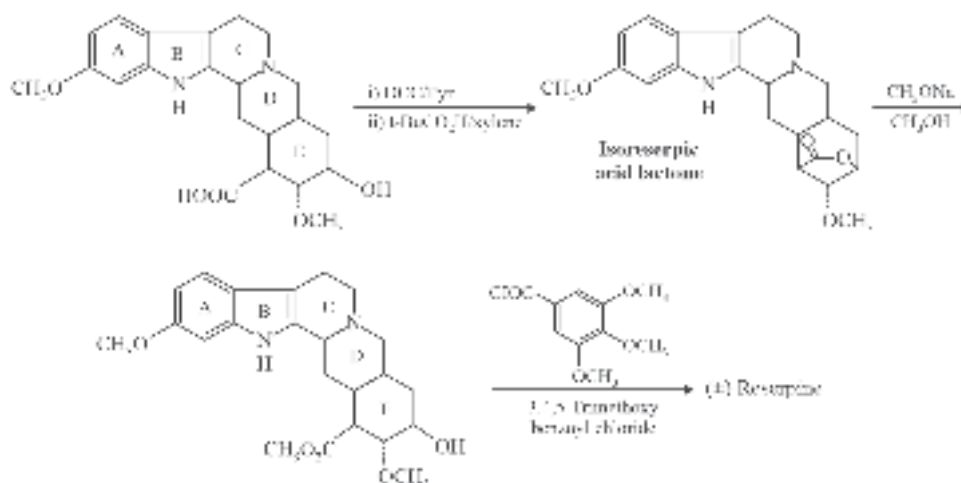


Fig. 3.12. Synthesis of reserpine

Spectral Data

UV: $\lambda_{\max}$ 296, 267, 216 nm (ϵ 9660, 15700 and 55700 respectively).

IR (cm^{-1}): 3480 (N–H stretching), 2840–3030 (C–H stretching), 1732 and 1713 (C = O stretching), 1265, 1240, 1220.

$^1\text{H-NMR}$ (CDCl_3) (δ): 7.70 (br, s, –NH), 7.33 (d, $J = 8$ Hz, H-9), 7.30 (s, benzoyl ring proton), 6.84 (s, H-12), 6.78 (d, $J = 8$ Hz, H-10), 4.48 (br s, H-3), 3.99 (9H, s, $\text{OCH}_3 \times 3$), 3.79 (3H, s, $\text{C}_{16}\text{–OCH}_3$), 3.46 (3H, s, $\text{C}_{17}\text{OCH}_3$), 3.29 (3H, s, $\text{C}_{11}\text{–OCH}_3$), 3.17 (m, H-5), 1.81 (ddd, H-14).

MS (m/e): 609 ($\text{M}^+ + \text{H}$), 579, 448, 409, 397, 301, 195, 174, 145.

2. Ergot Alkaloids

Ergot alkaloids are isolated from the dried sclerotium of the fungus *Claviceps purpurea* (Hypocreaceae). This fungus is a parasite on rye and wheat and other grains. Ingestion of bread contaminated with ergot, causes ergotism, also known as the **Devil's curse** or **St. Anthony's fire**, and has been a problem for centuries.

The ergot alkaloids are indole compounds that are biosynthetically derived from L-tryptophan and represent the largest group of nitrogenous fungal metabolites found in nature. Most of the natural ergot alkaloids possess the tetracyclic ergoline ring system as their characteristic structural feature (Fig. 3.13). In the majority of ergot alkaloid molecules, the ring system is methylated on nitrogen N-6 and substituted on C-8. Most of the ergot alkaloids have a double bond in position C-8, C-9 ($\Delta^{8,9}$ -ergolenes, C-5 and C-10 being the asymmetric centers) or in position C-9, C-10 ($\Delta^{9,10}$ -ergolenes, C-5 and C-8 being the asymmetric centers).

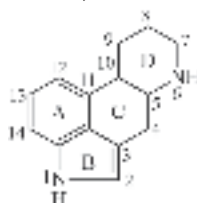


Fig. 3.13: Ergoline ring system

The classification of the ergot alkaloids are based on the type of substituent at C-8 and are divided into four groups:

- Clavine alkaloids
- Simple lysergic acid derivatives
- Ergopeptine alkaloids
- Ergopeptam alkaloids

Clavine Alkaloids

The clavines are hydroxy and dehydro derivatives of 6,8-dimethylergolenes and the corresponding ergolines. This group includes the chanoclavines with an open D-ring between N-6 and C-7. Figure 3.14 illustrates the structure of chanoclavine.

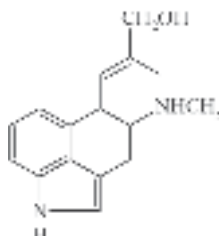


Fig. 3.14: Chanoclavine

The clavine type of alkaloids, such as agroclavine and elymoclavine, are generally regarded as precursors to the, other groups of ergot alkaloids in the biogenetic pathway. These alkaloids are among several of the ergot alkaloids; also isolated from higher plants, particularly the seeds of *Ipomoea violacea* and *Rivea corymbosa* ("ololiuqui," the Mexican morning glory), both members of the *Convolvulaceae* family. These alkaloids are not used pharmacologically, but agroclavine is a powerful uterine stimulant, and many of these ergot alkaloids are prolactin release inhibitors.

Simple Lysergic Acid Derivatives

The derivatives of lysergic acid are amides in which the amidic moiety is formed by a small peptide or an alkylamide. The derivatives of (+)-lysergic acid with 8 α -configuration are pharmacologically active. Nonpeptide amides of lysergic acids isolated from ergot fungi are ergometrine, lysergic acid amide, and paspalic acid (Fig. 3.15).

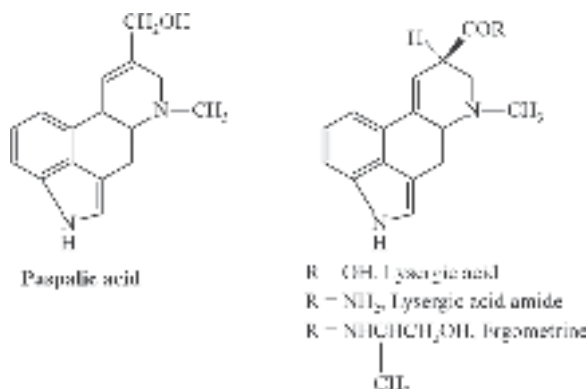


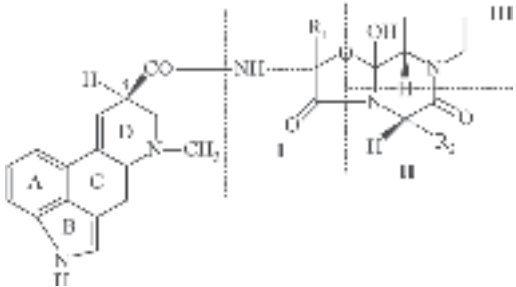
Fig. 3.15: Simple lysergic acid derivatives

Ergopeptine Alkaloids

The ergopeptines, also called cyclol ergot alkaloids (CEA) are composed of two parts, namely lysergic acid and a tripeptide moiety. Table 3.4 shows the general structure of the ergopeptines.

Their characteristic feature is the cyclol part which results from the reaction of α -hydroxy-amino acid adjacent to lysergic acid with a carboxyl group of proline. Amino acid, **III** of this tripeptide is 1-proline and is common to all the naturally occurring ergopeptines. Their molecular structures have been described by the exchangeability of the 1-amino acid, **I** and the 1-amino acid **II** between alanine, valine, phenylalanine, leucine, isoleucine, homoleucine, and α -aminobutyric acid. The groups of the ergopeptines formed by the combination of these amino acids are ergotamine, ergotoxine, ergoxine, and others (ergobalansine) (Table 3.4).

Table 3.4: Ergopeptine alkaloids

				
Group	R_1	Amino acid, <i>I</i>	R_2	Amino acid, <i>II</i>
Ergotamine group				
Ergotamine	CH ₃	1-alanine	CH ₂ Ph	1-phenylalanine
Ergovaline	CH ₃	1-alanine	Isopropyl	1-valine
Ergosine	CH ₃	1-alanine	Isobutyl	1-leucine
Ergotoxine group				
Ergocristine	Isopropyl	1-valine	CH ₂ Ph	1-phenylalanine
Ergocryptine	Isopropyl	1-valine	Isobutyl	1-leucine
Ergolabidine	Isopropyl	1-valine	2-CH ₃ - <i>n</i> -Butyl	1-homoleucine
Ergoxine group				
Ergostine	Ethyl	1-2-aminobutyric acid	CH ₂ Ph	1-phenylalanine
Ergonine	Ethyl	1-2-aminobutyric acid	CH ₂ Ph	1-phenylalanine
Ergoptine	Ethyl	1-2-aminobutyric acid	Isobutyl	1-phenylalanine
Other				
Ergobalansine	CH ₃	1-alanine	Isobutyl	1-leucine

Ergopeptam Alkaloids

Ergopeptams are noncyclol lactam, ergot alkaloids (LEA). Their structure is similar to ergopeptines except that the amino acid **III** is *d*-proline and the tripeptide chain is a noncyclol lactam (Fig. 3.16). The ergopeptams are further classified as ergotamams, ergoxams, ergotoxams, and ergo annams.

It is very important to note that all the ergot alkaloids yields either lysergic acid or isolysergic acid on the hydrolysis and most of the ergot alkaloids constitute isomeric pairs, like ergotamine and ergotamine; ergosine and ergosine.

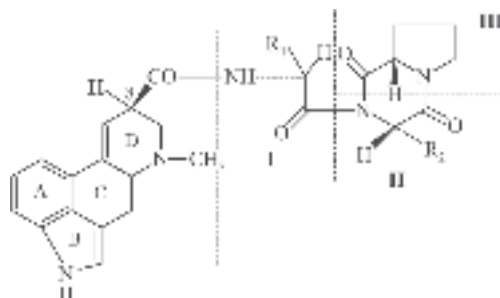
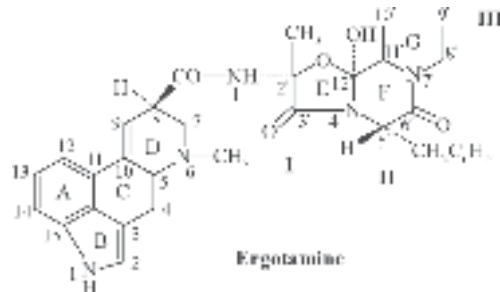


Fig. 3.16: General structure of ergopeptams

Each pair contains a levorotatory and physiologically active, and a strong dextro-rotatory and physiologically weak member. All the alkaloids obtained are interconvertible.

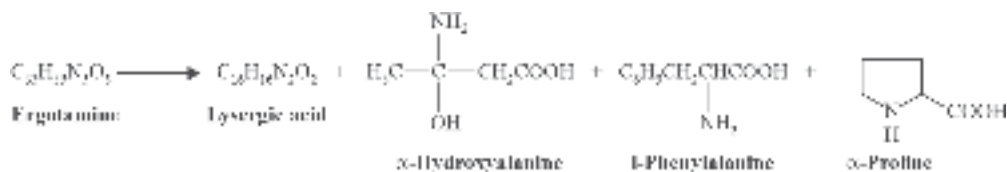
Ergotamine

It is the water insoluble lysergic acid derivatives, considered as peptide ergot alkaloids, first isolated in 1918, but its structure is not determined until 1951. Ergotamine occurs in ergot or *Secale cornutum* a product of the filamentous fungus, *Claviceps purpurea* which is grown parasitically on rye and other grasses and cereals, along with the several alkaloids. It is used as medicine due to its action on the uterus and on the vasomotor centre). It is an α -1 selective adrenergic agonist vasoconstrictor and so ergotamine, as its tartrate salt, is an analgesic specifically used for treatment of severe migraine headaches.



Structural elucidation of ergotamine

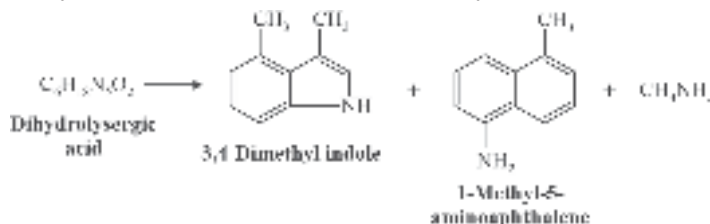
- (i) **Molecular formula:** It is found to be $C_{33}H_{35}N_5O_5$.
- (ii) **Degradation studies:** Ergotamine on alkaline hydrolysis gives *d*-lysergic acid as one of the principal products along with some other products (non-lysergic acid moiety). In case of ergotamine, other products are α -hydroxyalanine, *d*-proline and *I*-phenylalanine.



Hence, the complete structure of ergotamine can be studied under two heads, viz. structure of lysergic acid and structure of non-lysergic acid moiety.

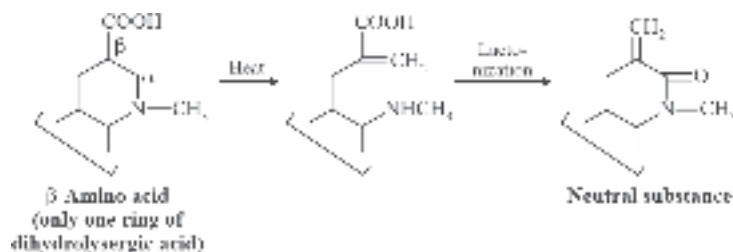
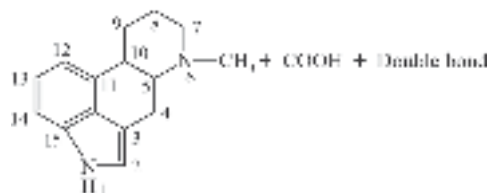
A. Structure of Lysergic Acid ($C_{16}H_{16}N_2O_2$)

- (i) By usual reactions, lysergic acid is found to contain a carboxylic group, a non-basic amino group, an N-CH₃ group and a double bond.
- (ii) 'On fusion with potassium hydroxide, dihydrolysergic acid yields 3,4-dimethyl-indole, 1-methyl-5-aminonaphthalene and methylamine.

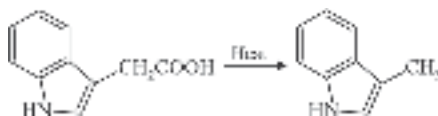


From this reaction, following conclusions can be drawn:

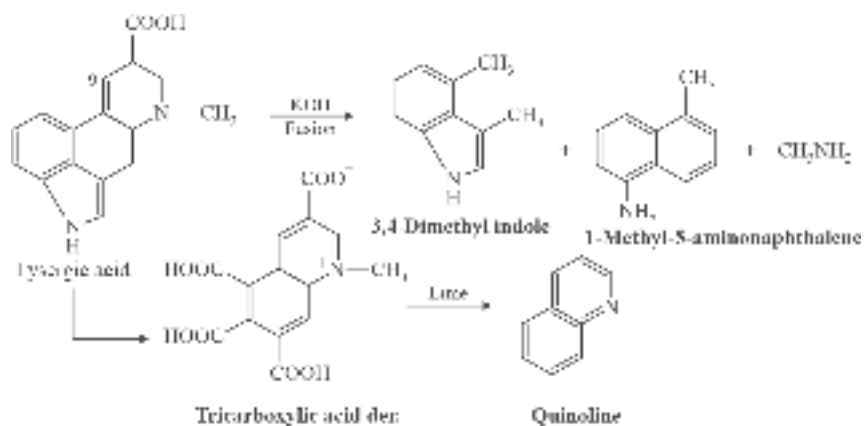
- (a) Presence of indole and naphthalene moieties.
- (b) Formation of CH₃NH₂ and 3,4-dimethyl indole suggests that 'N' atom of 1-methyl-5-aminonaphthalene is not its own but derived from indole nucleus of lysergic acid. This is predicted from the fact that lysergic acid contains two nitrogen atoms, one as -N-methyl (eliminated in the form of methyl amine) and other as -NH group (gives the formation of 3,4-dimethyl indole).
- (iii) Lysergic acid on oxidation yields tricarboxylic acid which on distillation yields quinoline, suggesting that lysergic acid possesses a quinoline nucleus, probably reduced.
- (iv) The above two reactions (ii and iii) establishes the presence of three types of ring structure in lysergic acid (by the formation of 3,4 dimethyl indole, 1-methyl-5-aminonaphthalene, quinoline derivative). This indicates that lysergic acid has tetracyclic of the following type in order to explain the formation of the following type in order to explain the formation of these cyclic products.
- (v) The comparison of UV spectra of lysergic and isolysergic acids with that of dihydrolysergic acid indicates that the additional double bond of lysergic acid is present in conjugation with the indole system, i.e. either between C-9 and C-10 position or between C-5 and C-10 position.
- (vi) The carboxylic group in lysergic acid is found to be present at C-8 position As it behaves like a β -amino acid as dihydrolysergic acid forms a neutral substance (shown below) on heating having one mole of water less than the dihydrolysergic acid itself. The neutral compound has easily reducible double bond and responds to keller reaction (gives blue color with conc. sulphuric acid and glacial acetic acid having traces of iron chloride).



In contrast, if lysergic acid is heated, the additional double bond which is formed in neutral substance, is found in conjugation with already present double bond and hence, in lysergic acid the double bond must be between C₉ and C₁₀.

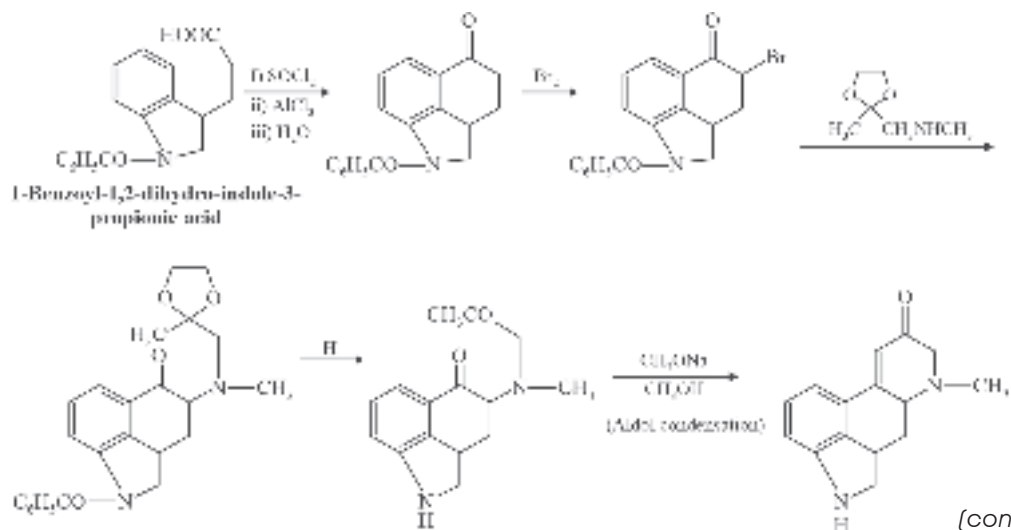


The little possibility of carboxylic group to be present at C-4 position, so as to give β -amino acid is discarded by the fact if it is present at C-4 position, dihydrolysergic acid would contain an indolyl acetic acid fragment which is readily decarboxylated, while the dihydrolysergic acid has high thermal stability. Thus, all the above mentioned reactions may be explained by assigning the below given structure to lysergic acid.

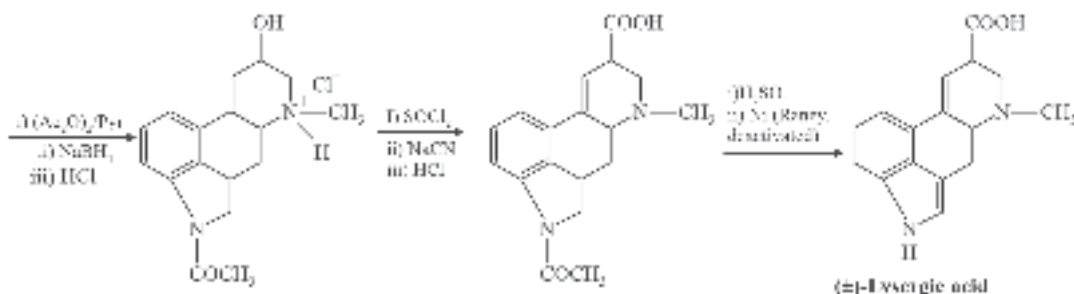


It should be noted from the formula of carboxylic acid that although it is tetracarboxylic acid, it is known as tricarboxylic acid because the 4th acidic group is marked by the positively charged quaternary nitrogen atom.

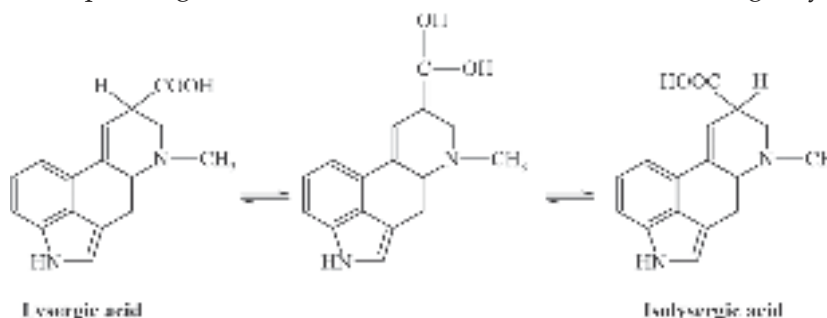
(viii) Finally, the above structure of lysergic acid is confirmed by its synthesis from 1-Benzoyl-1,2-dihydro-indole-3-propionic acid.



(contd.)



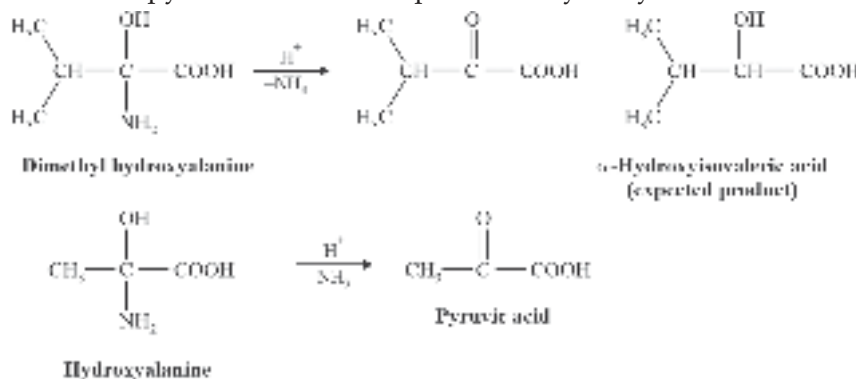
Isolysergic acid is diastereomer of lysergic acid at C-8. The two acids and of course their corresponding alkaloids are interconvertible in the following way:



B. Structure of Non-lysergic Acid Moiety

- (i) On hydrolysis, ergotamine produces lysergic acid, d-proline, phenylalanine and pyruvic acid. The formation of these amino acids indicates that the alkaloid contains peptide bonds, which on hydrolysis affords these amino acids.
- (ii) d-proline and phenylalanine are present as such in the alkaloid. but the α -keto acid, like pyruvic acid is not present as such since no free keto group is detected in the alkaloid. Moreover, the fact that keto acids are not present as such in ergot alkaloids, comes from the following observation:

Dihydroergocristine on hydrolysis yields dimethylpyruvic acid instead of the expected product α -hydroxyisovaleric acid, indicating that dimethyl pyruvic acid is actually present as α -hydroxy α -aminoisovaleric acids which decomposes to corresponding keto acid, and an equivalent ammonia upon hydrolysis. Hence, in ergotamine the pyruvic acid must be present as hydroxyalanine.

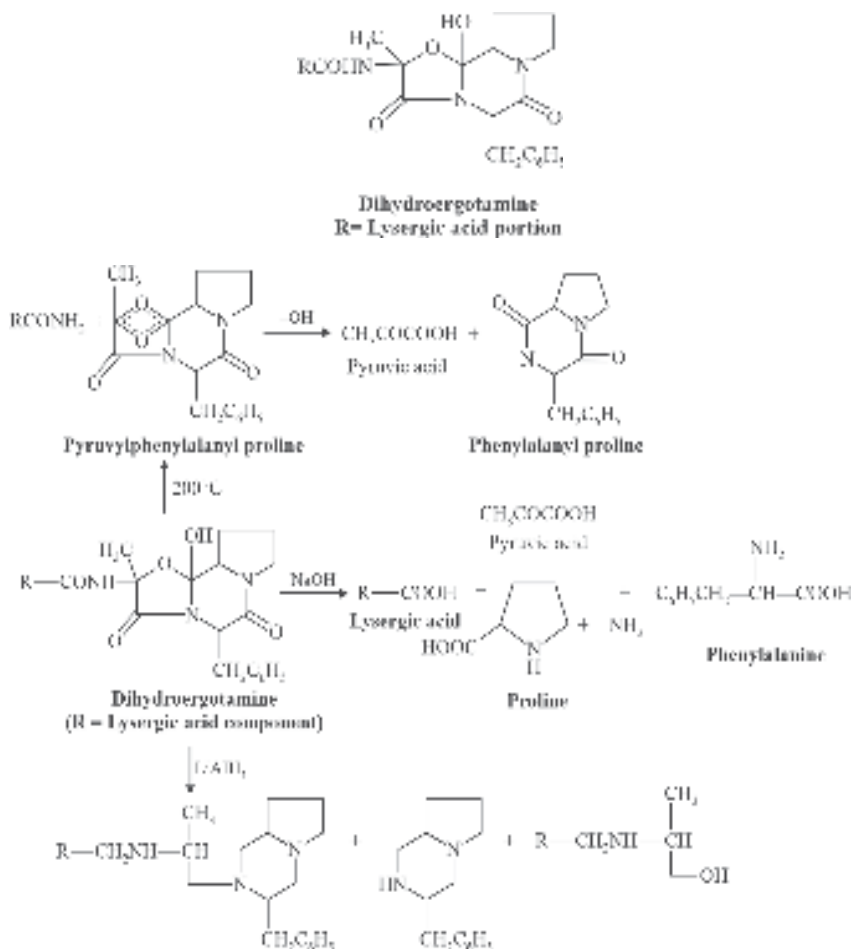


- (iii) As the non-lysergic acid moiety neither contains a free carboxyl group nor a basic amino group, it is predicted that the various amino acids are present in the form of a cyclic structure.

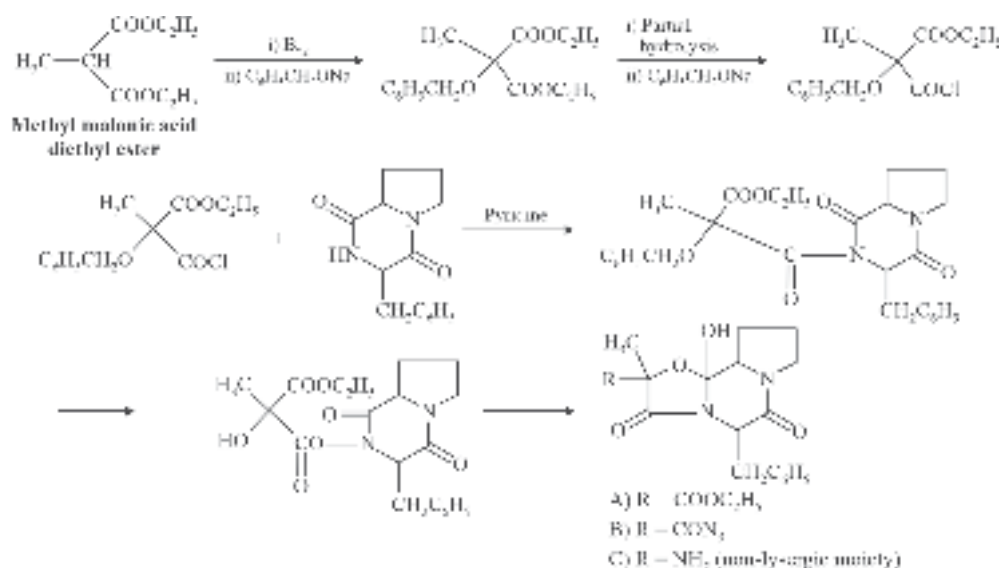
- Note:** The keto acid component on reduction with hydrazine gives fatty acid.

- The products of the above two reactions indicate the following sequence of amino acids in the nonlysergic acid moiety.

(vii) The above two reactions also produces a considerable quantity of diketopiperazine. This suggests that in the alkaloid, phenylalanine and proline units are linked with each other in a way to form a six membered ring along with five membered ring. Thus, the peptide or non-lysergic acid portion of the ergotamine may be written as below. The assigned structure can explain the formation of various products resulting from hydrolysis.

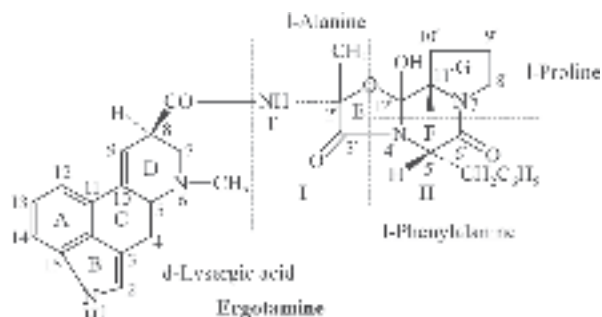


- (viii) The above structure is confirmed by synthesis, starting from methyl malonic acid. In the last step, controlled hydrolysis of compound, A ($R = \text{COOC}_2\text{H}_5$), followed by conversion to acid chloride and treatment with NaN_3 gives the acid azide, B ($R = \text{CON}_3$). The Curtius rearrangement of acid azide B by heating followed by hydrogenation yields the non-lysergic moiety, C.



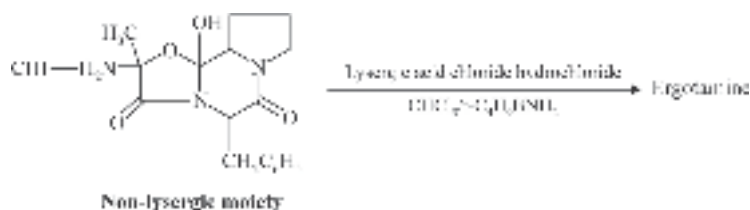
Linkage of Lysergic Acid with Non-lysergic Moiety

The lysergic acid is linked via carboxylic group to amino group present in non-lysergic moiety to give the structure of ergotamine.



Synthesis of Ergotamine

Finally, the above proposed structure of ergotamine is confirmed by its synthesis.



Spectral Data

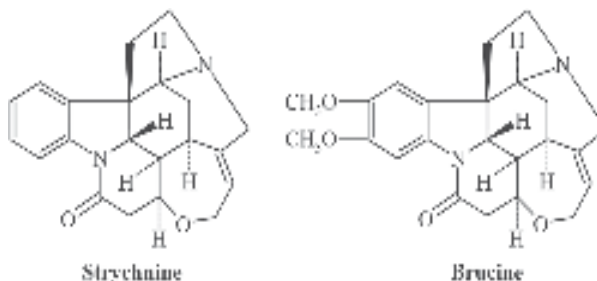
UV: $\lambda_{\max}$ 311 (ϵ 8260).

IR (KBr) (cm^{-1}): 3310, 3260, and 3130 ($>\text{NH}$ and OH), 1615 (amide-CO) and 1598 (aromatic vibration).

$^1\text{H-NMR}$ (DMSO-d_6) (δ): 10.98 (1H, s, indole NH), 9.25 (1H, d, CONH), 7.6 (4H, m, H-12, H-13, H-14, H-2), 7.2–7.4 (5H, m, Ar-H), 6.57 (m, H-9), 4.3 (1H, m, α -H-8), 4.0 (1H, s, OH), 3.10 (3H, s, N-CH₃), 2.2–2.6 (8H, m, $(\text{CH}_2)_4$), 1.12 (3H, d, C-2', CH₃).

3. Nux Vomica Alkaloids

The seeds of *Nux vomica* have commercial value. They contain 2.5–5% alkaloids among which the prominent ones are strychnine (often up to 30–50% of the total alkaloids) and brucine (dimethoxy strychnine). Strychnine and brucine contain quinolirine skeleton in addition to indole nucleus. The minor alkaloids accompanying are α - and β -colubrines, vomicine, N-oxystrychnine and psuedostrychnine.



Isolation of Strychnine and Brucine

- (i) The *nux vomica* seed powder is placed in a flask containing mixture of chloroform, ether, and 10% ammonia water (30:50:5).
- (ii) The mixture is frequently shaken for 45 minutes, and then filtered through cotton.
- (iii) The filtrate is transferred to a separatory funnel containing dilute H_2SO_4 and shaken well.
- (iv) The lower aqueous layer, which should have an acid reaction when tested with litmus paper is transferred into a second separatory funnel and washed with ether-chloroform mixture.
- (v) The acid extracts containing the alkaloids from the original drug is made alkaline with dilute ammonia water to liberate alkaloidal base.
- (vi) The aqueous layer is extracted by shaking with several portions of chloroform.
- (vii) The chloroform extract is washed with a little water, dried over anhydrous calcium chloride and evaporated off the solvent-chloroform in an evaporating dish.
- (viii) A little ethyl alcohol is added and then, at once evaporated off to get the residue, consisting of strychnine and brucine.

Note: The process for separating strychnine from brucine depends on the greater readiness by which brucine is nitrated with HNO_3 .

(Note: *The separation of brucine from strychnine is most conveniently effected by exposing the mixed alkaloids to the action of diluted nitric acid, which destroys brucine very rapidly while having no appreciable action on strychnine).*

- (ix) The alkaloidal mixture is dissolved in a dilute H_2SO_4 and the added concentrated HNO_3 .

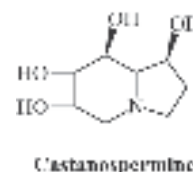
The addition of HNO_3 should cause the solution to attain a crimson color. After standing for exactly 15 minutes, the contents are transferred to a separating funnel and at once made alkaline with NaOH solution.

- (x) The strychnine is extracted with chloroform in a usual way. The chloroform is evaporated off to get strychnine.

Note: *Brucine may be separated from strychnine by virtue of the lesser solubility of its oxalate in dehydrated alcohol or of its hydriodide in water, or by the insolubility of strychnine chromate in water.*

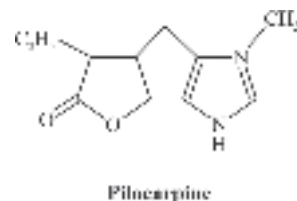
10. INDOLIZIDINE-BASED ALKALOIDS

Only a small number of indolizidine alkaloids are currently known, but they have recently become of pharmaceutical interest through the discovery of the tetrahydroxy alkaloids castanospermine and 6-epicastanospermine, which are possible lead compounds in the search for anti-AIDS drugs. Also, like the above, swainsonine, the toxic constituent of locoweeds and Australian *Swainsona* spp., is a powerful glycosidase inhibitor; this alkaloid is a trihydroxyindolizidine. Both alkaloids are biosynthesized from lysine *via* pipecolic acid.



11. IMIDAZOLE-BASED ALKALOIDS

The most important pharmaceutical examples of this group are the *Pilocarpus* alkaloids, isolated from *Pilocarpus microphyllus*. Pilocarpine is widely used as a cholinergic drug in ophthalmology. Possible biosynthetic routes to pilocarpine could involve either of the amino acids histidine or threonine.



12. PURINE-BASED ALKALOIDS

The purine derivatives, caffeine, theobromine and theophylline are usually referred to as purine alkaloids (refer to Chapter 9 for details).

13. STEROID-BASED ALKALOIDS

Alkaloids based on the steroid nucleus are not very widely distributed, being restricted to plants of the *Holarrhena*, *Solanum* and *Veratrum* species. Among the bases, there is relatively little variation in structural type, the following sub-groups (Fig. 3.17) may be distinguished.

- Derivatives of Δ^5 -pregnene, e.g. conessine and holarrhimine present mainly as free bases or their salts in plants of the *Holarrhena* species.
- Derivatives of solanidine solanine and the closely related bases [e.g. tomatidine], occurs as glycosides in *Solanum*, *Lycopersicum* and *Veratrum* species.
- Derivatives of the veratramine and jervine.

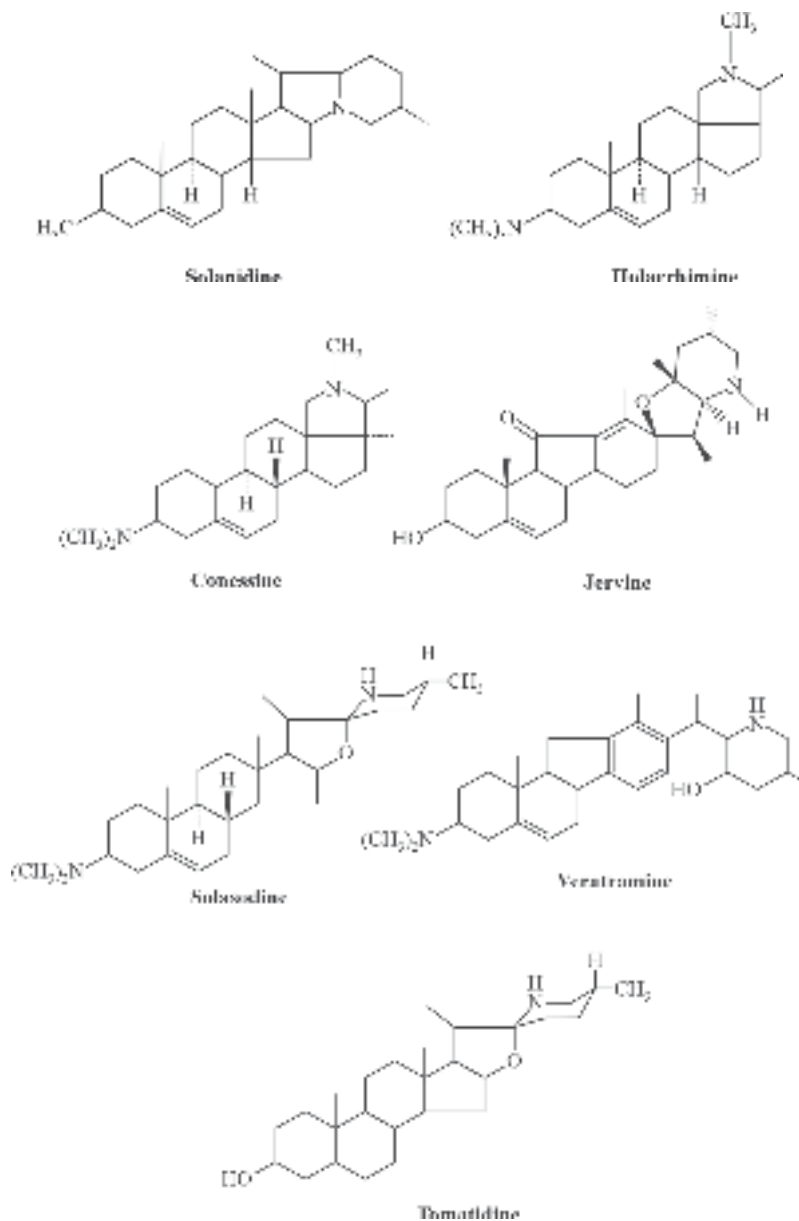


Fig. 3.17: Steroid-based alkaloids

(i) Derivative of Δ^5 -Pregnane

This group of alkaloid probably arises from pregnenolone by amination at either C-3 or C-20 position. Conessine is a common alkamine of the group and used as starting material for the synthesis of some hormones (e.g. aldosterone).

(ii) Derivatives of Solanidine and the Closely Related Bases

The alkaloids of this sub-group occur in many *Solanum* species, including *S. nigrum*, *S. tuberosum* (potato) and *S. lycopersicum* (tomato), and are responsible for the immunity to attack by insects of certain plants of these species. In the plants, the alkaloids present

generally as glycosides of a wide variety of sugars and a relatively small group of steroidal bases; a few of these simpler bases have been isolated directly from the plants but there is no real certainty that they occur in this way naturally. Hydrolysis of the glycosides readily gives the free base.

The base, **Solanidine** is present in the alkaloids like α , β and γ -solanine, α , β and γ -chaconine and solacauline. It has the molecular formula, $C_{27}H_{43}ON$ and contains an alcoholic hydroxyl group, a reducible double bond (and must thus be hexacyclic), a tertiary nitrogen that does not bear a methyl group and four C-methyl groups.

Demissidine or dihydrosolanidine (solanidan-3 β -ol) base, the aglycone of the alkaloid demissine, is identical with dihydrosolanidine (solanidan-3 β -ol) and has a *trans* A/B ring fusion. **Solasodine** base is obtained by the hydrolysis of the primary alkaloids solasonine, solasodamine and solamargine. It is a secondary base containing one alcoholic hydroxyl group and has the composition $C_{27}H_{43}O_2N_7$, but has only one double bond and must thus be hexacyclic.

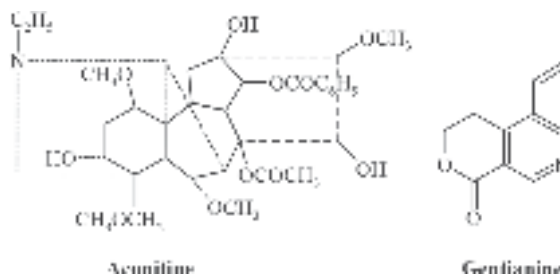
(iii) Derivatives of Veratramine and Jervine

Veratramine is a steroidal base obtained from the hydrolysis of veratrosine. It is the D-glucoside having the molecular formula of $C_{27}H_{29}O_2N$ and contains the following detectable groups; one secondary amino group, two secondary alcoholic hydroxyl groups, one reducible double bond, four C-methyl groups and an aromatic nucleus, and must therefore be pentacyclic.

Jervine base has been isolated directly from *Veratrum album*. It contains one secondary amino group, one secondary hydroxyl group, two double bonds one of which is present in α , β -unsaturated ketone system and inactive ether oxygen. Overall, it has hexacyclic structure. Jervine like veratramine can be oxidized to a base, jervone.

14. TERPENOID-BASED ALKALOIDS

Terpenoid based alkaloids are an assortment of amalgams or compounds that are obtained biosynthetically through different procedures that produce a number of group of terpenes like sesquiterpenoids, monoterpene, diterpenoids as well as steroids. Terpenoid-based alkaloids originate from the same prenyl units that biosynthesize terpene skeleton. However, during biosynthesis, a nitrogen atom is introduced in the form of β -aminoethanol, ethylamine or methylamine. Incidentally, this variety of alkaloids is found in an irregular manner in the plant kingdom. For instance, the monoterpene alkaloids like gentianine are usually found in the plant family Gentianaceae. Sesquiterpenoids, for instance, dendrobine are generally found in the Orchidaceae or cassinine in the Celastraceae. Diterpenoids like aconitine (*Aconitum napellus*) are found in the Ranunculaceae family.



SHORT AND LONG QUESTIONS

1. Write the general methods of isolation of alkaloids.
2. Give the methods and isolation and structure determination of alkaloids.
3. Elucidate the structure of papaverine in a systematic way.
4. Discuss the structural elucidation of atropine.
5. Write the structures of quinine, ephedrine, papaverine and nicotine.
6. Classify the alkaloids with suitable examples.
7. Explain the degradation reactions of alkaloids.
8. Classify the alkaloids. Write about the constitution of nicotine.
9. Explain the methods used for detection of OCH_3 groups and N-CH_3 groups in alkaloids.
10. Define the term 'alkaloid'. Give the general methods for the structure elucidation of alkaloids.
11. Give the synthesis of nicotine starting from ethyl nicotinate.
12. Enlist the ergot alkaloids. Give the suitable chemical evidences for the structure of ergotamine.
13. What are cinchona alkaloids? Discuss the structural elucidation of quinine.
14. Write a note on followings:
 - a. Indole alkaloids
 - b. Steroidal alkaloids
15. Write a note on followings:
 - a. Zerewitinoff active hydrogen determination
 - b. Khun-Roth oxidation method to determine the number of C-methyl groups
 - c. Hofmann exhaustive methylation method.
 - d. Herzig Mayer method and its significance
16. Classify the opium alkaloids. Give the chemical and synthetic evidence in favor of the structure of morphine.

MULTIPLE CHOICE QUESTIONS

1. Which alkaloid was first isolated from opium?
 - a. Morphine
 - b. Codeine
 - c. Papaverine
 - d. Thebaine
2. Which of the following is an example of pseudoalkaloid?
 - a. Morphine
 - b. Caffeine
 - c. Ephedrine
 - d. Reserpine
3. Which of the following is an example of protoalkaloid?
 - a. Morphine
 - b. Caffeine
 - c. Ephedrine
 - d. Reserpine

4. Which alkaloid is used for anti-cancer property?
 - a. Reserpine
 - b. Vincristine
 - c. Morphine
 - d. Emetine
5. Which alkaloid is used for anti-hypertensive property?
 - a. Reserpine
 - b. Vincristine
 - c. Morphine
 - d. Emetine
6. Alkaloid used in treatment of migraine is:
 - a. Atropine
 - b. Cocaine
 - c. Pilocarpine
 - d. Ergotamine
7. Which is true for alkaloids?
 - a. Basic in nature
 - b. Contain heterocyclic nitrogen ring
 - c. Both a and b
 - d. Pyridine derivatives

Answers

1. (a) 2. (b) 3. (c) 4. (b) 5. (a) 6. (d) 7. (c)

Chapter 4

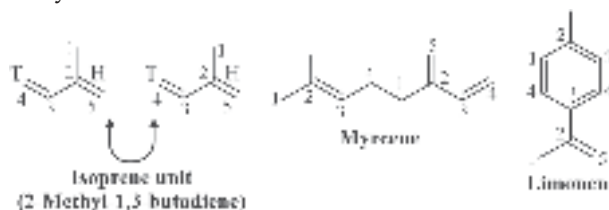
Chemistry of Terpenoids

The term *terpenes* originally was designated for a mixture of isomeric hydrocarbons of molecular formula, $C_{10}H_{16}$ occurring in turpentine oil and other essential oils. The term 'terpenes' was first coined by **Kekule** in 1866. At present, terpenes are considered as large and varied class of naturally occurring compounds, derived biosynthetically from isoprene (2-methyl-1,3-butadiene) units. These are commonly present in higher plants as constituents of essential oils, derived from one or more plant parts, such as flowers (e.g. rose, jasmine, clove, rosemary, lavender), leaves (e.g. mint, *Ocimum* spp., lemongrass), leaves and stems (e.g. geranium, cinnamon), bark (e.g. cinnamon), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, saussurea, valerian). seeds (e.g. fennel, coriander, caraway, dill, nutmeg), fruits (orange, lemon, juniper), rhizomes (e.g. ginger, curcuma) and gums or oleoresm exudations (e.g. balsam of Peru, balsam of Tolu, storax, myrrh, benzoin).

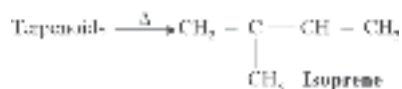
Some insects can also produce terpenes. These are also major components of resin. Most of the terpenes have cyclic structures and used as fragrance in perfumery, as constituent of flavors for spicing food, etc. Terpenes are technically only hydrocarbons, while terpenoids are oxygenated derivatives.

ISOPRENE RULE

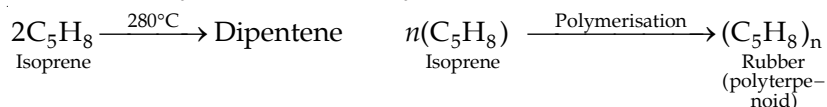
The isoprene rule, developed by **Ruzicka** in 1921, plays key role in structure determination. The isoprene unit is the fundamental building block of terpenes. These are represented by the general structural formula $(C_5H_8)_n$, where n is the number of linked isoprene units. These isoprene units are linked together via **H-H**, or **T-T** or **H-T** position but in most of naturally occurring terpenes, the isoprene units are linked head-to-tail or 1-4 link as shown in myrcene and limonene.



Hence, on thermal decomposition, almost all the terpenoids give isoprene as one of the products.



At the same time, isoprene can be converted into dipentene on heating at 280°C temperature or to be polymerized into polyterpenes (rubber).



CLASSIFICATION OF TERPENES

Terpenes classification is based on number of isoprene units linked together in a molecule; prefix represents the number of terpene unit present in a molecule. They are classified into mono-, sesqui-, di-, tri-, tetra-, poly-, etc. (Table 4.1) based on number of isoprene unit. Each class can be further subdivided into subclasses according to the number of rings present in the structure.

- (i) Acyclic terpenoids: They contain open structure.
- (ii) Monocyclic terpenoids: They contain one ring in the structure.
- (iii) Bicyclic terpenoids: They contain two rings in the structure.
- (iv) Tricyclic terpenoids: They contain three rings in the structure.
- (v) Tetracyclic terpenoids: They contain four rings in the structure.

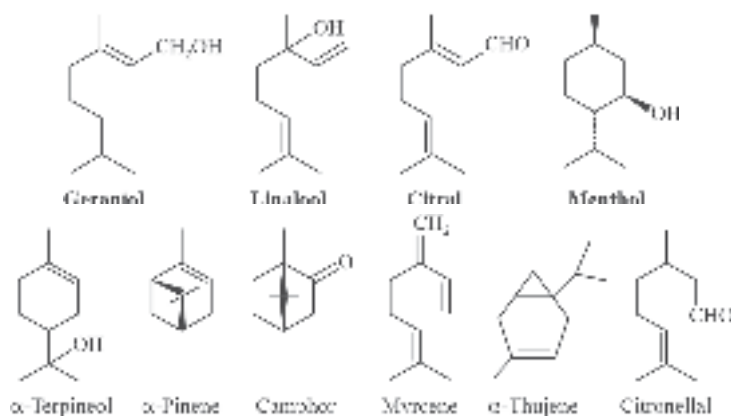
Table 4.1: Classification of terpenes

S. Terpenes No.	Isoprene units	No of carbon atoms	Examples
1. Monoterpenes	2	10	
<i>Acyclic</i>			Myrcene, citral, Geraniol
<i>Monocyclic</i>			Limonene, α -terpineol, Carvone, menthol, menthone
<i>Bicyclic</i>			
6 + 5 membered			Camphor
6 + 4 membered			α -Pinene
6 + 3 membered			α -Thujene
2. Sesquiterpenes	3	15	
<i>Acyclic</i>			Farnesol, Nerolidol, etc.
<i>Monocyclic</i>			Abscisic acid, Bisabolene, etc.
<i>Bicyclic</i>			Caryophyllene, Cadinene, etc.
3. Diterpenes	4	20	
<i>Acyclic</i>			Phytol
<i>Monocyclic</i>			Vitamin A
<i>Bicyclic</i>			Agathic acid
<i>Tricyclic</i>			Abietic acid
4. Sesterpenes	5	25	Heselenene, Sclerane
5. Triterpenes	6	30	Squalene, Lanosterol
6. Tetraterpenes	8	40	
a. Hydrocarbons			Carotenes and Lycopene
b. Xanthophylls			Zeaxanthin, Capsanthin, Violaxanthin, Fucoxanthin, Lutein
7. Polyterpenes	>100	>500	Rubber

A. Monoterpenes

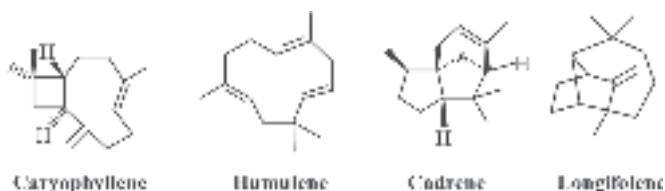
It consists of two isoprene units and has the molecular formula, $C_{10}H_{16}$. These are generally volatile natural products, obtained from higher plants. These are also known as essential oils and are widely used in perfumery and flavoring industries. For example, geraniol is a main constituent of geranium oil (*Pelargonium graveolens*) and its isomer, linalool is found in the oil of a garden herb, clary sage. Citral, a lemon oil component, is extracted from lemon grass oil (*Cymbopogon flexuosus*). Menthol is isolated from *Mentha arvensis*. It has significant commercial values and widely used to flavor sweets, tobacco and toothpaste. It is also used for local anaesthetic and refreshing effects.

The pine oil (turpentine) contains two monoterpenes, α -terpineol and α -pinene. Camphor is extracted from camphor tree, *Cinnamomum camphora*. It is used to protect cloths from moths. At present, α -pinene is a raw material for the commercial synthesis of camphor. Some of the other examples such as myrcenes, limonene, citronellal, are also member of monoterpenes. Thujene (or α -thujene) is a natural organic compound classified as a monoterpene. It is found in the essential oils of a variety of plants, and contributes pungency to the flavor of some herbs such as Summer savory.

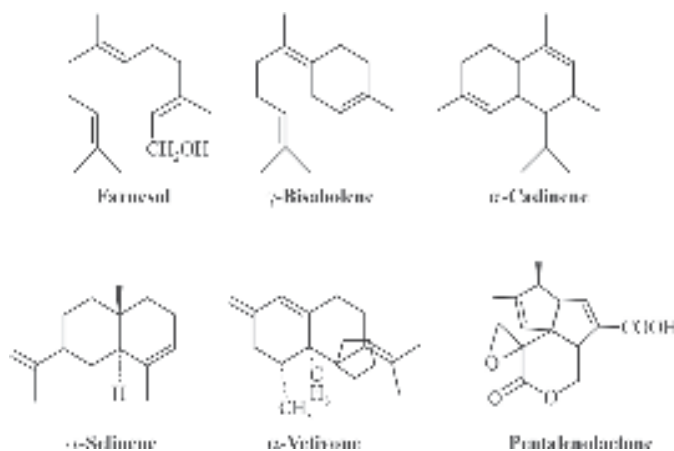


B. Sesquiterpenes

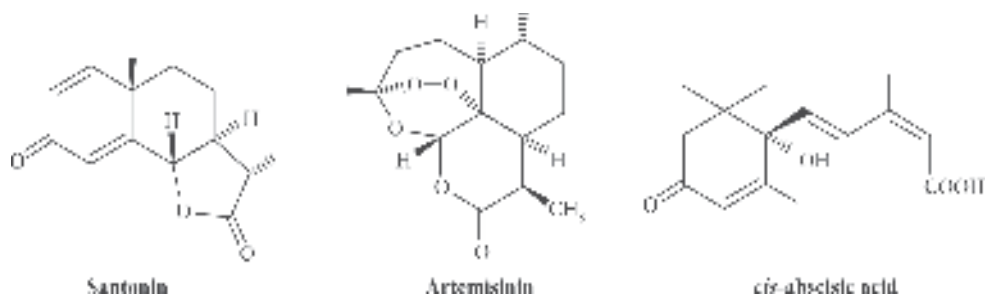
Sesquiterpenes are also generally obtained from the essential oils but from higher boiling point fractions. They contain three isoprene units and have the molecular formula, $C_{15}H_{24}$. For example, caryophyllene is isolated from oil of cloves, humulene from oil of hops, cadrene from cedar wood oil and longifolene from Indian turpentine oil (*Pinus ponderosa*).



There are some other examples of sesquiterpenes such as farnesol, bisabolene, cadinene, selinene, vetivone, and antibiotic, pentalenolactone, etc.

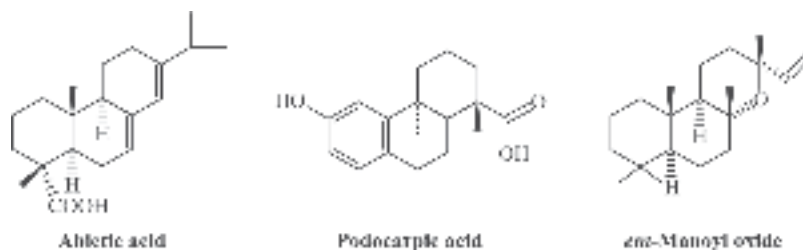


Sesquiterpenoid lactones such as santonin from *Artemisia maritima* (worm wood) is used in medicine for the elimination of intestinal worms. Derivatives of the Chinese drug qinghaosu, artemisinin, obtained from *Artemisia annua*, have recently been recommended by the World Health Organization for the treatment of resistant strains of malaria. This compound contains an unusual peroxide linkage which is associated with its biological activity. Absciscic acid, a plant hormone is also an example of sesquiterpenoid. It stimulates leaf fall and dormancy in plants.

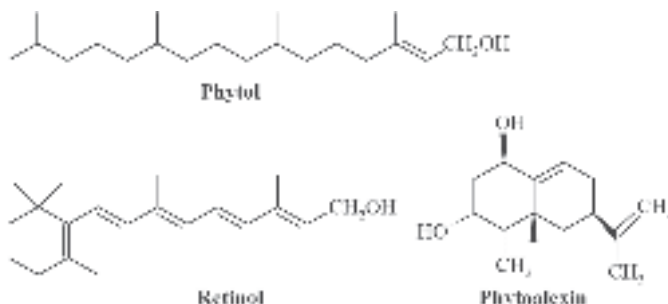


C. Diterpenes

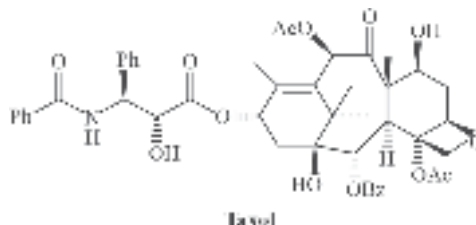
Diterpenes composed of four isoprene units, have the general molecular formula, $C_{20}H_{32}$. Some of diterpenes are wood resin product. For example, abietic acid (from *Pinus* and *Abies* species), podocarpic acid (from *Podocarpus cupressinum*) and neutral resin ent-manoyl oxide.



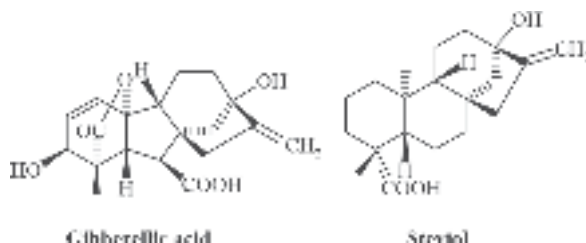
Biologically active compounds such as phytol, vitamin A (Retinol) and capsidiol (phytoalexin) have been also assigned as diterpenes.



Taxol (or paclitaxel) is a diterpenoid and is first isolated from the bark of the Pacific yew, *Taxus brevifolia*. It is widely used for the treatment of breast and ovarian cancer.

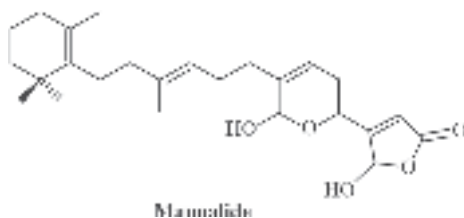


Steviol is the aglycone of the natural sweetener, stevioside which is obtained from the plant *Stevia rebaudiana*. Stevioside is used in some countries as a non-nutritive sweetener in low caloric drinks. On the other hands, some of the diterpenoids constituents of *Euphorbia* species have powerful skin irritant and co-carcinogenic properties. Plant hormone, gibberellic acid belongs to diterpenoid. It is also synthesized as a phytotoxin by the fungus, *Gibberella fujikuroi*. It is used in the malting step in beer manufacture to increase α -amylase production and also for the growing of seedless grapes.



D. Sesterpenes

These contain five isoprene units and have the molecular formula, C₂₅H₄₀. Sesterterpenes are most abundant in marine sponges. Manoalide is first discovered in 1980 by **Scheuer** from the marine sponge *Luffariella variabilis*. It shows antibacterial activity against *Streptomyces pyogenes* and *Staphylococcus aureus*.



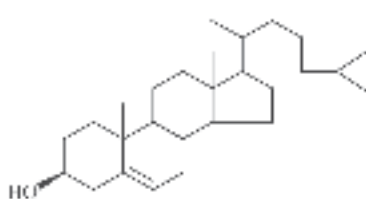
E. Triterpenes

Triterpenes contain six isoprene units having the molecular formula, $C_{30}H_{48}$. Squalene is a simple linear triterpene and is first isolated from fish liver oil.

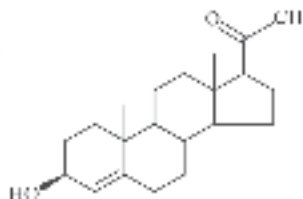


Squalene

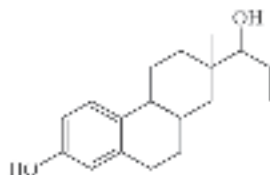
Subsequently, it has also been extracted from plant oils, and mammalian fats. Most of the members of this category contain tetracyclic structure.



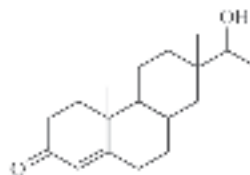
Cholesterol



Progesterone

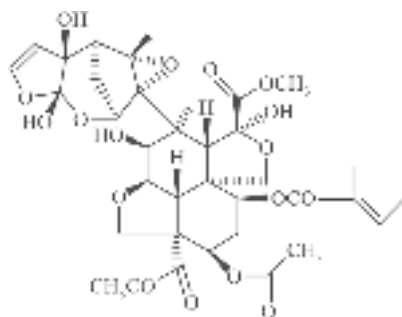


Oestradiol

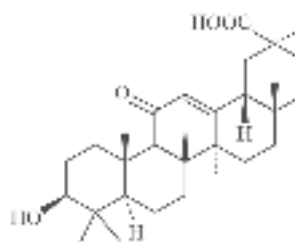


Testosterone

The cyclopentaperhydrophenanthrene backbone is common feature of all steroids and belongs to category tetracyclic triterpenes. Cholesterol is an important constituent of lipid membranes. Female steroid hormones such as progesterone, oestradiol and male hormone, testosterone also belong to triterpenes.



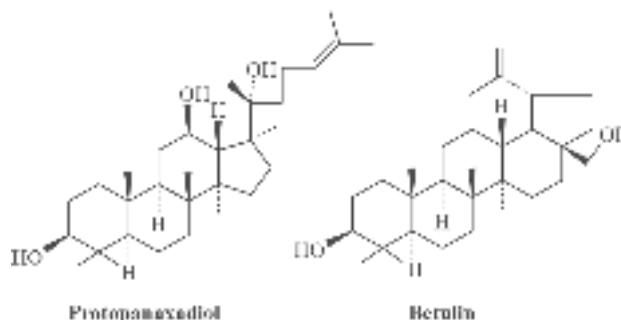
Azadirachtin



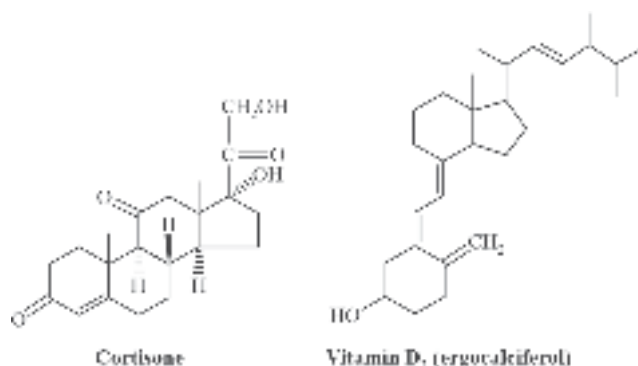
Glycyrrhetic acid

Azadirachtin, extracted from Neem tree, is a highly oxidized tetranortriterpenoid and shows strong insect antifeedant and growth inhibitor activity. Glycyrrhetic acid is a triterpene found in liquorice and has healing properties in the treatment of peptic ulcers.

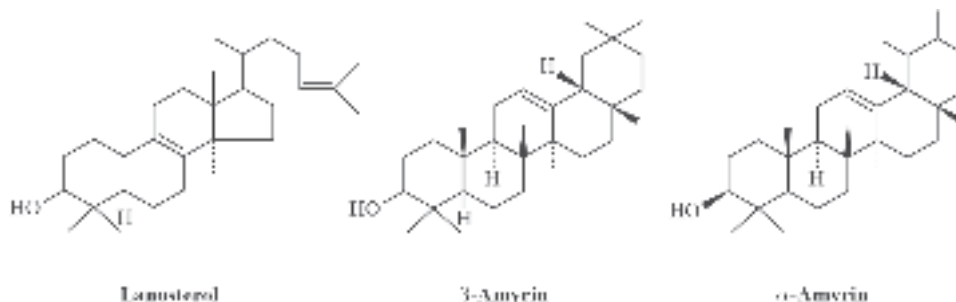
The traditional Chinese medicine, ginseng (*Panax ginseng*) contains glycosides of triterpenes such as protopanaxadiol. This is widely used in China and Russia for the treatment of number of diseases, e.g. insomnia, sexual impotence as a general restorative. Recently, derivatives of betulin (from the bark of beech tree) have shown interesting activity against the human immunodeficiency virus.



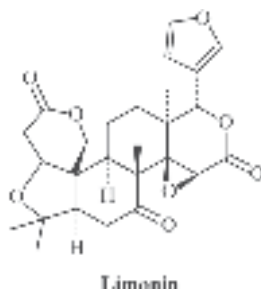
Moreover, corticosteroids such as cortisone are also triterpenes, possessing an immunosuppressive activity and can reduce inflammation. They are also used in the treatment of asthma, rheumatoid arthritis, etc. The vitamin D helps in the absorption of calcium and phosphate in intestine.



Lanosterol is a tetracyclic triterpene, present in wool fat and its ester is present in lanolin cream while the α - and β -amyrins are commonly found in wood resin and the bark of many trees.

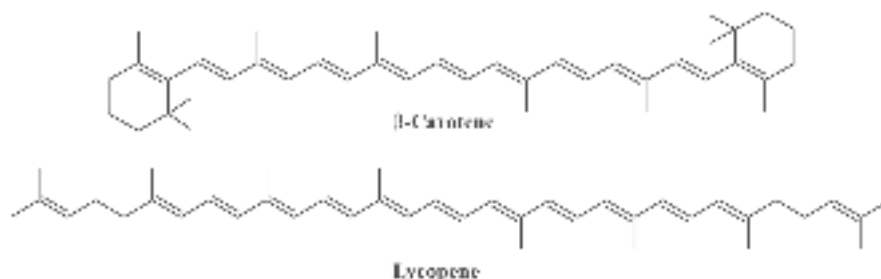


Many highly oxidized and degraded triterpenes are exemplified by limonin which is a bitter principle obtained from lemon and orange seeds.



F. Tetraterpenes (Carotenoids)

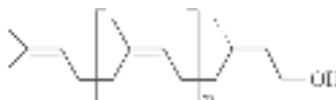
It is composed of eight isoprene units having molecular formula, $C_{40}H_{64}$. These are lipid soluble pigments widely distributed in all plants including bacteria. Carotenoids are present either in simple unsaturated hydrocarbon form or their oxygenated derivatives known as xanthophylls. Crocin obtained from *Crocus sativus* is only water soluble carotenoid present in plants, which on hydrolysis with acid yields crocetin dicarboxylic acid and glucose.



Some of the biologically active compounds like lycopene, monocyclic α -carotene and bicyclic α - and β -carotenes are common examples of tetraterpenes. Thus, the red color of carrot is due to the presence of α -carotene while the deep red pigment of tomato is due to the presence of lycopene. The carotenoids possess anti-oxidants properties. Moreover, carotenoids are precursors of vitamin A, which has vital role in vision process.

G. Polyterpenes

Polyterpenes are polymer in which several isoprene units are joined through head-to-tail fashion. Natural rubber (*Heva brasiliensis*) is best known example of this series.



BIOSYNTHETIC PATHWAY

Although, "isoprene rule" is developed by **Ruzicka** much earlier but the biosynthetic origin of the isoprene unit (C_5) is recently established. There are two most acceptable pathways: (i) Mevalonic acid pathway, and (ii) 1-Deoxyxylulose pathway.

(i) Mevalonic acid pathway

The first step is formation of (S)-3-hydroxyl-3-methylglutaryl co-enzyme A (HMG-CoA) by the condensation of acetyl co-enzyme A with acetoacetyl co-enzyme CoA. The next irreversible step involves the enzymatic reduction of (S)-3-hydroxyl-3-methylglutaryl co-enzyme A (HMG-CoA) with hydrogen from nicotinamide adenine dinucleotide phosphate (2X NADPH) to give (R)-mevalonic acid. Mevalonic acid undergoes two successive phosphorylation by adenosine triphosphate (2X ATP) to give the 5-pyrophosphate. This undergoes a trans elimination of the tertiary hydroxyl group and the carboxyl group to form 3-methylbut-3-enyl pyrophosphate (isopentenyl pyrophosphate, IPP). This is in equilibrium with dimethylallyl pyrophosphate (DMAPP) (Fig. 4.1).

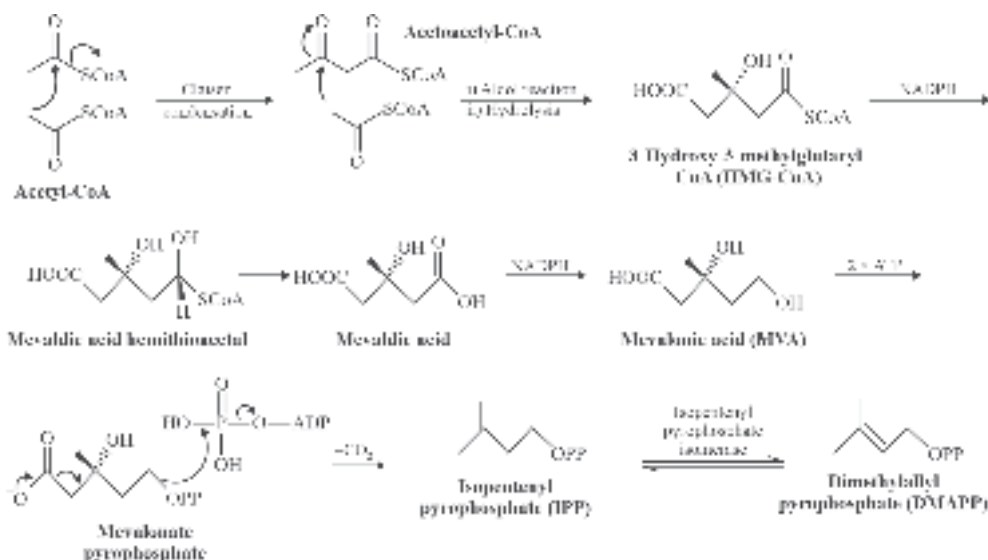


Fig. 4.1: Mevalonic acid pathway

(ii) 1-Deoxyxylulose Pathway

In this pathway, pyruvic acid (2-oxopropanoic acid) and glyceraldehyde monophosphate undergo condensation reaction to form 1-deoxyxylulose 5-P. The pinacol-type rearrangement of 1-deoxy D-xylulose SP, followed by reduction with NADPH yields di-alcohol, 2-C-methyl-D-erythritol 5-P which further undergoes a series of reactions to produce 3-methylbut-2-enyl pyrophosphate (isopentenyl pyrophosphate, IPP). Next step is a stereospecific and reversible isomerization of the double bond of IPP that produces 3-methylbut-2-enyl pyrophosphate (dimethylallyl pyrophosphate, DMAPP) (Fig. 4.2). This step is very important because it generates reactive allylic pyrophosphate which helps in joining of two isoprene units to form the C₁₀ geranyl pyrophosphate. In both pathways, equilibrium favors DMAPP over IPP.

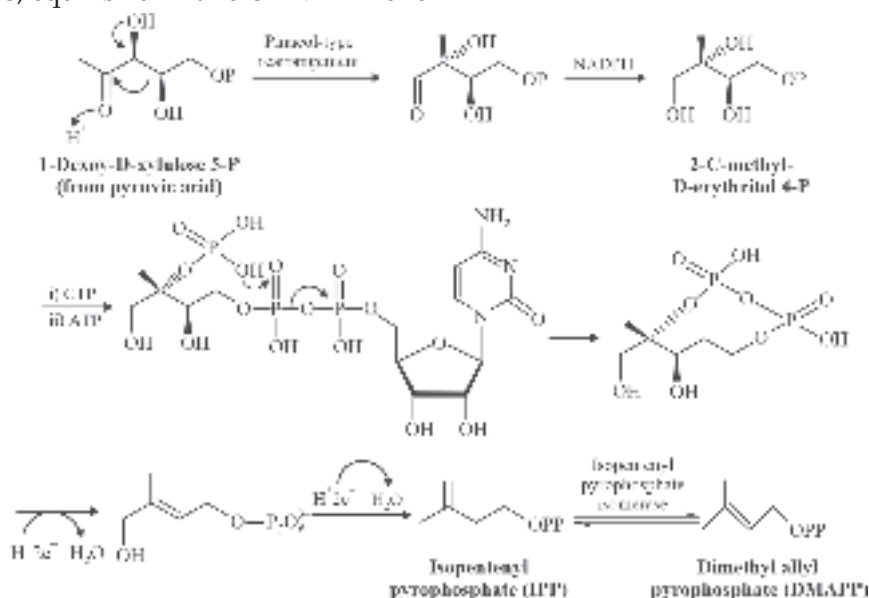


Fig. 4.2: Deoxyxylulose pathway

GENERAL APPROACH FOR THE BIOSYNTHESIS OF TERPENES

IPP and DMAPP are universal building blocks for the synthesis of various classes of terpenes. The enzyme, isopentenyl pyrophosphate isomerase catalyses reaction between IPP and DMAPP in presence of metal ion to produce geranylpyrophosphate (GPP), farnesyl pyrophosphate (FPP), geranylgeranylpyrophosphate (GGPP), and DMAPP; a precursor for mono-, sesqui-, di-, tri-, tetra-, and polyterpenes. The simple schematic representation is shown in Fig. 4.3.

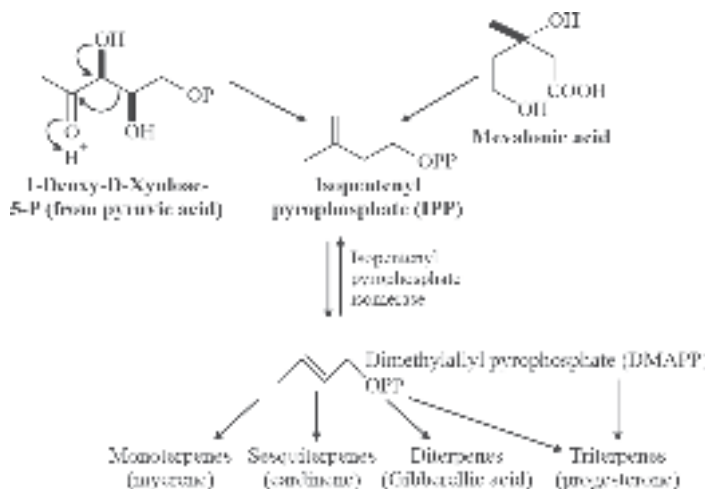
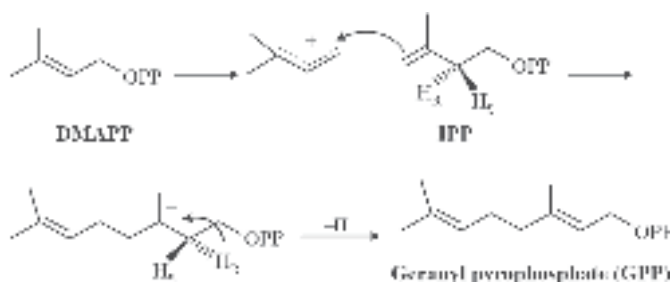


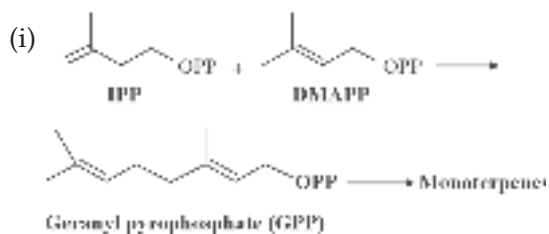
Fig. 4.3: Biosynthesis of terpenes

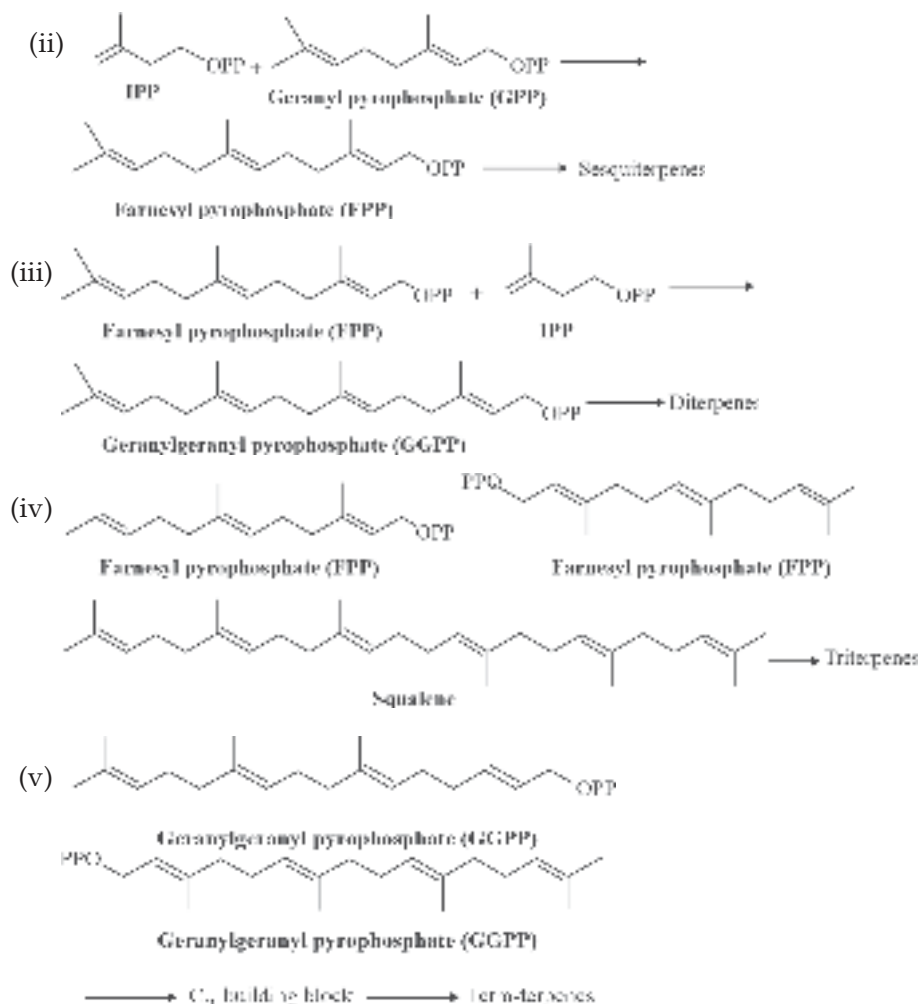
The mechanism for the synthesis of GPP, FPP, GGPP and squalene is very simple. In the presence of enzyme, pyrophosphate group is activated and acts as leaving group to generate an allylic-tertiary carbocation. The carbocation acts as electrophile and is attacked by the double bond of IPP, a second substrate to generate new carbocation which on stereospecific loss of proton produces various precursors for the synthesis of terpenes. This enzymatic reaction involves removal of the H_R-atom and formation of new C=C double bond with the E configuration. The overall mechanism is shown here:

Mechanism:



Thus, mono-, sesqui-, di-, and tetra-terpenes are biosynthesized using the following substrates:





Biosynthesis of Squalene

Squalene is a precursor of triterpenes and all steroids. Biosynthesis of squalene involves assembly of two molecules of farnesyl pyrophosphate joined together in a head-to-tail fashion (Fig. 4.4). In the presence of enzyme, pyrophosphate of one of the two farnesyl pyrophosphate is activated, resulting in the formation of allylic carbocation. This carbocation acts as electrophile and is attacked by double of second farnesyl pyrophosphate molecule. This reaction involves formation of cyclopropyl intermediate, pre-squalene pyrophosphate as a result of an alkylation of the double bond of one farnesyl unit by the pyrophosphate of the second molecule. During reaction, NADPH acts as H donor and replaces one hydrogen stereospecifically. Rearrangement and reduction of intermediate gives squalene.

Conversion of Squalene to Lanosterol

Squalene is a precursor for synthesis of lanosterol, a steroid commonly found in wood, fat and yeast. Biosynthesis of lanosterol involves epoxidation of squalene to squalene-2,3-epoxide by enzyme squalene epoxidase that uses molecular oxygen and NADPH as FAD. In the next step, squalene-2,3-epoxide is converted to a protosterol cation by enzyme

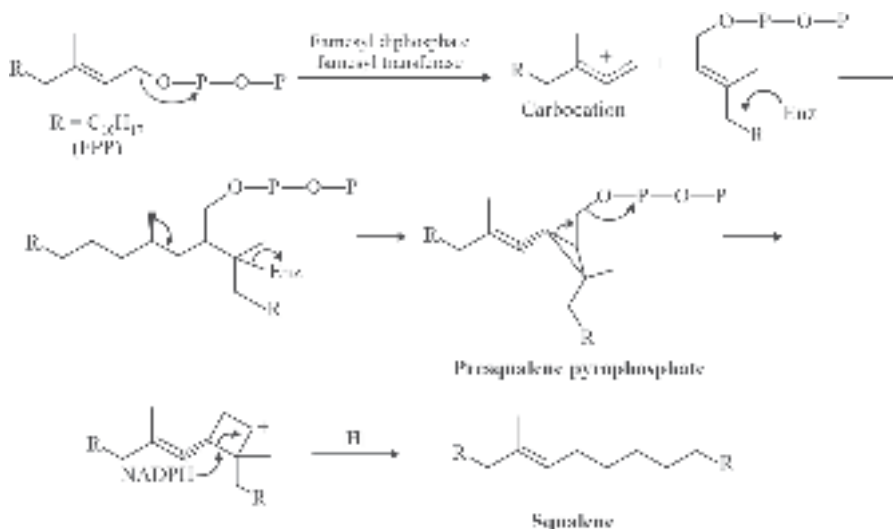


Fig. 4.4: Biosynthesis of squalene

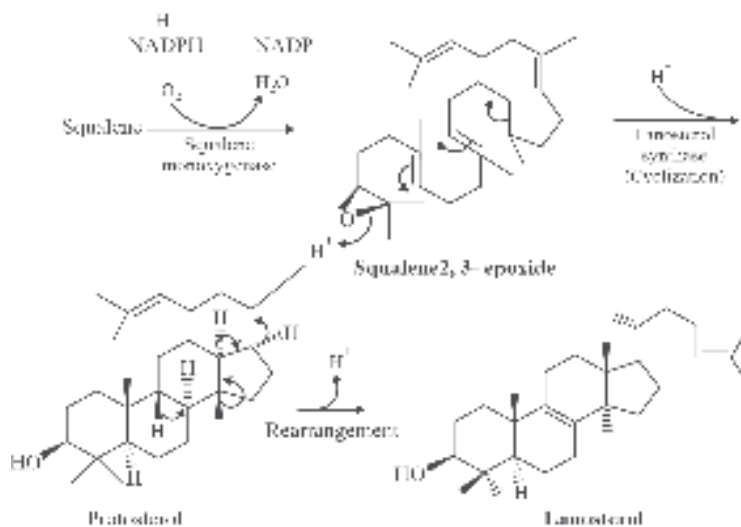


Fig. 4.5: Biosynthesis of lanosterol

protosterol synthase and finally to lanosterol by a stereospecific cyclization and rearrangement (hydride shifts) through series of intermediates (Fig. 4.5).

Conversion of Lanosterol to Cholesterol

Lanosterol is a precursor for the biosynthesis of cholesterol which in turn becomes precursor for steroid hormones, lipoproteins, bile acids, vitamin D. The conversion of lanosterol into cholesterol is very complex process and involves nineteen steps (Fig. 4.6). It requires total nine different enzymes from the endoplasmic reticulum. Out of nine, two enzymes catalyse multiple steps while three are used only two times in this transformation. Lanosterol has three methyl groups viz. at C-30, C-31, and C-32 position which are oxidized and removed as formic acid and two carbon dioxide molecule.

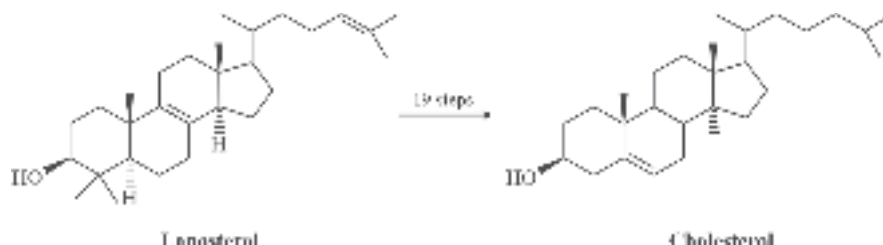


Fig. 4.6: Biosynthesis of cholesterol

Biosynthesis of Gibberellins

Gibberellins, tetracyclic diterpene acids, are group of plant growth hormones. It has significant influence on many physiological processes in higher plants. Eiichi Kurosawa discovered Gibberellin in 1926 while working on the cause of bakanae, the foolish seeding disease in rice, that is main cause of low yield of rice crops in Japan. Later on, it was first isolated by Teijiro Yabuta in 1935 from fungal strains, *Gibberella fujikuroi*. All gibberellins contains kaurene atom skeleton. These are tetracyclic diterpenoids, made up of 4-isoprenoids units.

Geranylgeranyl pyrophosphate (GGPP) is precursor of Gibberellins. Tetracyclic diterpenoid hydrocarbon, ent-kaurene is synthesized from GGPP by two step process. Oxidation of the hydrocarbon at C-19 and C-7 gives ent-7 α -hydroxykaurenoic acid. This is substrate for unique oxidative ring contraction to form gibberellins A₁₂ 7-aldehyde. The key feature in all pathways is loss of C-20 and the formation of the γ -lactone such as gibberellic acid (Fig. 4.7).

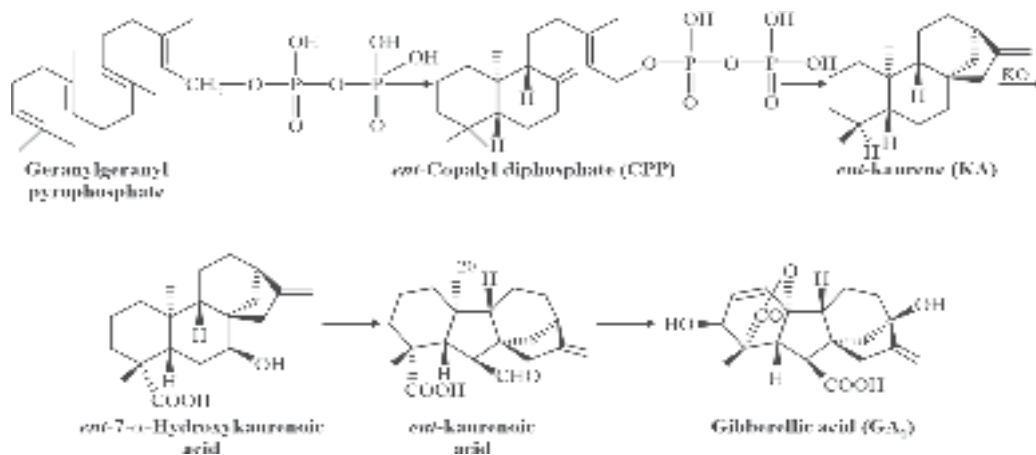


Fig. 4.7. Biosynthesis of gibberellin

ISOLATION OF TERPENOIDS

The essential oils are the common source for mono and sesquiterpenoids. Hence, their isolation is carried out in two steps:

- (i) Isolation of essential oils from plant parts.
- (ii) Separation of individual terpenoid from essential oils.

Isolation of Essential Oils from Plant Parts

1. Extraction by cold pressing

2. Hydro distillation/steam distillation
3. Solvent extraction
4. Enflourage method
5. Supercritical fluid method

1. Extraction by Cold Pressing

The isolation of essential oils by cold pressing or expression is applied only for citrus oils. The outer peel of the fruits is squeezed in presses, and the oil is decanted or centrifuged to separate it from water and cell debris.

2. Hydro Distillation/Steam Distillation

The steam distillation process consist of a distillation vessel containing the plant material. The steam is percolated through the flask with plants from the bottom and the oil evaporates. The emerging mixture of vaporized water and oil moves through a coil usually cooled with running water, and then condensed. The mixture of condensed water and essential oil is collected and separated by decantation or in rare cases, by centrifugation (Fig. 4.8). If necessary, the oil should be freed from dissolved and suspended water by treatment with anhydrous sodium sulphate. This serves to prevent subsequent hydrolysis of esters and other hydrolysable constituents of the oil, hence helping to preserve its odor and properties.

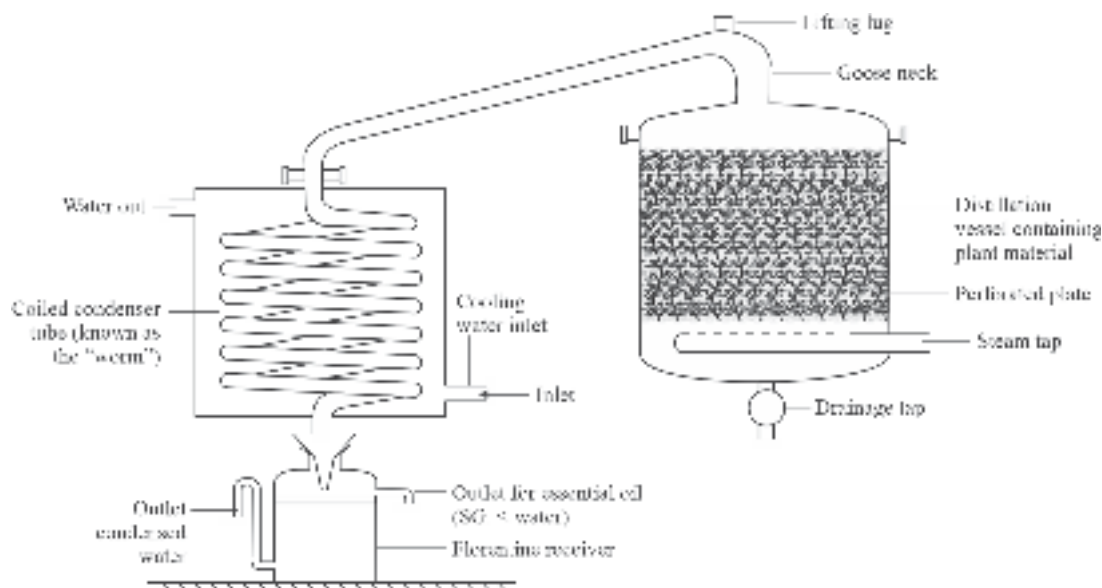


Fig. 4.8: Steam distillation process

In hydrodistillation, the plant material and water are put together in a vessel A (Fig. 4.9), and the mixture is allowed to boil. The vaporised mixture of water and essential oil condenses in the trap B and passes through a hexane layer where the oil dissolves and the condensed water returns back into vessel A. The distillation process usually continues for two to three hours. Usually the volatile oil, which is less affected by heat, is isolated by this method.

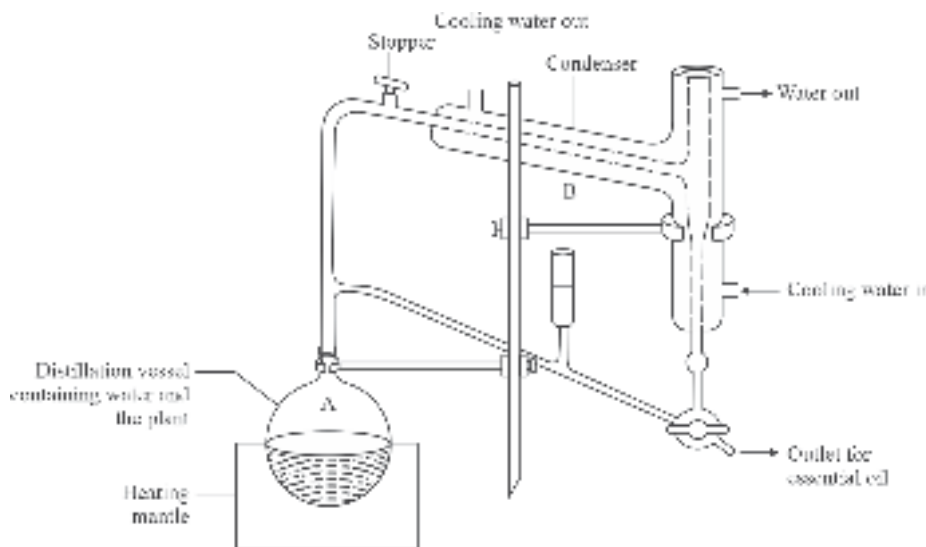


Fig. 4.9: Hydrodistillation apparatus

3. Solvent Extraction

This is very widely used extraction method in perfume industry for the production of volatile oil using lipid solvent like ether or benzene. This is very economical as there are very less deterioration of volatile oil and solvent is distilled off.

4. Enflurage Method

Often, the volatile oil content of fresh plant parts (flower petals) is so small that oil removal is not commercially feasible by the aforementioned methods. In such instances, an odorless fixed oil or fat or wax is spread in a thin layer on glass plates. The flower petals are placed on the fat for a few hours; then repeatedly, the oil petals are removed, and a new layer of petals is introduced. After the fat has absorbed as much fragrance as possible, the fat/wax layer is separated out from glass plate, digested with alcohol and cooled at 20°C to remove fat. The alcohol is then removed by distillation under reduced pressure to get essential oil. This process, known as enflurage, was formerly used extensively in the production of perfumes and still used.

5. Supercritical Fluid Method

At certain critical pressure and temperature, the gases behave like a liquid, which diffuse well through solids and acts as good solvent. The gases like CO₂ are chemically inert, non-inflammable, nontoxic, easy to eliminate, selective, readily available and do not cause any hydrolysis or rearrangement of component to be extracted. Hence, in spite of high cost this method is enormously spreading for supercritical fluid to liquid-liquid extraction. Nowadays, this method is used for production of decaffeinated coffee, nicotine less tobacco products and terpene less oils.

Separation of Individual Terpenoid from Essential Oils

A number of terpenoids are present in essential oil, obtained from the extraction. Definite physical and chemical methods can be used for the separation of individual terpenoid.

They are separated by fractional distillation. The terpenoid hydrocarbons distill over first followed by the oxygenated derivatives. More recently, different chromatographic techniques have been used both for isolation and separation of terpenoids. These terpenoids are present either in the form of hydrocarbon or their oxygenated derivative (alcohol, aldehyde, ketone, etc.). These are separated usually by two methods:

- (i) **Physical method:** In physical method, different chromatographic methods and fractional distillation are applied for separation of individual terpenoid.
- (ii) **Chemical method:** Various terpenes of diverse functional groups (Fig. 4.10) are present in essential oil. These are isolated on the basis of chemical reaction depending on the functional group present in terpenes.

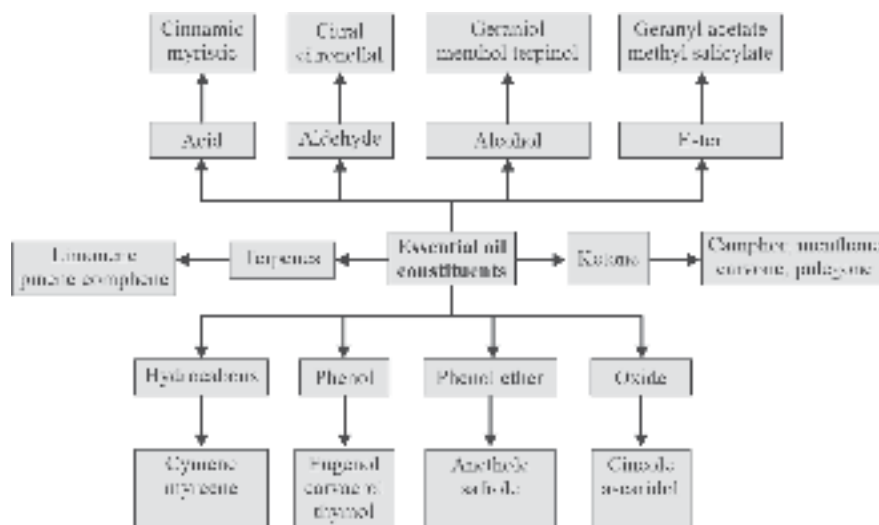
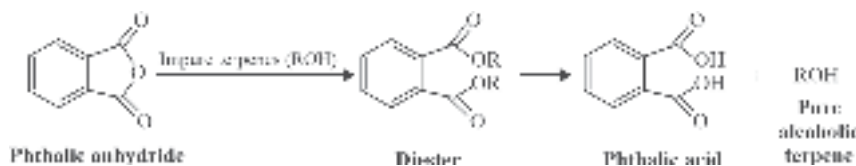


Fig. 4.10: Essential oil constituents

- a. **Separation of terpenoid hydrocarbon:** These are separated by using Tilden reagent composed of solution of nitrosyl chloride (NOCl) in chloroform. The terpenoid hydrocarbons on treatment with Tilden reagent forms crystalline adduct having sharp m.p., which is separated from volatile oil followed by hydrolysis or decomposed to get back the terpenoidal hydrocarbon.
- b. **Separation of terpenoid alcohol:** Terpenoid alcohols on reaction with phthalic anhydride forms di-ester, which precipitate out from volatile oil. These di-esters on treatment with NaHCO_3 in presence KOH, yields back terpene alcohol and phthalic acid.



- c. **Separation of terpenoid aldehyde and ketone:** Terpenoid aldehydes and ketones forms crystalline adduct on reaction with NaHSO_3 and phenyl hydrazines, etc. These crystalline adducts can be hydrolyzed to get back carbonyl compounds.

General Properties of Terpenoids

- Most of the terpenoids are colorless, steam volatile fragrant liquids which are lighter than water. A few of them are solids, e.g. camphor. All are soluble in organic solvent but usually insoluble in water. Most of them are optically active.
- They are open chain or cyclic unsaturated compounds, having one or more double bonds. Consequently they undergo addition reaction with hydrogen, halogen, acids, etc. A number of addition products are reported to have antiseptic properties.
- They undergo polymerization and dehydrogenation.
- They are easily oxidized nearly by all the oxidizing agents. On thermal decomposition, most of the terpenoids yields isoprene as one of the products.

Phytochemical Tests for Terpenoids

Salkowski test: 0.2 g of the extract of the whole plant sample is mixed with 2 ml of chloroform and then 3 ml of concentrated H_2SO_4 carefully added to form a layer. Formation of reddish brown coloration at the interface indicates the positive results for the presence of terpenoids.

Trichloroacetic acid test: On heating triterpenes with trichloroacetic acid, a distinct temperature dependant color appears.

Tetra nitromethane test: Tetra nitromethane gives yellow color with unsaturated compounds (steroids and terpenoids).

Zimmermann test: On heating a mixture of alcoholic solution of the compounds and *m*-dinitrobenzene in presence of alkali, violet color appears, which faded after dilution (for 3-oxo steroids and triterpenoids).

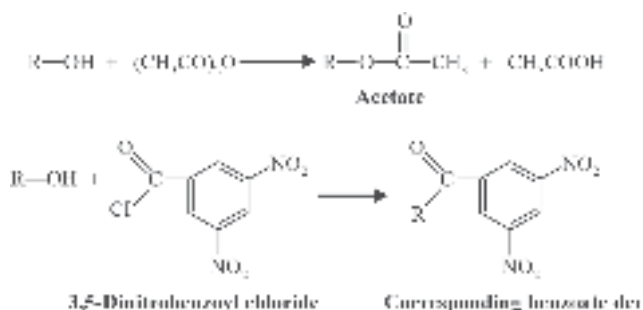
Pharmaceutical applications of volatile oils: Volatile oils containing terpenes, are used as flavoring agent, perfuming agent in pharmaceutical formulations, foods, beverages, and in cosmetic industries. These are also used as important medicinal agent for therapeutic purposes like:

- (i) Carminative, e.g. Umbelliferous fruits
- (ii) Anthelminthic, e.g. Chenopodium oil
- (iii) Diuretics, e.g. Juniper
- (iv) Antiseptic, e.g. Eucalyptus, Thyme
- (v) Counter irritant, e.g. Oil of winter green
- (vi) Local anesthetic, e.g. Clove
- (vii) Sedative, e.g. Jatamansi
- (viii) Local irritant, e.g. Turpentine
- (ix) Insect repellent, e.g. Citronella
- (x) Source of vitamin A, e.g. Lemon grass

General Methods of Structural Elucidation of Terpenoids

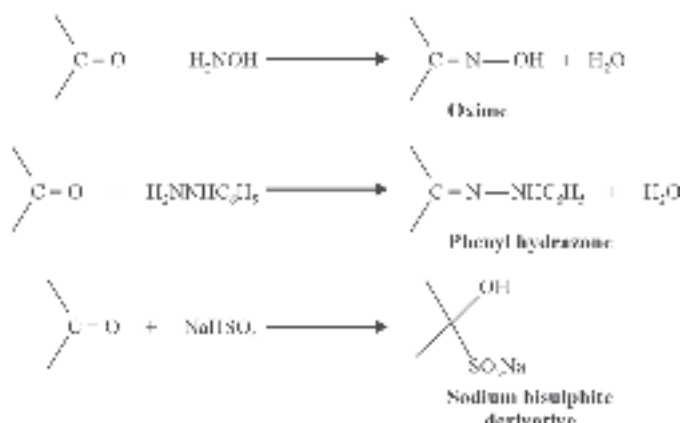
- (i) **Determination of molecular formulae:** It is determined by usual traditional method or by means of mass spectrometry.
- (ii) If terpenoid is optically active, its specific rotation can be measured for identification.
- (iii) Nature of oxygen atom present: If oxygen is present in terpenoids, its functional nature is determined. It is generally present as hydroxy, aldehyde, ketone or carboxylic groups.

- (a) **Presence of hydroxyl group:** Presence of $-OH$ group can be determined by the formation of acetates with acetic anhydride and benzoate with 3,5-dinitrobenzoyl chloride.



Primary alcoholic group undergoes esterification more readily than secondary and tertiary alcohols.

- (b) **Presence of $>C=O$ group:** Terpenoids containing carbonyl function form crystalline addition products like oxime, phenyl hydrazone and bisulphite, etc. with usual carbonyl reagents.

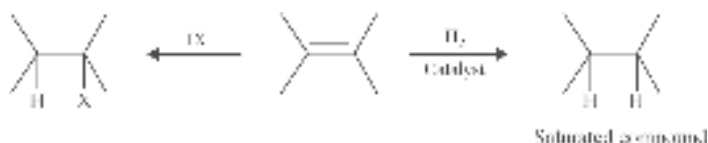


The carbonyl group present as aldehyde gives carboxylic acid on oxidation without loss of any carbon atom whereas the ketone on oxidation yields acid with lesser number of carbon atoms.

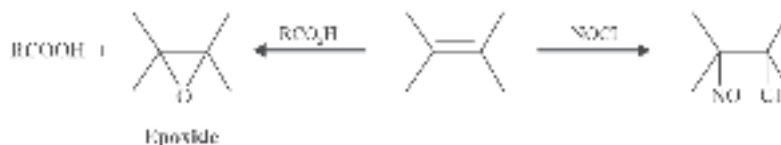
If ketone is a part of ring structure, it yields acid on oxidation having same number of carbon atoms (e.g. menthone).

- (iv) **C-Alkyl group:** It is detected and estimated by **Kuhn-Roth method** as mentioned in Chapter 3.
- (v) **Unsaturation:** The presence of olefinic double bond is confirmed by decoloration of bromine water or alkaline potassium permanganate solution, and number of double bond determination can be done by analysis of the bromide or by quantitative hydrogenation or by titration with monoperphthalic acid.

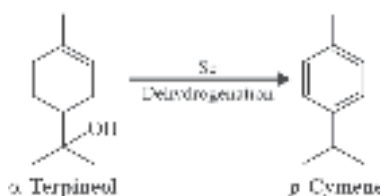
Presence of double bond also confirmed by means of catalytic hydrogenation or addition of halogen acids. Number of moles of HX ($X = Br$ or other halide atom) absorbed by one molecule is equal to number of double bonds present.



Addition of nitrosyl chloride (NOCl) (Tilden's reagent) and epoxide formation with peracid also gives idea about double bonds present in terpenoid molecule.



- (vi) **Dehydrogenation:** On dehydrogenation with sulphur, selenium, or palladium, terpenoids are converted to aromatic compounds. Examination of these products indicates the skeleton structure and position of side chain in the original terpenoids. For example, α -terpineol on selenium dehydrogenation yields *p*-cymene structure, indicates the presence of *p*-cymene skeleton or 6-membered ring containing methyl and isopropyl group at 1,4-position.



- (vii) **Oxidative degradation:** Oxidative degradation has been the parallel tool for elucidating the structure of terpenoids. Reagents for degradative oxidation are ozone, acid, neutral or alkaline potassium permanganate, chromic acid, sodium hypobromide, osmium tetroxide, nitric acid, lead tetra acetate and peroxy acids. Since oxidizing agents are selective, depending on a particular group to be oxidized, the oxidizing agent is chosen. With the help of structure of degradation products, original structure can be predicted.
- (viii) **Number of the rings present:** With the help of general formula of corresponding parent saturated hydrocarbon, number of rings present in that molecule can be determined (Table 4.2).

Table 4.2: Correlation of general formula with nature of compounds

General formula of parent saturated hydrocarbon	Type of structure
C_nH_{2n+2}	Acyclic
C_nH_{2n}	Monocyclic
C_nH_{2n-2}	Bicyclic
C_nH_{2n-4}	Tricyclic
C_nH_{2n-6}	Tetracyclic

For example, limonene ($C_{10}H_{16}$) consumes 2 moles of hydrogen to give tetrahydro limonene ($C_{10}H_{20}$) corresponding to the general formula, C_nH_{2n} . It means, limonene has monocyclic structure.

- (ix) **Spectroscopic studies:** All the spectroscopic methods are very helpful for the confirmation of structure of natural terpenoids and also structure of degradation products. The various methods for elucidating the structure of terpenoids are:
- (a) **UV spectroscopy:** For terpenes containing conjugated dienes or α,β -unsaturated ketones, UV spectroscopy is very useful tool. The values λ_{max} for various types of terpenoids have been calculated by applying Woodward's empirical

rules. There is generally good agreement between calculation and observed values. Isolated double bonds, α,β -unsaturated esters, acids, lactones also have characteristic maxima.

- (b) **IR spectroscopy:** IR spectroscopy is useful in detecting group such as hydroxyl group ($\sim 3400\text{ cm}^{-1}$) or an oxo group (saturated $1750\text{--}1700\text{ cm}^{-1}$). Isopropyl group, cis and trans also have characteristic absorption peaks in IR region.
- (c) **NMR spectroscopy:** This technique is useful to detect and identify double bonds, to determine the nature of end group and also the number of rings present, and also to reveal the orientation of methyl group in the relative position of double bonds.
- (d) **Mass spectrometry:** It is now being widely used as a means of elucidating structure of terpenoids. This technique is used for determining mol. wt., mol. formula, nature of functional groups present and relative positions of double bonds.
- (x) **X-ray analysis:** This is very helpful technique for elucidating structure and stereochemistry of terpenoids.
- (xi) **Synthesis:** Proposed structure is finally confirmed by synthesis. In terpenoid chemistry, many of the synthesis are ambiguous and in such cases analytical evidences are used in conjunction with the synthesis.

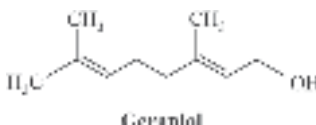
EXAMPLES OF STRUCTURAL ELUCIDATIONS

MONOTERPENES

Acyclic Compound

1. Geraniol

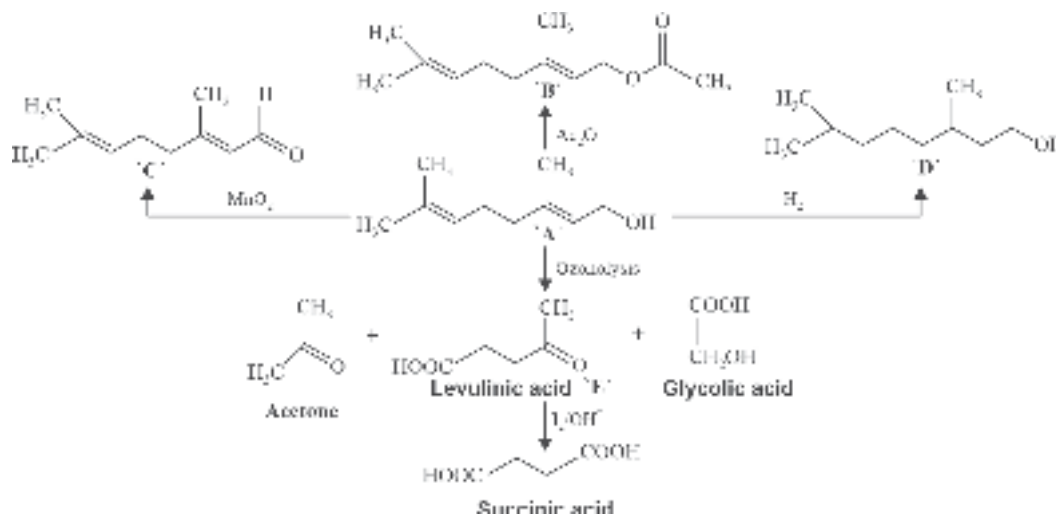
Geraniol, b.p. $229\text{--}230^\circ\text{C}$ is a monoterpene and an alcohol. It is the primary constituent of rose oil, palmarosa oil, and citronella oil (Java type). It also occurs in small quantities in geranium, temon, and many other essential oils. Geraniol is isolated from citronella and palmarosa oils. Fractional distillation of the oils, preceded by saponification of the esters, yields a fraction containing about 60% geraniol accompanied by small quantities of nerol, citronellol and citronellal. A product of a higher geraniol different and slightly different odor quality for use in fine fragrances is obtained by fractionating palmarosa oil after saponification of the geranyl esters.



It appears as a clear to pale-yellow oil that is insoluble in water, but soluble in most common organic solvents. It has a rose-like odor and is commonly used in perfumes.

Structural Elucidation

- (i) **Molecular formula:** The first step in structure elucidation is the determination of the correct molecular formula, which is found to be $\text{C}_{10}\text{H}_{18}\text{O}$, A.
- (ii) **Functional nature of oxygen:** The compound contains hydroxy group as it undergoes acetylation reaction with acetic anhydride to give monoacetylated product, B.



Scheme 4.1: Reactions of geraniol

The geraniol can be oxidized with MnO_2 to yield **C** ($\text{C}_{10}\text{H}_{16}\text{O}$), which forms an *orange-red* derivative with 2,4-dinitrophenylhydrazine (DNP). These observations suggest that the compound '**C**' is an α,β -unsaturated aldehyde or ketone. The compound, **C** does not answer the iodoform test (I_2/OH^-), but reduces Tollen's reagent, suggesting that it is not a methyl ketone, but an aldehyde.

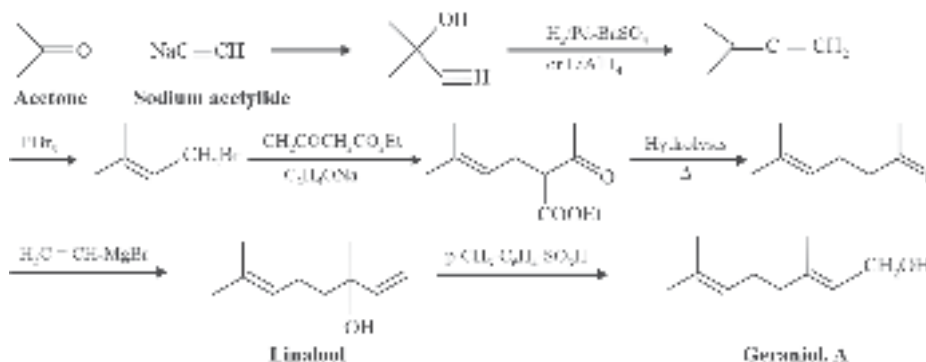
Note:

- (a) MnO_2 is a specific oxidant for allylic and benzylic alcohols.
- (b) DNP derivatives of saturated carbonyl compounds are pale yellow, whereas highly conjugated compounds like benzophenone give brick-red products with DNP.
- (iii) **Presence of double bonds:** The geraniol **A** on catalytic hydrogenation gives, α -*tetrahydro* derivative, **D** ($\text{C}_{10}\text{H}_{22}\text{O}$), showing the presence of two double bonds. The molecular formula of compound **D** also shows that it is a saturated alcohol. Therefore, **A** is a double unsaturated, acyclic allylic primary alcohol.
- (iv) **Structure of Geraniol:** Ozonolysis of Geraniol **A** followed by oxidative workup yields one molecule each of acetone, glycolic acid, and a keto carboxylic acid **E** ($\text{C}_5\text{H}_8\text{O}_2$). Compound **E** on treatment with I/OH^- gives succinic acid. The keto acid, **E** could therefore be identified as levulinic acid. The structure to geraniol can thus be logically assigned, **A** as shown in the Scheme 4.1 to explain the formation of these products.
- (v) Finally, the structure of geraniol is confirmed by synthesis (Scheme 4.2).

Spectral Data

UV: The UV spectrum of a methanolic solution of compound '**A**' shows an intense absorption at about 205 nm. In the UV spectrum of compound '**C**', there are two absorption maxima at 205 and 232 nm. The inferences are: The compound **A** has two similar, if not identical chromophores. In compound '**C**', one of these chromophores is retained while the other, responsible for the maximum at 232 nm, is an α,α -unsaturated aldehyde.

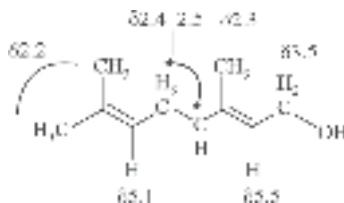
IR: The IR spectrum of compound '**A**' has a broad band at 3350 cm^{-1} which is absent in the spectrum of compound **C**. This band is due to a hydroxyl group. The spectra of



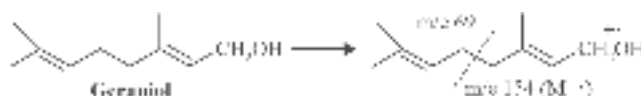
Scheme 4.2: Synthesis of geraniol

both compound A and C have bands at 3040 (C-H), 2920, 2860 and 1350 (CH_2 and CH_3 groups) cm^{-1} . These bands show that A and C possess an aliphatic skeletal framework with one or more double bonds: The most prominent absorption band in the spectrum of compound C is seen at 1680 cm^{-1} , which is absent in the spectrum of A. This band is due to the α,β -unsaturated carbonyl group. The presence of the CHO group in compound C is also indicated by a band at 2800 cm^{-1} .

$^1\text{H-NMR}$: The NMR spectra of A and C provide the most compelling evidence for their structures. The spectrum of compound A has signals at a 2.2 (s, 6H), 2.3 (s, 3H), 2.4–2.5 (m, 4H), 3.5 (d, 2H), 5.1 (t, 1H) and 5.3 (t, 1H). These data show the presence of three methyl groups on sp^2 carbons, two olefinic hydrogens (each on a carbon atom next to a CH_2 group as the signals appear as triplets), and a $\text{CH}_2\text{-CH}_2$ unit. The signal at delta 3.5 is due to the $-\text{CH}_2\text{OH}$ end group next to an sp^2 carbon. This signal shifts to delta 4.5 upon acetylation of A. In the NMR spectrum of C, the two proton signal at delta 3.5 is missing, and instead, there is a one proton doublet at delta 9.3, due to the aldehydic proton.



MS (m/e): The Scheme 4.3 gives the mass spectral fragmentation pattern of geraniol A. Besides the molecular ion signal at m/z 154, there is a prominent peak at m/e 153. A strong peak at m/e 69 appears due to loss of terminal isopentenyl group by allylic cleavage. These peaks give strong support to the structure assigned to the geraniol.

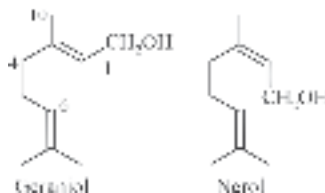


Scheme 4.3: Mass fragmentation pattern of geraniol

Stereochemistry

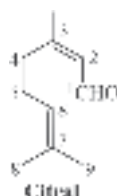
The geraniol is a mixture of two *cis-trans* isomers. Both are properly named—geraniol (*trans*) and nerol (*cis*). The two configurational isomers (diastereomers) have separate

existence. However, upon oxidation, both geraniol and nerol give a mixture of aldehydes (citral) which is an inseparable mixture of geranial (citral-a) and neral (citral-b).



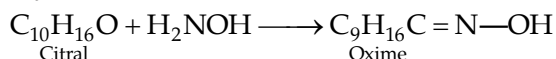
2. Citral

Citral, b.p. 77°C is an acyclic monoterpenoid. It is pale yellow liquid having strong lemon like odor and can be obtained by fractional distillation under reduced pressure from lemongrass oil.

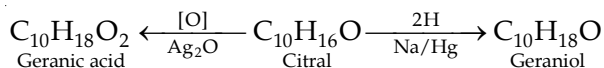


Structural Elucidation of Citral

- (i) **Molecular formula:** It is found to be $C_{10}H_{16}O$.
- (ii) **Functional nature of oxygen atom:** Formation of oxime of citral indicates the presence of carbonyl group in citral molecule.

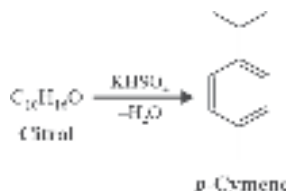


On reduction with Na/Hg it gives an alcohol called geraniol and on oxidation with silver oxide, it gives a monocarboxylic acid, called geranic acid without loss of any carbon atom.



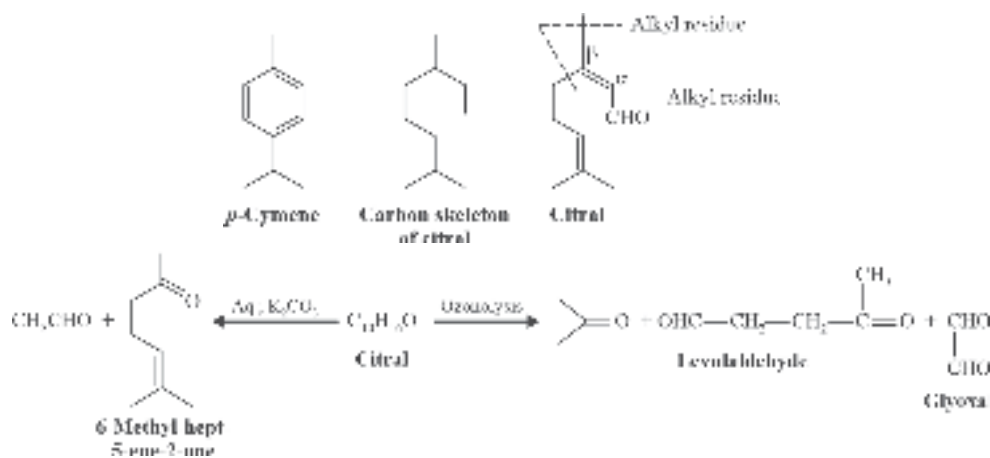
Both of these reaction reveal that carbonyl group in citral is therefore an aldehyde group. Citral reduces Fehling's solution, further confirming the presence of aldehydic group.

- (iii) **Presence of double bonds:** It adds on two molecule of Br_2 , so shows the presence of two double bonds. Corresponding saturated hydrocarbon of citral (mol. formulae $C_{10}H_{22}$) corresponds to the general formula C_nH_{2n+2} for acyclic compounds, indicating that citral must be an acyclic compound.
- (iv) **Presence of *p*-cymene skeleton:** Citral gives *p*-cymene on dehydration with potassium hydrogen sulphate. This indicates the presence of *p*-cymene skeleton.



- (v) **Structure of Citral:** On ozonolysis, it gives acetone, levulaldehyde and glyoxal. Citral on boiling with aqueous potassium carbonate solution gives 6-methyl hept-5-ene-2-one and acetaldehyde. Formation of *p*-cymene and product obtained from

the ozonolysis and reaction with potassium carbonate solution reveals that citral is formed by the joining of two isoprene units in the head to tail fashion. On the basis of above evidences, following structure is proposed for citral.



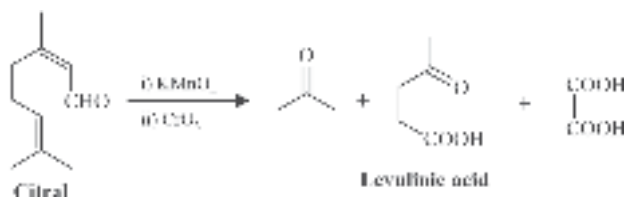
(vi) The above structure is supported from calculation of its λ_{max} by following **Woodward's rule**. The basic value for acyclic aldehyde: 215 nm.

The increments due to two β alkyl residues $2 \times 12 = 24$ nm.

Total value = 239 nm.

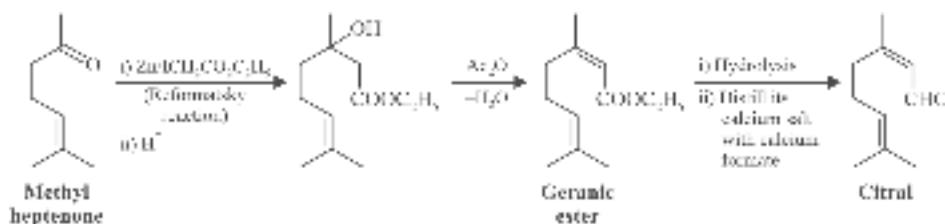
The theoretical value nearly coincides with the practical value, 238 nm.

(vi) The above structure is further supported by the degradation of citral on treatment with alkaline KMnO_4 , followed by chromic acid. It appears that citral is product of aldol condensation of these products.



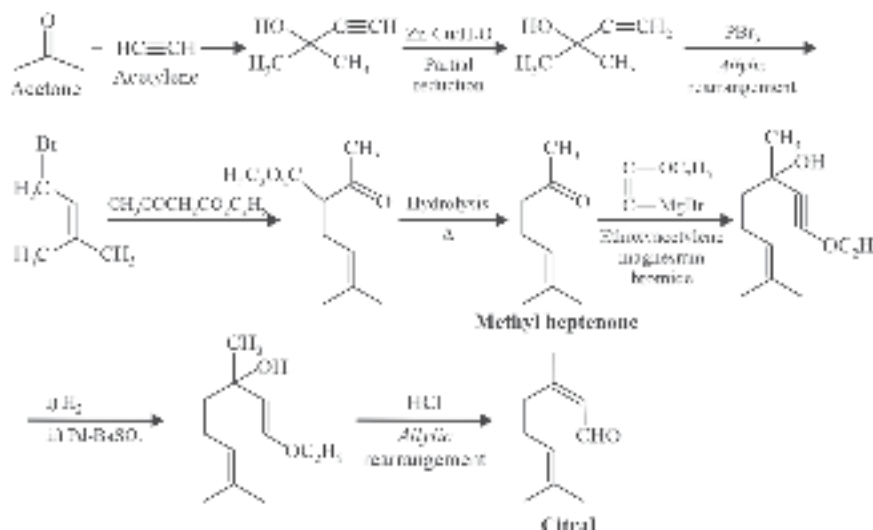
(vii) Finally, the assigned structure of citral is confirmed by its synthesis.

(a) **Barbier-Bouveault-Tiemann's synthesis:** In this synthesis, methyl heptenone is converted to geranic ester by using **Reformatsky's reaction**. Geranic ester is then converted to citral by distilling a mixture of calcium salts of geranic and calcium formate.



(b) **Arens-Van drop's synthesis:** This synthesis involves condensation of acetone with acetylene in the presence of liquid ammonia. Condensation product is then reduced and treated with PBr_3 , allylic rearrangement takes place. The

rearranged product so obtained is treated with sodium salt of acetoacetic ester and then hydrolysed to yield methyl heptenone. The later compound on condensation with ethoxy acetylene magnesium bromide, followed by the partial reduction and acidification gives citral by allylic rearrangement (Scheme 4.4).



Scheme 4.4: Synthesis of citral

Spectral Data

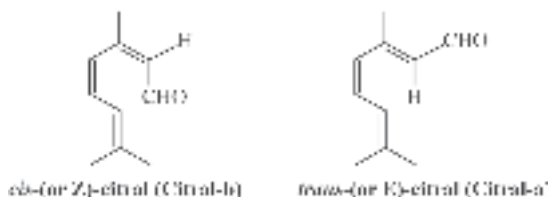
UV: $\lambda_{\max}$ 236 nm (ϵ 16,300).

IR (cm^{-1}): 1665, 1625, 1603, 1398, 1190, 1117.

$^1\text{H-NMR}$ (δ): 1.65 (6H, d, C-7 methyl), 2.10 (4H, s, $(\text{CH}_2)_2$) 2.15 (3H, s, C-3 methyl), 5.0 (1H, d, H-2), 9.84 (1H, d, H-1) .

MS (m/e): 69 (100), 41, 84, 94, 109, 67, 83, 81.

Stereochemistry: Two geometrical isomers occur in nature. The existence of the two isomeric citrals in nature has been confirmed chemically by the formation of two different semicarbazones and formation of geraniol and nerol on reduction. The *trans*-cital-a gives geraniol while *cis*-cital-b gives nerol.



Monocyclic Compound

3. Menthol and Menthone

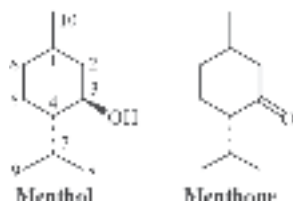
Menthol is the major constituent of *Mentha Piperita*. It is optically active. Only (–) form of menthol is found naturally. It is a colorless solid, m.p. 43°C and used as an antiseptic and anesthetic. Menthol (also called peppermint camphor or mint camphor) is the major

constituent of peppermint oil and is responsible for its odor and taste and the cooling sensation when applied to the skin. It is ingredient in cold balms.

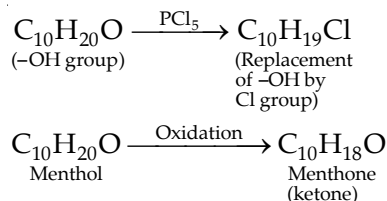
Menthone is a naturally occurring organic compound with a molecular formula $C_{10}H_{18}O$. Menthone is a monoterpene and a ketone. It is structurally related to menthol which has a secondary alcohol in place of the carbonyl. Menthone is used in perfumery and cosmetics for its characteristic aromatic and minty odor.

Structural Elucidation of Menthol and Menthone

- (i) **Mol. formula:** The molecular formula of menthol and menthone is determined as $C_{10}H_{20}O$ and $C_{10}H_{18}O$, respectively.



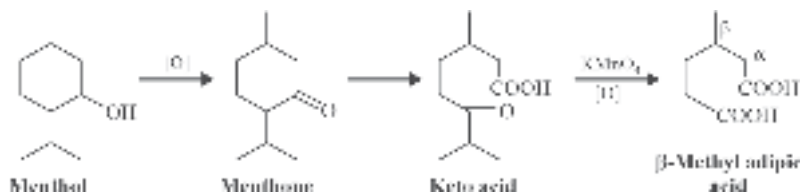
- (ii) **Functional nature of oxygen:** Menthone forms esters readily with acids and oxidized to yield ketone it means that it must possess alcoholic group, which is secondary in nature.



- (iii) **Presence of p-Cymene skeleton:** Menthol on dehydration, followed by dehydrogenation produces p-cymene. It shows the presence of p-Cymene nucleus in menthol.

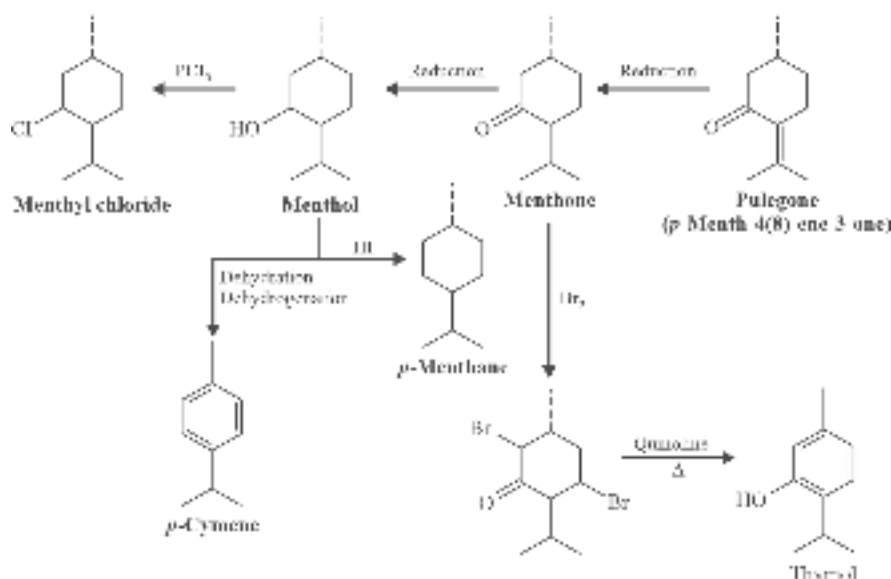


- (iv) **Structure of Menthone method:** Menthone on oxidation with $KMnO_4$ yields keto acid, $C_{10}H_{18}O_3$ which readily oxidizes to 3-methyladipic acid. These reactions can be explained by considering the following structure of menthol.



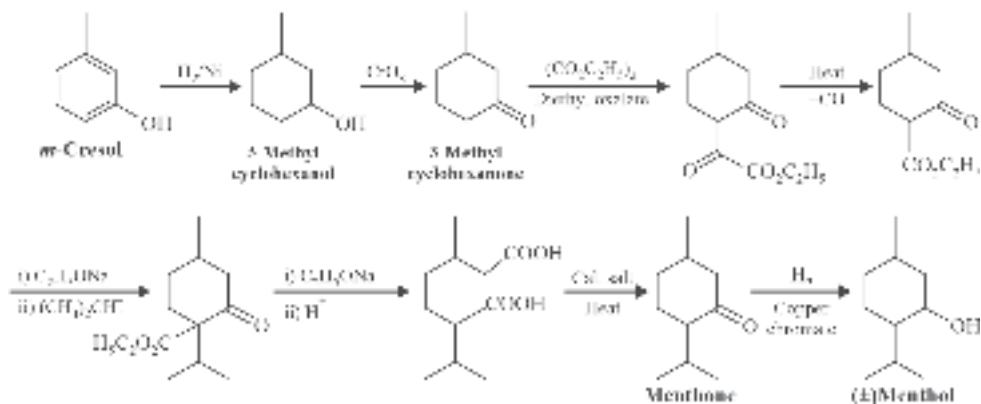
- (v) Pulegone on reduction yielded menthone which on further reduction produces menthol. Menthol is converted to p-cymene by dehydration followed dehydro-

genation. Menthone on bromination followed by heating with quinoline gives the thymol.



Thus, the correlation of pulegone with menthol proved the structure of menthol and menthone.

(vi) Finally, the structure of menthone and menthol has been confirmed by its synthesis from *m*-cresol (Scheme 4.5).



Scheme 4.5: Synthesis of menthone/menthol from *m*-cresol

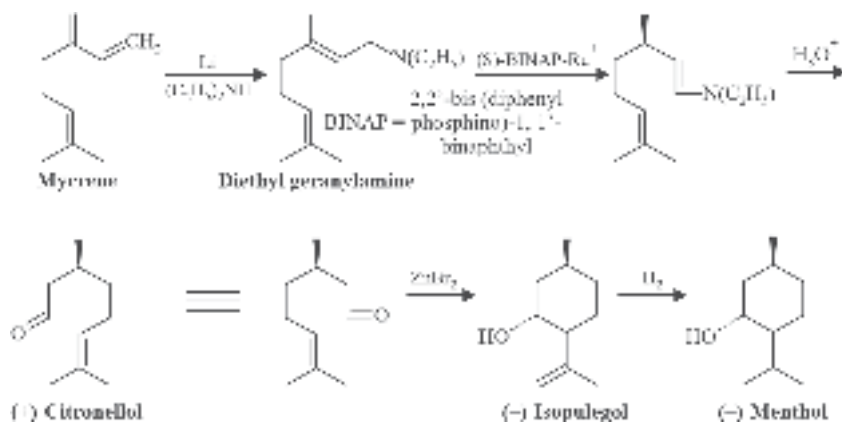
Synthesis of Menthol from Myrcene (Scheme 4.6)

Spectral Data

IR (cm^{-1}): 3333, 1048, 1028, 994, 977.

$^1\text{H-NMR}$ (δ): 0.82 (3H, d, C-1 methyl), 0.90 and 0.93 (6H, d, C-7 methyl), 1.82 (1H, m, H-3), 3.42 (1H, m, s, OH).

MS (m/e): 141, 138, 123, 95, 71 (100), 55, 43, 41.

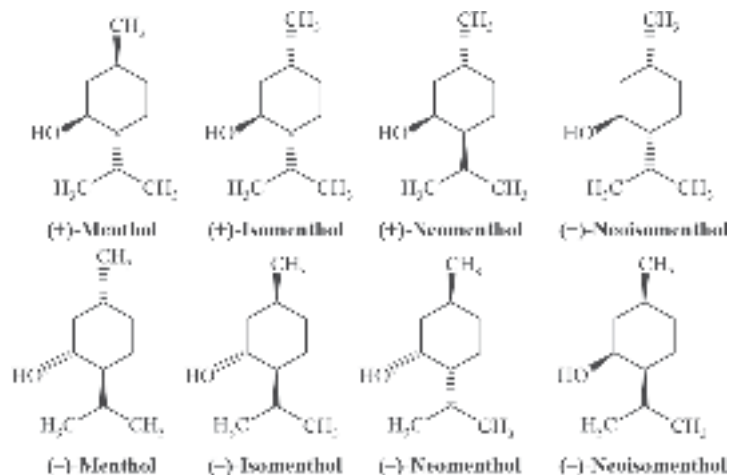


Scheme 4.6: Synthesis of menthol from myrcene

Stereochemistry in Menthol

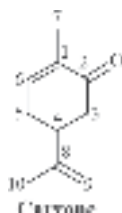
Since menthol has 3 stereocenters and has a total of 8 stereoisomers.

Natural menthol exists as one pure stereo isomer, nearly always the (1*R*, 2*S*, 5*R*) form. The eight possible stereoisomers are:



4. Carvone

Carvone is found naturally in many essential oils, but is most abundant in the oils from seeds of caraway (*Carum carvi*) and dill. It is liquid ketone, b.p. 231°C, soluble in alcohol and water and optically active. Both dextro and levo forms found naturally.



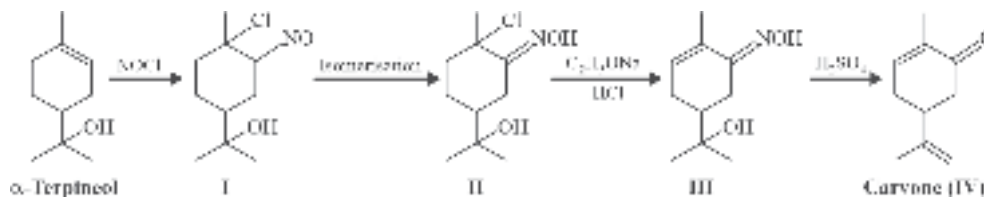
Structural Elucidation of Carvone

(i) **Molecular formula:** It is found to be $\text{C}_{10}\text{H}_{14}\text{O}$.

- (ii) **Presence of double bonds:** It contains two double bonds as it forms tetrabromo derivative on bromination with the consumption of two bromine molecules.
- (iii) **Presence of keto group:** The usual reaction with carbonyl reagent, (e.g. hydroxylamine HCl) indicates the presence of ketonic group in the carvone.
- (iv) By taking into consideration of two double bond and ketonic group, its parent saturated molecular formula becomes $C_{10}H_{20}$ which corresponds to monocyclic compound (C_nH_{2n}).



- (v) On heating with phosphoric acid, carvone forms Carvacrol (2-hydroxy-4-isopropyltoluene), indicating the presence of *p*-cymene structure and presence of keto group at ortho position to methyl group.
- (vi) **Position of two double bonds:** The position of two double bonds is assigned on the basis of its synthesis from α -terpineol and limonene.
 - (a) Synthesis from α -terpineol.

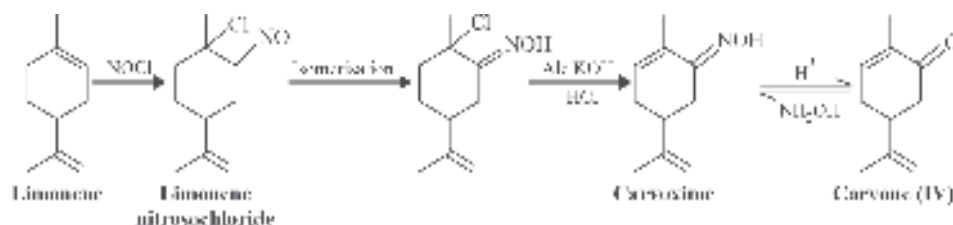


The above sequence of reactions can be explained as follows:

- (a) Addition of nitrosyl chloride to double bond at 1,6-position in α -terpineol produces α -terpineol nitrosochloride, **I**.
- (b) The nitrosochloride rearranges spontaneously to the oximino compound, **II**. This rearrangement also proves the orientation of addition of $NOCl$ to the double bond. Addition in usual way does not give an oxime.
- (c) Removal of a HCl molecule from compound, **II** gives compound **III**, which on warming with dilute sulphuric acid, loses a molecule of water with simultaneous hydrolysis of the oxime to give carvone (IV) (*p*-menth-6,8-diene-2-one).

It indicates that carvone has same carbon skeleton as α -terpineol and also confirms the position of keto group.

- (d) Synthesis from limonene.



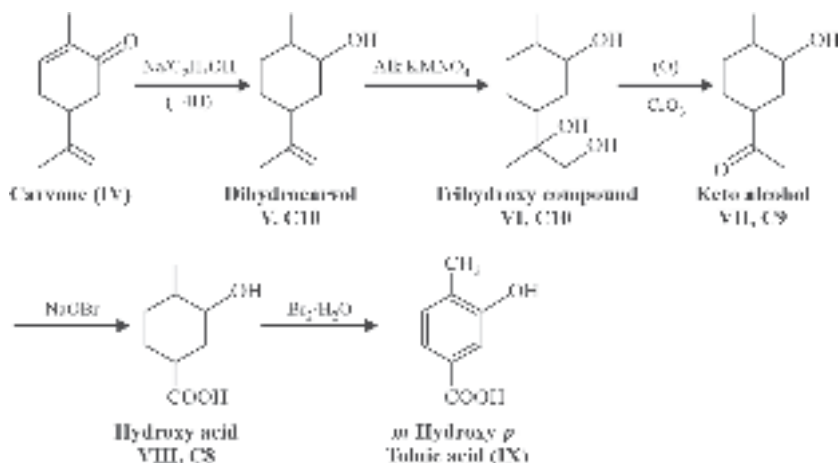
The limonene on reaction with nitrosyl chloride gives the limonene nitrosochloride, which gives carvoxime. The carvoxime on acid treatment gives the carvone. The same carvoxime can be formed from the reaction of carvone with hydroxyl amine.

- (vii) The position of the double bonds at C-8 and 9 positions can be further confirmed on the basis of degradation of carvone as follows:

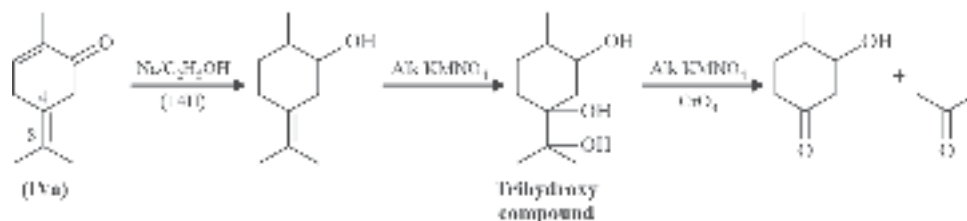


- Carvone (IV) on reduction with sodium and ethanol gives dihydrocarvol, $\text{C}_{10}\text{H}_{18}\text{O}$ (V), which is a secondary alcohol and has one double bond, one of two double bonds and the keto group have been reduced.
- Reaction with alkaline KMnO_4 hydroxylates the double bond of dihydrocarvol (V), to produce a trihydroxy compound, $\text{C}_{10}\text{H}_{20}\text{O}_3$ (VI).
- Chromic acid oxidation of trihydroxy compound (VI) results in cleavage of the glycol bond to give a keto alcohol, $\text{C}_9\text{H}_{16}\text{O}_2$ (VII).
- Treatment of VII with NaOBr losses one carbon atom to give a compound $\text{C}_8\text{H}_{14}\text{O}_3$ (VIII), which is found to be a hydroxyl monocarboxylic acid. Further, since one carbon atom is lost in the formation of VIII, its precursor (VII) must be a methyl ketone.
- Finally, dehydrogenation of (VIII) by heating with bromine water gives *m*-hydroxy-*p*-toluic acid (IX), a simple known compound.

The above reactions can be explained by assuming the double bond present at C-8 and 9 position. On the basis of above reactions, the structure IV is assigned to carvone.

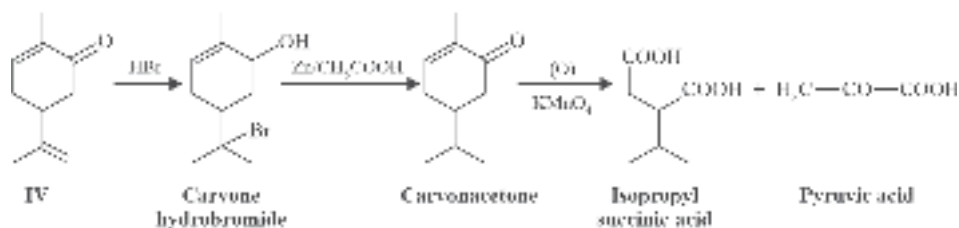


In case, this double bond is in C-4 and C-8 positions (IVa) then compound VIII and IX cannot be obtained, since three carbon atoms would be lost during the oxidation.

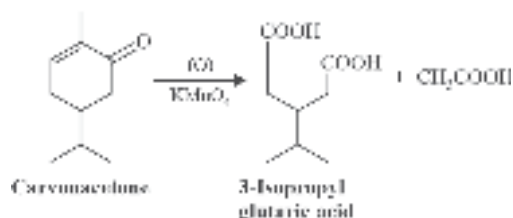


Further, the structure IV has a chiral centre, whereas IVa is symmetric and so cannot exhibit optical activity. Since carvone is known to show optical activity, structure IV is possible one.

It further gives the confirmation of double bond is present at C-6 position. The position C-6 of the second double bond in the carvone is further established by the formation of isopropyl succinic acid and pyruvic acid in its graded oxidation as shown below:



The formation of these products can only be explained if there is a double bond at C-6 position in the ring. In case double bond is in the C-7 position, formic acid (not pyruvic acid) will be obtained.



Further support for the double bond in the C-6 position is obtained by the oxidation of Carvonacetone (obtained above) with KMnO_4 solution to give 3-isopropylglutaric acid and acetic acid.

On the basis of the above facts, the structure of carvone is established as *p*-menth-6,8-diene-2-one(IV).

Spectral Data

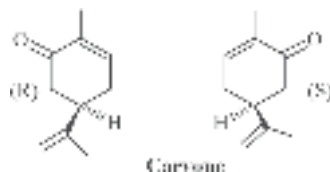
UV: λ_{max} 235 nm (ϵ 19000).

IR (cm^{-1}): 1650–1720 (conjugated ketone).

$^1\text{H-NMR}$ (δ): 1.2 (3H, s, CH_3 -1) 1.26 (3H, s, CH_3 -8) 5.22 (=CH) (olefinic proton), 6.5 ($-\text{CH}=\text{C}(\text{CH}_3)-\text{C}=\text{O}$) (Characteristic to α,β -unsaturated ketone).

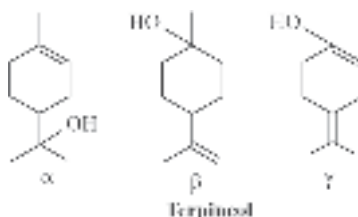
Stereochemistry

Carvone exists as a pair of enantiomers. (R)-(-)-carvone smells like spearmint, where as (S)-(+)-carvone smells like caraway.



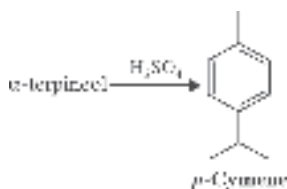
5. α -Terpineol

Terpineol is a naturally occurring monoterpene alcohol that has been isolated from a variety of sources such as cajuput oil, pine oil, and petit grain oil. There are three isomers, α -, β -, and γ -terpineol, the last two differing only by the location of the double bond. Terpineol is usually a mixture of these isomers with α -terpineol, b.p. 219°C as the major constituent.



Structural Elucidation α -Terpineol

- (i) **Molecular formula:** It is found to be $\text{C}_{10}\text{H}_{18}\text{O}$.
- (ii) **Presence of double bond:** It contains one double bond, confirmed by its reaction with bromine to form dibromide.
- (iii) **Nature of Oxygen:** Oxygen atom present is found to be present as tertiary alcoholic group.
- (iv) The saturated hydrocarbon of α -terpineol has the molecular formula $\text{C}_{10}\text{H}_{20}$. This corresponds to the general formula, C_nH_{2n} . It means that it is a monocyclic monoterpenoid.
- (v) **Presence of *p*-Cymene nucleus:** On heating with sulphuric acid, it forms *p*-cymene, indicating the presence of *p*-cymene skeleton.



- (vi) Oxidation of α -terpineol with 1% KMnO_4 solution gives a trihydroxy compound, $\text{C}_{10}\text{H}_{20}\text{O}_3$. This on oxidation with chromic acid produces a compound with molecular formula $\text{C}_{10}\text{H}_{16}\text{O}_3$. This compound is shown to be a lactone of monocarboxylic acid. There is no loss of carbon atom in its formation. It indicates that the double bond must be present in a ring of α -terpineol. Further warming with alkaline

Synthesis of Terpenylic Acid

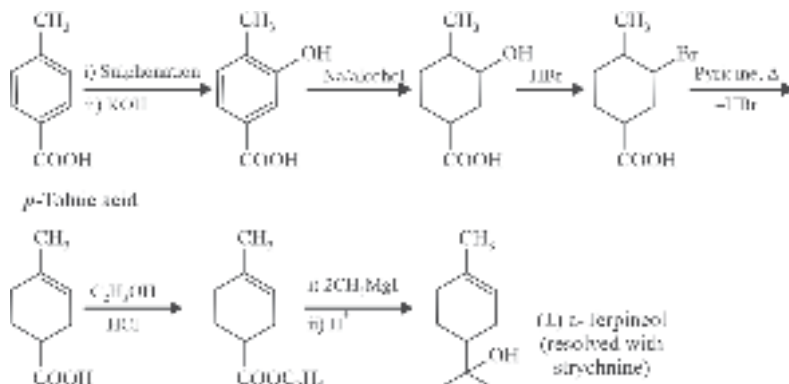
$$\begin{array}{c}
 \text{CH}_3\text{COCH}_2\text{COOC}_2\text{H}_5 \\
 \text{ethyl acetoacetate}
 \end{array}
 \xrightarrow[\text{-HCl-CH}_2\text{COOH}]{\text{(1) CH}_3\text{I}/\Delta}
 \begin{array}{c}
 \text{CH}_3\text{CO}-\text{CH}-\text{COOC}_2\text{H}_5 \\
 | \\
 \text{CH}_2-\text{COOC}_2\text{H}_5
 \end{array}
 \xrightarrow[\text{EtOCH}_2\text{COOEt}]{\text{(2) I}_2/\Delta}
 \begin{array}{c}
 \text{COOC}_2\text{H}_5 \\
 | \\
 \text{CH}_3\text{CO}-\text{C}-\text{CH}_2-\text{COOC}_2\text{H}_5 \\
 | \\
 \text{CH}_2-\text{COOC}_2\text{H}_5
 \end{array}
 \xrightarrow[\text{in H}^+, \text{Heat}]{\text{EtOAc, KOH}}
 \begin{array}{c}
 \text{HOOC}-\text{C}-\text{CH}_2-\text{CH}_2-\text{COOH} \\
 | \\
 \text{CH}_2-\text{COOH}
 \end{array}
 \xrightarrow[\text{to II'}]{\text{(3) CH}_3\text{MgI}}
 \begin{array}{c}
 \text{CH}_3 \\
 | \\
 \text{HOOC}-\text{C}-\text{CH}-\text{CH}_2-\text{COOH} \\
 | \quad | \\
 \text{OH} \quad \text{CH}_2-\text{COOH}
 \end{array}
 \xrightarrow{-\text{H}_2\text{O}}
 \begin{array}{c}
 \text{HOOC} \quad \text{CO} \\
 \diagdown \quad / \\
 \text{C} \\
 / \quad \backslash \\
 \text{CH}_2 \quad \text{CH}_2
 \end{array}$$

Terpenylic acid

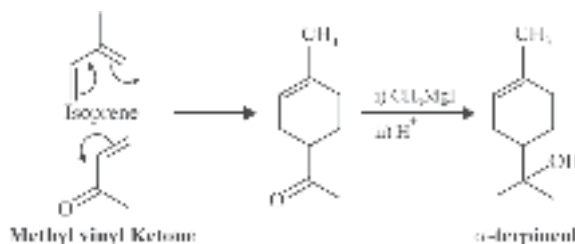
$$\text{CH}_3\text{COCH}_2\text{CH}_2\text{CO}_2\text{C}_2\text{H}_5 \xrightarrow[\text{7) CH}_3\text{CH}_2\text{CH}_2\text{NH}_2]{\text{1) Na, 2) SO}_2} \text{C}_2\text{H}_5\text{O}_2\text{C}-\text{C}(\text{CH}_3)(\text{CH}_2\text{CH}_2\text{CO}_2\text{C}_2\text{H}_5)=\text{CH}_2 \xrightarrow[\text{5) H}]{\text{3) CH}_3\text{MgI}} \left[\text{HOOC}-\text{C}(\text{CH}_3)(\text{CH}_2\text{CH}_2\text{COOH})=\text{CH}_2 \right] \xrightarrow{-\text{H}_2\text{O}} \text{HOOC}-\text{C}(\text{CH}_3)(\text{CH}_2\text{CH}_2\text{COOH})-\text{CH}=\text{CH}_2$$

Tetrablenic acid

Finally, the assigned structure of α -terpineol is confirmed by its synthesis (Scheme 4.7) from *p*-toluic acid.



It can also be synthesized by much simpler way from Diels-Alder reaction of isoprene with methyl vinyl ketone as per **Alder** and **Vogt** method.



Spectral Data

IR (cm^{-1}): 3363 (OH), 1674 (double bond), 1157, and 1126 (C–O, stretching and bending).

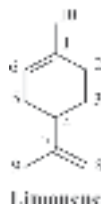
$^1\text{H-NMR}$: 1.24 (6H, $2 \times \text{CH}_3$), 1.71 (1H, Ring-H), 1.82 (3H, CH_3), 1.74, 1.49 (2H, Ring- CH_3), 2.04, 1.79 (2H, Ring- CH_2), 2.01, 1.91 (2H, Ring- CH_2), 3.65 (1H, OH), 5.3 (1H $_2$ = CH).

MS (m/e): 154[M^+]: 136(20), 121(55), 99(5), 93(75), 81(17), 68(6), 59(100), 43(16).

6. Limonene

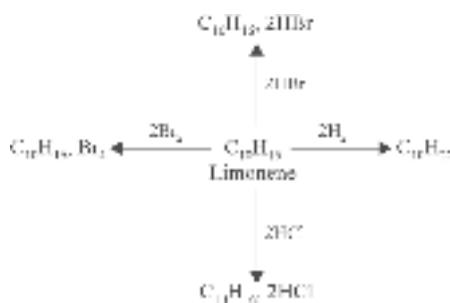
Limonene, b.p. 176°C is a colorless liquid hydrocarbon classified as a cyclic monoterpene. The more common D-isomer has a strong smell of oranges. It is used in chemical synthesis as a precursor to carvone and as a renewably-based solvent in cleaning products.

Limonene takes its name from the lemon, as the rind of the lemon, like other citrus fruits, contains considerable amounts of this monocyclic terpene, which contributes to their odor. Limonene is a chiral molecule, and biological sources produce only one enantiomer. The principal industrial source, citrus fruit, contains D-limonene (+)-limonene), which is the 5(R)-enantiomer. Racemic limonene is known as dipentene. D-Limonene is obtained commercially from citrus fruits through two primary methods: centrifugal separation or steam distillation.

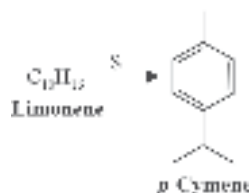


Structural elucidation of limonene

- (i) **Molecular formula:** It is found to be $\text{C}_{10}\text{H}_{16}$.
- (ii) **Presence of two double bonds:** Limonene adds on two molecules of bromine to form crystalline tetrabromo products. It also forms dihydrogen bromide by reacting with two moles of HBr. All these reactions indicate the presence of two double bonds.

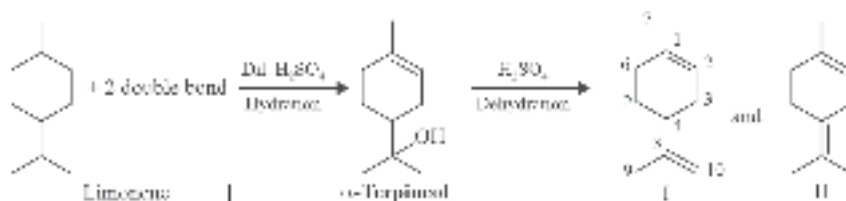


- (iii) On catalytic hydrogenation, it consumes two molecules of H_2 to form *p*-menthane. The *p*-menthane is a saturated compound, corresponding to molecular formula, $C_{10}H_{20}$. The *p*-menthane has a cyclic structure, therefore limonene also has the cyclic structure.
- (iv) **Presence of *p*-Cymene skeleton:** On dehydration with sulphur, limonene yields *p*-cymene confirming the latter skeleton in its structure.



- (v) Now, the only problem is to assign the position of the two double bonds in the fully saturated nucleus of *p*-menthane. The position of the two double bonds was established with reference to the structure of α -terpineol and carvone.

Limonene on hydration with dilute H_2SO_4 gives α -terpineol, which on dehydration yields back the limonene. Now, since the structure of α -terpineol is known, the possible dehydration products may be written as I or II.

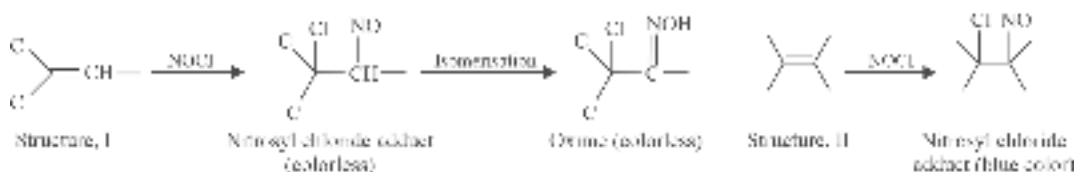


Thus, either I or II must be limonene and since, both the structure (I and II) have one double bond in between C-1 and C-2 position.

It is further confirmed by the oxidation of limonene with $KMnO_4$ to terpenylic and terebic acid (also obtained the oxidation of α -terpineol) during which only the double bond between C-1 and C-2 is affected.

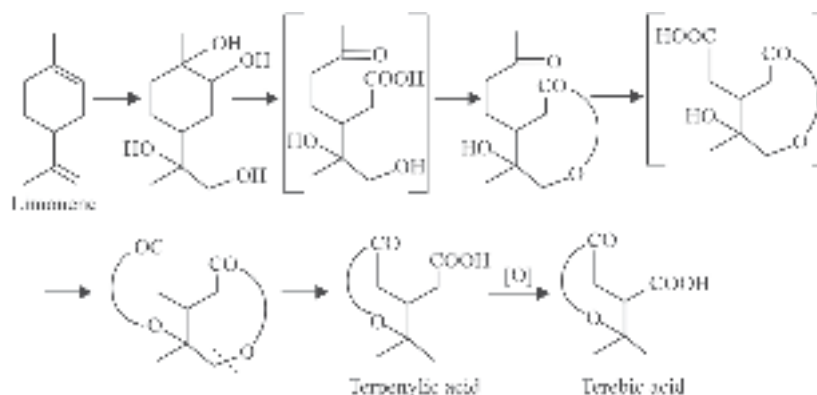
The second bond in the limonene is found to be between C-8 and C-10 position by the following two facts:

- (a) As limonene is optically active it must have at least one asymmetric carbon atom which is possible only in structure I (C-4 is asymmetric), while in the structure II, there is no asymmetrical carbon atom and hence, it must be optically inactive, and of course found to be so and identified as terpinolene.
- (b) Limonene on treated with nitrosyl chloride gives colorless product indicating that the double bond at which reagent is added is constituted by a tertiary and secondary or primary carbon atom. Hence, in limonene a double bond cannot be between C-4 and C-8 position as in II which will give the blue colored product with **Tilden's** reagent and hence limonene must be I.

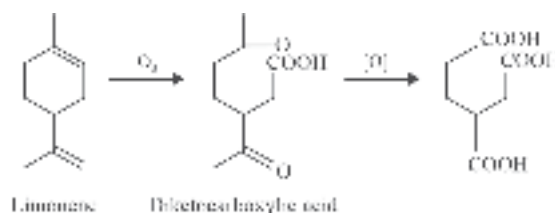


(vi) Thus, limonene must be I which explain all its reaction, viz, hydration to α -terpineol, oxidation to terpenylic and terebic acids, ozonolysis to a diketomono-carboxylic acid, and conversion to carvone through colorless carvoxime.

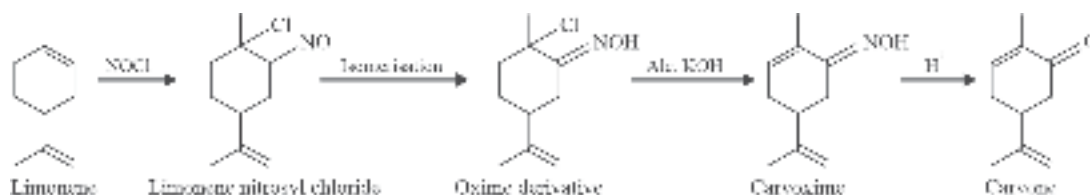
(a) Oxidation to terpenylic and terebic acid.



(b) Ozonolysis to diketocarboxylic acid.

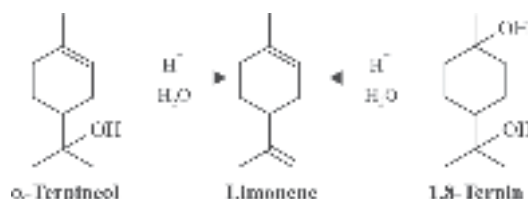


(c) Formation of carvone.



(vii) **Synthesis of limonene:**

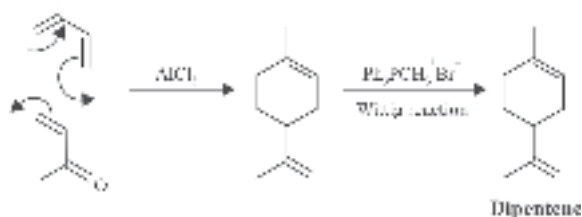
(a) Limonene may be obtained by the dehydration of 1,8-terpin or α -terpineol.



(b) Dipentene mixture of two (d, l) isomers is obtained although in poor yield by the Diels-Alder dimerisation of isoprene in a sealed tube. The reaction is reversible and the dipentene may be decomposed to isoprene in 60% yield by pyrolysis.



(c) The Diel's-Alder reaction between isoprene and methylvinyl ketone followed by a simple Wittig reaction gives the dipentene.



Spectral Data

UV: $\lambda_{\max}$ 207 nm.

IR (cm^{-1}): 3100, 1630.

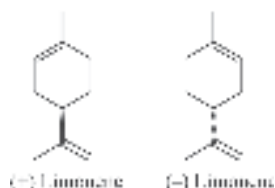
$^1\text{H-NMR}$ (δ): 1.2 (3H, s, CH_3 -1), 1.31 (3H, s, CH_3 -9), 1.5 (1H, m, C-5a), 1.71 (1H, m, C-5e), 1.9 (1H, m, C-6a), 2.1 (1H, m, C-6b), 1.99 (1H, m, C-3a), 2.1 (1H, m, C-3e), 4.7 (2H, s, C-8 methylene).

MS (m/e): 136 (M^+), 121 (M-15), 107 (M-29), 93 (M-43), 68 (base peak) (**Retro-Diels-Alder** reaction).

Stereochemistry in Limonene

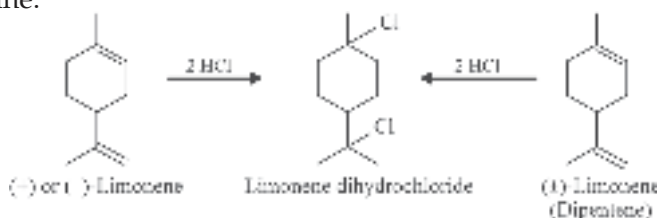
Limonene exists in two enantiomers that are characterized by specific rotation; $[\alpha]_D$ values of D and L-limonene are +123 and -123, respectively.

In addition, the two enantiomers differ in the aroma: orange for (+) limonene and lemon for (-) limonene.



The connection between limonene and dipentene is shown by the fact that (+) or (-) limonene on addition of two molecules of HCl in presence of moisture forms limonene dihydrochloride which is identical to dipentene dihydrochloride.

Limonene can be regenerated by heating this with sodium acetate in acetic acid or boiling with aniline.

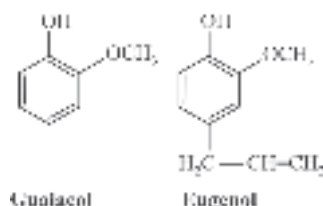


7. Eugenol

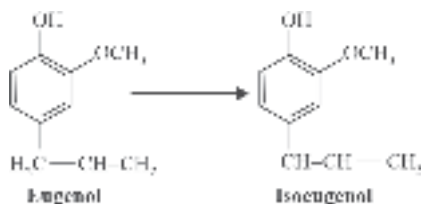
Eugenol is the principal ingredient of clove oil and responsible for its characteristic spicy fragrance. It also occurs in many other essential oils but mainly eugenol is isolated from clove oil. Eugenol is used in perfumes, mouth washes, etc. It is also used as an insect attractant and dental analgesic.

Structural Elucidation of Eugenol

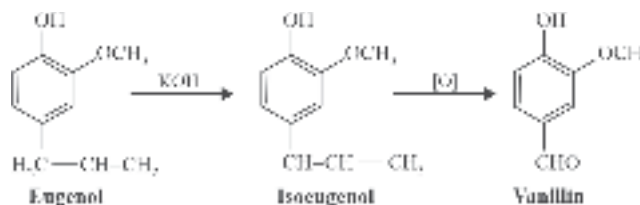
- (i) **Molecular formula:** It is found to be $C_{10}H_{12}O_2$.
- (ii) Eugenol is phenolic ether (α -allyl guaiacol) having b.p. 254°C . Guaiacol is also a phenolic ether containing one $-\text{OCH}_3$ group ortho to phenolic hydroxyl group. In Eugenol, one more allyl group is present other than a hydroxyl and methoxy group at C-4 position. Thus, structure of eugenol may be written as shown here.



- (iii) On heating with ethanolic potassium hydroxide, eugenol isomerizes to isoeugenol which is 4-propenyl guaiacol. Isoeugenol, b.p. 267.5°C , also occurs naturally.



- (iv) Presence and position of one $-\text{OH}$, one $-\text{OCH}_3$ and side chain in eugenol is also confirmed by its conversion to vanillin which is *p*-Hydroxy-*m*-methoxybenzaldehyde. Eugenol first isomerizes to isoeugenol which is oxidized to vanillin.



Spectral Data

UV: λ_{max} 279 nm.

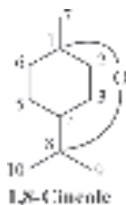
IR (cm^{-1}): 3400, 1630.

$^1\text{H-NMR}$ (δ): 3.32 (2H, m, benzylic protons), 3.38 (3H, s, OCH_3), 5.0 (1H, OH) 5.45 to 5.63 (3H, m, allylic protons), 6.6-6.75 (3H, aromatic protons).

MS (m/e): 164 (M^+), 149 ($\text{M}^+ - \text{CH}_3$), 137 ($\text{M} - \text{CH}=\text{CH}_2$), 123 ($\text{M} - \text{CH}_2\text{CH}=\text{CH}_2$).

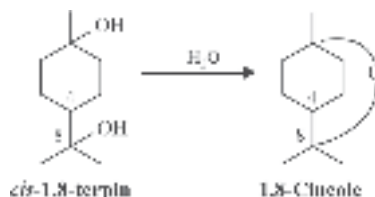
8. 1,8-Cineole

1,8-Cineole, also known as Eucalyptol, is a naturally occurring monoterpene. It is colorless cyclic ether, b.p. 176°C. It has a fresh camphor-like smell and a spicy, cooling taste. It is insoluble in water, but miscible with ether, ethanol and chloroform. Because of its pleasant spicy aroma and taste, eucalyptol is used in flavorings, fragrances, and cosmetics.



Structural Elucidation of 1,8-Cineole

- (i) Its molecular formula is $C_{10}H_{18}O$. It is isomeric with α -terpineol but contains neither hydroxyl group nor a double bond.
- (ii) The oxygen atom is inert in nature because it is unreactive to sodium metal or by usual reducing agents. This suggests the adjustment of oxygen atom in ether form.
- (iii) It is formed by dehydration reaction of *cis*-1,8-terpin. On the basis of this reaction, structure is assigned to 1,8-cineole.



- (iv) Further support comes from the following sequence of reaction. 1,8-cineole on oxidation with $KMnO_4$ form cineolic acid (dicarboxylic acid) which on distillation with acetic anhydride forms cineolic anhydride. The cineolic anhydride on distillation forms 6-methyl hept-5-ene-2-one.



Spectral Data

UV (methanol): $\lambda_{\max}$ 262 nm.

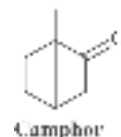
IR (cm^{-1}): 2175 (O-H), 2985 (C-H), 1375 (C-H), 1168 (C-O).

^1H -NMR (CD_3OD) (δ): 1.03 (s, 3H, H-7), 1.21 (s, 3H, H-10), 1.29 (s, 3H, H-9), 1.54–1.61 (m, 3H, H-2a, H-6a, H-6b), 1.74 (m, 1H, H-5a), 1.81 (m, 1H, H-4), 2.08 (m, 1H, H-2b), 2.18 (m, 1H, H-5b).

MS (m/e): 154 (M^+), 139 ($M-CH_3$), 136 ($M-H_2O$).

Bicyclic Terpenoidal Compounds

9. Camphor

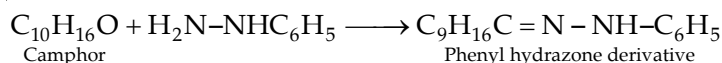


It is solid having m.p. 180°C optically active; the (+) and (–) forms occur in nature. It is obtained by steam distillation of wood, leaves or bark of a tree known as the camphor laurel, or *Cinnamomum camphora*. It sublimes at room temperature.

It is used in pharmaceutical preparation because of its analgesic, stimulant for heart muscles, expectorant and antiseptic properties. It is used in manufacture of celluloid, smokeless powder and explosives. It is also used as moth repellent.

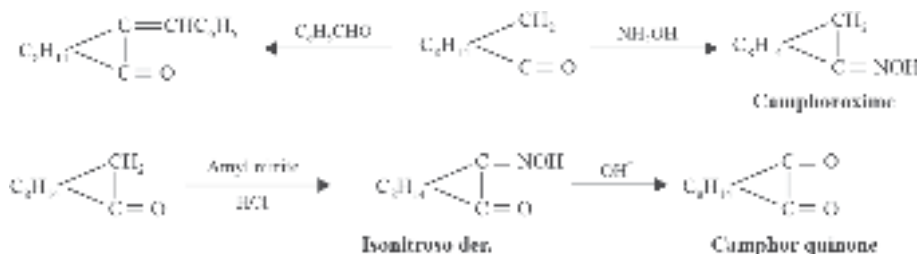
Structural Elucidation of Camphor

- (i) **Molecular formula:** By usual method. It is found to be $C_{10}H_{16}O$.
- (ii) **Saturated nature:** General reactions like formation of mono substituted products (mono-bromo-monochlorocamphor) show that it is saturated.
- (iii) **Nature of oxygen atom:** Nature of oxygen atom in camphor is found to be ketonic as it forms oxime on reaction with hydroxyl amine, and phenyl hydrazone derivative with phenyl hydrazine.



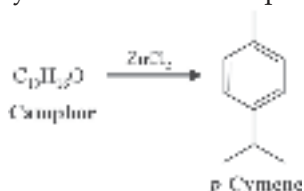
Camphor when oxidized with nitric acid yields a dicarboxylic acid called camphoric acid, without loss of carbon atoms. On reduction with sodium amalgam it gives secondary alcohol; borneol. Thus, oxo function in camphor is cyclic ketone.

Reaction of camphor with amyl nitrite and HCl resulted in an isonitroso derivative to reveal the presence of a methylene group alpha to carbonyl group. Hydrolysis of isonitroso compound gives camphor-quinone, which supported the presence of methylene group adjacent to carbonyl functional group.

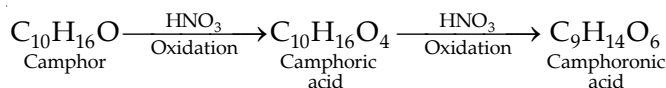


Presence of methylene group adjacent to keto function is also supported by the formation of benzylidene derivative on reaction with benzaldehyde.

- (iv) **Presence of bicyclic system:** Molecular formula of saturated parent hydrocarbon of camphor is $C_{10}H_{18}$ corresponds to the general formula of a bicyclic compound (C_nH_{2n-2}).
- (v) **Presence of a six membered ring:** On distillation with zinc chloride, it yields *p*-cymene. Formation of *p*-cymene confirms the presence of six-membered ring.



- (vi) **Nature of carbon skeleton:** Bredt assigned the correct formula to camphor on the basis of above facts and also on the basis of oxidation products obtained from camphor. Oxidation of camphor with nitric acid gives camphoric acid, $C_{10}H_{16}O_4$ and further oxidation of camphoric acid gives camphoronic acid, $C_9H_{14}O_6$.



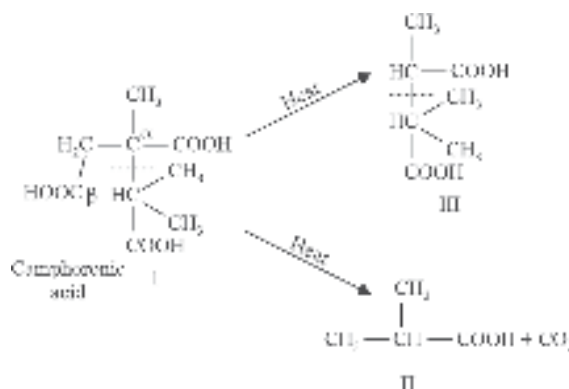
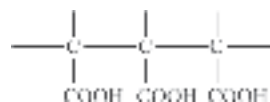
Camphoric acid is saturated dicarboxylic acid, with the same number of carbon atoms as camphor. It suggest that keto group is present in one of ring, and ring containing keto group is opened in the formation of camphoric acid. Thus, camphoric acid should be a monocyclic compound.

Camphoronic acid is tricarboxylic acid. In order to determine the structure of camphor, the structures of camphoric acid and camphoronic acid should be known.

- (vii) **Structure of camphoronic acid and camphoric acid:**

- (a) Camphoronic, $C_9H_{14}O_6$ is found to be saturated tricarboxylic acid, and on distillation at atmospheric pressure, it gives isobutyric acid II, trimethyl succinic acid III, and carbon dioxide. But it does not undergo decarboxylation under ordinary condition, which shows that three carboxylic groups are attached to the different carbon atoms.

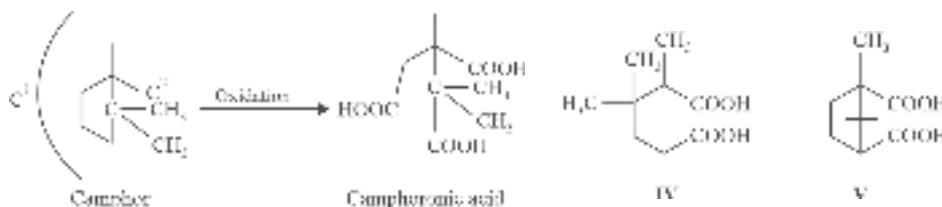
To explain the formation of these products, Bredt suggested that camphoronic acid is α,α,β -trimethyl tricarboxylic acid (I).



Above proposed structure for camphoronic acid is confirmed by its synthesis given by Perkin junior and Thorpe (1897). Camphoric acid is found to be saturated dicarboxylic acid. The three methyl groups are present in camphoric acid as present in above structure of camphoronic acid, I. So, camphoric acid is $(CH_3)_3C_5H_5(COOH)_2$. The saturated hydrocarbon of this (C_5H_{10}) corresponds to the general formula C_nH_{2n} . Thus, camphoric acid is cyclopentane derivative and oxidation of camphoric acid to camphoronic acid may be written as:

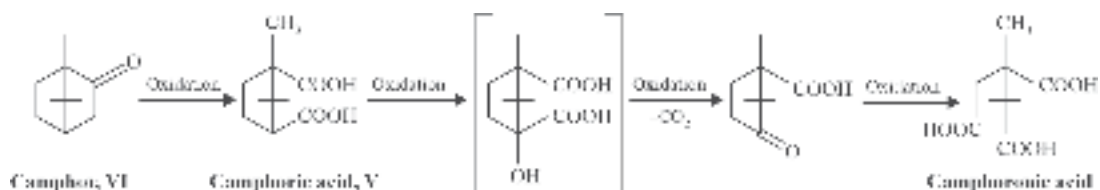
Camphoric acid forms only one mono-bromo-derivative, it means one α -hydrogen is there in camphoric acid. Thus, the carbon atom of one carboxylic acid must be ^1C . But question arises what should be the position of other $-COOH$ group, when cyclopentane ring open on oxidation. It opens with loss of

one carbon atom to give camphoronic acid. So two structures (IV) and (V) are proposed for camphoric acid.

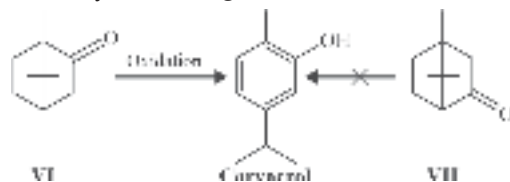


The structure (V) accounts for all the facts given in the foregoing discussion.

(viii) **Structure of camphor:** Bredt therefore proposes the structure (V) for camphoric acid and structure (VI) for of Camphor. With this, the complete sequence of reactions can be explained.

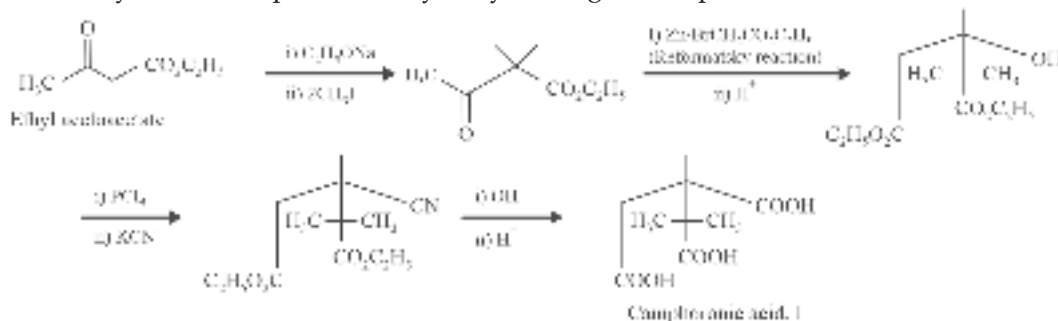


Bredt also proposes structure (VII) for the camphor, but he rejected (VII) in favor of (VI) because camphor gives carvacrol when distilled with iodine. Formation of which can be explained by assuming the structure (VI) for camphor.

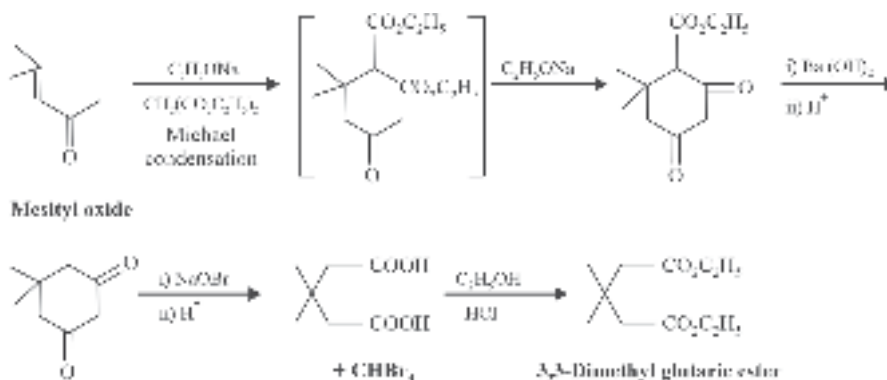


(ix) **Synthesis:** Finally, structure was confirmed by the synthesis. All the deductions of Bredt are confirmed by the synthesis of camphoronic acid, camphoric acid and Camphor.

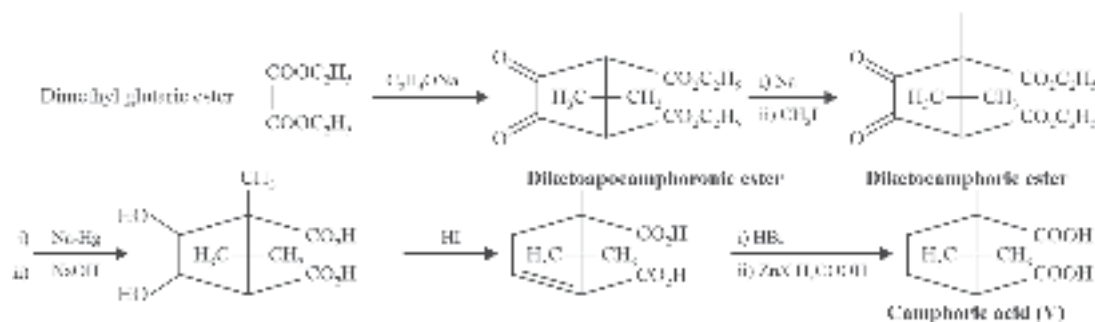
(a) **Synthesis of (±)-Camphoronic acid:** This synthesis is given by Perkin junior and Thorpe (1987). In this synthesis, ethyl acetoacetate is converted to its dimethyl derivative, which undergoes Reformatsky reaction with ethyl acetate derivative. The product so obtained is converted into halide and then into cyanide. Last product is hydrolyzed to give camphoronic acid.



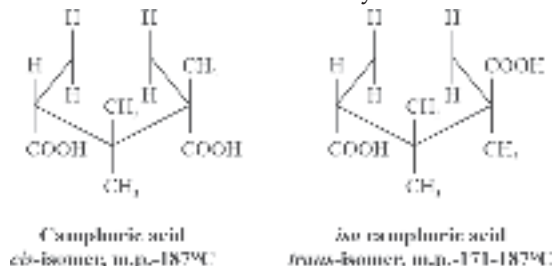
(b) **Synthesis of (±)-Camphoric acid:** This is given by Komppa (1903). He first synthesized 3,3-dimethyl glutaric acid from mesityl oxide and ethyl malonate as follows.



Komppa (1903) then prepared camphoric acid from 3,3-dimethylglutaric acid as follows:

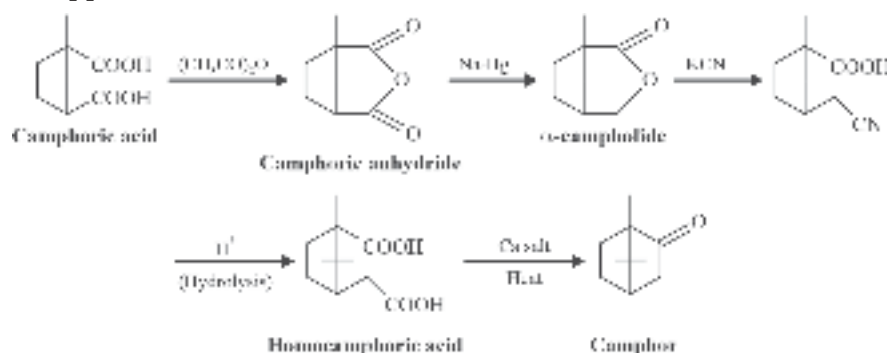


Camphoric acid can exist in two geometrical isomeric forms, *cis* and *trans*. The *cis* form is camphoric acid and form anhydride and the *trans* form is isocamphoric acid and does not form anhydride on heating.

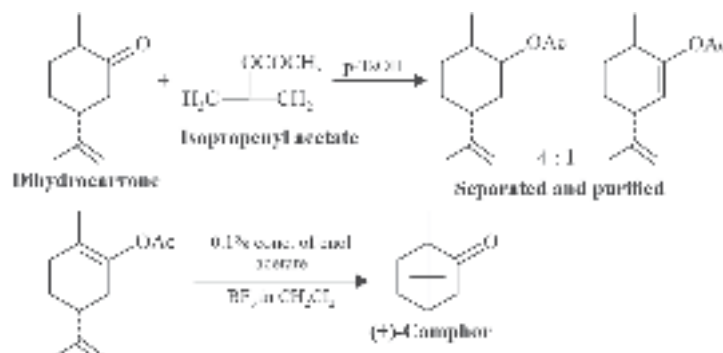


Synthesis of Camphor

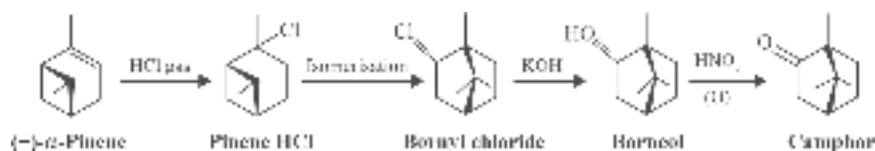
- (i) Haller gave this synthesis of camphor from camphoric acid, which was synthesized by Komppa.



- (ii) Another two step synthesis of camphor from dihydrocarvone was given by Money et al.



- (iii) Commercially, camphor is synthesized from α -pinene (given below).



Difference between natural and synthetic camphor is illustrated in Table 4.3.

Table 4.3: Difference between natural and synthetic camphor

Property	Natural camphor	Synthetic camphor
Optical rotation	Optically active (d or l)	Optically inactive (dl)
Test for chloride	Negative	Positive
Gentle heating	Yellow to green to blue	Yellow
Heating	Color (due to other volatile contaminants)	Yellow

Spectral Data

UV (methanol): λ_{max} 288 (ϵ 36).

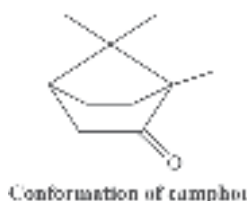
IR (cm^{-1}): 1740, 1422, 1050.

$^1\text{H-NMR}$ (δ): 0.9 (3H, CH_3), 1.09 (3H, CH_3), 1.19 (3H, 3CH_2), 2.0 (4H, $2 \times \text{CH}_2$), 2.2 (1H, CH), 2.5 (2H, CH_2CO).

MS (m/e): 152 (M^+), 109, 108, 95.8, 83, 69.

Stereochemistry

Though camphor has two dissimilar chiral centers but only one pair of enantiomers is possible, since only cis fusion of the gem dimethylmethylene to cyclohexane ring is possible. Transfusion of the gem dimethylmethylene bridges to the cyclohexane ring is impossible. Boat conformation with cis fusion of gem dimethylmethylene is shown below.



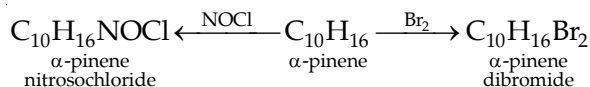
10. α -Pinene

α -Pinene is an organic compound of the bicyclic monoterpene class, one of two isomers of pinene. It is found in the oils of many species of many coniferous trees, notably the pine. It is also found in the essential oil of rosemary.

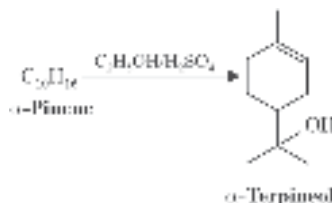


Structural elucidation of α -Pinene

- (i) **Molecular formula:** It is found to be α -pinene is $C_{10}H_{16}$.
- (ii) **Presence of one double bond:** It is indicated by the following sequence of reactions:
 - (a) α -pinene on treatment with bromine produce α -pinene dibromide with the consumption of one bromine molecule.
 - (b) α -pinene on treatment with nitrosyl chloride to produce α -pinene nitrosochloride.



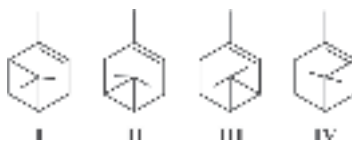
- (iii) The molecular formula of its saturated hydrocarbon $C_{10}H_{18}$ corresponds for bicyclic compounds (C_nH_{2n-2}).
- (iv) α -pinene on treatment with alcoholic H_2SO_4 gives α -terpineol. This indicates that α -pinene possess one six membered ring having the double bond like α -terpineol.



Formation of terpineol concludes:

- (a) Presence of six membered ring having the double bond at C-1 (2)-position like terpineol.
- (b) Presence of methyl group at C-1 position.
- (c) C-8 position contains hydroxyl group in terpineol while it is absent in α -pinene. This indicates that position C-8 is involved in the formation of second ring.
- (d) The second ring contains gem-dimethyl group.

On the basis of above facts, following four structures can be drawn having bicyclic structure.

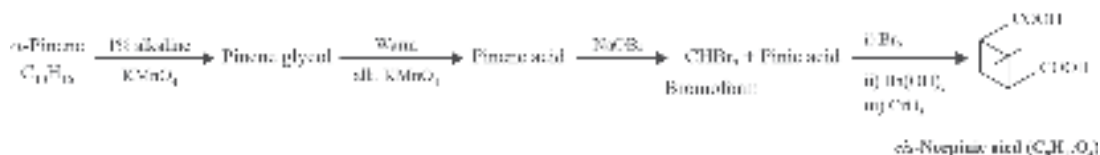


Out of four structures, one structure **IV** can be discarded on the basis of Bredt rule.

According to Bredt's rule, bridge head system cannot be formed on the carbon atom having double bond.

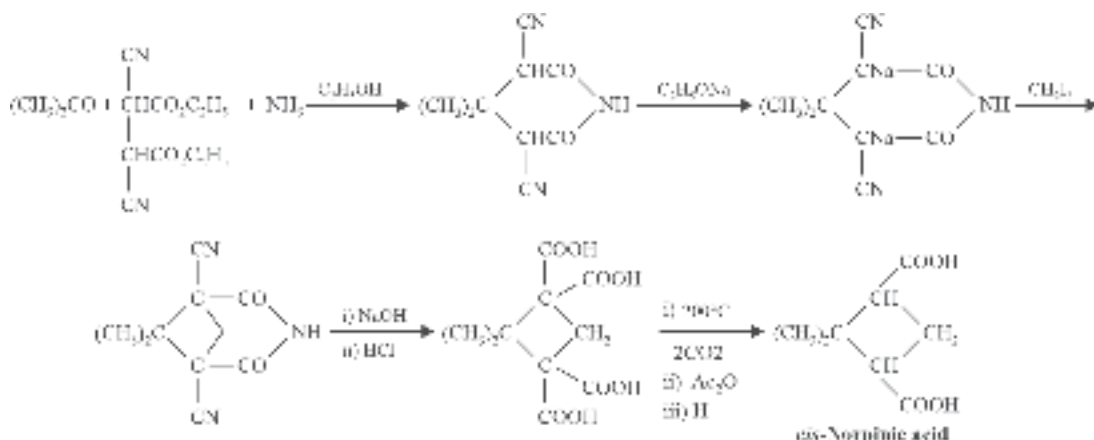
Out of remaining three structures, one structure contains four membered ring and two structures possess three membered rings system in bicyclic system. The nature of second ring structure is proved by oxidative degradation.

- (v) **Oxidative degradation of α -pinene (nature of second ring):** Baeyer concluded that the second ring is four membered ring in α -pinene on the basis of following series of reaction:

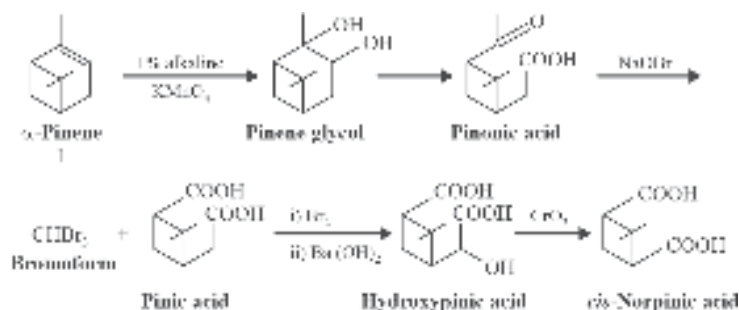


- Conversion of α -pinene to pinene glycol indicates the hydroxylation of double bond present in six membered ring.
- Oxidation of pinene glycol to pinonic acid, this show that the six membered ring having glycolic group and double bond is oxidized.
- Formation of bromoform suggests the presence of the acetyl group in previous compound, pinonic acid.
- Final oxidation product, *cis*-norpinic acid is saturated dicarboxylic acid. Its formula may be written as $C_6H_{10}(COOH)_2$. As already stated, gem-dimethyl group ($-CHMe_2$) is present in ring other than six membered. Hence, the molecular formula may be modified as $C_4H_4(CH_3)_2(COOH)_2$. There are two substituents in *cis*-norpinic acid—gem-dimethyl group and two carboxylic groups. Hence, unsubstituted saturated structure corresponds to C_4H_8 , means the second ring is four membered (cyclobutane). The position of two carboxylic groups and gem-dimethyl group is assigned on the basis of synthesis from the reaction of diethyl cyanoacetate with acetone and ammonia.

Synthesis of *cis*-Norpinic acid:



- (vi) On the basis of above discussion, the structure, **I** is assigned to α -pinene (as predicted above which explains all the sequence of reaction steps.



(vii) Finally, the proposed structure of α -pinene is confirmed by its synthesis (Scheme 4.8) from *cis*-norpinic acid.

Spectral Data

IR (cm^{-1}): 1667, 1220, 1127, 1013, 952, 885, 784.

$^1\text{H-NMR}$ (δ): 0.85 and 1.28 (6H, s, C-7 methyls), 1.65 (3H, dd, C-1 methyl), 1.82 (1H, $\text{C}_2\text{-H}$), 1.97 (1H, $\text{C}_4\text{-H}$), 5.78 (1H, m, H-2).

MS (m/e): 136 (M^+), 121, 94, 93 (100), 92, 91, 79, 77, 41.

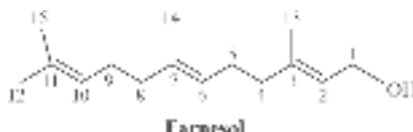
Stereochemistry

Pinene has two chiral centers but only one pair of enantiomer is known instead of two. It is due to the fact that only *cis* fusion of the gem-dimethyl methylene to cyclohexane ring is possible. *Trans* fusion of the *gem* dimethylmethylene bridges to the cyclohexane ring is impossible.

SESQUITERPENES

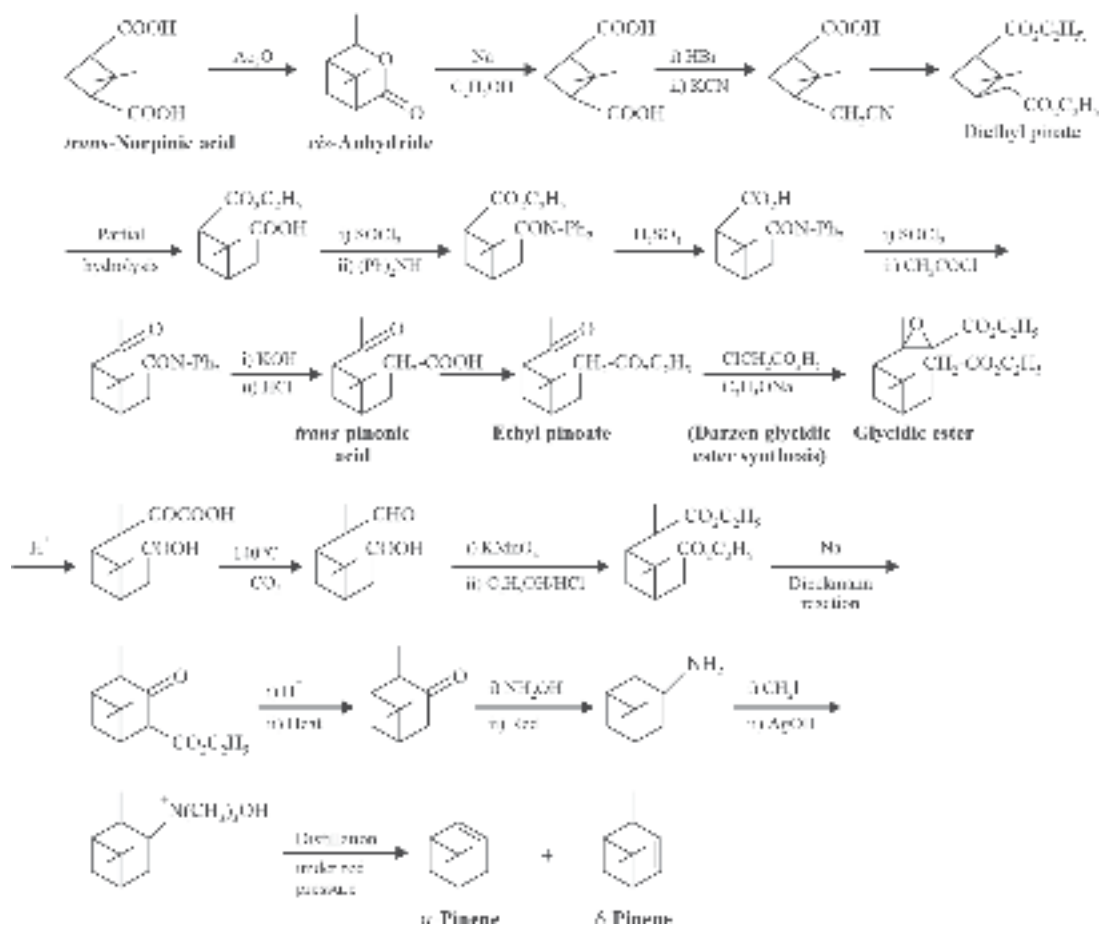
11. Farnesol

Farnesol is a naturally occurring acyclic sesquiterpene alcohol, found as a colorless liquid. It is extracted from the flowers of acacia. It is insoluble in water, but miscible with Farnesol oils. It is the building block of most, and possibly all, acyclic sesquiterpenoids and is an important starting compound for organic synthesis.

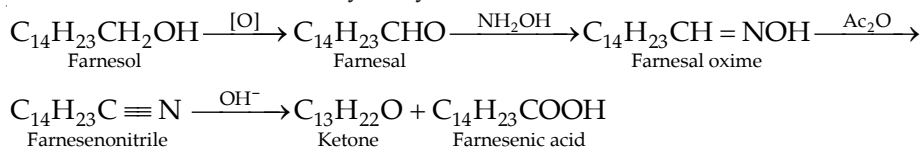


Structural elucidation of farnesol

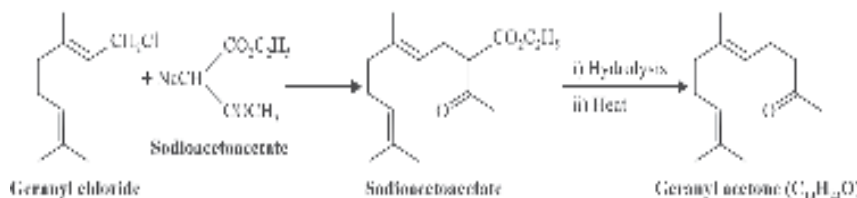
- (i) **Molecular formula:** It is found to be $\text{C}_{15}\text{H}_{26}\text{O}$.
- (ii) **Presence of double bonds:** It forms saturated derivative with the consumption of 3 molecules of hydrogen which indicates the presence of 3 double bonds. The molecular formulae of saturated compound is $\text{C}_{15}\text{H}_{32}$, corresponds to $\text{C}_n\text{H}_{2n+2}$, general formulae for acyclic compound.
- (iii) **Presence of hydroxyl group:** Usual reactions show the presence of hydroxyl group which is primary in nature because it gives aldehyde, farnesal on oxidation.
- (iv) **Structure of Farnesal:** Farnesal on oxidation yields farnesal (aldehyde) which on reaction with hydroxylamine produces farnesal oxime. The oxime on dehydration

Scheme 4.8: Synthesis of α -pinene

reaction with acetic anhydride gives nitrile. The nitrile gives a mixture of farnesic acid and ketone on alkaline hydrolysis.

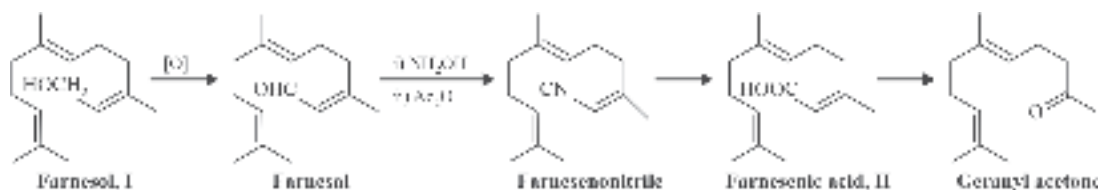


The ketone, $C_{13}H_{22}O$ obtained through the sequence of above reactions is found to identical with α,β -dihydropsuedo ionone (geranyl acetone), can be obtained from geranyl chloride and sodium acetoacetate.

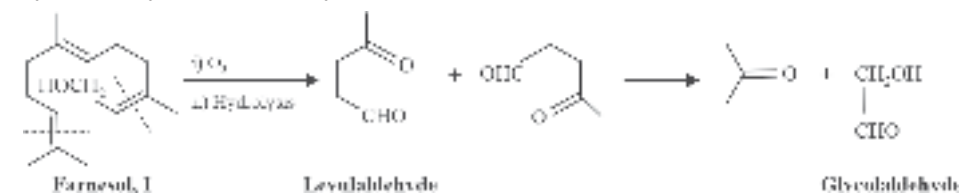


During the formation of keto compound from its precursor Farnesenitrile, there is loss of two carbon atoms (characteristic for α,β -unsaturated carbonyl

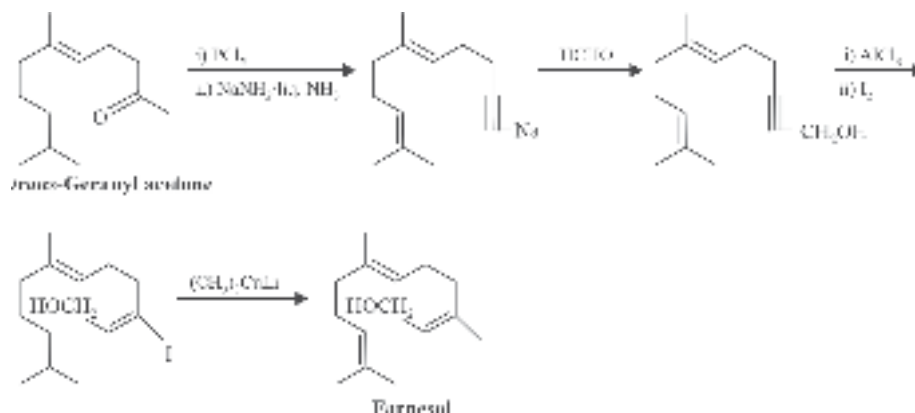
compounds). It reveals that farnesenic acid contains α,β -unsaturated carbonyl group and structure, II is assigned to this acid. The structure, I is assigned to farnesol on the basis of formation of farnesenic acid.



The assigned structure I also explains the formation of acetone, levulaldehyde and glycoaldehyde on ozonolysis.



(v) The assigned structure is confirmed from synthesis from geranyl acetone (Corey et al.).



Spectral Data

UV: λ_{max} 192–196 nm, ϵ_{max} 28500.

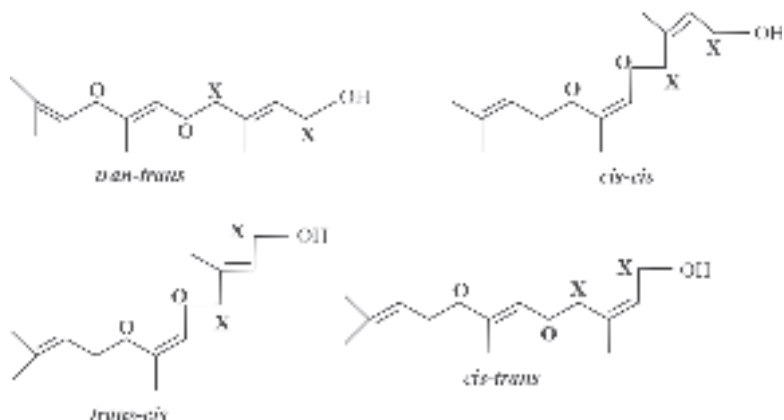
IR (cm^{-1}): 3350, 2980, 1665, 1448, 1180.

$^1\text{H-NMR}$ (δ): 1.60 (3H, s, CH_3), 1.62 (s, CH_3), 1.68 (3H, s, CH_3), 1.69 (3H, s, CH_3), 1.97–2.16 (8H, m, $4 \times \text{CH}_2$), 5.14 (t, $J = 6.6$ Hz, H-6), 5.38 (t, $J = 6.6$ Hz, H-2) and 5.46 (t, $J = 6.8$ Hz, H-10), 9.12 (s, OH).

MS (m/e): 222 (M^+), 84, 77, 69 (base peak, arising from loss of the isopentenyl end group by allylic cleavage), 41.

Stereochemistry

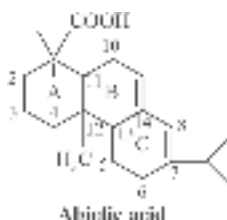
There are number of geometrical isomers of farnesol. The prefixes *cis* and *trans* are used to denote the position of methylene groups (in the main chain) with respect to each other for each double bond (in the chain) and at the end of the chain the position of the functional group with respect to methylene group of the chain (*o* and *x* denote each pair).



DITERPENES

12. Abietic acid

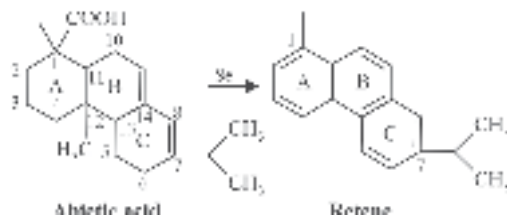
Abietic acid (also known as sylvic acid), a resin acid, is the primary irritant found in pine wood and resin, isolated from rosin or colophony (via isomerisation). It belongs to the diterpene group of organic compounds.



Abietic acid is soluble in alcohols, acetone, and ether. It was isolated by Baup in 1826 from the resin of *Pinus oabies*. Commercial abietic acid is usually a glassy or partly crystalline, yellowish solid having m.p. 172–175°C. It is used in lacquers, varnishes, and soaps, and for the analysis of resins.

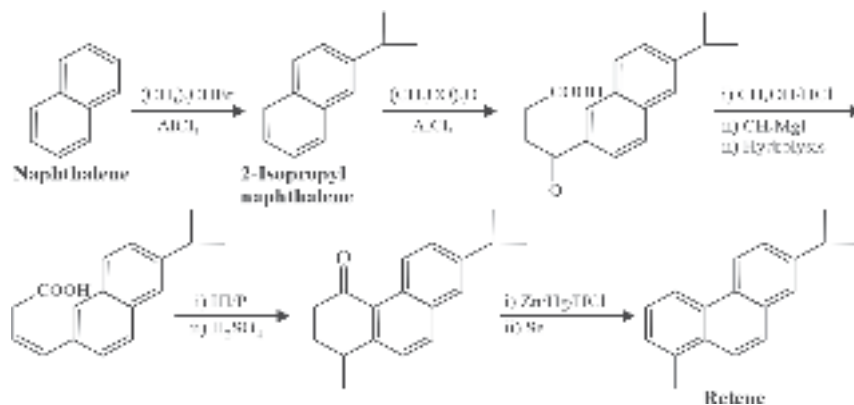
Structural elucidation of abietic acid, m.p. 172–175°C

- (i) **Molecular formula:** It is found to be $\text{C}_{20}\text{H}_{30}\text{O}_2$.
- (ii) Usual reactions show that it is a monocarboxylic acid.

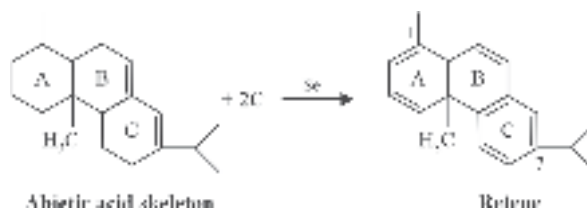


Nature of carbon skeleton: Dehydrogenation of abietic acid with sulphur, selenium or palladium-charcoal gives retene, which is identified as 1-methyl-7-isopropylphenanthrene by series of degradation steps.

The structure of retene is confirmed by synthesis from naphthalene which revealed that abietic acid has tricyclic structure.



On the basis of retene formation from abietic acid, same skeleton is suggested.



Number of Double Bond

Catalytic hydrogenation of abietic acid gives tetrahydroabietic acid, $\text{C}_{20}\text{H}_{34}\text{O}_2$. Thus abietic acid contains two double bonds. The parent hydrocarbon has molecular formula, $\text{C}_{19}\text{H}_{34}$ (excluding the carboxyl group as a substituent), which corresponds to $\text{C}_n\text{H}_{2n-4}$, indicating the tricyclic nature of abietic acid.

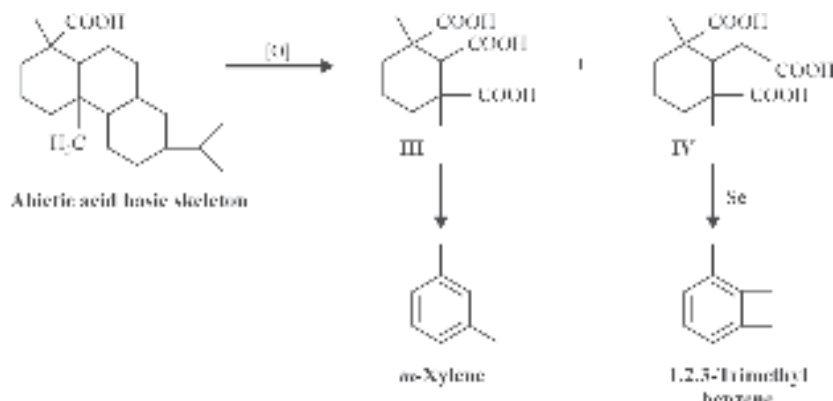
Position of Angular Methyl Group and Carboxylic Group

Formation of retene (18 carbons) takes place with the loss of 2 carbon atoms from abietic acid (20 carbons). It is suspected that in dehydrogenation reaction, angular methyl group and carboxyl group are eliminated.

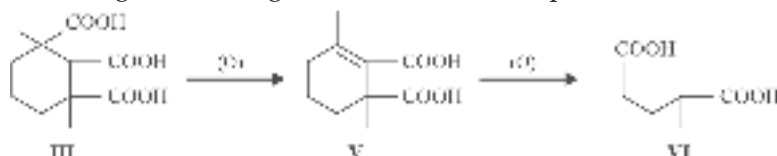
Abietic acid is difficult to esterify. This indicates that carboxylic group is attached to tertiary carbon atom. This is also supported by the chemical test. It gives carbon monoxide (CO) on warming with concentrated sulphuric acid, which is a characteristic test for carboxylic group attached to tertiary carbon atom.

Oxidation of abietic acid with KMnO_4 gives a mixture of products; among which are two different tricarboxylic acids, $\text{C}_{11}\text{H}_{16}\text{O}_6$ (III) and $\text{C}_{12}\text{H}_{18}\text{O}_6$ (IV). The compound III on dehydrogenation with selenium forms *m*-xylene and compound IV forms hemimellitene (1,2,3-trimethyl benzene). In both the cases, there is loss of three carbon atoms, and if we assume that these are three carboxyl groups, then two methyl groups in III and IV must be in the meta position. Furthermore, since III and IV each contain methyl group originally present in abietic acid (C-1 position), acids (III) and IV must contain ring A of abietic acid. This suggests the presence of an angular methyl group at C-12 position which can be expected to be eliminated from this position in dehydrogenation reaction (this 12-methyl group is meta to 1-methyl group). Vocke (1932) showed that acid (III) evolves two molecules of CO_2 when warm with concentrated sulphuric acid; this indicates that compound III contains two carboxyl groups attached to tertiary carbon atoms. These results can be explained by assuming that one carboxyl group in III is that in abietic

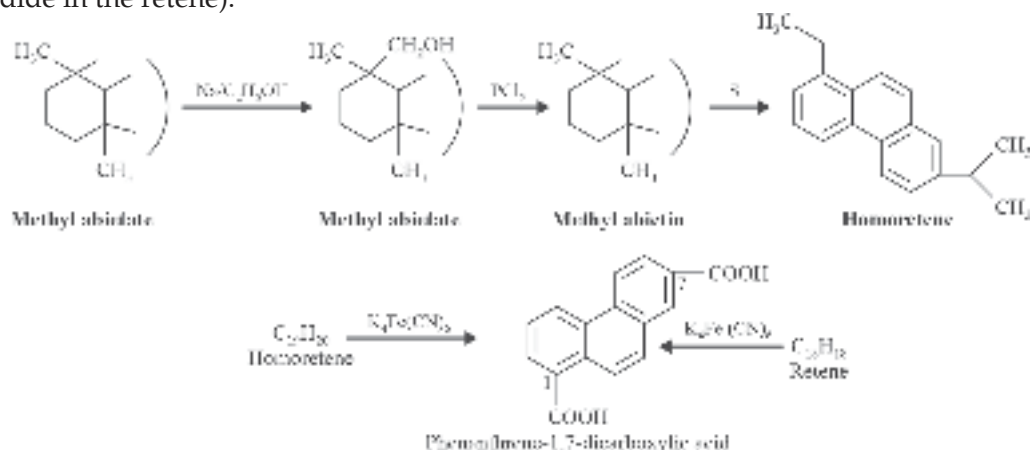
acid, and since in both cases this carboxyl group is attached to a tertiary carbon atom, the most likely position of this group is 1 (in abietic acid). Accepting these assumptions, the oxidation of abietic acid may be formulated as follows: by assuming basic skeleton to abietic acid.



Vocke subjected **III** to oxidative degradation and obtained a dicarboxylic acid **V**, which on further oxidation gave 2-methylglutaric acid (**VI**). He has formulated the reaction sequence by assuming the below given structure to compound, **III**.



The position of the carboxyl group at C-1 in abietic acid has been confirmed by **Ruzicka** et al. Methyl abietate, $C_{19}H_{29}CO_2CH_3$, on reduction with Na/C_2H_5OH forms abietinol, $C_{19}H_{29}CH_2OH$ which on treatment with PCl_5 loses a water molecule to form methylabietin, $C_{20}H_{30}$. This on distillation with sulphur forms homoretene, $C_{19}H_{20}$. Homoretene contains one CH_2 group more than retene and on oxidation with alkaline potassium ferricyanide, gives phenanthrene-1,7-dicarboxylic acid, the identical product obtained from the oxidation of retene under similar condition. These results can only be explained by assuming that homoretene has an ethyl group at position, 1 (instead of the methyl group in retene), i.e. homoretene is 1-ethyl-7-isopropylphenanthrene. This has been confirmed by synthesis (ethylmagnesium iodide is used instead of methylmagnesium iodide in the retene).

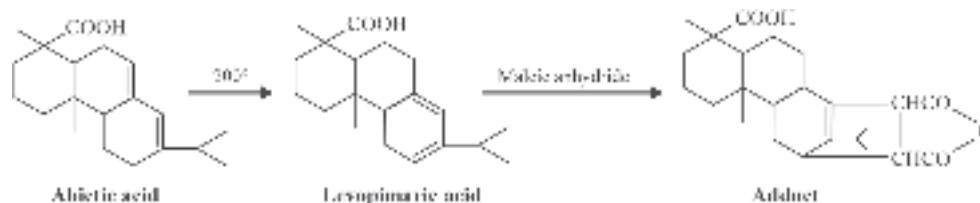


Position of two double bonds

As abietic acid forms an adduct with maleic anhydride at above 100°C , means two double bonds are conjugated. It is also shown that levopimaric acid also forms the same adduct at room temperature. It appears that abietic acid isomerises to levopimaric acid at above 100°C , and then forms the adduct. Abietic acid shows a maximum at 238 nm ($\epsilon\ 16000$) in UV region. This indicates that two double bonds are conjugated, since the basic value for homoannular diene is 253 nm , it may therefore be concluded that the two double bonds are not in the same ring. This is supported by the fact that levopimaric acid has $\lambda_{\text{max}}\ 272.5$ ($\epsilon\ 7000$), a value in agreement with the two double bonds being in the same ring in this compound (calculated value for the structure is $253 + 4 \times 5 = 273\text{ nm}$).

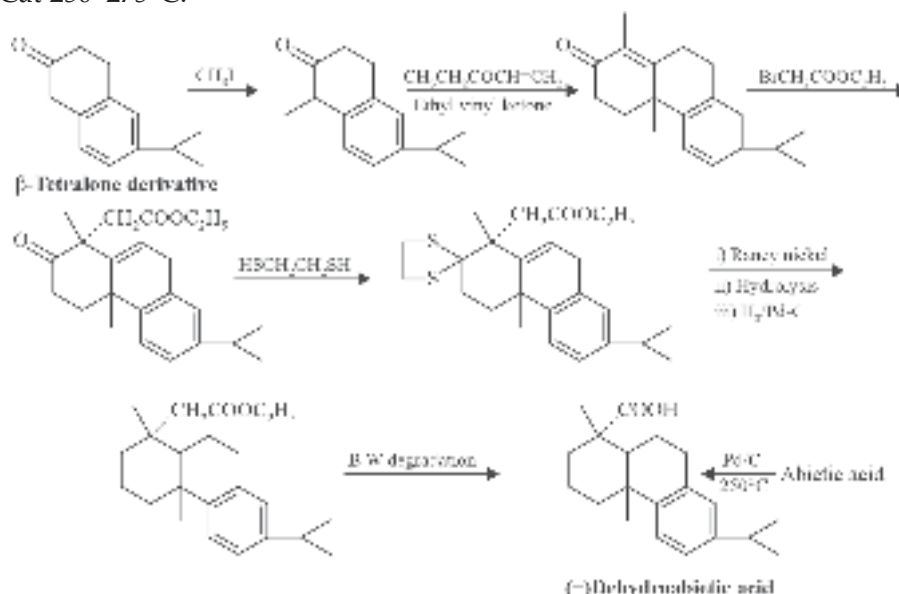
Oxidation of abietic acid with KMnO_4 gives, among other products, isobutyric acid. This suggests that one double bond is in the ring C and the 6:7 or 7:8-position. If the double bond is in the 6:7-position, then the double bond, which "is conjugated with it, must be in the same ring (5:13 or 8, 14); if 7:8, then the other double bond could be in the same ring, but it could also be in the ring B. Since, the two double bonds are in different rings, their positions are 7:8 and 14:9. Further evidence for these positions is afforded by the fact that oxidation of abietic acid gives acids III and IV, in which ring A is intact.

On the basis of above evidences, the formation of adduct is explained.



Synthesis

The final structure of abietic acid is proposed by the synthesis of dehydroabietic acid from β -tetralone derivative. It can be prepared on heating from abietic acid in presence of Pd/Cat $250\text{--}275^{\circ}\text{C}$.



Spectral Data

UV: 235, 241.5, 250 nm (E_{max} -21500, 23000, 15000 respectively).

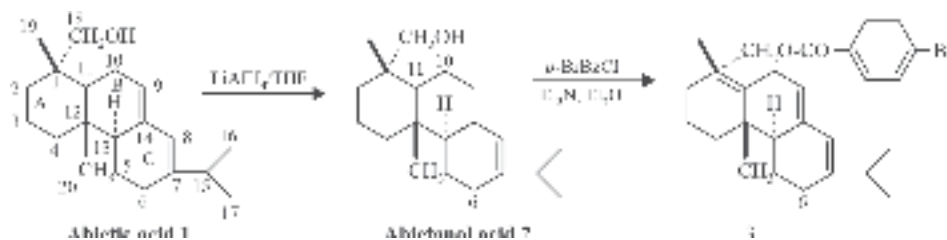
IR (cm⁻¹): 1735, 1465, 1435, 1250, 900.

¹H-NMR (methyl ester, δ): 0.99 (3H, s, CH₃-7), 1.02 (3H, s, CH₃-12), 1.26 (3H, d, CH₃-1), 5.37 (1H, t, H-13), 5.77 (1H, s, H-8).

MS (methyl ester, m/e): 313 (100), 254, 121, 104, 93, 91, 79.

Stereochemistry

The abietic acid contains four chiral centers: C-1, C-11, C-12 and C-13 positions. Stereochemical features is determined by preparing simple derivatives of abietic acid: these include the p-bromo ester derivative (3) of the abietanol (2) obtained by standard reduction of abietic acid (1). A single crystal X-ray study established the connectivity and the absolute configuration of (3) (Scheme 4.9), thereby confining the absolute configuration of (-)-abietic acid as 1R, 11R, 13R, 12R. The molecule exhibits a trans anti 6/6/6 tricyclic hydrocarbon skeleton in which the cyclohexane ring A has a typical chair form. Cyclohexane rings Band C, containing conjugated double bonds, have half-chair conformations. Thus, the relative stereochemistry is transfusion for the A/B ring junction, anti between C-13 hydrogen and C-12 methyl (phenanthrene numbering), and coplanar for the B/C ring junction. The ester linkage is located at C-1 and the isopropyl group at C-7. The structure is unsolvated. Bond lengths and angles lie in the ranges normally observed for such sterically non-strained molecules.



Scheme 4.9

TRITERPENES

13. Squalene

Squalene, b.p. 240–242°C a polyunsaturated triterpene hydrocarbon is a key precursor for the biosynthesis of cholesterol, bile acids, steroid hormones, and vitamin D in the plants and human body. Squalene is the main constituent of shark liver oil, yeast and many vegetable oils and it has also been reported from higher plants. Squalene is used in cosmetics industry as moisturizing agent and as emollient because it serves as natural oxidant that protects cells from free radicals and reactive oxygen species. More recently it is also used as an immunologic adjuvant in vaccines. Squalene has shown hypocholesterolaemic activity and preventive and therapeutic efficacy on tumor proliferation.

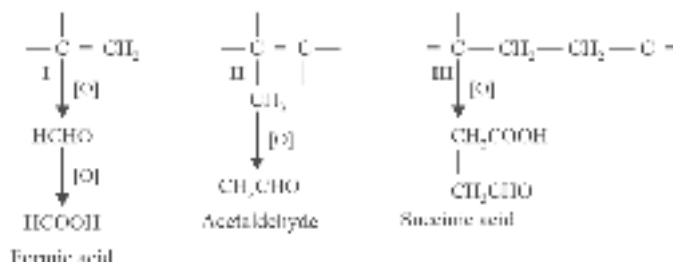
Role in Steroid Synthesis

Squalene is the biochemical precursor to the whole family of steroids. Oxidation (via squalene monooxygenase) of one of the terminal double bonds of squalene yields 2, 3-

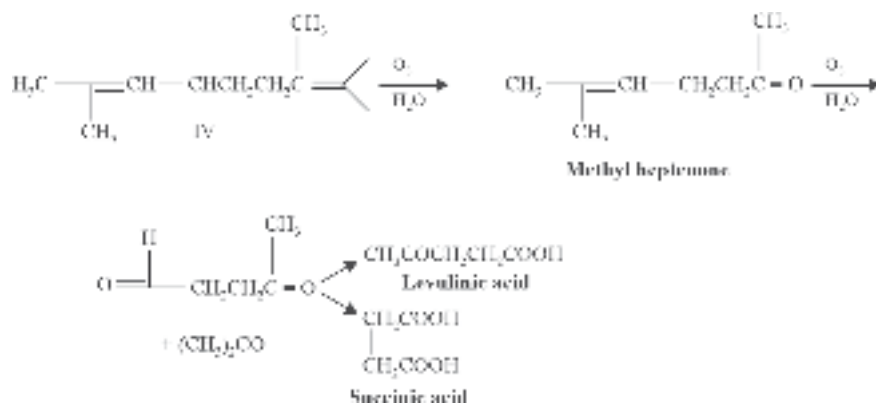
squalene oxide, which undergoes enzyme-catalyzed cyclization to afford lanosterol, which is then elaborated into cholesterol and other steroids.

Structure Elucidation of Squalene

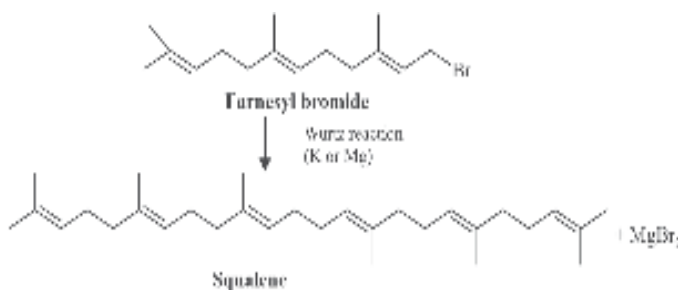
- (i) **Molecular formula:** It is found to be $C_{30}H_{50}$.
- (ii) **Presence of double bonds:** The consumption of six moles of hydrogen to form perhydro-squalene indicates the presence of six double bonds.
- (iii) **Presence of rings:** The completely saturated squalene, i.e. perhydrosqualene has the molecular formula $C_{30}H_{62}$ which corresponds to C_nH_{2n+2} for acyclic compounds. Hence, squalene is an open chain compound.
- (iv) Squalene cannot be reduced by sodium and amyl alcohol, which indicates the absence of conjugated double bond present in the molecules.
- (v) **Isoprene rule:** On treatment with boiling acetic anhydride containing 1% sulphuric acid or formic acid, squalene gets cyclized to give bicyclic, tricyclic and finally tetracyclic compounds having two, three or four double bonds. This reaction suggests that squalene is a terpenoid.



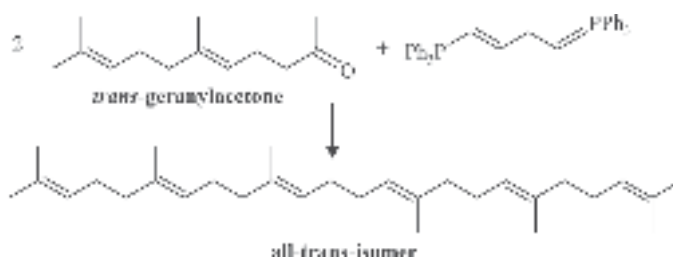
- (vi) On oxidation with chromyl chloride in CCl_4 , squalene gives formaldehyde, acetaldehyde and succinic acid. The formation of these products suggests the presence of fragments having structure I, II, and III respectively.
- (vii) **Ozonolysis:** The ozonolysis gives the carbon dioxide, formaldehyde, formic acid, succinic acid, levulinic acid and methylheptenone. The formation of levulinic acid can only be explained if basic structure as given in structure IV is present in squalene. However, structure IV does not explain the formation of ozonolysis products such as CO_2 , HCHO, and HCOOH.



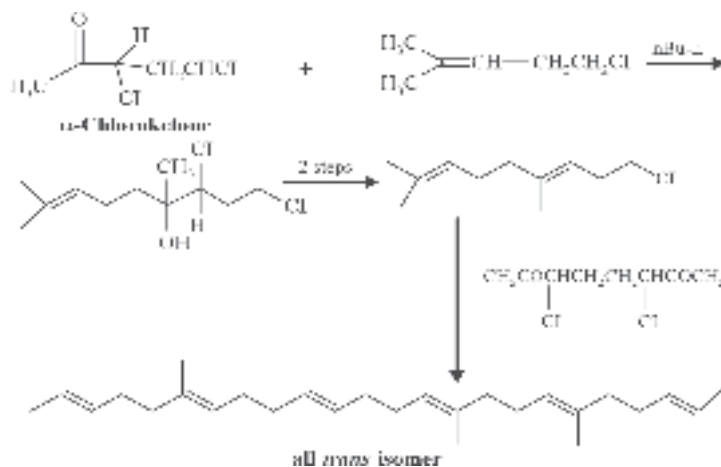
- (viii) Perhydrosqualene is observed to be identical with the product obtained from hexahydro-farnesyl bromide through Wurtz reaction. On this basis, Karrer et al. synthesize squalene from farnesyl bromide.



- (ix) Squalene forms thiourea inclusion complex which indicates that it is all *trans* stereoisomer. This is also supported by X-ray crystallographic studies.
- (x) The Whiting et al. synthesized all-*trans*-squalene through Wittig reaction from *trans*-geranylacetone via the thiourea complex in 12.5% yield. This is found identical with natural squalene.



- (xi) Cornforth et al. also synthesized all-*trans*-squalene using a general stereoselective synthesis from α -chloroketone.



Spectral Data

IR (KBr) (cm^{-1}): 2925, 1670, 1449, 1379.

$^1\text{H-NMR}$ (CDCl_3) (δ): 5.09 (olefinic H), 2.04 ($\text{CH}_2\text{-CH=C}$), 2.0 ($\text{CH}_2\text{-CMe=C}$), 1.68 (*cis* allylic Me), and 1.63 (*trans* allylic Me).

EI-MS (m/e): 410(M^+) (4), 341 (7), 273 (3), 149 (32), 136 (48), 121 (43), 109 (34), 95 (49), 81 (79), 69 (100), 67 (39), 41 (54).

SHORT AND LONG QUESTIONS

1. Explain the general methods of isolation of terpenoids.
2. Discuss the constitution of menthol and menthone.
3. Write the methods of isolation and classification of terpenes.
4. Write about classification of terpenoids.
5. Discuss the constitution of citral.
6. Discuss the constitution of camphor.
7. a. What is isoprene rule?
b. Discuss the medicinal importance of terpenoids.
8. Give the synthesis of carvone from alpha terpineol.
9. What is Bredt's rule. Discuss the chemistry of alpha pinene.
10. What are bicyclic terpenoids? Give the few examples of bicyclic terpenoids belonging to different class. Discuss the structural elucidation of camphor.
11. How limonene is isolated. Explain the structural elucidation of limonene. Mention its stereochemistry.
12. Give the synthesis of carvone.
13. a. Discuss the isoprene rule
b. How will you determine nature of oxygen atom in terpenoids. Explain it with examples.

MULTIPLE CHOICE QUESTIONS (TICK MARK THE APPROPRIATE CHOICE)

1. The example of a cyclic monoterpene is:
a. Alpha pinene
b. Geraniol
c. Limonene
d. Cadinene
2. Ketone group containing plant constituents is:
a. Neral
b. Camphor
c. Citronellal
d. Linalool
3. A cyclic compound with general formula, C_nH_{2n-4} has:
a. 2 rings
b. 3 rings
c. 4 rings
d. 1 ring

Answers

1. (c) 2. (b) 3. (b)

Chapter 5

Lipids—Structure, Analysis and Function

Lipids are a diverse range of compounds for which no agreed definition exists. Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, F and K), monoglycerides, diglycerides, phospholipids, and others. Lipids are insoluble in water but soluble in nonpolar organic solvents, such as hydrocarbons, chloroform, benzene, ethers and alcohol. The lipids together with carbohydrates and proteins constitute the principal structural component of living cells.

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment.

Tests for Lipids

Biological lipids originate entirely or in part from two distinct types of biochemical subunits or “building blocks”: ketoacyl and isoprene groups. These can be detected by using following generalized tests.

- **Lipids emulsion test:** Lipids are insoluble in water, but dissolve in ethanol. This characteristic property is used in the emulsion test. Do not start by dissolving the sample in water, but instead shake some of the test sample with about 4–5 ml of ethanol. Decant the liquid into a test tube of water, leaving any undissolved substances behind. If there are lipids dissolved in the ethanol, they will precipitate in the water, forming a cloudy white emulsion.
- **Grease spot test/Brown paper test:** Lipids leave translucent spots (grease spots) on unglazed brown paper bags.
- **Sudan red test:** Sudan red is a fat-soluble dye that stains lipids red. The staining with Sudan red dye can show the amount and the location of lipids.

Biological Functions of Lipids

1. **Foundation of cell membranes:** Lipids are a major building block of cell walls and cell membrane of every living cell. Cells are surrounded by a thin layer of lipids. The layer is made-up of a special type of lipid that has both hydrophobic and hydrophilic properties. The hydrophilic ends of these molecules face the water-filled environment inside the cells and the watery environment outside the cells. A hydrophobic region exists inside the two layers. The membrane that surrounds the cells is rich in proteins and other lipids such as cholesterol.

2. **Energy storage:** The main function of lipids is energy storage. Because lipids pack in more energy—in the form of calories—than protein or carbohydrates, they are an ideal way to store energy. The human body produces nine calories when burning a gram of fat; a gram of glucose only yields four calories of energy when burned.
3. **Transmission of information in cells (signal transduction):** Certain types of lipids—such as phospholipids—perform an important function in the body by carrying messages between cells. Platelet-activating factor, an important lipid that causes blood platelets to stick together, also plays a critical role in inflammation and helps the body produce an immune response to invaders. Certain types of lipids also form a protective layer around nerve endings that also helps in connecting nerve impulses.
4. **Cellular metabolism:** Lipids supply essential lipid-soluble vitamins and bile acids that are required in relatively high amounts during growth and life process. The fat-soluble vitamins A, D, E, and K are required for metabolism, usually as coenzymes. The bile acids are required for absorption of fats.
5. **Hormones:** Lipids are the foundation of hormones. For example, cholesterol forms the basis of all sex hormones in the body, including testosterone, estrogen and progesterone.

CLASSIFICATION OF LIPIDS

The biologically important lipids can be classified into three types namely simple lipids, compound lipids and derived lipids (Fig. 5.1). Simple lipids contain C, H, and O, and compound (compound lipids) contain one or more additional elements, such as phosphorus, nitrogen, or sulphur. Derived lipids are obtained from hydrolysis of simple and compound lipids.

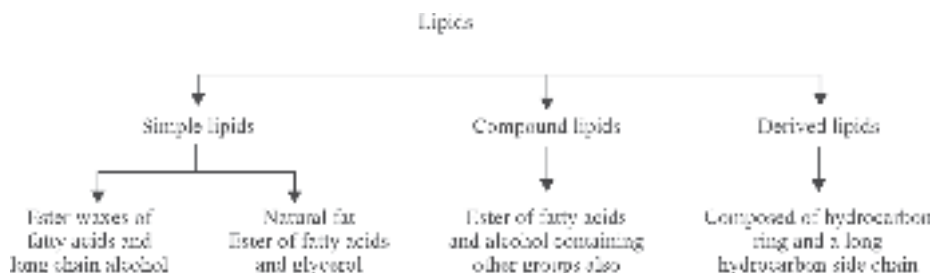


Fig. 5.1: Classification of lipids

A. Simple Lipids

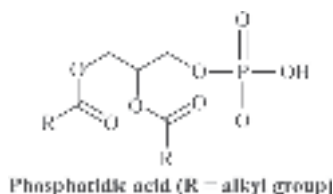
1. **Triglycerides, neutral fats:** These are found in adipose tissue, butter fat, lard, fish oils, olive oil, com oil, etc. These are esters of fatty acids with glycerol. Three molecules of fatty acids combine with one molecule of glycerol in fat molecule. The fatty acid may be different in different fat.
2. **Waxes:** Beeswax, camuba, carnauba oil, and lanolin are the example of waxes. These are composed of esters of fatty acids with alcohol other than glycerol.

B. Compound Lipids (Complex Lipids)

Compound lipids are esters of fatty acid containing certain groups in addition to alcohol and fatty acid.

Among the compound lipids, important structural types are phosphoglycerides, phosphosphingol lipids, and glycolipids.

The parent phosphoglyceride, phosphatidic acid (PA), is similar in structure to a triglyceride except that the 3-hydroxyl group of the glycerol component is esterified to phosphoric acid rather than to fatty acid (FA). Further esterification of the phosphoric acid of PA with a variety of small, hydroxyl-containing molecules leads to a series of derived phosphoglycerides, including phosphatidyl choline (PC) commonly known as lecithin), phosphatidyl ethanolamine (PE) (commonly known as cephalin), and phosphatidyl serine (PS) (see Table 5.4).



The phosphosphingolipids are derived from sphingosine, a long-chain dialcohol with an amino group. The formation of an amide with an FA at one point along this chain yields ceramide. Esterification of a ceramide derivative with phosphorylcholine yields sphingomyelin, which is the major phosphosphingolipid. If ceramide is linked to a simple sugar, a cerebroside glycolipid is formed. Further addition of several amino sugars yields the lipids called gangliosides.

C. Derived lipids

These lipids contain glycerol and other alcohols. This class of lipids includes steroid hormones, ketone bodies, hydrocarbons, fatty acids, fatty alcohols, mono and diglycerides, terpenes and carotenoids. These are sometimes present as waste products of metabolism.

A. SIMPLE LIPIDS

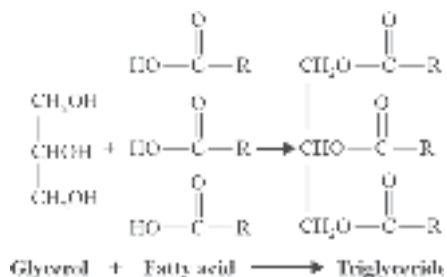
1. Fats and Oils

Oils and fats are esters of fatty acids and glycerol. A fatty acid is an organic acid ending in a carboxyl (COOH) group. The various fatty acids differ in combination of carbon and hydrogen atoms. Saturated fats are literally saturated with hydrogen molecules, while mono-unsaturated and polyunsaturated fats have substantially fewer molecules of hydrogen attached. Both fats and oil belong to the class of esters known as glycerides or triglycerides but main difference between them is that oils are liquid at room temperature (20°C) while fats are solids or semi solids at room temperature. Oils contain higher proportion of glycerides of unsaturated fatty acids while fats contain high proportion of saturated.

Triglycerides

Triglycerides are a type of fat in the bloodstream and fat tissue. The triglyceride molecule is the ester of the chemical glycerol (tri = three molecules of fatty acid + glyceride = glycerol) esterified with three molecules of fatty acids. To be absorbed, these parts are broken apart in the small intestine, and afterwards are reassembled with cholesterol to form chylomicrons. This is the source of energy for cells in the body. Fat cells and liver cells are used as storage sites and release chylomicrons when the body needs the energy.

Triglycerides are measured along with cholesterol as part of a blood lipid profile test. Normal triglyceride levels are below 150. Levels above 200 are considered high. Elevated triglyceride level is a risk factor for atherosclerosis, the narrowing of arteries with the buildup of fatty plaques that may lead to heart attack, stroke, and peripheral artery disease. Markedly elevated triglyceride levels may also cause fatty liver disease and pancreatitis.



Types of Triglycerides

A triglyceride comprises three fatty acids attached to glycerol. The three types of fatty acids that compose triglycerides are saturated, monounsaturated and polyunsaturated fatty acids. Accordingly, depending on the type of fatty acid, there are three types of triglycerides, or fats; (i) saturated, (ii) monounsaturated and (iii) polyunsaturated triglycerides.

(i) Saturated Triglycerides

In saturated triglycerides, or saturated fats, most of the fatty acids (e.g. palmitic acid, stearic acid, etc.) are saturated in nature. It means that each carbon atom has the maximum number of attached hydrogen atoms. Saturated fats are the biggest dietary cause of high LDL, or 'bad', cholesterol levels. Saturated fats should represent at most only 10% of the total calorie intake. Saturated fats are found in many animal products such as butter, cheese, whole milk, ice cream, cream and fatty meats.

(ii) Monounsaturated Triglycerides

Most of the fatty acids are monounsaturated in monounsaturated triglycerides; one pair of hydrogen atoms in the middle of the molecule is missing (e.g. oleic acid). Monounsaturated fats are a type of unsaturated fat. Eating unsaturated fats instead of saturated fats can help in lowering the blood cholesterol. Foods with high levels of monounsaturated fats include vegetable oils such as olive oil, canola oil, peanut oil and sesame oil.

(iii) Polyunsaturated Triglycerides

Most of the fatty acids are polyunsaturated in polyunsaturated fats. Polyunsaturated fatty acids have two or more pairs of hydrogen atoms short of saturation; two examples are ω -3 and ω -6 fatty acids. Polyunsaturated fat is another type of unsaturated fat, which can help in lowering cholesterol levels. Foods that contain polyunsaturated fats include many vegetable oils and fatty fish such as salmon, mackerel, herring and trout.

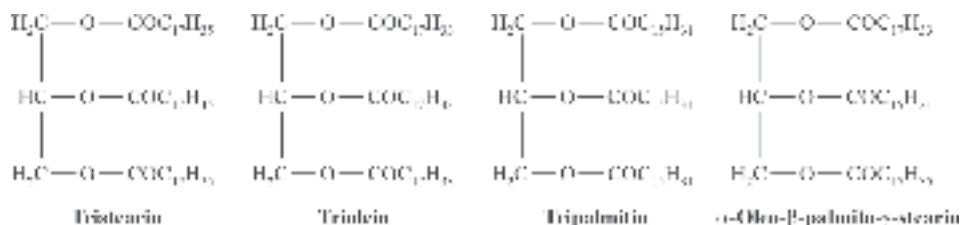
Depending upon the fatty acid residues in glycerol, there are two types of fats, namely simple triglycerides and mixed triglycerides.

(a) Simple triglycerides

If all three fatty acids are the same, the triglyceride is called simple triglycerides. For example, in olive oil, the fatty acid is oleic acid then it was named as triolein. Similarly, tristearin contains three stearic acid residues. Oils contain glycerides of unsaturated fatty acids, while fats contain glycerides of saturated fatty acids.

(b) Mixed triglycerides

If more than one fatty acid is esterified with glycerol then the fat is named as mixed triglycerides. The name of the fat depends upon the substituted fatty acid and its position. Mixed triglycerides exhibit isomerism depending on the position occupied by the different fatty acid residues. Oleo-palmito-stearin is an example of mixed glyceride.

**Types of Fatty Acids**

Fatty acids can also be classified according to *chain length of carbon atoms*.

Short chain fatty acids: 2–4 carbon atoms.

Medium chain fatty acids: 6–10 carbon atoms.

Long chain fatty acids: 12–26 carbon atoms.

or on the basis of unsaturation

Fatty acids may be divided as follows:

- (i) Monounsaturated (monoenoic) acids: These contain one double bond.
- (ii) Polyunsaturated (polyenoic) acids: These contain two or more double bonds.
- (iii) Eicosanoids: These compounds are derived from eicosa-(20-carbon) polyenoic fatty acids. The prostaglandins, leukotrienes (LTs), etc. are the example of eicosanoids.

or essential vs those can be biosynthesized into the body.

Linoleic and linolenic acids are two examples of essential fatty acids.

or into *cis* and *trans* fatty acids

or as Omega fatty acids:

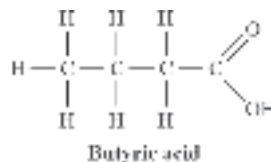
- ω -3
- ω -6
- ω -9

or as hydroxy or cyclic fatty acids.

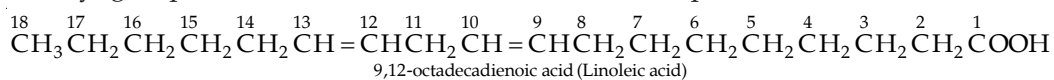
Saturated, monounsaturated and polyunsaturated fatty acids: Fatty acids consist of the elements carbon (C), hydrogen (H) and oxygen (O), arranged as a carbon chain skeleton with a carboxyl group ($-\text{COOH}$) at one end. Saturated fatty acids (SFAs) have all the hydrogen that the carbon atoms can hold, and therefore, have no double bonds between the carbons. Monounsaturated fatty acids (MUFAs) have only one double bond.

Polyunsaturated fatty acids (PUFAs) have more than one double bond.

Butyric acid (butanoic acid) is one of the saturated short-chain fatty acids responsible for the characteristic flavor of butter. This structural formula shows four bonds for every carbon atom and can also be represented as the equivalent line formulas:



In unsaturated fatty acids, the numbers at the beginning of the scientific names indicate the locations of the double bonds. By convention, the carbon of the carboxyl group is carbon number one. Greek numeric prefixes such as *di*, *tri*, *tetra*, *penta*, *hexa*, etc. are used as multipliers and to describe the length of carbon chains containing more than four atoms-. Thus, "9,12-octadecadienoic acid" indicates that there is an 18-carbon chain (octa deca) with two double bonds (di en) located at carbons 9 and 12, with carbon 1 constituting a carboxyl group (oic acid). The structural formula corresponds to:



This would be abbreviated as:



Fatty acids are frequently represented by a notation such as C18:2 indicate that the fatty acid consists of an 18-carbon chain and 2 double bonds. Although, this could refer to any of several possible fatty acid isomers with this chemical composition, it implies the naturally-occurring fatty acid with these characteristics, i.e. linoleic acid. Double bonds are said to be "conjugated" when they are separated from each other by one single bond, e.g. $(-\text{CH}=\text{CH}-\text{CH}=\text{CH}-)$. The term "conjugated linoleic acid" (CLA) refers to several C 18:2 linoleic acid variants such as 9,11-CLA and 10, 12-CLA which correspond to 9,11-octadecadienoic acid and 10, 12-octadecadienoic acid. The principal dietary isomer of CLA is *cis*-9, *trans*- 11 CLA, also known as rumenic acid. CLA is found naturally in meats, eggs, cheese, milk and yogurt.



Length of free fatty acid chains: Fatty acid chains differ by length, often categorized as short, medium, or long.

- Short-chain fatty acids (SCFA) are fatty acids with aliphatic tails of fewer than six carbons (i.e butyric acid).
- Medium-chain fatty acids (MCFA) is fatty acids with aliphatic tails of 6–12 carbons, which can form medium-chain triglycerides.
- Long-chain fatty acids (LCFA) are fatty acids with aliphatic tails longer than 12 carbons.
- Very-long-chain fatty acids (VLCFA) are fatty acids with aliphatic tails longer than 22 carbons.

Table 5.1 describes the chemical names and description of common fatty acids.

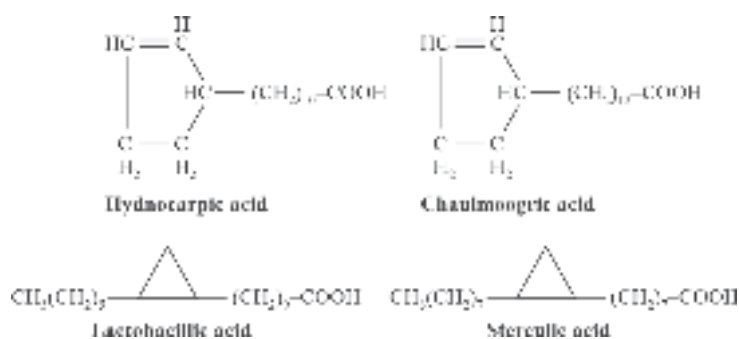
Cyclic Fatty Acids

These are rare in occurrence. Chaulmoogra oil, obtained from the plant, *Hydnocarpus kurzii* and used in the treatment of leprosy, contains two such acids hydnocarpic and

Table 5.1: Chemical names and description of common fatty acids

<i>Common name</i>	<i>Carbon atoms</i>	<i>Double bonds</i>	<i>Scientific name</i>	<i>Sources</i>
Butyric acid	4	0	Butanoic acid	Butter fat
Caproic acid	6	0	Hexanoic acid	Butter fat
Caprylic acid	8	0	Octanoic acid	Coconut oil
Capric acid	10	0	Decanoic acid	Coconut oil
Lauric acid	12	0	Dodecanoic acid	Coconut oil
Myristic acid	14	0	Tetradecanoic acid	Palm kernel oil
Palmitic acid	16	0	Hexadecanoic acid	Palm oil
Palmitoleic acid	16	0	9-Hexadecenoic acid	Animal fats
Stearic acid	18	0	Octadecanoic acid	Animal fats
Oleic acid	18	1	9-Octadecenoic acid	Olive oil
Ricinoleic acid	18	1	12-Hydroxy-9-octadecenoic acid	Castor oil
Vaccenic acid	18	1	11-Octadecenoic acid	Butter fat
Linoleic acid	18	2	9,12-Octadecadienoic acid	Grape seed oil
α -Linolenic acid (ALA)	18	3	9,12,15-Octadecatrienoic acid	Flaxseed (linseed) oil
γ -Linolenic acid (GLA)	18	3	6,9,12-Octadecatrienoic acid	Borage oil
Arachidic acid	20	0	Eicosanoic acid peanut oil	Fish oil
Gadoleic acid	20	1	9-Eicosenoic acid	Fish oil
Arachidonic acid (AA)	20	4	5,8,11,14-Eicosatetraenoic acid	Liver fats
Eicosapentaenoic acid (EPA)	20	5	5,8,11,14,17-Eicosapentaenoic acid	Fish oil
Behenic acid	22	0	Docosanoic acid	Rapeseed oil
Erucic acid	22	1	13-Docosenoic acid	Rapeseed oil
DHA	22	6	4,7,10,13,16,19-docosahexaenoic acid	Fish oil
Lignoceric acid	24	0	Tetracosanoic acid	Small amounts in most fats

chaulmoogric acids. They are named so with the word 'cyclic' due to presence of cyclic structure.

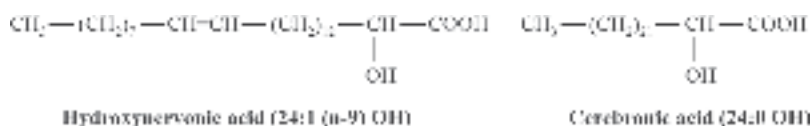


Lipids from the lactobacilli contain a fatty acid, lactobacillic acid, with a cyclopropyl group.

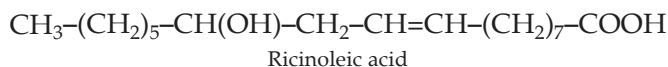
Similarly, sterculic acid from plant sources has a comparable structure, with a suggested relationship to oleic acid. It may be derived from oleic acid by the addition of a methylene group across the double bond in a manner that the unsaturated nature is not altered, unlike the lactobacillic acid.

Hydroxy Fatty Acids

2-Hydroxytetracosanoic acid (cerebronic acid) and 2-hydroxy-15-tetracosenoic acid (hydroxynervonic acid) are constituents of the ceramide part of cerebrosides (glycosphingolipids found mainly in nervous tissue and in little amount in plants).



Ricinoic acid (12-hydroxy-9-cis-octadecenoic acid) is an unsaturated omega-9 fatty acid that naturally occurs in mature Castor plant (*Ricinus communis* L., Euphorbiaceae) seeds.



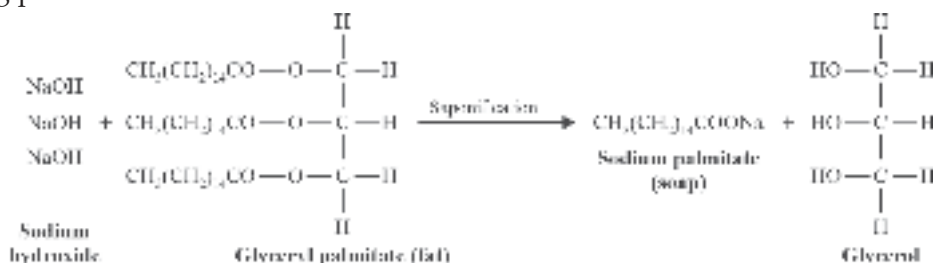
Roles of Fatty acids

Fatty acids have four major biological roles:

- (i) These are used to make glycerophospholipids and sphingolipids, essential components of biological membranes.
- (ii) Numerous proteins are covalently modified by fatty acids. Myristate and palmitate are directly attached to some proteins, while phosphatidylinositol is covalently linked to the C-terminus of other proteins via a complex glycosylated structure.
- (iii) Fatty acids act as fuel molecules, being stored as triacylglycerols, and broken down to generate energy.
- (iv) Derivatives of fatty acids serve as hormones (such as the prostaglandins) and intracellular second messengers (such as DAG and IP3).

Saponification of Fatty Acids

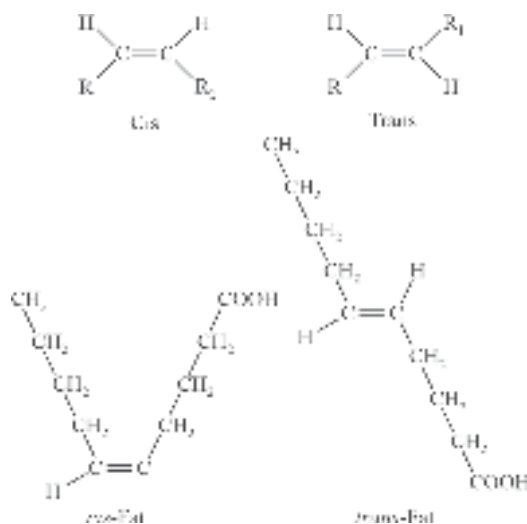
The hydrolysis of fats by alkali is called as saponification. This reaction results in the formation of glycerol and salts of fatty acids which are called as soaps. The soaps are of two types namely hard and soft. Hard soaps such as the common bar soaps are the sodium salts of the higher fatty acids. Soft soaps are the potassium salts of higher fatty acids and are marketed as semisolids or pastes. The fatty acid salts of calcium, magnesium, zinc and lead are, however, insoluble in water. Calcium soaps are used industrially as lubricating greases. Zinc soaps are employed in the manufacture of talcum powder and other cosmetics. Lead and magnesium soaps are used in paint industries to hasten the drying process.



Trans Fats

Double bonds bind carbon atoms tightly and prevent rotation of the carbon atoms along the bond axis. This gives rise to *conjugational isomers* in which are arrangements of

atoms can only be changed by breaking the bonds. Hence, two types of double bonds exist in nature *cis* and *trans*. As shown on the left *cis* double bonds have the hydrogens on the same side while *trans* has them on opposite sides.



Double *cis* bonds cause the fatty acid molecule to bend or kink. Kinks in the molecule do not allow the molecules to stack nicely together. As a result, the strength of the van der Waals forces is not as great as in saturated fatty acids. Hence, these type of molecules are liquid at room temperature.

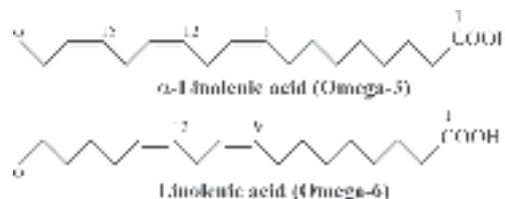
Molecules with *cis* double bonds are more likely to react with oxygen in the air than molecules with *trans* double bonds to form shorter chain molecules with unpleasant smells. At the same time, *trans* fatty acids, like saturated fatty acids (SFA), raise LDL (or “bad”) cholesterol levels in the blood, thereby increasing the risk of coronary heart disease. Therefore, food companies are faced with a dilemma. If they use healthier unsaturated fats, they face the risk of the food spoiling. The vast majority of *trans* fats in the food are manufactured by adding hydrogen bonds to unsaturated fats. This makes the fat more stable, so it does not spoil as quickly. These fats are usually referred to as “hydrogenated fats” or “partially hydrogenated fats”. “Fully hydrogenated fats” should not contain significant amounts of *trans* fats.

Omega-3 Fatty Acids

Omega-3 (ω -3) and omega-6 (ω -6) fatty acids are unsaturated “Essential Fatty Acids” (EFAs) that must be included in the diet because the human metabolism cannot biosynthesise them from other fatty acids. These fatty acids use the Greek alphabet (α , β , γ , ..., ω) to identify the location of the double bonds. The “alpha” carbon is the carbon closest to the carboxyl group (carbon number 2), and the “omega” is the last carbon of the chain because omega is the last letter of the Greek alphabet. Linoleic acid is an omega-6 fatty acid because it has a double bond six carbons away from the “omega” carbon. Linoleic acid plays an important role in lowering cholesterol levels.

α -Linolenic acid is an omega-3 fatty acid because it has a double bond three carbons away from the “omega” carbon. By subtracting the highest double-bond locant in the scientific name from the number of carbons (represented as ‘n’ in Table 5.2) in the fatty

acid, we can obtain its classification. For example, in arachidonic acid, we subtract 14 from 20 to obtain 6; therefore, it is an omega-6 fatty acid. This type of terminology is sometimes applied to oleic acid which is an omega-9 fatty acid.



DHA (docosahexaenoic acid) and AA (arachidonic acid) are both crucial to the optimal development of the brain and eyes. The importance of DHA and AA in infant nutrition is well established, and both substances are routinely added to infant formulas. Excessive amounts of omega-6 polyunsaturated fatty acids and a very high omega-6/omega-3 ratio have been linked with pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases. The ratio of omega-6 to omega-3 in modern diets is approximately 15:1, whereas ratios of 2:1 to 4:1 have been associated with reduced mortality from cardiovascular disease, suppressed inflammation in patients with rheumatoid arthritis, and decreased risk of breast cancer. Some researchers have suggested that there is not very strong evidence for the benefits of these ratios, and that it may be better to increase the consumption of omega-3 fatty acids rather than decrease the consumption of omega-6 fatty acids because a reduction of polyunsaturated fats in the diet would increase the incidence of cardiovascular disease.

Sources of Omega-6 and Omega-3 Fatty Acids

1. **Omega-3 fats** are found in fish, sea food, lean meat, plant foods (such as cereal grains, seeds, nuts, legumes, dark green leafy vegetables, and figs), plant oils including soybean, canola, linseed and walnut. Oily fish (such as mackerel, salmon, trout, tuna, sardines, herring and gemfish) have more omega-3 fatty acids than white fish.
2. **Omega-6 fats** are found primarily in nuts, seeds and plant oils, such as corn, soy and safflower.

Benefits of Omega Fatty Acids

The benefits of omega fats in the diet seem to include:

1. Lowering blood cholesterol levels, which reduces one important risk factor in coronary heart disease (Omega 6 and 3).
2. Producing anti-inflammatory compounds called leukotrienes, which can help ease a variety of inflammatory disorders, such as psoriasis, rheumatoid arthritis, ulcerative colitis and inflammation of blood vessel walls associated with atherosclerosis (Omega 3).
3. Contributing to the normal development of the foetal brain.

The deficiency of essential fatty acids could lead to:

- Skin eruptions
- Loss of hair
- Liver degeneration
- Susceptibility to infections
- Poor wound healing
- Male sterility
- Arthritis

- Growth retardation
- Circulatory problems

Table 5.2 lists several different names for the most common *n*-3 fatty acids found in nature.

Table 5.2: List of *n*-3 fatty acids

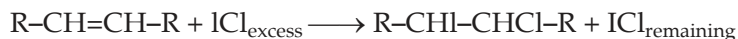
<i>Common name</i>	<i>Lipid name</i>	<i>Chemical name</i>
Hexadecatrienoic acid (HTA)	16:3 (n-3)	all- <i>cis</i> -7,10,13-hexadecatrienoic acid
α -Linolenic acid (ALA)	18:3 (n-3)	all- <i>cis</i> -9,12,15-octadecatrienoic acid
Stearidonic acid (SDA)	18:4 (n-3)	all- <i>cis</i> -6,9,12,15-octadecatetraenoic acid
Eicosatrienoic acid (ETE)	20:3 (n-3)	all- <i>cis</i> -11,14,17-eicosatrienoic acid
Eicosatetraenoic acid (ETA)	20:4 (n-3)	all- <i>cis</i> -8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA)	20:5 (n-3)	all- <i>cis</i> -5,8,11,14,17-eicosapentaenoic acid
Heneicosapentaenoic acid (HPA)	21:5 (n-3)	all- <i>cis</i> -6,9,12,15,18-heneicosapentaenoic acid
Docosapentaenoic acid (DPA)	22:5 (n-3)	all- <i>cis</i> -7,10,13,16,19-docosapentaenoic acid
Docosahexaenoic acid (DHA)	22:6 (n-3)	all- <i>cis</i> -4,7,10,13,16,19-docosahexaenoic acid
Tetracosapentaenoic acid	24:5 (n-3)	all- <i>cis</i> -9,12,15,18,21-tetracosapentaenoic acid
Tetracosahexaenoic acid (Nisinic acid)	24:6 (n-3)	all- <i>cis</i> -6,9,12,15,18,21-tetracosahexaenoic acid

ANALYSIS OF FATS AND OILS

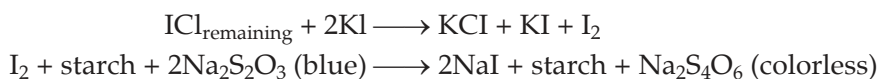
A number of chemical methods have been developed to provide information about the type of lipids present in edible fats and oils. These techniques are much cruder than chromatography techniques, because they only give information about the average properties of the lipid components present, e.g. the average molecular weight, degree of unsaturation or amount of acids present. Nevertheless, they are simple to perform and do not require expensive and complicated apparatus, and so they are widely used in industry and research.

1. Iodine Value

The iodine value gives a measure of the average *degree of unsaturation* of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value *is* expressed as the grams of iodine absorbed per 100 g of lipid. One of the most commonly used methods for determining the iodine value of lipids is “Wijs method”. The lipid to be analyzed is weighed and dissolved in a suitable organic solvent, to which a known excess of iodine chloride is added. Some of the ICl reacts with the double bonds in the unsaturated lipids, while the rest remains:



The amount of ICl that has reacted is determined by measuring the amount of ICl remaining after the reaction has gone to completion ($\text{ICl}_{\text{reacted}} = \text{ICl}_{\text{excess}} - \text{ICl}_{\text{remaining}}$). The amount of ICl remaining is determined by adding excess potassium iodide to the solution to liberate iodine, and then titrating the liberated iodine with a standard sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) solution in the presence of starch to determine the concentration of iodine released:



Iodine itself has a reddish brown color, but this is often not intense enough to be used as a good indication of the end-point of the reaction. For this reason, starch is usually used as an indicator because it forms a molecular complex with the iodine that has a deep blue color. Initially, starch is added to the solution that contains the iodine and the solution goes a dark blue. Then, the solution is titrated with standard sodium thiosulphate solution. *When there is even traces of I_2 remaining in the solution, blue color persists, but once all of the I_2 has been converted to I^- , it turns colorless.* Thus, a change in solution color, from blue to colorless can be used as the end-point of the titration. The concentration of C=C in the original sample can therefore be calculated by measuring the amount of sodium thiosulphate needed to complete the titration. A blank titration is performed to omit out any error.

The approximate weight, in 'g' of substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated with smaller quantity of substance.

Formula:

$$\text{Iodine value} = \frac{(\text{blank titer} - \text{sample titer}) \text{ ml} \times N \times 1.269}{\text{Weight of sample (g)}}$$

where

N = Normality of sodium thiosulphate solution

The higher the degree of unsaturation, the more the iodine absorbed. and the higher is the iodine value. The iodine value is used to obtain a measure of the average degree of unsaturation of oils, and to follow processes such as hydrogenation and oxidation that involve changes in the degree of unsaturation.

Standard iodine value: Linseed oil 175–202; Coconut oil 8.4; Cotton seed oil 103–111.

2. Saponification Number

The *saponification number* (SN) is a measure of the *average molecular weight* of the triacylglycerols in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali:



The saponification number is defined as the mg of KOH required to saponify one gram of fat. The lipid is first extracted with chloroform-methanol mixture and then dissolved in an ethanol solution which contains a known excess of KOH. This solution is then heated so that the reaction goes to completion. The unreacted KOH is then determined by adding an indicator and titrating the sample with standard HCl solution. The blank titration is performed to omit out any error. The saponification number is then calculated from a knowledge of the weight of sample and the amount of KOH which reacted. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present. The ester of fatty acid of lower molecular weight requires more amount of alkali for saponification. The SN is inversely proportional to mean of molecular weight of fatty acids.

As per IP the normality of each alcoholic KOH and HCl solution used for determination of saponification number is 0.5 N.

Formula:

$$\text{Saponification number} = \frac{(b - a)}{\text{Weight of sample}} \times 28.05$$

b = Volume of potassium hydroxide consumed in blank titration.

a = Volume of potassium hydroxide consumed in titration with sample.

Standard saponification number: Mustard oil 174; Coconut oil 250–265; Butter fat 233–240.

3. Acid Value

The acid value is a measure of the amount of *free acids* present in a given amount of fat. The lipids are extracted from the food sample and then dissolved in a mixture of ether and alcohol solution containing an indicator. This solution is then titrated with alkali (KOH) until a pinkish color appears. The acid value is defined as the mg of KOH necessary to neutralize the fatty acids present in 1 g of lipid. The acid value may be overestimated if other acid components are present in the system, e.g. amino acids or acid phosphates.

The acid value is often a good measure of the break down of the triacylglycerols into free fatty acids, which has an adverse effect on the quality of many lipids.

Formula:

$$\text{Acid value} = \frac{b}{\text{Weight of sample}} \times 5.61$$

b = Volume of 0.1 N potassium hydroxide solution consumed in titration with sample

Standard acid value: Castor oil = 0.12–0.80; Olive oil = 0.3–1.0; Sesame oil = 9.8.

4. Determination of Reichert-Meissl Value, Polenske and Kirschner Value

Milk fat content in margarine is estimated by the determination of the following values which are obtained by specifically defined empirical methods.

Reichert-Meissl (R) value: The 'R' value is the number of ml of 0.1 N aqueous alkali solution required to neutralize the water-soluble volatile fatty acids, distilled from 5 g of the oil or fat under the precise conditions specified in the method.

Polenske value (P): The Polenske value (P) is the number of ml of 0.1 N aqueous alkali solution, required to neutralize the water-insoluble volatile fatty acids distilled from 5 g of the oil or fat under the precise conditions specified in the method.

Kirschner value (K): The Kirschner value (K) is the number of ml of 0.1 N aqueous alkali solution required to neutralize the water-soluble volatile fatty acids which form water-soluble silver salts distilled from 5 g of the oil or fat under the precise conditions specified in the method.

The tests are based on the quantitative measurement of low molecular weight fatty acids (C_4 – C_{14}) that are predominant in butter.

Principle of the method

Margarine fat is saponified. The fatty acids are then liberated from the soap and distilled in a standard apparatus under specified conditions. The water-insoluble fatty acids are filtered off, dissolved in ethanol and titrated. The 'P' value is calculated from the amount of alkali solution required. The water-soluble fatty acids are then titrated with barium hydroxide solution, and 'R' value is calculated from the amount of the solution required in the titration. The neutralized solution is then treated with silver sulphate. After separation of the insoluble silver salts by filtration, the filtrate containing the water-soluble silver salts is acidified, distilled in the standard apparatus under the same specified conditions and titrated to determine 'K' value. The percentage of milk fat in margarine

fat is then calculated according to a formula based on (K) and (P). This process does not determine the total quantities of volatile fatty acids, soluble and insoluble in water, present in combination in the fat. The amounts of these acids actually determined by the process are dependent on strict adherence to the dimensions of the apparatus and the details of procedure.

Procedure

Hydrolysis and distillation

- (i) Weigh $5 + 0.01$ g of the fat into a Polenske flask.
- (ii) Add 20 g of glycerol and 2 ml of the 50% w/v sodium hydroxide solution. Protect the burette containing the NaOH solution from carbon dioxide, and wipe its nozzle clean from carbonate deposit before withdrawing solution for the tests; reject the first few drops withdrawn from the burette.
- (iii) Heat the flask over a naked flame, with continuous mixing, until the fat including any drops adhering to the upper parts of the flask, is saponified, and the liquid becomes perfectly clear; avoid over-heating during this saponification to avoid discoloration. Cover the flask with a watch glass.
- (iv) Make a blank test without fat, but using the same quantities of reagents and following the same procedure, again avoid over-heating during the heating with sodium hydroxide; such over-heating would be indicated by darkening of the solution.
- (v) Measure 93 ml of boiling distilled water, which has been vigorously boiled for 15 minutes, into a 100 ml graduated cylinder. When the soap is sufficiently cool to permit addition of the water without loss, but before the soap has solidified, add the water, draining the cylinder for 5 seconds, and dissolve the soap. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating over-heating) repeat the saponification with a fresh sample of the fat.
- (vi) Add 0.1 g of powdered pumice, followed by 50 ml of the dilute sulphuric acid, and connect the flask at once with the distillation apparatus. Heat the flask without boiling its contents, until the insoluble acids are completely melted, then increase the flame and distil 110 ml in between 19 and 21 minutes. Adjust the water flowing in the condenser at a sufficient speed to maintain the temperature of the collecting distillate between 18°C and 21°C .
- (vii) When the distillate reaches the 110 ml mark. remove the flame and replace the 110 ml flask by a cylinder of about 25 ml capacity, to catch drainings. Close the 110 ml flask with its stopper, and without mixing the contents, place it in water at 15°C for 10 minutes so as to immerse the 110 ml mark below the level of water. Remove the flask from the water, dry the outside and invert the flask carefully, avoiding wetting the stopper with the insoluble acids. Mix the distillate by four or five double inversions, without violent shaking. Filter through a dry 9 cm open-texture filter paper (a Whatman No. 4 or 41) which fits snugly into a funnel. Reject the first running and collect 100 ml in a dry flask, cork the flask and retain the filtrate for titration as under Determination (a) on page 243. The filtrate should be free from insoluble fatty acids.
- (viii) Where liquid, insoluble fatty acids pass through the filter, receive the filtrate in a separating funnel, and after separation draw off the lower (aqueous) layer, leaving behind insoluble acids which have risen to the surface. Add these to the main bulk of the insoluble acids.

- (ix) Detach the still-head and wash the condenser with three successive 15 ml portions of cold distilled water, passing each washing separately through the cylinder, the 110 ml flask, the filter and the separating funnel, nearly filling the paper each time and draining each washing before filtering the next. Discard the washings.
- (x) Dissolve the insoluble acids by three similar washings of the condenser, the cylinder, and the filter with 15 ml of neutralized ethanol, collecting the solution in the 110 ml flask and draining the ethanol after each washing. Cork the flask, and retain the solution for titration, as under Determination (b).

Determinations of different values

(a) Reichert-Meissl (R) Value or Soluble Volatile Acid

Pour 100 ml of the filtrate containing the soluble volatile acids into a previously dried titration flask, add 0.1 ml of phenolphthalein indicator and titrate with the barium hydroxide solution until the liquid becomes pink, rinsing the 110 ml flask with the nearly neutralized liquid towards the end of the titration. Note the actual volume of barium hydroxide solution used; drain the 100 ml flask into the titration flask. close with a cork and continue as under determination (c).

(b) Polenske or Insoluble Volatile Acid, Value (P)

Titrate the alcoholic solution of the insoluble volatile acids after addition of 0.25 ml of phenolphthalein indicator with the 0.1 N barium hydroxide solution until the solution becomes pink.

(c) Kirschner Value (K)

Add 0.5 g of finely powdered silver sulphate to the neutralized solution from (a) above. Allow the flask to stand in the dark for one hour with occasional shaking and filter the contents in the dark through a dry filter. Transfer 100 ml of the filtrate to a dry Polenske flask, add 35 ml of cold distilled water, recently boiled for 15 minutes, 10 ml of the dilute sulphuric acid and a loosely wound 5 mm coil of 30 cm of aluminium wire (about 1 mm thick or 18 to 20 Standard Wire Gauge) or 0.1 g of pumice powder.

Connect the flask with the standard apparatus and repeat the process as described above, i.e. the distillation of 110 ml in 19–21 minutes, the mixing (but without the cooling for 10 minutes), and the filtration and the titration of 100 ml of the filtrate with the barium hydroxide solution.

Calculation and expression of results

Reichert–Meissl (R) value = $1.10 (T_1 - T_2)$

Polenske (P) value = $T_3 - T_4$

$$\text{Kirschner value (K)} = \frac{121(100 + T_1)(T_5 - T_6)}{10000}$$

where

T_1 = ml of 0.1 N barium hydroxide solution used for sample under (a)

T_2 = ml of 0.1 N barium hydroxide solution used for blank under (a)

T_3 = ml of 0.1 N barium hydroxide solution used for sample under (b)

T_4 = ml of 0.1 N barium hydroxide solution used for blank under (b)

T_5 = ml of 0.1 N barium hydroxide solution used for sample under (c)

T_6 = ml of 0.1 N barium hydroxide solution used for blank under (c)

$$\text{Percentage milk fat in margarine fat} = \frac{K - 0.1P - 0.24}{0.244}$$

where

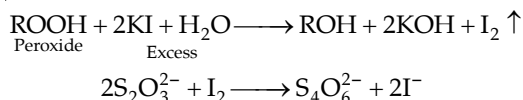
K = Kirschner value

P = Polenske value

5. Determination of Peroxide Value

Peroxides (R-OOH) are the primary reaction products formed in the initial stages of oxidation. Therefore, peroxide value (PV) is a very sensitive indicator of the early stages of oxidative deterioration of fats and oils. PV therefore provides a means of predicting the risk of the development of flavor rancidity. The PV is defined as reactive oxygen contents, expressed in terms of milliequivalents (mEq) of free iodine per kg of fat. There are numerous analytical procedures for the measurement of peroxide value.

The most common method is based on the iodometric titration originally reported by **Lea** and **Wheeler**, which measure the iodine produced from potassium iodide on reaction with peroxides present in the oil.



Principle

When a rancid fat or oil sample is treated with potassium iodide after dissolving in an appropriate solvent, peroxides present in the fat liberate iodine. The test is a volumetric one where I_2 , formed from KI in the presence of peroxide is determined by titrating with standard sodium thiosulphate solution using starch mucilage as indicator.

Procedure

- (i) Weigh accurately (by difference) 5 g of fat or oil sample in the-Iodine flask.
- (ii) Add 25 ml of solvent and displace the air with nitrogen gas.
- (iii) Add 1 ml of KI solution, stopper the flask, and allow it to stand for 1 min (with gentle shaking).
- (iv) Add 35 ml of distilled water and a few drops of starch indicator. Appearance of blue color on addition of starch indicates presence of free iodine.
- (v) Titrate the liberated iodine with 0.01 N or 0.1 N sodium-thiosulphate until the blue color just vanishes.
- (vi) Carry out a blank determination simultaneously (omitting oil).
- (vii) Calculate Peroxide value using following equation:

$$\text{PV (mEq / kg)} = \frac{N \times V_S - V_B \times 1000}{\text{Weight of sample (g)}}$$

where,

N = normality of sodium thiosulphate

V_S = sodium thiosulphate consumed by sample (ml)

V_B = sodium thiosulphate consumed by blank (ml)

6. Acetyl Number

The number of milligrams of KOH required to neutralize the acetic acid released by saponification of one gram of fat after it has been acetylated is known as acetyl number. Some of the fatty acid residues in fats contain hydroxyl groups. In order to determine the proportion of these, they are acetylated by means of acetic anhydride. Thus, an acetyl group is introduced wherever a free $-OH$ group is present. After washing out the excess of acetic anhydride and acetic acid liberated, the acetylated fat is dried and weighed and the acetic acid in combination with the fat determined by titration of liberated acetic acid from acetylated fat or oil with standard alkali after it has been set free (Fig. 5.2). The acetyl number is thus a measure of the number of hydroxyl group present. Acetyl number can be used to detect adulteration.

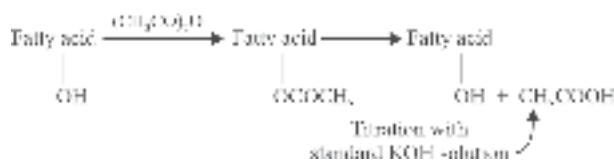


Fig. 5.2: Determination of acetyl number

$$\text{Acetyl number} = 1335 (b - a) / (1335 - a)$$

where a = Saponification value of the substance

b = Saponification value of acetylated substance

Castor oil, because of its high content of ricinoleic acid, has a high acetyl number.

Castor oil 146–150

Peanut oil 3.5

Cod liver oil 1.1

Olive oil 10.5

Cotton seed oil 21–25

7. Melting Point of Fat by Open Capillary Tube Method

The temperature at which the oil or fat softens or becomes sufficiently fluid to slip or run is determined by the open-tube capillary-slip method. Fats do not melt sharply because they contain different types of fatty acids with different melting points. The melting point of fat is increased with the increase in the degree of saturation and chain length of fatty acid. Unsaturated bonds produce kinks in the fatty acid chain and therefore allow very loose molecular packing. This facilitates faster slipping away of molecules, thereby resulting in low melting point. Melting point also depends on isomeric forms and polymorphism in fatty acids. *Trans* isomers of fatty acids (that usually form during hydrogenation process) have higher melting point because the chains are less kinked.

Procedure

- (i) Melt the sample and introduce a capillary tube into the molten sample, so that a column of the sample, about 10 mm long, is sucked into the tube.
- (ii) Chill the tube containing the sample immediately by touching the tube, against a piece of ice until the fat solidifies.
- (iii) Place the tube in a small beaker and hold it for one hour either in a refrigerator or in water maintained at $4-10^\circ\text{C}$.
- (iv) Remove the tube and attach with a rubber band to the thermometer bulb, so that the lower end of the capillary tube and the thermometer bulb are at the same level.

- (v) Take water at 10°C in the Thiele tube and immerse the thermometer with the capillary tube containing the sample of fat.
- (vi) Gradually increase the temperature by heating at the side-tube of the Thiele tube at the rate of 2°C per min, till the temperature reaches 25°C, and thereafter at the rate of 0.5°C per min.
- (vii) Note the temperature of the water when the sample column begins to rise in the capillary tube.
- (viii) Report the average of two such separate determinations as the melting point, provided that the readings do not differ by more than 0.5°C.

8. Baudouin Test

The development of pink color with furfural solution in the presence of hydrochloric acid indicates the presence of sesame oil. The color is produced on account of reaction with sesamolin present in sesame oil. This test is useful in the detection of adulteration of dairy ghee with vanaspati ghee. The test is based on the color reaction between sesamolin (a compound present in sesame oil) and furfural. In Nepal, use of sesame oil in vanaspati is mandatory. Dairy ghee containing sesamolin gives a positive Baudouin test, thereby indicating the presence of vanaspati ghee.

Procedure

- (i) Take 5 ml of the oil or melted fat in a 25 ml measuring cylinder (or test tube) provided with a glass stopper.
- (ii) Add 5 ml of concentrated hydrochloric acid.
- (iii) Add 0.4 ml of furfural solution.
- (iv) Insert the glass stopper and shake vigorously for two minutes.
- (v) Keep the measuring cylinder to separate out the mixture into two layers. The development of pink color in the lower acid layer indicates presence of sesame oil.
- (vi) Confirm it by adding 5 ml of water and shake again.
- (vii) If the color in acid layer persists, sesame oil is present; if the color disappears, it is absent.

9. Hexabromide Test

The formation of a precipitate of hexabromide when the oil in chloroform is treated with bromine followed by alcohol and ether in cold condition indicates the presence of linseed oil. This test is of importance for detecting adulteration of edible oil with linseed oil (which is inedible). The test is based on the formation of insoluble polybromides when unsaturated fatty acids are brominated. Di- and tetrabromides that result from oleic and linoleic acids are soluble and therefore do not interfere with this visual test.

Procedure

- (i) Pipette 1 ml of oil into a boiling tube (wide-mouthed, 100 ml capacity).
- (ii) Add 5 ml chloroform and about 1 ml of bromine dropwise till the mixture becomes deep red in color.
- (iii) Cool the test tube in an ice water-bath.

- (iv) Add about 1.5 ml of rectified spirit dropwise while shaking the mixture until the precipitate which is first formed just dissolves.
- (v) Add 10 ml diethyl ether.
- (vi) Mix the contents and place the tube within the ice water-bath for 20 minutes.
- (vii) Appearance of precipitates indicates the presence of linseed oil.

RANCIDITY

Rancidification is the chemical decomposition of fats, oils and other lipids. When these processes occur in food, undesirable odors and flavors can result. In some cases, however, the flavors can be desirable (as in aged cheeses) (Fig. 5.3). Rancidification can also detract from the nutritional value of the food. Some vitamins are highly sensitive to degradation.

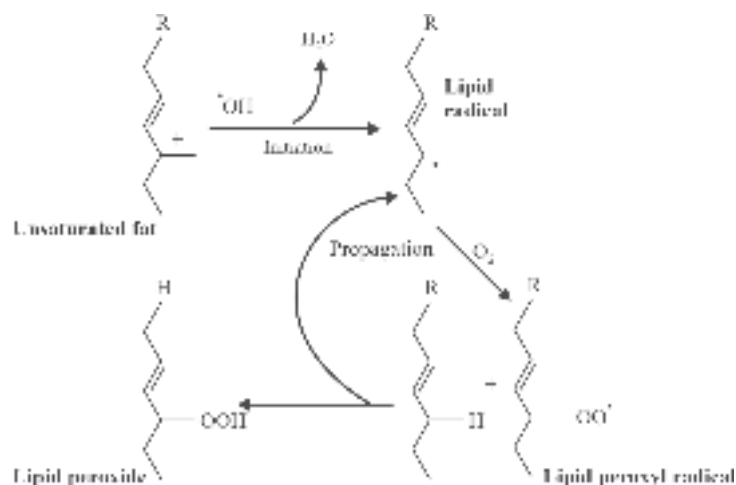


Fig. 5.3: Free radical pathway for the first phase of the oxidative rancidification of fats

Causes of Rancidity

Three causes for rancidification are recognized:

1. Hydrolytic rancidity occurs when water splits fatty acid chains away from the glycerol backbone in triglycerides (fats). The chemical term is ester hydrolysis. Usually this hydrolysis process goes unnoticed, since most fatty acids are odorless and tasteless. When, however, the triglyceride is derived from short chain fatty acids, the released carboxylic acid can confer strong flavors and odors. A particular problem arises with butter, which contains triglycerides with a high content of butyric acid derivatives.
2. Oxidative rancidity is associated with the degradation by oxygen in the air. Essentially, the food or fatty acid is damaged by a free radical process which is both oxygen and temperature driven (oxidation). As a result, the fatty acid is broken down into smaller, lighter and more volatile fatty acids. Via a free radical process, the double bonds of an unsaturated fatty acid can undergo cleavage, releasing volatile aldehydes and ketones that give the bad rancid taste and smell to the fat. This process can be suppressed by the exclusion of oxygen or by the addition of antioxidants. Oxidation primarily occurs with unsaturated fats.

3. Microbial rancidity refers to a process in which microorganisms, such as bacteria, use their enzymes such as lipases to break down fat. This pathway can be prevented by sterilization.

Role of Antioxidants in Preventing the Rancidity

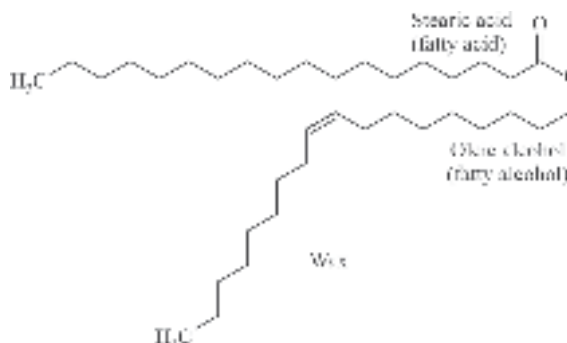
- Antioxidants are often added to fat-containing foods in order to retard the development of rancidity due to oxidation.
- Natural anti-oxidants include flavonoids, polyphenols, ascorbic acid (vitamin C) and tocopherols (vitamin E).
- Synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl 3,4,5-trihydroxybenzoate also known as propyl gallate and ethoxyquin.
- The natural antioxidants tend to be short-lived, so synthetic antioxidants are used when a longer shelf life is needed.
- The effectiveness of water-soluble antioxidants is limited in preventing direct oxidation within fats, but is valuable in intercepting free radicals that travel through the watery parts of foods.
- A combination of water-soluble and fat-soluble antioxidants is ideal, usually in the ratio of fat to water.
- In addition, rancidification can be decreased, but not completely eliminated, by storing fats and oils in a cool, dark place with little exposure to oxygen or free radicals, since heat and light accelerate the rate of reaction of fats with oxygen. (Oxidative rancidity or autooxidation is a chemical reaction with a low activation energy consequently the rate of reaction is not significantly reduced by cold storage).
- Do not add fresh oil to vessels containing old oil. The old oil will trigger a reaction and the new oil will become rancid far more rapidly than if the oil was stored in a clean empty vessel. Avoid using vessels that are wet, this will also speed up the problems associated with oxidation, allow tanks to drain and dry adequately before use.

2. Waxes

Waxes are esters of long chain fatty alcohols (reduced fatty acids) and fatty acids. Camauba wax, lanolin, beeswax, spermaceti, etc. are the examples of naturally occurring waxes.



The word “wax” originates from the old English word “Weax” meaning “the material of the honeycomb”, i.e. beeswax. The fatty acids range in between 14 and 36 carbon atoms and the alcohols range from 16 to 36 carbon atoms. Some of the alcohols are listed in Table 5.3. The term ‘wax’ is sometimes used wrongly. For example, Japan wax and myrtle wax are not true waxes but glycerides as these give glycerol and palmitic acid on saponification.

**Table 5.3:** Commonly occurring fatty alcohol in waxes

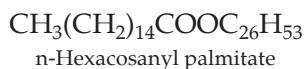
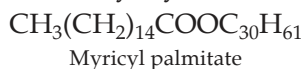
Name	Formula	m.p. °C	Occurrence
Cetyl alcohol	C ₁₆ H ₃₃ OH	50	Spermaceti
Ceryl alcohol	C ₂₆ H ₆₁ OH	79	Chinese wax
Myricyl alcohol	C ₃₀ H ₆₁ OH	88	Beeswax
Cholesterol	C ₂₇ H ₄₅ OH	148–150	Animal oil
Isocholesterol	C ₂₇ H ₄₅ OH	137–148	Wool wax
Phytosterol	C ₂₉ H ₄₉ OH	135–148	Vegetable oil
Montanyl	C ₂₈ H ₅₇ OH	–	Cotton wax alcohol
Gossypol alcohol	C ₃₀ H ₆₁ OH	82–88	Cotton alcohol

Waxes are saponified with great difficulty than fats and are not attacked by lipase. Although, waxes may be saponified by prolonged boiling with alcoholic KOH, they are more easily saponified by treating a solution of the wax in petroleum ether with absolute alcohol and metallic sodium, i.e. with sodium ethoxide. The saponification products of waxes are water soluble soaps while the water insoluble long chain alcohols appear in the “unsaponifiable matter” fraction. Waxes contain about 31–55% of the unsaponifiable matter while fats and oils contain only 1–2% unsaponifiable matter. They are classified into:

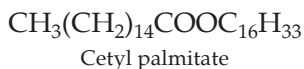
- A. Naturally occurring waxes.
- B. Synthetic waxes.

A. Naturally occurring waxes

Beeswax: It contains esters derived from alcohols having 24–30 carbon atoms. For example palmitate of myricyl alcohol and n-hexacosanol.



Spermaceti: It is obtained from the head of the sperm whale. It is rich in ester of cetyl alcohol and palmitic acid. It is used in the manufacture of candle.



Sperm oil: It is a liquid wax and occurs with spermaceti in the sperm whale. It is a valuable lubricant used for delicate instruments such as watches. It does not become gummy like other normal oils.

Carnauba wax: It is the hardest known wax. It consists mainly of fatty acids esterified with tetracosanol and tetratriacontanol. It is found in the leaves of the carnauba palm,

Copemicia prunifera of Brazil. It is used as an ingredient in the manufacture of various wax polishes. Because waxes are very inert chemically; they make an excellent protective coating.

Lanolin: It is obtained from wool and is used in making ointments and other pharmaceutical formulations. It is an emollient with moisturizing properties and an emulsifier with high water-absorption capabilities.

Montan wax: Montan is a mineral wax, derived from lignite primarily in German lignite mines. The material is refined and may be esterified before being emulsified. Montan can be used as a Carnauba substitute in many compositions due to its toughness, high melting point and emulsifiability. It is light in color with good polishability.

Paraffin wax: Paraffin wax is a byproduct of petroleum refining. Paraffin is a soft, low molecular weight straight chain hydrocarbon, low melting (120°F – 160°F / 49°C – 71°C) wax. Paraffin emulsions are used for water resistance, anti-blocking release and barrier properties in paint and coatings.

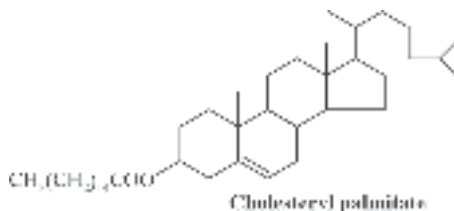
Microcrystalline wax: It is similar to paraffin wax but has higher melting point due to presence of cyclic saturated hydrocarbons in addition to normal alkanes.

Miscellaneous: The waxes from the conifers, contain polymers formed by the ester linking of many omega hydroxy acids such as Juniperic acid with each other.



Juniperic acid

There are certain waxes which have a characteristic odor. This is due to the presence of hydroxy acids in the form of lactones in them. For example, a wax named ambretolide, which is extracted from the seeds of lady's finger, *Ambelmoschus esculentus*, has a characteristic musky smell. Lipids of marine organisms such as starfish, squid and shark contain fatty alcohols that are long chain alkyl ethers of glycerol. Chinese wax is the secretion of an insect. Some of the waxes found in skin, are esters of hydroxylated fatty acids and open chain alcohols. A wax found in blood plasma is found to have cholesteryl palmitate, i.e. ester of cholesterol and palmitic acid.



B. Synthetic waxes

Synthetic waxes are produced primarily from ethylene. These materials are less variable than natural products and less inclined to price fluctuations since supply is not dependent on weather, rain fall, etc. which can affect production. Some examples of synthetic waxes are given here.

Polyethylene wax: Polyethylene waxes are manufactured from ethylene, generally produced from natural gas. The polyethylene we use is either oxidized or co-polymerized with acrylic acid to give the polyethylene chemical functionality which allows it to be emulsified. Polyethylene is classified as either high density polyethylene (HDPE) or low density polyethylene (LOPE). HDPE has higher melting (110°C – 140°C / 230°F – 284°F) and is harder. HOPE is used to improve rub resistance, slip and antiblocking. LDPE has lower melting (100°C – 110°C / 212°F – 230°F) and is softer. LOPE is used for lubricity, and rub resistance.

Polypropylene wax: Polypropylene wax is generally polymerized from propylene and then either maleated or oxidized to give chemical functionality so that it is more easily emulsified. Polypropylenes are hard materials with molecular weights from 10,000–60,000+ and have high melting points (120°C–160°C/248°F–320°F). Polypropylenes are used to lower coefficient of friction in floor finishes and water-borne coatings.

Tetrafluoroethylene (PTFE) Wax: PTFE is a fluorocarbon polymer. PTFE has extreme heat resistance 330°C/626°F. The fluorine component gives this product additional release, slip and rubs characteristics. Many fluorinated products also help with grease and oil resistance.

Ethylene-acrylic acid (EAA): EAA co-polymer properties are more resin like than wax. These polymers have high 20% acrylic acid content. These products are high molecular weight with excellent adhesion to a wide range of substrates. EAA dispersions are film formers with good barrier properties. They can be crosslinked with a wide range of crosslinkers to increase their resistance properties. Because of their broad FDA approvals these products can be used in food packaging applications.

Difference between Fat and Wax

- Fats (and oils) are triglycerides (or triacylglycerols). They consist of glycerol combined with three fatty acid molecules. The fatty acids are linked to the glycerol by ester links.
- Waxes are esters of an alcohol other than glycerol and a single long chain acid.
- Waxes often contain varying amount of hydrocarbons

Physiological Importance of Waxes

1. The most important physiological function of waxes is as a protective agent on the surfaces of animals and plants.
2. Waxes are found on the surface of feathers and hair with the result they remain soft and pliable.
3. Waxes prevent aquatic animals from becoming wet. Because of this, water related birds like duck able to maintain dryness.
4. Waxy coating on the surface of plants protects them from excessive loss of moisture. Hence, desert plants like palm and cactus can live for long periods with rain.
5. Waxy coating prevents the infection of plants from fungi and bacteria, which can cause diseases.
6. Waxy coating on several fruits like apples and citrus fruits prevent them from drying out and thus these fruits can be stored for long period of time. The waxy covering also protects such fruits from organism which cause rot.

B. COMPOUND OR COMPLEX LIPIDS

These are lipids, which contain an inorganic or organic group in addition to fatty acids and glycerol. It is divided into two types:

1. PHOSPHOLIPIDS

These are lipids containing a phosphate group. A phospholipid molecule has a strongly nonpolar and hydrophobic (water insoluble) tail region represented by fatty acid chains

and a strongly polar or hydrophilic (water soluble) head region represented by the phosphate group (Fig. 5.4). Due to this property, a phospholipid placed in water, forms a lipid bilayer in a characteristic manner. Such an arrangement forms a basic component of plasma membranes. Due to its amphipathic nature and insolubility in water, phospholipids are ideal compounds for forming the biological membrane.

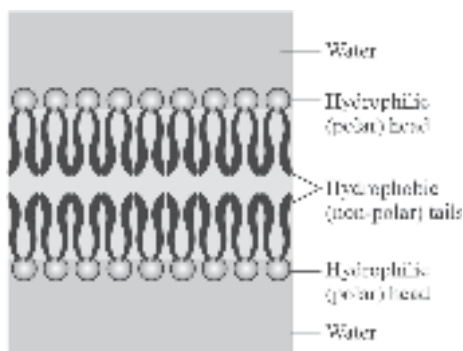


Fig. 5.4: Representation of phospholipid structure

Functions of Phospholipids

(i) Structural Role

Phospholipids participate in the lipoprotein complexes which are thought to constitute the matrix of cell walls and membranes, the myelin sheath, and specialised structures such as mitochondria and microsomes. In this role, they impart certain physical characteristics like high permeability towards certain nonpolar molecules and lysis by surface active agents like detergents, bile salts, etc.

(ii) Role in enzyme action

Certain enzymes require tightly bound phospholipids for their actions, e.g. mitochondrial enzyme system involved in oxidative phosphorylation.

(iii) Role in blood coagulation

Phospholipids play an important part in the blood coagulation process. They are required at the stage of conversion of prothrombin by active factor X and in the activation of factor VIII by activated factor IX. Platelets provide the chief source of phospholipids and that part of total lipid content which contribute to intrinsic blood coagulation process. This lipid content is called platelet factor 3.

(iv) Role in lipid absorption in intestine

Lecithin lowers the surface tension of water and aids in emulsification of lipid water mixture, a prerequisite in digestion and absorption of lipids from gastrointestinal tract.

(v) Role in transport of lipids from Intestines

Exogenous triglycerides is carried as lipoprotein complex, chylomicrons, in which phospholipids takes an active part.

(vi) Role in transport of lipids from liver

Endogenous triglycerides are carried from liver to various tissues as lipoprotein complex pre-lipoprotein. Phospholipid is required for the formation of the lipoprotein complex.

(vii) Role in electron transport

Probably phospholipid helps to couple oxidation with phosphorylation and maintains electron transport enzymes in active conformation and proper relative positions.

(viii) Lipotropic action of lecithin

Choline acts as a lipotropic agent as it can prevent formation of fatty liver. As lecithin can provide choline, it acts as a lipotropic agent.

(ix) Ion transport and secretion

Phospholipids are in some way implicated in the mechanism of secretion. It is suggested by the observation that phospholipids, especially phosphatidic acid and phosphorinositides turnover is proportional to the rate of secretion of cells liberating such products as hormones, enzymes, mucins and other proteins.

(x) Membrane phospholipids

Phospholipids of membrane are hydrolyzed by phospholipase A₂ and provide the unsaturated fatty acid arachidonic acid, which is utilized for synthesis of prostaglandins and leukotrienes.

(xi) Insulation

Phospholipids of myelin sheaths provide the insulation around the nerve fibers.

(xii) Cofactor

Phospholipids are required as a cofactor for the activity of the enzyme lipoprotein lipase and triacylglycerol lipase.

(xiii) Role in hormone action

Some signal must provide communication between the hormone receptor on the plasma membrane and intracellular calcium reservoirs. The best candidate appears to be the products of phosphatidylinositides metabolism. Phosphatidylinositol-4, 5-bisphosphate is hydrolyzed to myo-inositol 1,4,5-triphosphate and diacylglycerol through the action of a phosphodiesterase. Myo-inositol triphosphate releases calcium ions from a variety of membrane and organelle preparations.

Chemistry and Classification of Phospholipids

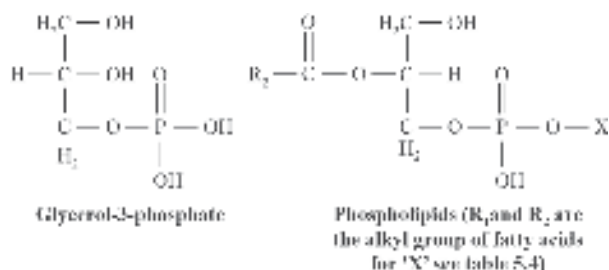
Phospholipids are of two types, namely glycerophospholipids and sphingolipids, depending upon the nature of the alcohol. If glycerol present as alcohol then they are referred as glycerophospholipids or glycerophospholipids. Similarly, if sphingosine present as alcohol then these are known as sphingophospholipids or sphingolipids.

A. Glycerophospholipids

These are phospholipids or phosphatides which consist of an alcohol such as glycerol, one or two molecules of fatty acid, and a phosphoric acid compound. In other way, these are triglyceride derivatives in which one fatty acid has been replaced by a phosphate group and one of several nitrogen-containing molecules. They are found in all plants and animals and include such substances as lecithin, cephalin, etc. Lecithin is a significant constituent of brain and nervous tissue consisting of a mixture of diglycerides of stearic, palmitic, and oleic acids, linked to the choline ester of phosphoric acid.

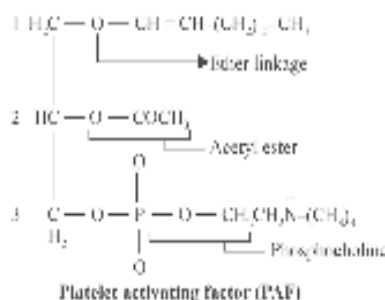
Glycerophospholipid structures

Phospholipids are synthesized by esterification of an alcohol to the phosphate of phosphatidic acid (1,2-diacylglycerol 3-phosphate). Most of phospholipids have a saturated fatty acid on C-1 and an unsaturated fatty acid on C-2 of the glycerol backbone. The most commonly added alcohols are serine, ethanol amine and choline. These also contain nitrogen that may be positively charged, whereas, glycerol and inositol do not. Based on the type of alcohol (X), the major classifications of phospholipids are shown in Table 5.4.



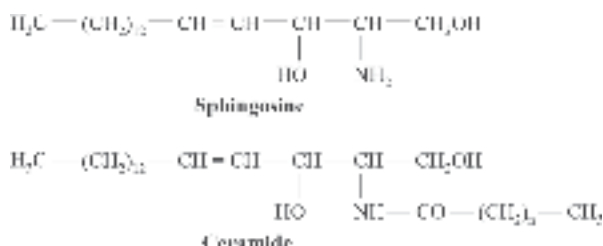
Plasmalogens

Plasmalogens are glycerol ether phospholipids. These are of two types, alkyl ether and alkenyl ether. Three major classes of plasmalogens have been identified: choline, ethanolamine and serine plasmalogens. Ethanolamine plasmalogen is prevalent in myelin. Choline plasmalogen is abundant in cardiac tissue. One particular choline plasmalogen (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) has been identified as an extremely powerful biological mediator, capable of inducing cellular responses at concentrations as low as 10^{-11} M. This molecule is called platelet activating factor (PAF).



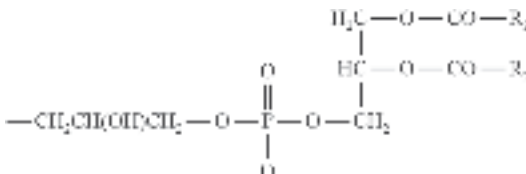
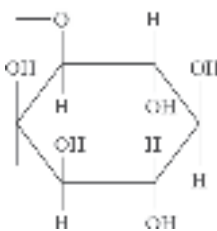
B. Spingophospholipids

The sphingophospholipids, like the phospholipids, are composed of a polar head group and two nonpolar tails. The core of sphingolipids is the long-chain amino alcohol, sphingosine. Amino acylation, with a long chain fatty acid, at carbon-2 of sphingosine yields a ceramide. It is a simple compound of sphingophospholipids.

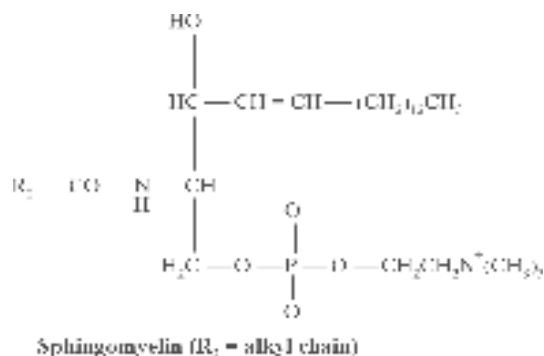


The sphingolipids include the sphingomyelins and glycosphingolipids (the cerebro-sides, sulphatides, globosides and gangliosides).

Table 5.4: Structures of glycerophospholipids

<i>Name of X-OH</i>	<i>Formula of X</i>	<i>Name of Glycerophospholipids</i>
1. Water	-H	Phosphatidic acid
2. Ethanolamine	-CH ₂ CH ₂ NH ₃ ⁺	Phosphatidylethanolamine (PE), also known as cephalin
3. Choline	-CH ₂ CH ₂ N(CH ₃) ₃ ⁺	Phosphatidylcholine (PC) (Lecithin)
4. Serine	-CH ₂ CH(NH ₃ ⁺)COO ⁻	Phosphatidylserine (PS)
5. Glycerol	-CH ₂ CH(OH)CH ₂ OH	Phosphatidylglycerol (PG)
6. Phosphatidyl glycerol		Di-phosphatidylglycerol (DPG)
7. Myoinositol		Phosphatidylinositol (PI)

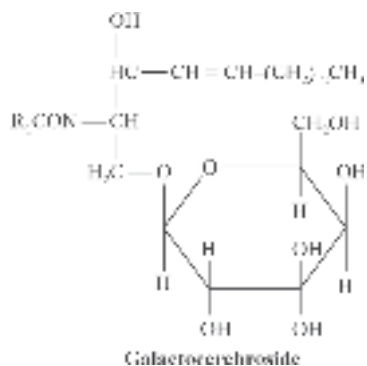
- (a) **Sphingomyelins** are a component of all membranes but are particularly abundant in the myelin sheath. The sphingomyelins are synthesized by the transfer of phosphorylcholine from phosphatidylcholine to a ceramide structure in a reaction catalyzed by sphingomyelin synthase.



- (a) **Glycosphingolipids** or **glycolipids**, are composed of a ceramide backbone with a wide variety of carbohydrate groups (mono- or oligosaccharides), attached to carbon-1 of sphingosine. Glycerol and phosphate are absent in glycosphingolipids. The four principal classes of glycosphingolipids are the cerebro-sides, sulphatides, globosides and gangliosides.

(i) Cerebrosides (Glycosphingolipids)

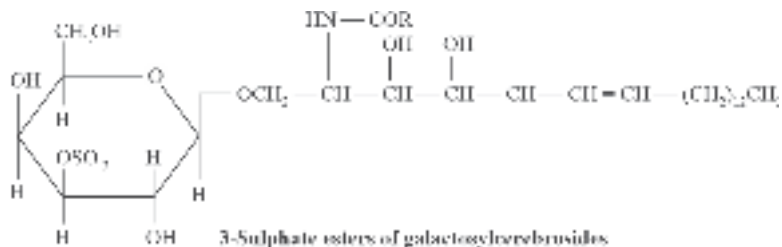
Cerebrosides have a single sugar group linked to ceramide. The most common of these is galactose (galactocerebrosides), with a minor level of glucose (glucocerebrosides).



There is no glycerol, no phosphoric acid and no nitrogenous base. Thus, on hydrolysis, cerebroside yields a sugar, usually galactose but sometimes glucose, a high molecular weight fatty acid and the alcohol, spingosine or dihydrospingosine.

(ii) Sulphatides

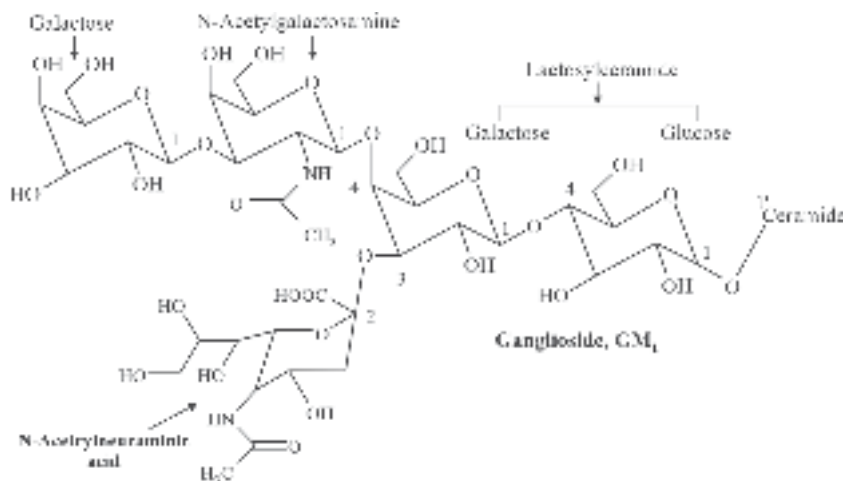
The sulphuric acid esters of galactocerebrosides are the sulphatides. Sulphatides are synthesized from galactocerebrosides and activated sulphate, **3'-phosphoadenosine-5'-phosphosulphate (PAPS)**.

**(iii) Globosides**

Globosides represent cerebrosides that contain additional carbohydrates, predominantly galactose, glucose or GalNAc.

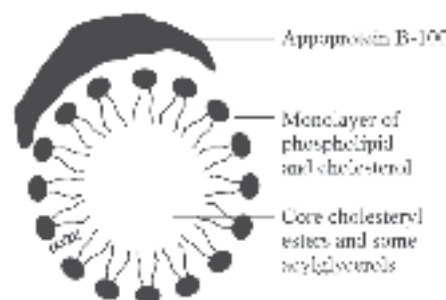
(iv) Gangliosides

The name **ganglioside** was first applied by the German scientist Ernst Klenk in 1942 to lipids newly isolated from ganglion cells of brain. Ganglioside molecule is composed of a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids (e.g. N-acetylneuraminic acid, NANA) linked on the sugar chain. The 60+ known gangliosides differ mainly in the position and number of NANA residues. They are conveniently defined by a short-hand nomenclature system proposed by Svennerholm, in which M, D, T and Q refer to mono-, di-, tri- and tetrasialogangliosides, respectively, and the numbers 1, 2, 3, etc. refer to the order of migration of the gangliosides on thin-layer chromatography. For example, the order of migration of monosialogangliosides is GM3 > GM2 > GM1 (sometimes defined by subscripts, e.g. GM₁ or GM1).



2. LIPOPROTEINS

Lipids, such as cholesterol and triglycerides, are insoluble in plasma. As a result, circulating lipid is bound to lipoproteins that transport the lipid to various tissues for energy utilization, lipid deposition, steroid hormone production, and bile acid formation. The lipoprotein consists of lipid (esterified and unesterified cholesterol, triglycerides, and phospholipids), and protein. The protein components of the lipoprotein are known as apolipoproteins or apoproteins. The different apolipoproteins serve as cofactors for enzymes and ligands for receptors. The general structure of a lipoprotein includes the core, consisting of a droplet of triacylglycerols and/or cholesteryl esters, a surface monolayer of phospholipid, unesterified cholesterol and specific proteins (apolipoproteins, e.g. apoprotein B-100 in low density lipoprotein). Lipoproteins differ in their content of proteins and lipids.



They are classified into five divisions based on their density:

- **Chylomicron** (largest; lowest in density due to high lipid/protein ratio; highest in triacylglycerols as % of weight).
- **VLDL** (very low density lipoprotein; 2nd highest in triacylglycerols as % of weight).
- **IDL** (intermediate density lipoprotein); it carries cholesterol esters and triglycerides.
- **LDL** (low density lipoprotein, highest in cholesteryl esters as % of weight).
- **HDL** (high density lipoprotein, highest in density due to high protein/lipid ratio).

C. DERIVED LIPIDS

These lipids are obtained on hydrolysis of simple and complex lipids. This class of lipids includes steroid hormones, ketone bodies, hydrocarbons, fatty acids, fatty alcohols, mono and diglycerides, terpenes and fat soluble vitamins.

SHORT AND LONG QUESTIONS

1. Discuss the chemical composition of fixed oils with examples.
2. Write the importance of acid value, iodine value and saponification value.
3. What are oils and fats? Discuss the analysis of oils.
4. What are lipids? Explain the differences between fixed oils and fats in chemical structure.
5. Write a note on following:
 - a. Phospholipids
 - b. Chemistry of wax
 - c. Omega fatty acids
 - d. Rancidity of oils and fats
6. Classify the fatty acids with examples.
7. Define the saponification value. Give the method of estimation of saponification value of oil. Mention its significance.
8. Write the significance of:
 - a. Acid value
 - b. Acetyl number
 - c. Saponification number
 - d. Iodine number.
9. Give the components and the biological function of each of the followings:
 - a. Triglycerides
 - b. Phosphatidyl choline
 - c. Sphingomyelin
 - d. GM1- Ganglioside
10. Enumerate the derived lipids and give a detailed account of any two of them.
11. Why do saturated fatty acids have a higher boiling point than the unsaturated fatty acids with the same number of carbon atoms?
12. What are ω -6 and ω -3 fatty acid? Give examples in support of your answer and discuss their biological significance.
13. Explain the terms:
 - a. Saponification
 - b. Rancidity
 - c. Lipid peroxidation
14. Give example of each of the followings:
 - a. Cyclic fatty acid
 - b. Branched chain fatty acid
 - c. Transfatty acid
 - d. Pentaenoic acid
 - e. Saturated fatty acid with 20 carbon atoms

MULTIPLE CHOICE QUESTIONS

1. What is the solubility of lipids in water?
 - a. Soluble
 - b. Partially soluble

- c. Insoluble
 - d. Partially insoluble
2. Which of the following is an example of unsaturated fatty acids?
 - a. Lauric or dodecanoic
 - b. Linoleic or octadecatrienoic
 - c. Palmitic or hexadecenoic
 - d. Myristic or tetradecanoic
 3. Which reagent is used in saponification?
 - a. Ammonia
 - b. Acetic acid
 - c. NaOH/KOH
 - d. Butanone
 4. Which of the following phospholipid is considered as a major constituent of nervous tissue?
 - a. Glycerophospholipid
 - b. Plasmalogen
 - c. Inositol
 - d. Sphingomyelin
 5. Identify the lowest density lipoprotein among the following?
 - a. HDL
 - b. LDL
 - c. VLDL
 - d. Chylomicrons
 6. Beta-oxidation of fatty acid is commonly seen in which of the following options?
 - a. Peroxisome, mitochondria and ER
 - b. Peroxisome and mitochondria
 - c. Only mitochondria
 - d. Only peroxisome
 7. Which type of prosthetic group is present in glycolipids?
 - a. Carbohydrate
 - b. Protein
 - c. Phosphoric acid
 - d. None of the above

Answer

1. (c) 2. (b) 3. (c) 4. (d) 5. (d) 6. (a) 7. (a)

Chapter 6

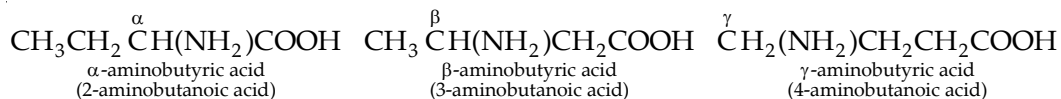
Amino Acids, Proteins and Peptides

AMINO ACIDS

Amino acids are group of organic molecules that consist of a basic amino group ($-\text{NH}_2$), an acidic carboxyl group ($-\text{COOH}$), and an organic, R group (or side chain) that is unique to each amino acid. They are building blocks of proteins and play many critical roles in the body. More than 100 amino acids have been isolated and identified but only 25 are obtained upon hydrolysis of typical proteins. All 25 except 2 are α -amino acids; the two exceptions are proline and hydroxy proline, which are imino acids. Only 20 amino acids are of general occurrence because they are usually found in all proteins.

Nomenclature

Amino acids contain both $-\text{NH}_2$ and $-\text{COOH}$ groups. The position of the amino group with respect to the carboxylic group is usually denoted by Greek symbols α , β , γ , etc. as illustrated below (IUPAC names are given in brackets):



An α -amino acid has the generic formula $\text{H}_2\text{NCHRCOOH}$, where R is an organic substituent, the amino group is attached to the carbon atom immediately adjacent to the carboxylate group (the α -carbon). Other types of amino acid exist when the amino group is attached to a different carbon atom; for example, in γ -amino acids (such as γ -amino-butyric acid) the carbon atom to which the amino group attaches is separated from the carboxylate group by two other carbon atoms. The various α -amino acids differ in which side-chain (R-group) is attached to their α -carbon and can vary in size from just one hydrogen atom in glycine to a large heterocyclic group in tryptophan.

In IUPAC name of an amino acid, the position of NH_2 is indicated by the number of carbon on which it lies, assigning the position of COOH as '1'. Thus, aminopropionic acid is named as 2-aminopropionic acid.

Classification: Types of Amino Acids

There are twenty amino acids that are used to form proteins in the human body. These are called the proteinogenic amino acids. The classification of amino acids as essential or nonessential does not reflect its importance, because all 20 amino acids are necessary for health. An **essential** or **indispensable** amino acid cannot be made by the body and so must be supplied by food. These include isoleucine, leucine, lysine, methionine,

phenylalanine, threonine, tryptophan, and valine. Another amino acid, L-histidine is considered **semi-essential** because the body does not always require dietary sources of it. Conditionally, essential amino acid means, they are not normally required in the diet but can become essential under special circumstances.

Nonessential amino acids are made by the body from the essential amino acids or normal breakdown of proteins. The nonessential amino acids are arginine, alanine, asparagines, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, and tyrosine.

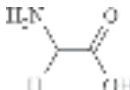
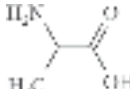
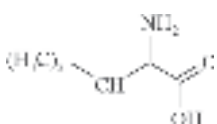
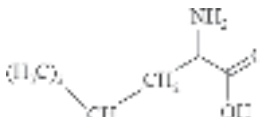
These nine essential amino acids perform a number of important functions in the body:

1. **Phenylalanine:** It is precursor for the neurotransmitter—tyrosine, dopamine, epinephrine and norepinephrine.
2. **Valine:** It stimulate muscle growth and is involved in energy production.
3. **Threonine:** It is a principal component of collagen and elastin.
4. **Tryptophan:** It is a precursor for serotonin, which regulate appetite sleep, etc.
5. **Leucine:** It is critical for protein synthesis.
6. **Methionine:** It has important role in metabolism and detoxification.
7. **Isoleucine:** It is important for immune function.
8. **Lysine:** It plays an important role in protein synthesis.
9. **Histidine:** It is used to produce histamine, neurotransmitter.

There are different classification systems but the classification, which is usually followed is presented here. These are classified on the basis of reactivity in water into acidic, basic and neutral amino acids as given in Table 6.1 .

- (i) **Neutral amino acid:** They contain one $-NH_2$ and one $-COOH$ group.
- (ii) **Acidic amino acid:** They contain one $-NH_2$ and two $-COOH$ group.
- (iii) **Basic amino acid:** They contain two $-NH_2$ and one $-COOH$ group.

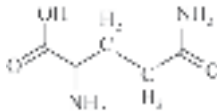
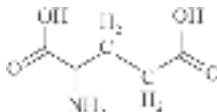
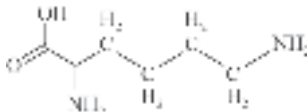
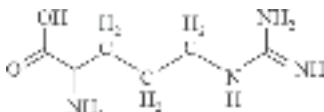
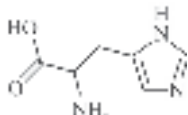
Table 6.1: Classification of amino acids

<i>Amino acid (abbreviated form)</i>	<i>Systematic name</i>	<i>Structure</i>
Neutral amino acids		
Glycine [Gly (G)]	Aminoacetic acid	
Alanine [Ala (A)]	α -aminopropionic acid	
Valine [Val (V)]	α -aminoisovaleric acid	
Leucine [Leu (L)]	α -aminoisocaproic acid	

(Contd.)

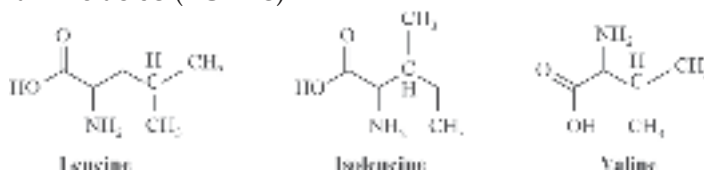
<i>Amino acid (abbreviated form)</i>	<i>Systematic name</i>	<i>Structure</i>
Isoleucine [Ile (I)]	α -amino- β -methylvaleric acid	
Proline [Pro (P)]	Pyrolidine α -carboxylic acid	
Phenylalanine [Phe (F)]	α -amino- β -phenyl propionic acid	
Tryptophan [Trp (W)]	α -amino- β -indole propanoic acid	
Cysteine [Cys (C)]	α -amino- β -mercaptopropionic acid	
Methionine [Met (M)]	α -amino- γ -methylthio-n-butyric acid	
Serine [Ser (S)]	α -amino- β -hydroxypropionic acid	
Threonine [Thr (T)]	α -amino- β -hydroxy-n-butyric acid	
Tyrosine [Tyr (γ)]	α -amino- <i>p</i> -hydroxyhydrocinnamic acid	
Acidic amino acids		
Asparagine [Asn (N)]	α -aminosuccinamic acid	
Aspartic acid [Asp (D)]	Aminosuccinic acid	

(Contd.)

Amino acid (abbreviated form)	Systematic name	Structure
Glutamine [Gln (Q)]	2-amino-4-carbamoyl butanoic acid	
Glutamic acid [Glu (E)]	α -aminoglutaric acid	
Basic amino acids		
Lysine [Lys (K)]	α,δ -diamino- <i>n</i> -caproic acid	
Arginine [Arg (R)]	α -amino- δ -guanidino- <i>n</i> -valeric acid	
Histidine [His (H)]	α -amino- β -imidazolepropionic acid	

Branched-chain Amino Acids (BCAAs)

A carbon atom is able to form up to four covalent bonds. When one carbon is bonded to more than two other carbons it forms a branch. Leucine, isoleucine, and valine are called branched-chain amino acids (BCAAs).

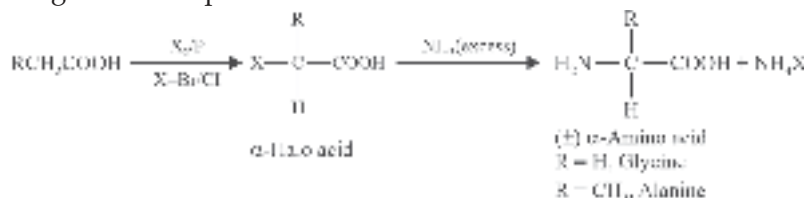


They are so named because their side-chains have a branch.

The combination of these three BCAAs makes up approximately one-third of skeletal muscle in the human body.

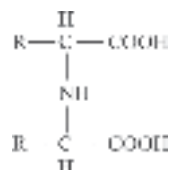
Methods of preparation of amino acids: Various methods of preparation of amino acids are as follows.

- 1. Amination of α -halogen substituted acids:** Amination of α -halo (chloro or bromo) acids, obtained by direct halogenation of carboxylic acids, with aqueous or liquid ammonia gives the respective amino acid.



This amination may, however, be applied for the synthesis of alanine, glycine, serine, threonine, valine, leucine and norleucine. All amino acids obtained in this method are in the form of ($\pm$) racemic mixture.

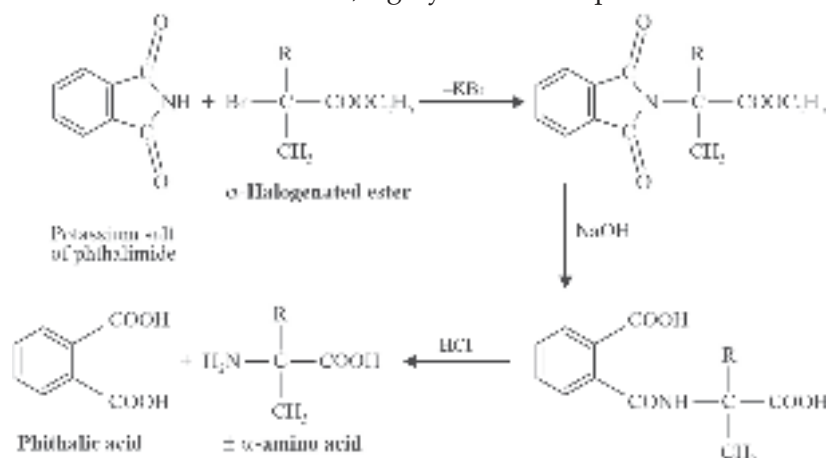
Since this method requires an excess of ammonia, and so formation of side products are also expected as given below. Due to formation of side products, there is a poor yield.



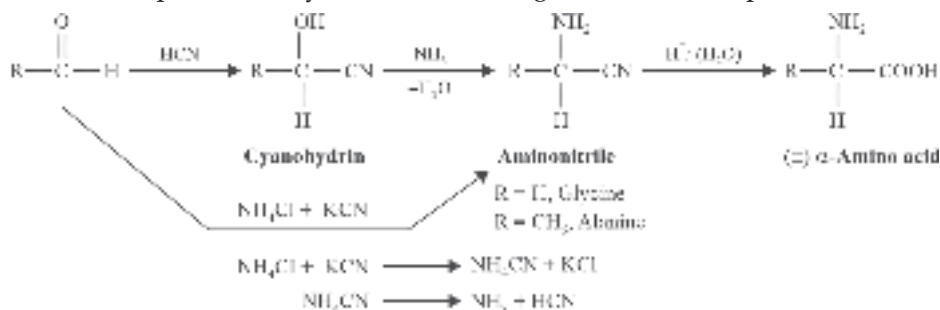
Hence, ammonia may be replaced by hexamethylene tetramine to prevent the formation of primary/secondary amines as side product.

2. **Gabriel's phthalimide synthesis:** This synthesis involves the treatment of halogenated ester with potassium salt of phthalimide and subsequent hydrolysis of the product.

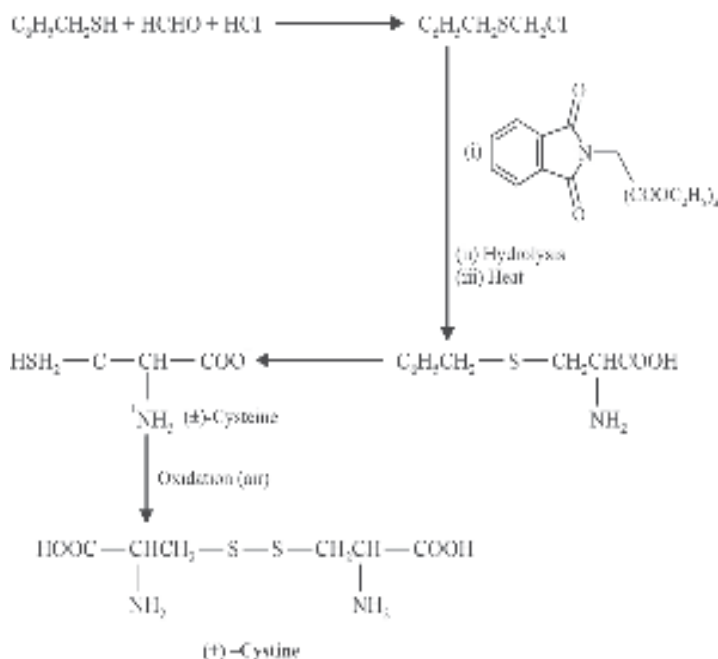
Better yield is obtained in this method than the above. It may also be used for the preparation of acidic amino acids, e.g. synthesis of aspartic acid.



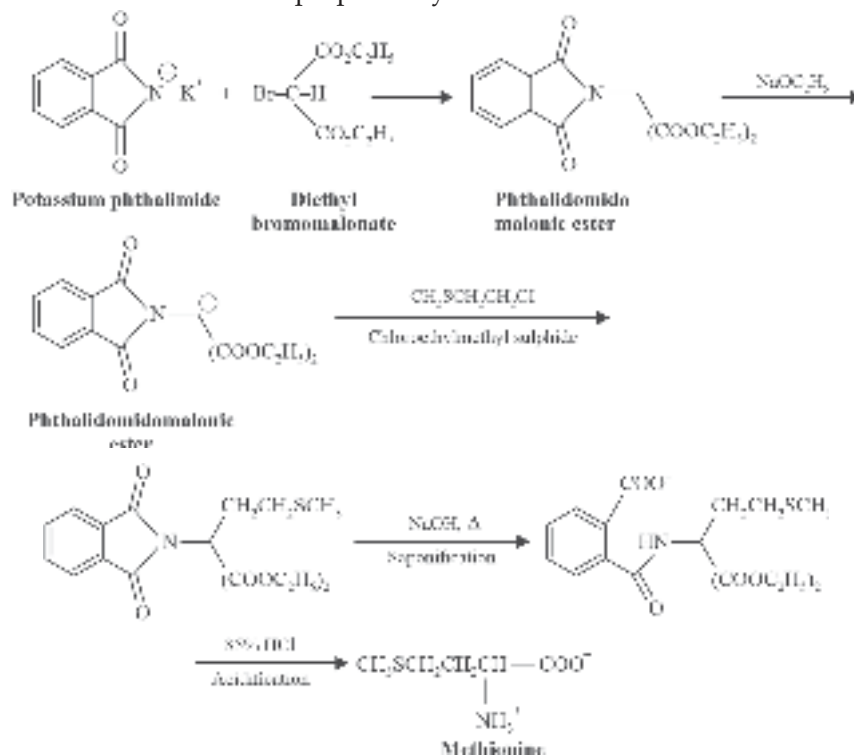
3. **Strecker's synthesis:** In this synthesis, a cyanohydrin, obtained from the reaction of aldehyde with hydrogen cyanide, is treated with concentrated ammonia and the resulting aminonitrile is then hydrolysed with an acid to get amino acid. Aminonitrile may also be prepared in one step by taking a mixture of ammonium chloride and potassium cyanide and treating it with oxo compound.



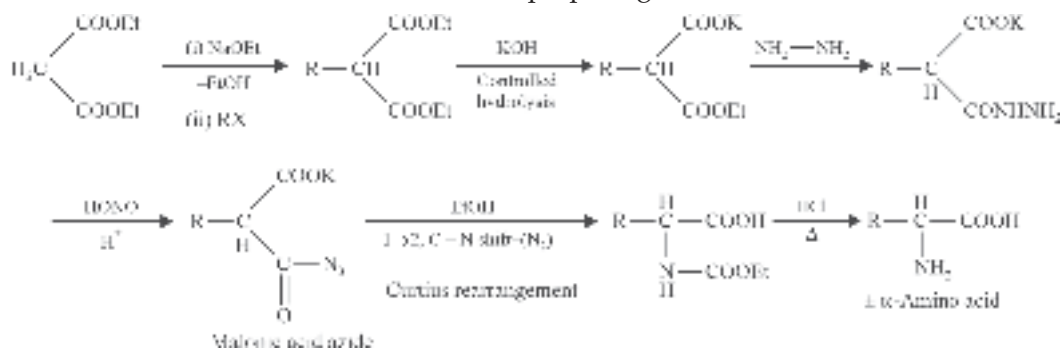
This method is useful for the preparation of glycine, alanine, serine, valine, methionine, leucine, isoleucine, norleucine, phenylalanine and glutamic acid.



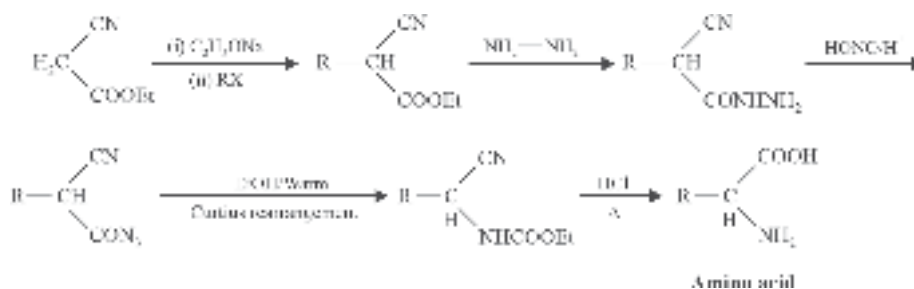
Methionine can also be prepared by this method.



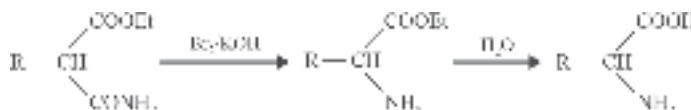
(iv) **Curtius reaction:** In this reaction in addition to malonic ester, cyanoacetic ester and ester amides also are used for preparing amino acids.



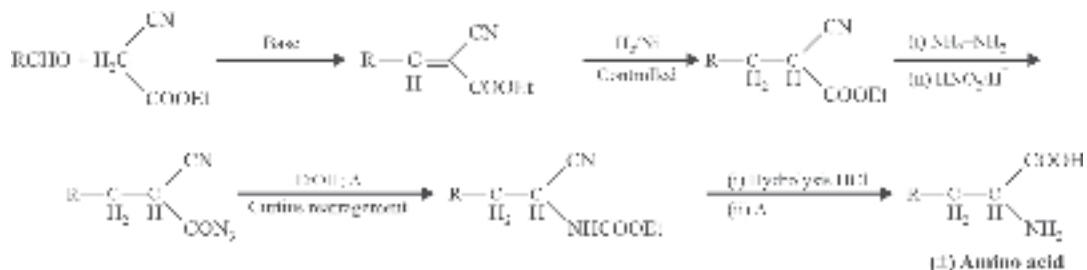
Glycine, alanine, phenylalanine and valine can be prepared by this method. By using cyanoacetic ester, phenylalanine and tyrosine can be conveniently prepared.



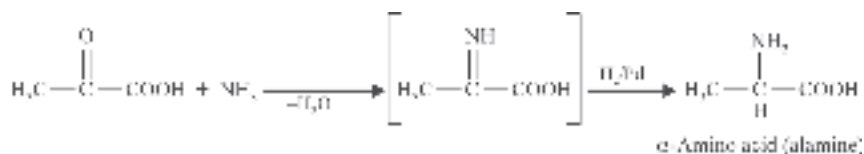
(v) **Hoffmann's degradation method:** This is another variation of malonic ester synthesis, which involves the degradation of ester amides.



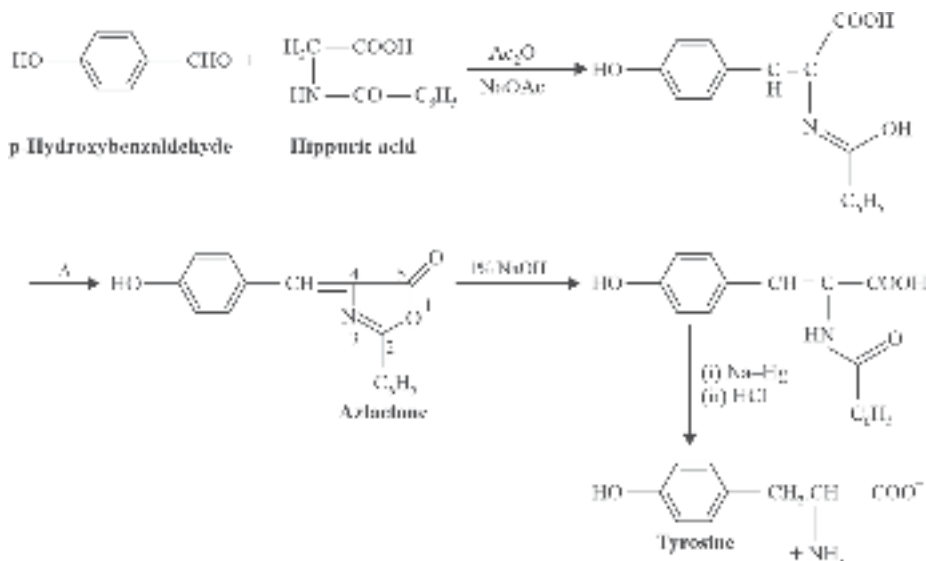
(vi) **Darapsky synthesis:** This method involves the condensation of cyanoacetic ester with an aldehyde.



5. **Reductive ammonolysis of α -keto acids [Koop synthesis]:** The treatment of α -keto acids with ammonia forms an imine, which on catalytic reduction gives amino acid.



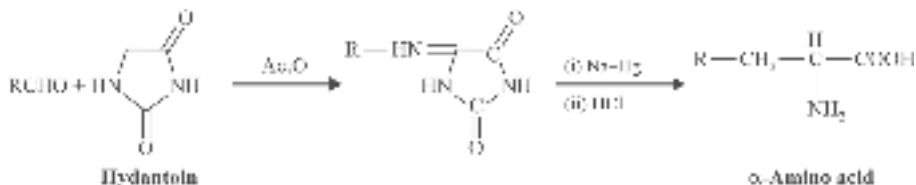
6. **Erlenmeyer azlactone synthesis:** Azlactones are prepared by heating an aromatic aldehyde with benzoyl glycine (hippuric acid) in presence of acetic anhydride and sodium acetate.



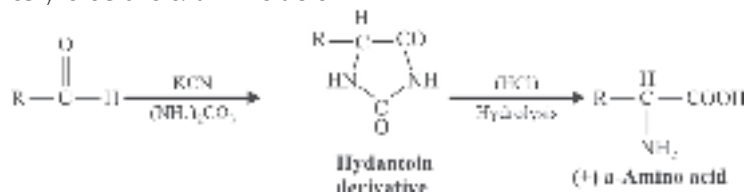
Benzoyl glycine may be replaced by acetyl or any other N-acyl glycine. An aliphatic aldehyde also condenses with benzoyl glycine, if lead acetate is used in place of sodium acetate.

Azlactone synthesis is a convenient method for preparing phenylalanine, tyrosine and tryptophan.

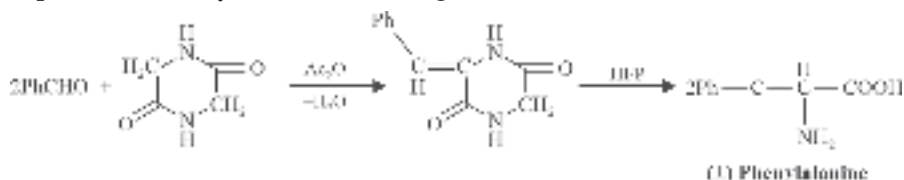
7. **Hydantoin synthesis:** In this method, aromatic aldehydes condense with hydantoin, and the product is then reduced and hydrated in the usual way to give α -amino acid.



8. **Bucherer's hydantoin synthesis:** In the modified method, carbonyl compound is first converted into 5-substituted hydantoin by means of ammonium carbonate and potassium cyanide in aqueous ethanol solution. Hydrolysis of hydantoin derivatives yields the α -amino acid.



9. **Synthesis via diketo piperazine:** This involves the condensation of aromatic aldehyde with diketo piperazine and product formed is heated with red phosphorous and hydroiodic acid to get amino acid.



Phenylalanine, tyrosine and methionine may be prepared by this method.

Resolution: Generally, all the synthetic methods produce racemic mixture of alpha amino acids. If pure L or D enantiomers are desired, it is necessary to resolve these racemic mixtures. A common method of resolving racemates is by diastereomeric salt formation with a pure chiral acid (e.g. tartaric acid) or base (brucine). This is illustrated for a generic amino acid in the Fig. 6.1.

In the initial display, the carboxylic acid function contributes to diastereomeric salt formation. The racemic amino acid is first converted to a benzoyl or acetyl derivative to remove the basic character of the amino group. Next, an ammonium salt is formed by combining the carboxylic acid with an optically pure amine, such as brucine (a relative of strychnine). The structure of this amine is not shown, because it is not a critical factor in the logical progression of steps. Since the amino acid moiety is racemic and the base is a single enantiomer (levorotatory in this example), an equimolar mixture of diastereomeric salts is formed. Diastereomers may be separated by crystallization, chromatography or other physical manipulation, and in this way one of the isomers may be isolated for further treatment, in this illustration, it is the (+):(-) diastereomer (Fig. 6.1a). Finally the salt is broken by acid treatment, giving the resolved (+)-amino acid derivative together with the recovered resolving agent (the optically active amine). Of course, the same procedure could be used to obtain the (-)-enantiomer of the amino acid (Fig. 6.1a).

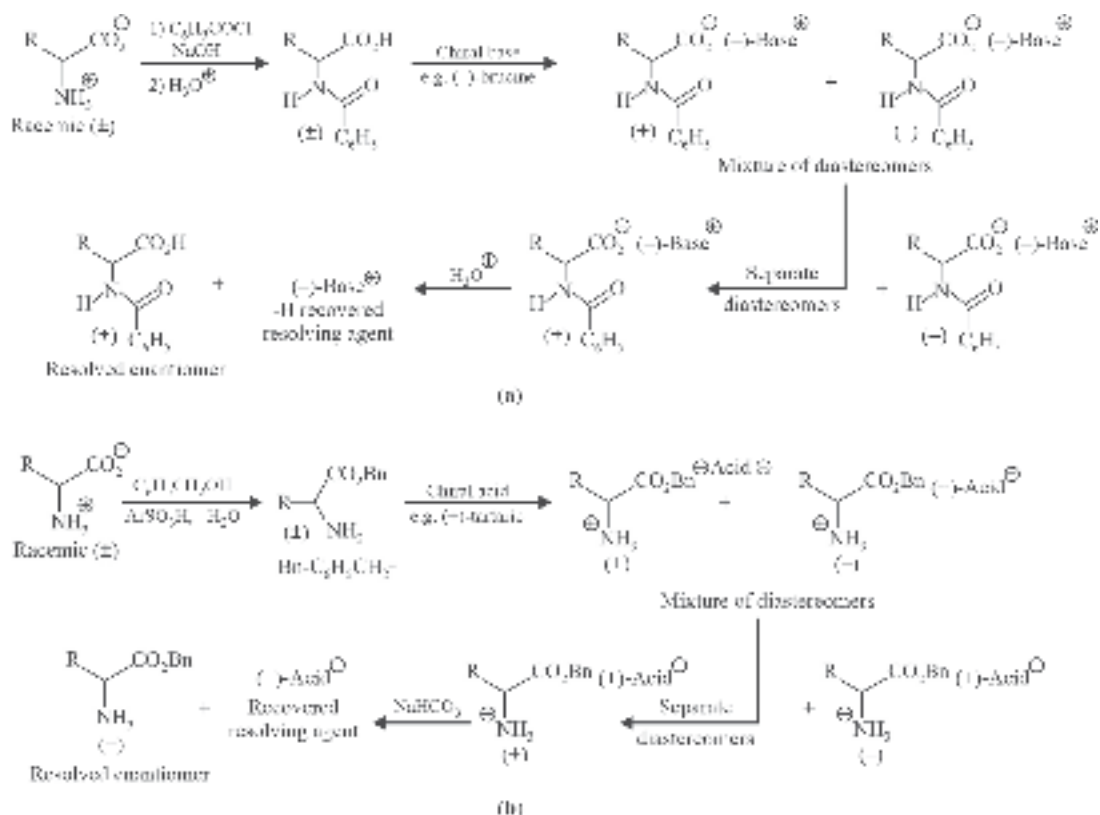


Fig. 6.1: Resolution of racemic mixture by using brucine base (a) and tartaric acid (b)

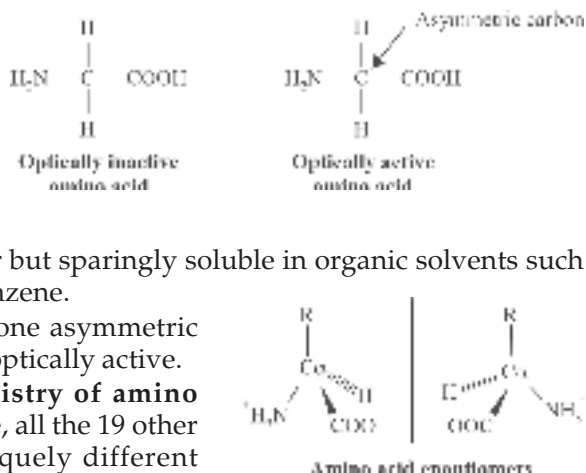
In the resolution of racemic amino acids by using tartaric acid is initiated by making benzyl derivative. The rest of procedure is almost same as shown in Fig. 6.1b. Resolution of amino acid derivatives may also be achieved by enzymatic discrimination in the hydrolysis of amides. For example, an amino cyclase enzyme (isolated from pig kidneys) cleaves an amide derivative of natural L-amino acid much faster than it does with the D-enantiomer.

GENERAL PROPERTIES OF AMINO ACIDS

1. Physical Properties

- Amino acids are colorless, crystalline, stable, high melting solids having sweet taste. These melt with decomposition at high temperature, but a few have tendency to sublime.
- They are generally soluble in water but sparingly soluble in organic solvents such as petroleum ether, ethanol and benzene.
- All α -amino acids contain atleast one asymmetric carbon (except glycine) and so are optically active.

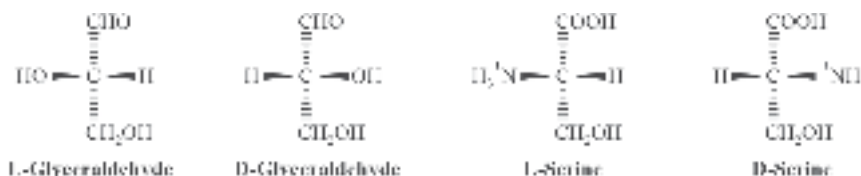
Optical activity and stereochemistry of amino acids: With the exception of glycine, all the 19 other common amino acids have a uniquely different



functional group on the central tetrahedral α -carbon (i.e. C_α). The C_α is termed "chiral" to indicate four different constituents and that the C_α is asymmetric. Since the C_α is asymmetric there exists two possible, nonsuperimposable, mirror images of the amino acids:

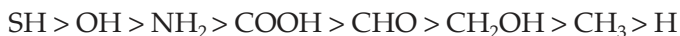
These two different structures distinguished by D, L system or R, S system.

The D, L system: Glyceraldehyde contains a chiral carbon, and therefore, there are two enantiomers of this molecule. One is labeled the "L" form, and the other the "D" form. This is the frame of reference used to describe amino acid enantiomers as being either the "L" or "D" form. For reference, the 'D' and 'L' isomers of serine are shown here:

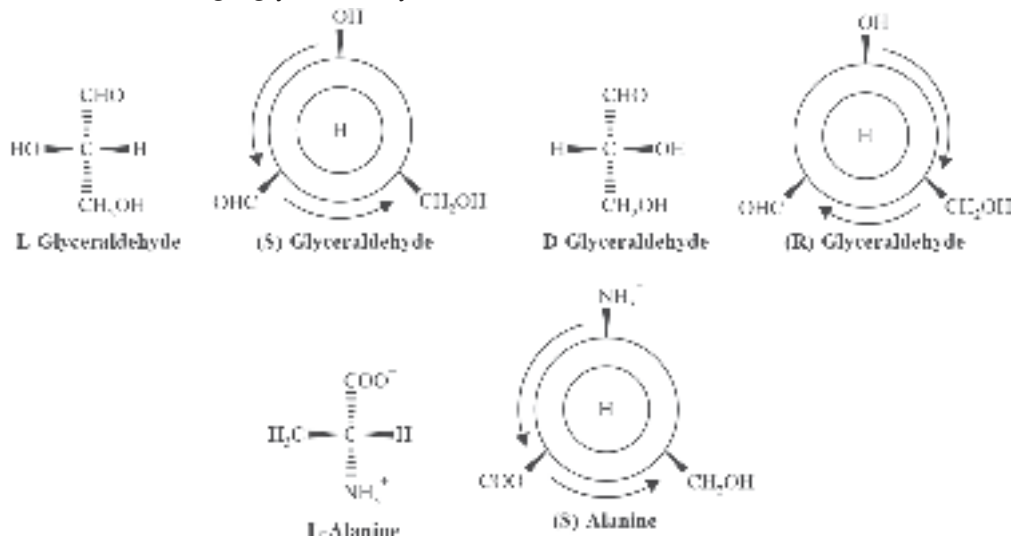


Even though the two enantiomers would seem to be essentially equivalent to each other, **all common amino acids are found in the "L" enantiomer** in living systems.

The R, S system of naming chiral centers: The D, L nomenclature serves well for simple systems with only a single asymmetric carbon atom, ambiguities can arise for more complex molecules. A more rigorous nomenclature termed as the "R, S system" was introduced by Cahn, Ingold and Prelog, which allows for the unambiguous description of molecules with multiple chiral centers. The four substituents around a chiral carbon atom are rank ordered, with atoms of a higher atomic number being ranked above those with a lower atomic number. If two of the bound atoms are identical, the functional groups are rank ordered in the following priority:

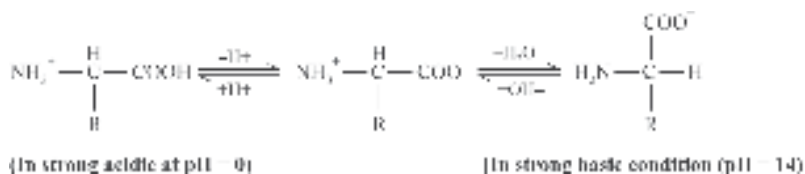


Identify the functional group with the lowest priority. View the chiral center down the bond from the chiral group to the lowest priority atom. Assign priorities to the three other functional groups connected to the chiral center, using the above ranking. If the priorities to these other groups go in a clockwise rotation, the chirality is "R". If the priorities of these other groups go counterclockwise, the chirality is "S" (note that this assignment has nothing to do with optical activity, and is not using D-glyceraldehyde as a reference molecule).



$\begin{array}{c} \text{COOH} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{H} \\ \\ \text{H}_2\text{C} - \text{C} - \text{H} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{COOH} \\ \\ \text{H} - \text{C} - \text{NH}_3^+ \\ \\ \text{H} - \text{C} - \text{CH}_3 \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{COOH} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{H} \\ \\ \text{H} - \text{C} - \text{CH}_3 \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{COOH} \\ \\ \text{H} - \text{C} - \text{NH}_3^+ \\ \\ \text{H}_2\text{C} - \text{C} - \text{H} \\ \\ \text{C}_2\text{H}_5 \end{array}$
L-Isolucine (2S, 3S)-Isolucine	D-Isolucine (2R, 3R)-Isolucine	L-Alloisoleucine (2S, 3R)-Isolucine	D-Alloisoleucine (2R, 3S)-Isolucine

In neutral solution, an amino acid will be present in the following species, which are in equilibrium.



The iso electric point of an amino acid can be calculated as follows:

$$\begin{array}{ccc} \text{NH}_3^+ - \text{Z} - \text{COOH} \rightleftharpoons \text{NH}_3^+ - \text{Z} - \text{COO}^- + \text{H}^+ & \text{NH}_3^+ - \text{Z} - \text{COO}^- \rightleftharpoons \text{NH}_2 - \text{Z} - \text{COO}^- + \text{H}^+ \\ \text{C.A.} & \text{D.I.} & \text{D.I.} & \text{C.B.} \\ K_1 = \frac{[\text{D.I.}][\text{H}^+]}{[\text{C.A.}]} : K_2 = \frac{[\text{C.B.}][\text{H}^+]}{[\text{D.I.}]} & \text{CA} = \frac{[\text{D.I.}][\text{H}^+]}{K_1} : \text{CB} = \frac{K_2 [\text{D.I.}]}{[\text{H}^+]} \end{array}$$

At the isoelectric point (pHi), [D.I] is a maximum and since the net charge is zero.

$$\begin{aligned}
 [\text{CA}] &= [\text{CB}] \\
 \frac{[\text{D.I.}](\text{Hi}^+)}{K_1} &= \frac{K_2[\text{D.I.}]}{[\text{Hi}^+]} \\
 [\text{Hi}^+]^2 &= K_1 K_2 \\
 2\text{pHi} &= \text{p}K_1 + \text{p}K_2 \\
 \text{pHi} &= \frac{(\text{p}K_1 + \text{p}K_2)}{2}
 \end{aligned}$$

e.g. for glycine :

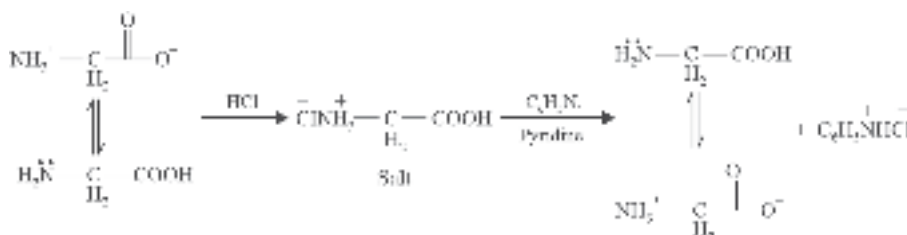
$$\text{p}K_1 = 2.4, \text{p}K_2 = 9.6, \text{pHi} = 2.4 + 9.6 / 2 = 6.0$$

2. Chemical Properties

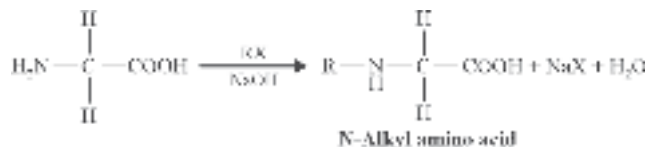
Reactive groups in amino acids include $-\text{NH}_2$ and $-\text{COOH}$ groups and groups present on side chains.

(a) Reaction due to $-\text{NH}_2$ group

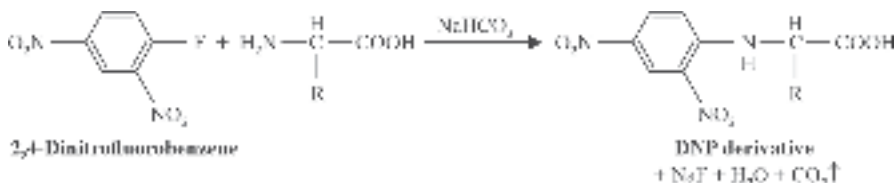
- (i) **Salt formation with strong acids:** Amino acids form salt with strong mineral acids. These salts are usually less soluble in water, but solution becomes strongly acidic. Free amino acids may be liberated from these salts by means of strong base like pyridine.



- (ii) **Alkylation:** In basic solution, amino group can displace the halogen of alkyl halides.



- (iii) **Arylation:** In solution, the amino group can also displace halogen of acyl halide. For example, amino acids form DNP derivative with 2,4-dinitrofluorobenzene. This reaction is useful for the determination of N-terminal amino acid.



- (iv) **Acylation and benzoylation:** Amino acids may be acylated with acid chloride or anhydride, which blocks the amino group and acylated amino group behaves as typical organic acid.

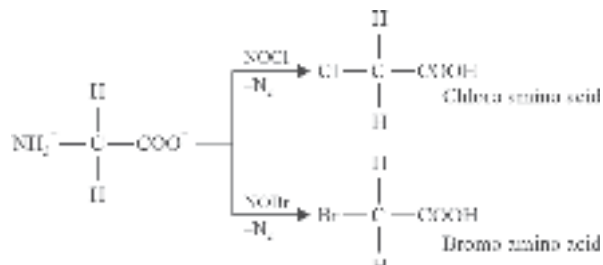
Similarly, amino acid can react with benzoyl chloride to yield benzoyl derivatives.



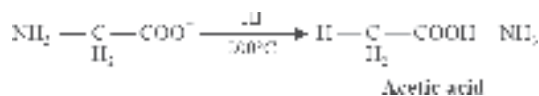
- (v) **Reaction with nitrous acid:** By the action of nitrous acid, amino acid is converted to hydroxyl acid with the liberation of nitrogen. Measurement of the nitrogen evolved, forms the basis of estimation of amino acids (**van Slyke method**).



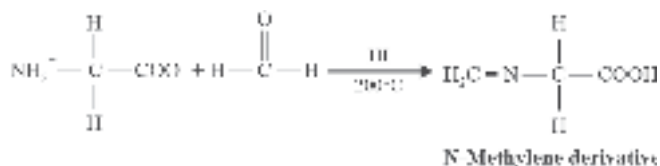
- (vi) **Reaction with nitrosyl chloride or bromide:** On reaction with nitrosyl chloride or bromide, amino acid forms chloro or bromo acids, respectively.



- (vii) **Reaction with hydroiodic acid:** On heating with hydroiodic acid at about 200°C, the amino group from amino acid is eliminated to produce the corresponding fatty acid.



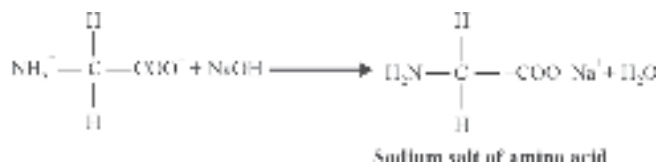
- (viii) **Condensation with formaldehyde:** Amino acid forms N-methylene derivative with formaldehyde.



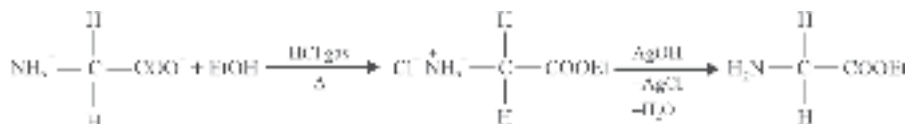
This N-methylene derivative containing a free COOH group can be titrated against standard alkali. This reaction, known as **Sorenson formal reaction**, is used for the estimation of amino acids.

b. Reaction due to -COOH group

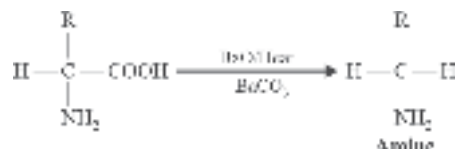
(i) **Formation of salt with bases:** Amino acids forms salts with strong bases.



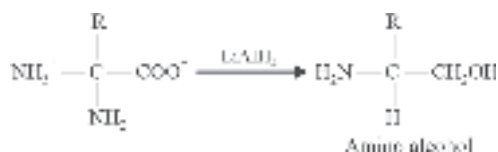
(ii) **Ester formation:** When heated with an alcohol in the presence of dry hydrogen chloride, they form respective ester hydrochloride. Free ester is obtained by the action of silver hydroxide or aqueous sodium carbonate solution on them.



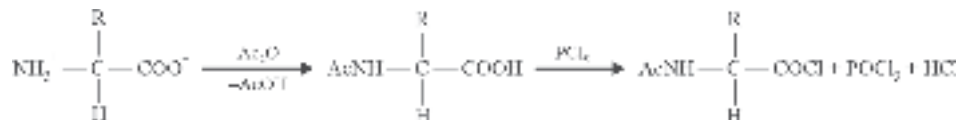
(iii) **Decarboxylation:** Amino acids may be decarboxylated by dry distillation with acids, bases, barium oxide or specific enzymes to give corresponding amine.



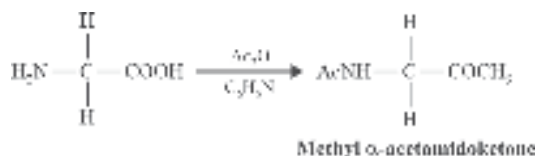
(iv) **Reduction:** Amino acids on reduction with LiAlH_4 give corresponding amino alcohols.



(v) **Formation of acid chloride:** In this reaction, amino acid in acetyl chloride reacts with phosphorous pentachloride to give corresponding acid chloride.

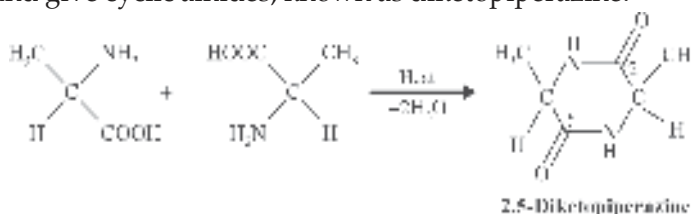


(vi) **Dakin-West reaction:** On treatment with acid anhydride in pyridine solution, amino acids are converted to methyl α -acetamidoketone. The reaction is referred to as the **Dakin-West reaction**.

**(c) Reactions due to both amino and carboxyl groups**

(i) **Action of heat:** On heating amino acids behaves as hydroxyl acids.

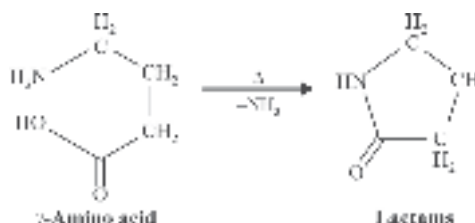
- (a) α -Amino acids lose two molecules of water between two molecules of amino acids and give cyclic amides, known as diketopiperazine.



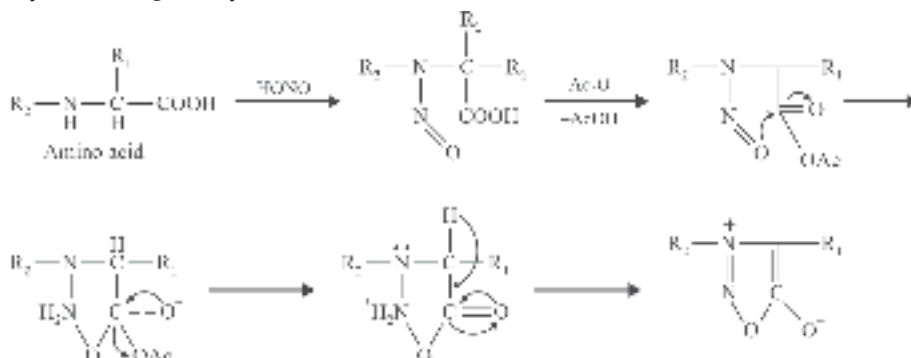
- (b) β -Amino acids eliminate a molecule of ammonia and yield α,β -unsaturated acids.



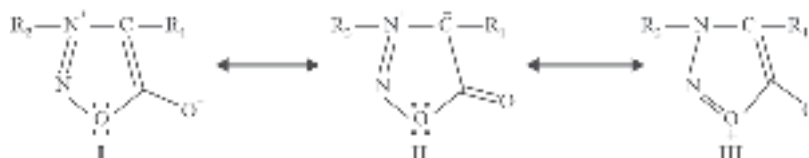
- (c) γ and δ -amino acids by losing one molecule of water within a molecule form cyclic amides called, lactams.



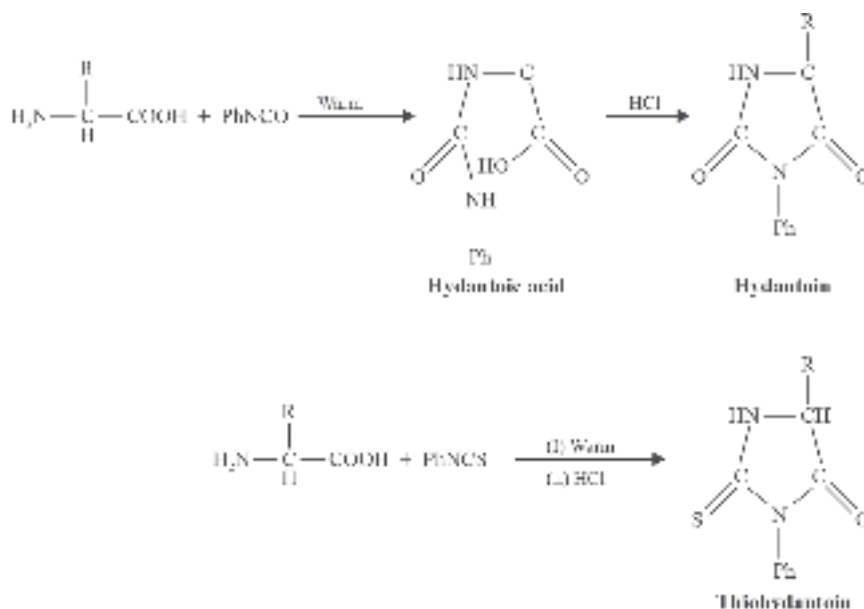
- (ii) **Action of nitrous acid:** N-alkyl or N-aryl amino acids form N-nitroso derivatives with nitrous acid and these derivatives dehydrate in the presence of acetic anhydride to give 'sydnones'.



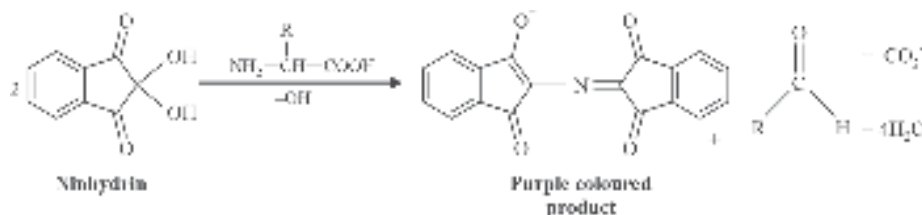
Although sydnones look like β -lactones, they are very stable because of having aromatic sextet. Sydnones are best represented as resonance hybrid of following three structures.



- (iii) **Reaction with phenyl isocyanate and phenyl thiocyanate:** Amino acids with phenyl isocyanate form phenyl hydantoic acids, which on treatment with hydrochloric acid easily form hydantoins.

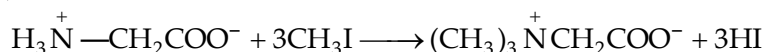


(iv) **Ninhydrin test:** In addition to common reactions of amines and carboxylic acids, common α -amino acids except proline undergo a unique reactions with ninhydrin to form purple colored imino derivatives.



(d) Formation of Betaines

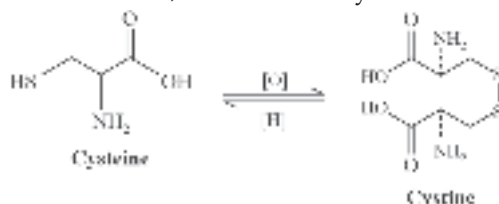
These are trialkyl derivatives of the amino acids. It is prepared by heating glycine with methyl iodide in methanolic solution.



Originally the term betaine was used to describe trimethyl glycine. In humans, it produces carnitine and promotes cellular reproduction and liver functions.

(e) Miscellaneous Reaction

Oxidative coupling: The mild oxidant, iodine reacts selectively with certain amino acid side chain groups. These include the phenolic ring in tyrosine and the heterocyclic ring in tryptophan and histidine. All of these yield products of electrophilic iodination. In addition, the sulphur groups in cysteine and methionine are also oxidized by iodine. Cysteine is a thiol and like most thiols, it is oxidatively dimerized to a disulphide, cystine.



PROTEIN

The term 'protein' is introduced by Mulder (1839). Proteins are nitrogenous substances occurring in the protoplasm of all animal and plant cells. Their composition varies with the source; carbon, 45–46%; hydrogen, 6–9%; oxygen, 12–30%; nitrogen, 10–32%; sulphur, 0.2–0.3%. Other elements may also be present, e.g. Phosphorous (nucleoproteins), Iron (haemoglobin).

There is no sharp dividing line between peptide, polypeptides and proteins. In general, they differ in physical and chemical properties which can be correlated with the difference in molecular size. Both groups often exhibit physiological activity, behaving, e.g. enzymes, hormones, growth factor, etc.

Proteins are amphoteric; they behave as an anion or a cation depending on the pH of the solution. At some definite pH, characteristic for each protein, the positive and negative charge is exactly balanced, i.e. no net change on the protein molecule, and the molecules do not migrate in an electric field. In this condition the protein is said to be at its isoelectric point and at this pH the protein has its least solubility, i.e. it is most readily precipitated. The amphoteric nature of the proteins is due to the presence of a large number of free acidic and basic groups arising from the amino acid units in the molecule. The osmotic pressure and viscosity of the protein solution are also a minimum at the isoelectric point.

All proteins are optically active, may be coagulated and precipitated from aqueous solution by heat, addition of acids, alkali salts, organic solvents etc. Protein in this precipitated state are said to be denatured, and the process of reaching this state is known as '**Denaturation**'. Denaturation occurs most readily near the isoelectric point. Denaturation of proteins involves the disruption and possible destruction of both secondary and tertiary structures (unfolding of protein molecule). After denaturation, loss of optical rotation and biological activity occurs, e.g. enzymes becomes inactive when denatured.

Denaturation is generally irreversible; but in many cases, the process has been reversed. This reversal of denaturation has been called **renaturation** or refolding. When denaturation is effected by heat, renaturation does not usually result on rapid cooling. If, however, cooling is carried out very slowly, renaturation often occurs. In these circumstances, the process of renaturation has been referred to as annealing.

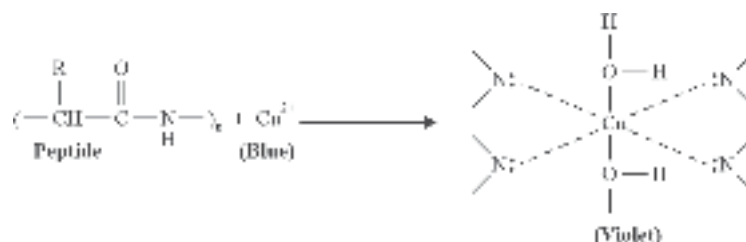
Tests for Amino Acids and Proteins

Proteins exhibit a variety of color reactions:

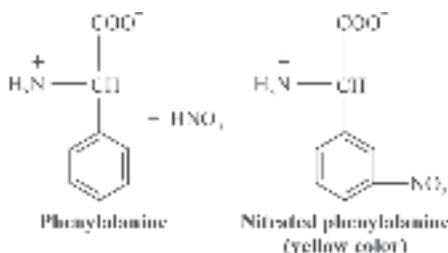
1. **Biuret test:** Alkaline solution of protein and dilute copper sulphate solution gives red or violet color.

Principle: Copper (II) ions, in basic solution form complex with the nitrogen atoms of four peptide (amide) bonds to form violet colored complex products. A general illustration of the reaction is given here:

These peptide bonds do not have to be in any order or any sequence in the protein. Any four peptide bonds can coordinate with the copper ion to form the color. A **positive reaction** for the biuret test is the formation of the violet color that indicates the **presence of peptide bonds**. Solutions without peptide bonds (such as free amino acids) do not give the violet color and will test negative in the biuret test for the presence of peptide bonds.

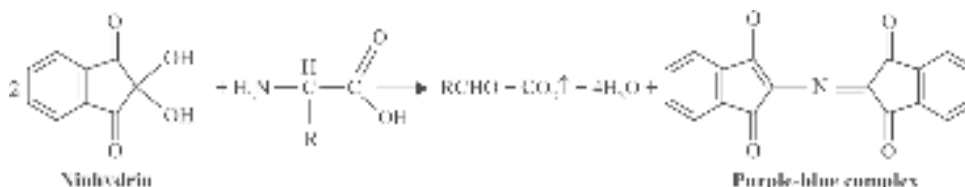


2. **Xanthoproteic reaction:** Protein solution and concentrated nitric acid on warming gives a yellow precipitate which changes to orange color in alkaline conditions.



Principle: Concentrated nitric acid (HNO_3) reacts with phenyl groups (aromatic rings) to produce a yellow color. The amino acids tyrosine, tryptophan and phenylalanine and any proteins that have significant numbers of tyrosine and phenylalanine in their primary sequence generate a yellow color. It is observed that nitric acid on reaction with skin gives yellow color due to reaction with phenyl groups. This is not a toxic reaction, but the yellow color does not disappear until the skin cells are replaced.

3. **Ninhydrin test:** Protein on boiling with dilute ninhydrin solution gives violet coloration.

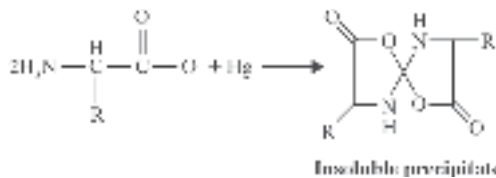


Principle: Molecules with a free $-NH_2$ group (a free amino group) form a purple-blue color with the ninhydrin reagent. If all amino groups are part of a peptide bond, no purple color will result.

A positive ninhydrin reaction is the purple-blue color, which indicates the presence of free amino groups. Only FREE amino groups (like those in free amino acids and protein side chains) give a positive reaction with the ninhydrin reagent. An exception is the amino acid proline, which contains a secondary amino group (instead of the usual primary amino group found in most amino acid and amino acid side chains), produces an orange color instead of the purple-blue.

4. **Millon's test:** Millon's reagent [mercuric nitrate in concentrated nitric acid having traces of nitrous acid] on adding to protein solution gives a white precipitate which on further heating produces a red precipitate due to the presence of phenolic group. Consequently, the protein containing tyrosine (due to phenolic group) gives a positive test of pink to dark-red color.

5. **Heavy metal ions test:** Heavy metal ions can react with proteins and some amino acids, causing them to precipitate (heavy metals can denature proteins by disrupting the secondary, tertiary, and quaternary structures of proteins). The heavy metals capable of doing this are: Ag^+ , Cd^{+2} , Cu^{+2} , Fe^{+3} , Hg^{+2} , Pb^{+2} , Sb^{+3} . The following diagram is an example of amino acids either free or in a peptide chain, reacting with heavy metal, mercury. This property of proteins (given as raw eggs) is utilized for the treatment of heavy metal poisoning.
6. **Hopkin-Cole test:** It is specific test for detecting tryptophan. The indole ring of tryptophan reacts with glyoxalic acid in presence of concentrated H_2SO_4 to give a purple color. Glacial acetic acid which has been exposed to the light, always contain glyoxalic acid, $\text{CHO}\cdot\text{COOH}$. Hence, few ml of glacial acetic acid containing glyoxalic acid is added to 1–2 drops of the amino acid solution.
7. **Nitroprusside test:** This test is characteristic for protein having $-\text{SH}$ groups. It involves the reaction of protein with sodium nitroprusside to impart transient red color.
8. **Sakaguchi test:** Guanidine in alkaline solutions gives a red color with reagent which contains α -naphthol and sodium hypobromite. The reaction is given by arginine and by proteins that contain this amino acids.
9. **Isatin test:** Imino acids such as proline and hydroxy proline condense with isatin reagent under alkaline condition to yield the blue colored adduct.



PEPTIDES/POLYPEPTIDES

Polypeptide is a chain of amino acids. Amino acids are bonded together with peptide bond to polypeptide. The control and regulatory mechanisms for many biological processes are dependent on **peptides**/proteins, derived from α -amino acids. In addition, many modern medicines (e.g. anticancer drugs, hypotensive drugs, etc.) are now produced from peptides or derivatives of peptides with undermentioned advantages.

Advantages of Utilizing Peptides as Therapeutic Molecules

- High activity
- High specificity
- Unique 3D characteristics
- No accumulation in organs
- Low toxicity
- Less immunogenic than antibodies.

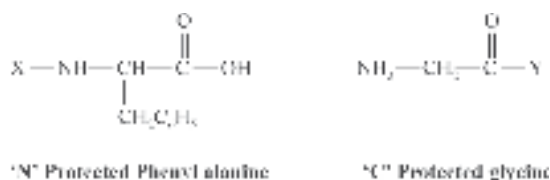
They can be made synthetically, by recombinant methods or by chemical modification of an isolated natural product.

For these reasons α -amino acids and peptide chemistry has become a central technology in organic chemistry, biochemistry, biotechnology and medical chemistry.

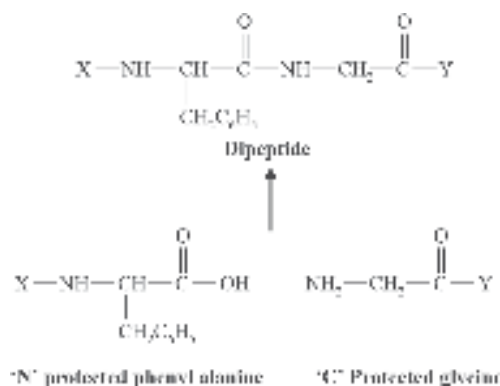
Making peptide bonds between amino acids to form peptide is not difficult. The challenge lies in connecting amino acids in the correct and desired sequence. Random peptide bond formation in a mixture of phenylalanine and glycine, for example, will give four dipeptides.



In a general strategy, first step is to limit the number of possibilities by “protecting” the nitrogen of one amino acid and the carboxyl group of the other amino acid.



Then, coupling is done of these two protected amino acids to form dipeptide.



Chemical Synthesis of Peptide

1. Solid-phase peptide synthesis
2. Solution phase peptide synthesis

1. Solid-phase Peptide Synthesis (SPPS)

Solid-phase peptide synthesis (SPPS), developed by **Merrifield**, has proven to be the method of choice for efficiently producing peptides and small protein (Fig. 6.2). Essentially, an N^α-derivatized amino acid is attached to a commercially available, insoluble (i.e. solid) support via a **linker** moiety. Solid support is available in bead form. The N^α-protecting group is then removed (deprotection) and the amino acid-linker-support complex is thoroughly washed with solvent. The next amino acid (which is also N^α protected) is then coupled to the amino acid-linker-support complex as either a preactivated species or directly (in situ) in the presence of activator. After this reaction is complete, the nascent oligopeptide-linker-support complex is washed with solvent, thus removing unreacted material. The deprotection-couple cycle is repeated until the desired sequence of amino acids is generated. Finally, the peptide-linker-support complex is cleaved to obtain the peptide as a free acid or an amide, depending on the chemical nature of the linker. Ideally, the cleavage reagent should also remove the amino acid side-chain-protecting groups, which are stable to the N^α-deprotection conditions.

There are **two main protocols** that have been used for the solid-phase chemical synthesis of proteins.

The **first protocol** uses the tertiary-butyloxycarbonyl (Boc) group for N^α-amino protection (Fig. 6.3). The Boc group is typically removed by trifluoroacetic acid (TFA) and the free N terminus is neutralized by tertiary amine. The peptide is removed from the resin with a relatively strong acid, usually hydrogen fluoride. Side-chain-protecting

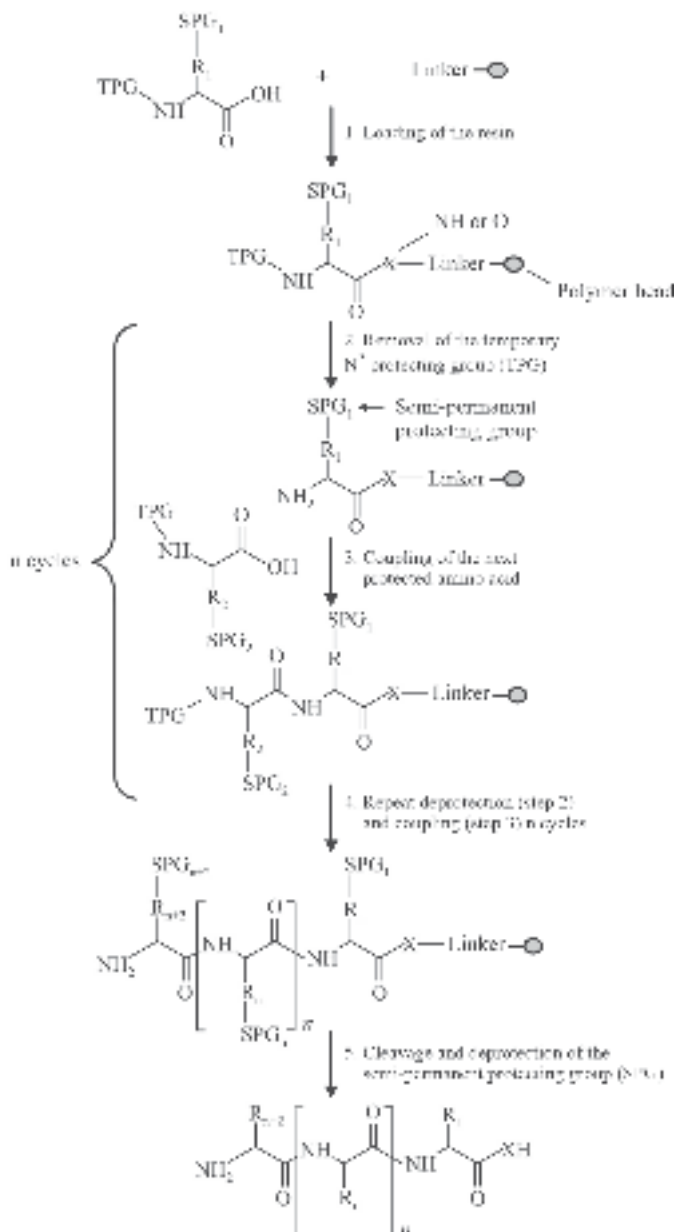


Fig. 6.2: Schematic representation of solid phase peptide synthesis

groups include ether, ester and urethane derivatives, based on benzyl alcohol, fine-tuned either with electron-donating methoxy or methyl groups, or with electron-withdrawing halogens to give the proper level of acid stability or lability. Alternatively, ether and ester derivatives based on cyclopentyl or cyclohexyl alcohol are sometimes used, because they moderate certain side reactions. Side-chain-protecting groups are specifically designed to be stable to repeated cycles of Boc removal, yet to be cleanly cleaved by halogen fluoride.

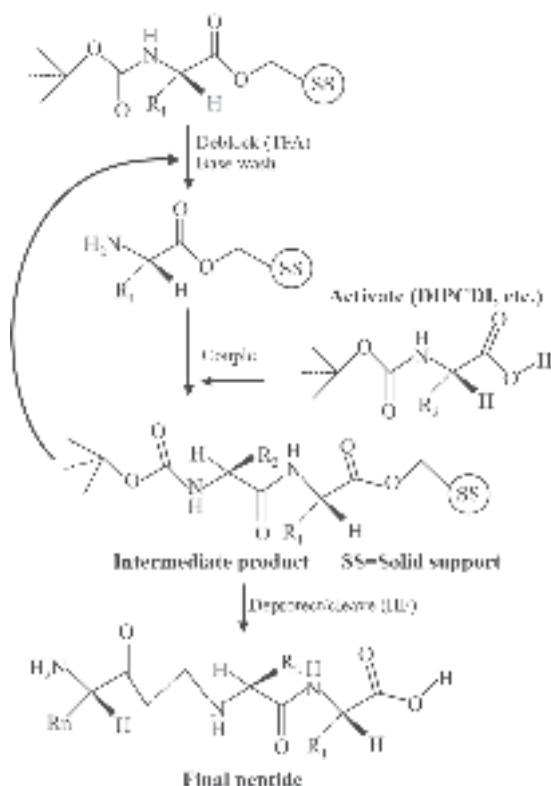
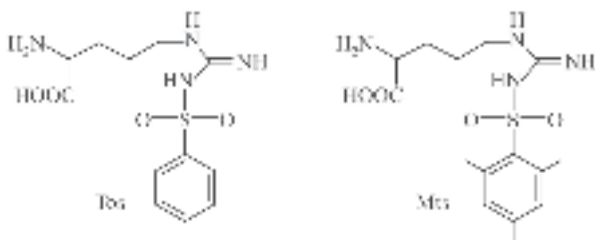


Fig. 6.3: Boc chemistry

Side Chain Protecting Groups for Boc Strategy

Amino Acid

Arg: Toluosulphonyl (Tos) or Mesitylen-2-sulphonyl-group (Mts) cleavage: HF/anisole (Tos), thioanisole (Mts).

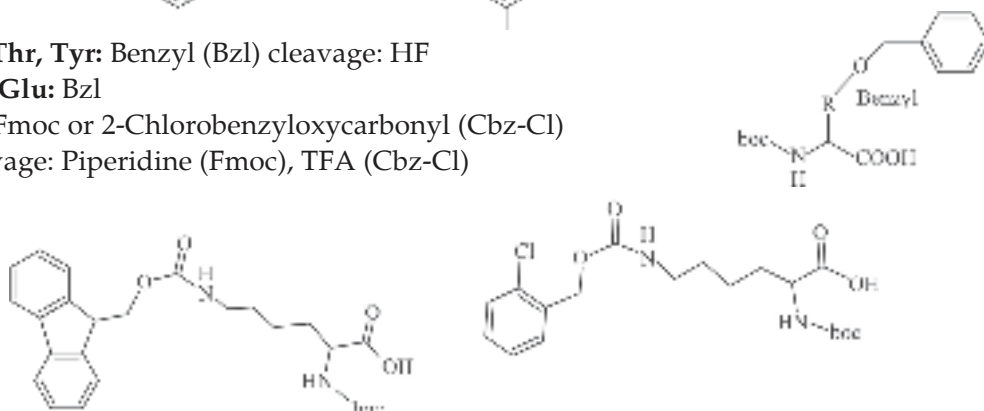


Ser, Thr, Tyr: Benzyl (Bzl) cleavage: HF

Asp, Glu: Bzl

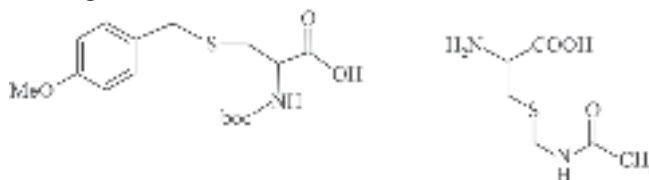
Lys: Fmoc or 2-Chlorobenzyloxycarbonyl (Cbz-Cl)

Cleavage: Piperidine (Fmoc), TFA (Cbz-Cl)



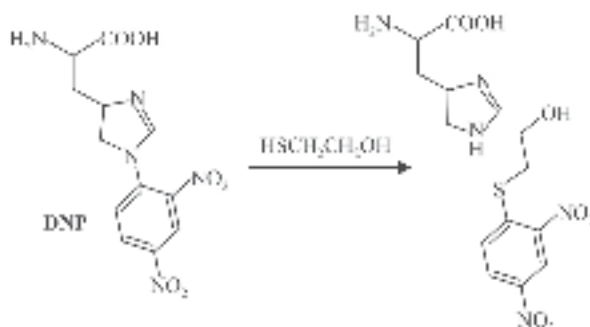
Cys: Acetamidomethyl (Acm) or 4-Methoxybenzyl (MeOBzl)

Cleavage: Hg^{2+} - or Ag^{+} -salt (Acm), HF (MeOBzl)



His: Dinitrophenyl group (DNP)

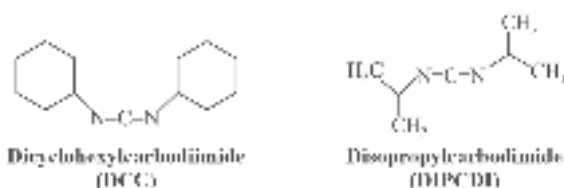
Cleavage: Thioles



Amide formation from the reaction of an amino group with a carboxylic acid is slow. Amide bond formation (peptide coupling) can be accelerated if the carboxylic acid is **activated**. Carboxyl group activation of the incoming amino acid utilizes one of the following method.

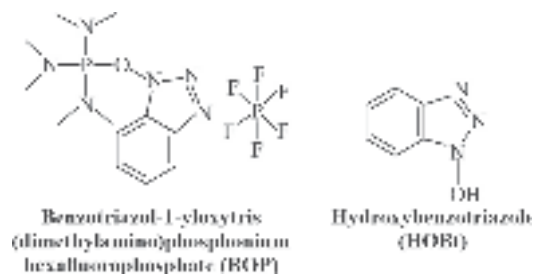
1. DCC/DIPCDI

Dicyclohexylcarbodiimide (DCC) or diisopropylcarbo-diimide (DIPCDI) can be used either with or without HOBt in a solution of DCM: DMF (di-chloromethane and dimethyl-formamide).



2. BOP/HOBt/NMM/HOAt

The amino acid is mixed with the BOP (Castro's reagent or (benzotriazol-yloxy) tris-(dimethylamino) phosphonium hexa-fluoro-phosphate), HOBt (1-hydroxybenzotriazole) and sufficient NMM (N-methyl-morpholine) to ionize 50% of the HOBt.



Note: The use of carbodiimide-mediated couplings is extended significantly by the use of either 1-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt) as an additive, either of which accelerates carbodiimide-mediated couplings, suppresses racemization, and inhibits dehydration of the carboxamide side chains of Asn and Gln to the corresponding nitriles.

3. Active Esters

These are usually pentafluorophenyl esters (OPfp) of the amino acids. The pentafluorophenyl esters of serine and threonine are oils and therefore are employed in the more convenient form of the dihydrooxobenzotriazine ester (ODhbt) (Fig. 6.3).

Advantages and Disadvantages of Boc-strategy

Bmoc

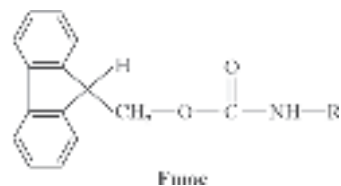
Advantages

- Easy to introduce.
- Boc-amino acids are stable at room temperature for extended periods (but storage at 4°C is recommended).
- Deprotection with TFA is rapid.
- Successful strategy for many peptide synthesis applications.
- Good coupling results.

Disadvantages

- Temporary and permanent (side chain) protecting groups are both acid labile → side chain deprotection during repeated TFA treatment can occur.
- Repeated TFA-mediated N- α -deprotection over the course of a long synthesis may lead to modification and/or degradation of sensitive peptide sequences.
- Difficulties for fragile peptides that do not survive the relatively harsh final HF cleavage.
- Boc-strategy requires the use of “dangerous” HF and expensive laboratory apparatus.
- Side reactions are possible:
t-Bu⁺ reacts with nucleophilic side chains like trptophan, tyrosine, methionine, histidine.
→ side chain protecting groups/adding of scavengers (1,2-Ethanedithiole) to the deprotection reagent.

The **second protocol** in peptide synthesis uses the 9-fluorenylmethoxycarbonyl (**Fmoc**) group for N-amino protection (Fig. 6.4). The **Fmoc** group is usually removed with piperidine in N, N-dimethylformamide or N-methylpyrrolidone. Side-chain protection that is compatible with N- α -Fmoc protection is provided primarily by ether, ester and urethane derivatives based on t-butanol. These derivatives are removed at the same time as the appropriate anchoring linkages by the use of TFA. The milder conditions of the Fmoc protocol have led to its being preferred by peptide laboratories but certain deleterious side reactions are more prevalent in the Fmoc protocol.



Fmoc Chemistry

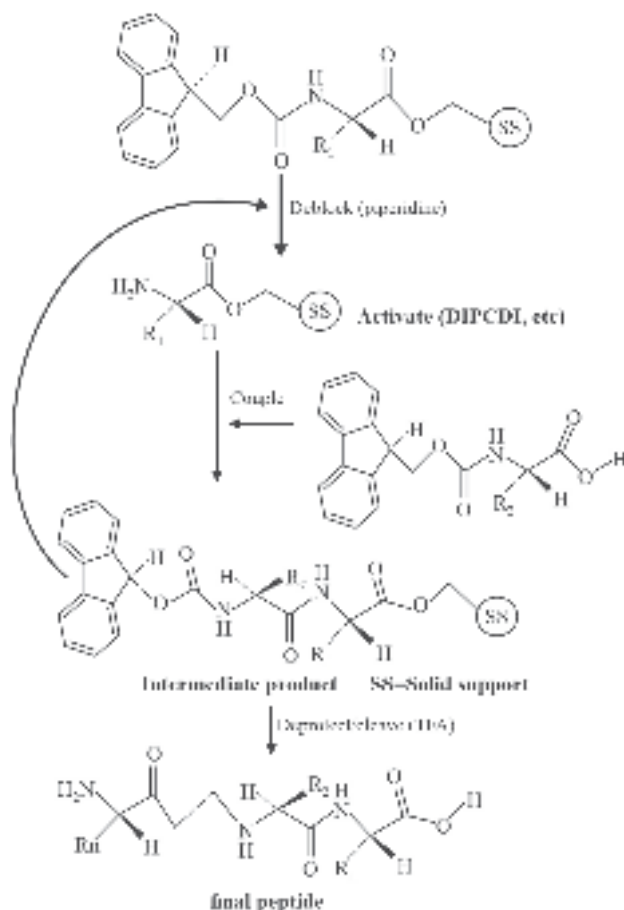


Fig. 6.4: Fmoc chemistry

Advantages and Disadvantages of Fmoc-strategy

Fmoc

Advantages

- Orthogonal protection scheme.
- Fmoc-amino acids are easy to prepare in crystalline form in high yield and stable when stored at 4°C.
- Milder reaction condition: Mild base (piperidine) for N- α -deprotection, TFA only for the final resin cleavage and deprotection.
- Progress of each deprotection reaction can be followed by real time spectrophotometric monitoring the release of the cleaved Fmoc-group at 300–320 nm.

Disadvantages

- Piperidine: Harmful vapor, toxic.
- Side reactions:
 - Aspartimide formation at Asp-X residues like Asp-Gly, -Ser-Thr, -Asn, -Gln.
 - Linker-bound C-terminal Cys undergoes significant racemisation (ca. 0, 5%) with each cycle of Piperidine-treatment.

Activation of the incoming amino acid employs DPCDI in a DCM:DMF solution and may also include HOBT.

A comparison of Fmoc and tBoc technology

<i>Fmoc</i>	<i>tBoc</i>
Polar polyamide or polysterene supports	Polystyrene support
Fmoc α -amino protection	t-Boc α -amino protection
t-Butyl side-chain protection	Benzyl side-chain protection
Mild base deprotection (piperidine)	Acid (TFA) deprotection
No neutralization step	Neutralization step required
Performed active esters or in situ activation	In situ activation required
TFA cleavage/deprotection	HF cleavage/deprotection
Single solvent system: Dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP)	Multi-solvent
Continuous flow or batch reaction methods	Batch reaction method most common
On-line UV monitoring option	Automated monitoring not available
Range of linkers to generate free acids, amides, and fully protected peptides	Multiple resins to generate free acids and amides
Double coupling not needed but can be used	Double coupling may be used
Capping not normally recommended	Capping normally recommended

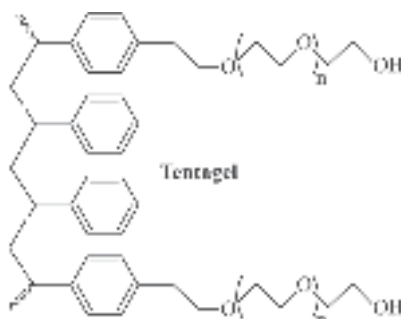
Solid Support

The solid support is a copolymer of styrene and divinylbenzene. Cross-linking with divinylbenzene simply provides a more rigid polymer.

Treating the polymeric support with chloromethyl methyl ether ($\text{ClCH}_2\text{OCH}_3$) and SnCl_4 places ClCH_2 side chains on some of the benzene rings.

The chloromethylated resin is treated with the Boc-protected C-terminal amino acid. Nucleophilic substitution occurs, and the Boc-protected amino acid is bound to the resin as an ester.

Tentagel is a polystyrene-polyethylene glycol graft polymer. It may have CH_2OH , CH_2NH_2 as active functional group.



Linkers

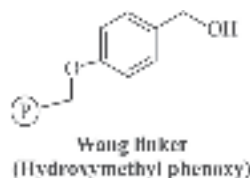
The purpose of the linker is:

- Provide a reversible linkage between the synthetic peptide chain and the solid support (SS).

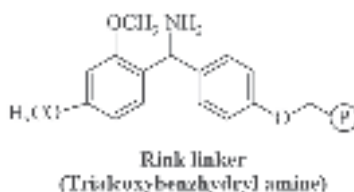
- Protect the C-terminal α -carboxyl group during the process of chain extension.
- Most linkers release peptide acid or amides upon treatment with TFA.
- Some linkers permit the cleavage with nucleophiles, used for the preparation of C-terminal modified peptides such as esters secondary amides, aldehydes, alcohol.

There are three types of commonly used linkers:

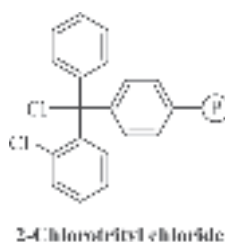
- Hydroxymethyl-based linkers.



- Aminomethyl-based linkers.



- Trityl chloride-based linkers.



2. Solution Phase Peptide Synthesis

This technique has been used for synthesis of small peptides composed by only a few amino acid residues. Its main advantage is that the intermediate products can be isolated and purified after each step of synthesis, deprotected and recombined to obtain larger peptides of the desired sequence. This technique is highly flexible with respect to the chemistry of coupling and the combination of the peptidic blocks. New strategies for synthesis in solution have been developed, going from the design of functional groups for the side chains and condensation of fragments for the synthesis of large molecules (Nishiuchi et al., 1988) to the use of new coupling reagents.

Comparison of peptide synthesis

<i>Solution synthesis</i>	<i>Solid phase synthesis</i>
Optimisation of reaction conditions and yields	Anchoring of the peptide chain on a resin
Purification of every step	Use of excess reagents removed by filtration and washing procedures
Time consuming	Simple, rapid and repetitive steps
Synthesis of small peptides	Synthesis of long peptides
Useful for large-scale manufacturing	Possible automation
	High yields

Classification of Proteins

Several arbitrary classifications of the proteins are in use. For example:

I. According to Solubility

- (a) **Fibrous proteins:** These are insoluble in common solvents but are soluble in concentrated acids and alkalis. These are highly resistant to digestion by proteolytic enzymes. These are proteins appearing as fibers made of linear molecules that are arranged roughly parallel to the fiber axis. The long linear molecules of proteins are held together by intermolecular hydrogen bonds. Examples: silk, wool, skin, hair, horn, nails, quills, connective tissue and bone.
- (b) **Globular proteins:** These are soluble in water and in dilute acids, alkali and salts. These proteins are more highly branched and cross-linked condensation products of basic or acidic amino acids. The polypeptide chains in this type of proteins are held together by cross-linked groups. Example: Enzymes, oxygen-carrying proteins, protein hormones, etc.

II. On the Basis of Increasing Complexity into their Structures

This is a more common method of classification, according to which proteins may be divided into three main groups.

- 1. **Simple proteins:** These give amino acids or their derivatives on hydrolysis. These are including the following groups:
 - (a) **Albumins:** Soluble in water, acids and alkalis, coagulated by heat and precipitated by saturated salt solution like ammonium sulphate. These are deficient in glycine. Some albumins are serum albumin, egg albumin and lactalbumin.
 - (b) **Globulins:** These are insoluble in water, but are soluble in dilute salt solution and in dilute solutions of strong inorganic acids and alkalis and precipitated by ammonia solution, coagulated by heat. Some globulins are serum globulins, tissue globulins and vegetable globulins.
 - (c) **Prolamins:** Insoluble in water, soluble in dilute acids and alkalis and contain large amount of proline. Example: Zein from maize, gliadine from wheat, hordein from barley.
 - (d) **Glutenins:** Insoluble in water, soluble in dilute acid and alkalis coagulated by heat, rich in arginine, proline and glutamic acid. Example: Glutenin from wheat, oryzenin from rice.
 - (e) **Scleroproteins (albuminoids):** Insoluble in water, soluble in concentrated acids and alkalis. Not attacked by enzyme. Example: Keratin from hair, hoof, fibroin from silk.Submembers of albuminoids are:
 - (i) **Collagens:** Present in skin, tendons and bones, they form gelatins, which is water soluble.
 - (ii) **Elastins:** Present in tendons and arteries, not converted to gelatin.
- (f) **Basic protein:** They are strongly basic and fall into two groups:
 - (i) **Histones:** These are soluble in water or dilute acid, not coagulated by heat, contain large amount of histidine and arginine, low in cystine or methionine. These are proteins of nucleic acid and haemoglobin, etc.

- (ii) **Protamines:** Less basic than histones, soluble in water, dilute acids and dilute ammonia, not coagulated by heat, precipitated from ethanol. They contain large amount of arginine and occur in various nucleic acids.
2. **Conjugated protein:** They contain non-protein group attached to protein part, called prosthetic group. These groups may be separated from protein part by careful hydrolysis. Some sub-members are:
- (a) **Nucleoprotein:** The prosthetic group is nucleic acid.
 - (b) **Chromoproteins:** The prosthetic group is colored chromophoric group in chromoproteins. The γ -chlorophyll and haemoglobin are the examples of this category.
 - (c) **Glycoproteins:** They contain carbohydrate prosthetic group. They are also known as mucoproteins, e.g. egg albumin, serum albumin and certain serum globulin.
 - (d) **Phosphoproteins:** In these, prosthetic group possesses phosphoric acid in some form other than in nucleic acids.
 - (e) **Lipoproteins:** Prosthetic groups are phospholipids and cholesterol.
 - (f) **Metalloproteins:** In these proteins, metal is an integral part of the structure. Generally, iron, magnesium, copper and manganese, e.g. haemoglobin and chlorophyll.
3. **Derived proteins:** These are the degraded products, obtained by the action of acids, alkalies or enzymes on proteins.

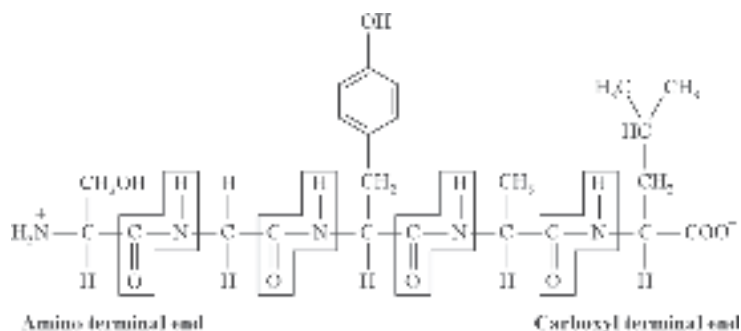


Structure of Proteins

It can be divided into four subheadings: (i) Primary structure of proteins; (ii) Secondary structure of proteins; (iii) Tertiary structure of proteins; (iv) Quaternary structure of proteins.

Primary structure of proteins: It is concerned with the sequence of amino acids in peptide chain. In every polypeptide, there is a specific sequence of amino acids. Biological activity of polypeptides depends on the sequence of amino acid. If only one amino acid in the sequence is changed, then total biological activity of amino acid may be changed. Biosynthesis of proteins is regulated by nucleic acids (DNA, RNA). In hemoglobin, there is a specific sequence of 574 amino acids. If only one amino acid is replaced it becomes defective haemoglobin, which may produces sickle cell anemia disease.

The 'amino end' is said to be 'N'-terminal and the 'carboxyl end' is said to be 'C'-terminal. The general method of writing the sequence of amino acids in a peptide is with the terminal amino group on the left. For example:



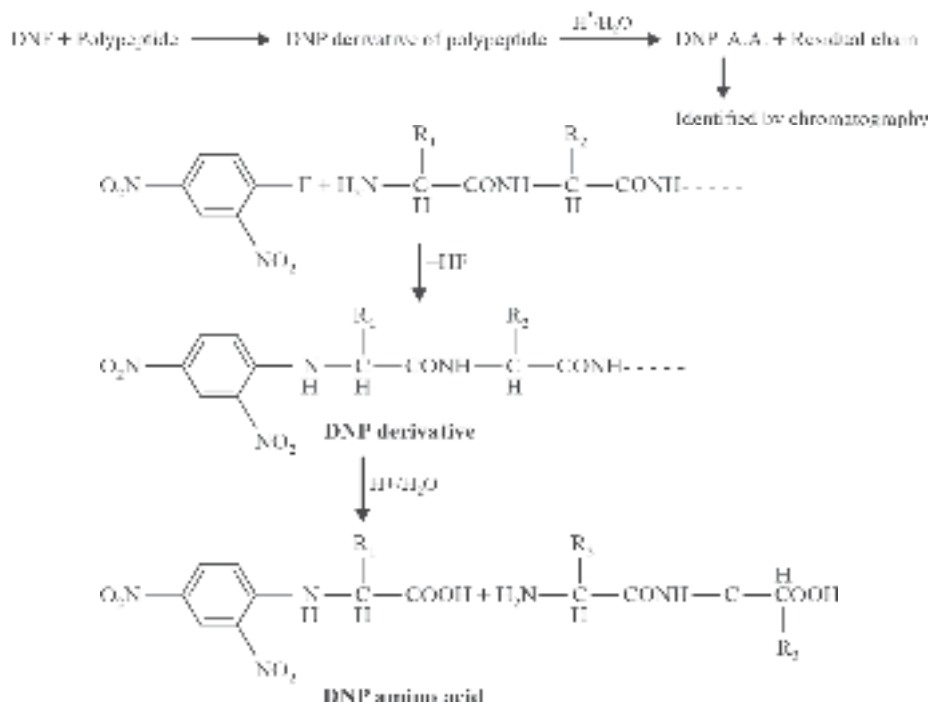
Determination of primary structure of proteins: Following steps are involved in the determination of structure of protein.

1. Isolation of protein in pure state.
2. Find out that whether the protein consists of only one peptide chain or composed of sub units.
3. Complete hydrolysis of protein into their constituent amino acid.
4. Minimum mol. wt determination from percentage composition of amino acid.
5. End or terminal group analysis to determine sequence.

Terminal group analysis: There are two types of analysis - N-terminal and C-terminal.

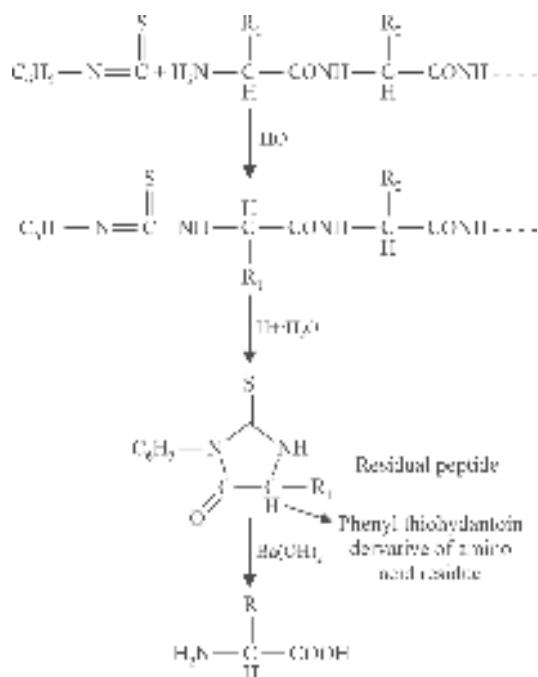
A. N-Terminal Analysis

- (i) **Sangers method:** This sequencing technique utilizes the compound, 2,4-dinitrofluorobenzene (DNF), which reacts with N-terminal residue of polypeptide chain under alkaline condition. The derivatized amino acid can be separated by hydrolysis. It will be labeled with dinitrobenzene group that imparts yellow color to the amino acid. Separation of modified amino acid (DNP-derivative) by electrophoresis and comparison with migration of DNP-derivative standards allows for the identification of N-terminal amino acid.

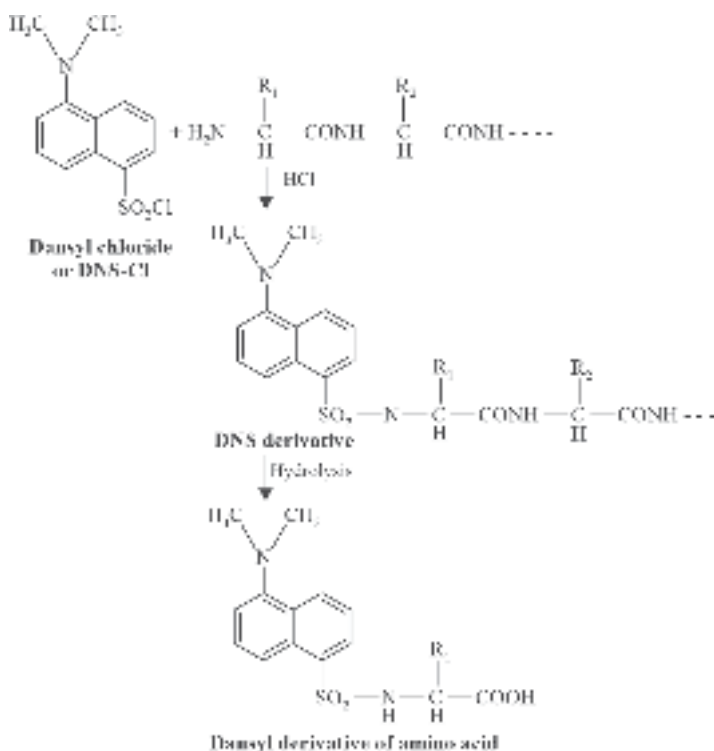


Note: In this process, complete hydrolysis takes place so only N-terminal acid is identified.

- (ii) **Edman's method:** The utility of Edman method is that it allows for additional amino acid sequence to be obtained from the N-terminal inward. Using this method, it is possible to obtain entire sequence of peptides by repetition of this technique. In this, phenylisocyanate reacts with the N-terminal, residue under alkaline conditions. The resultant phenylthiocarbonyl derivatized amino acid is hydrolysed in acid. The hydrolysis reaction results in rearrangement to release N-terminal residue to a phenylthiohydantoin derivative. As in Sanger's method, N-terminal residue is tagged with an identifiable marker, however, the added advantage of the Edman's process is that the remainder of the peptide chain is intact. The entire sequence is repeated to obtain the sequence of the peptide.



- (iii) **Dansyl method:** Like DNF, dansyl chloride (1-dimethylaminonaphthalene-1-sulphonyl chloride) reacts with N-terminal residue under alkaline conditions. Analysis of the modified amino acid is carried out similarly to the Sanger's method except that dansylated amino acids are detected by fluorescence. This imparts higher sensitivity to this technique in comparison to Sanger's method.



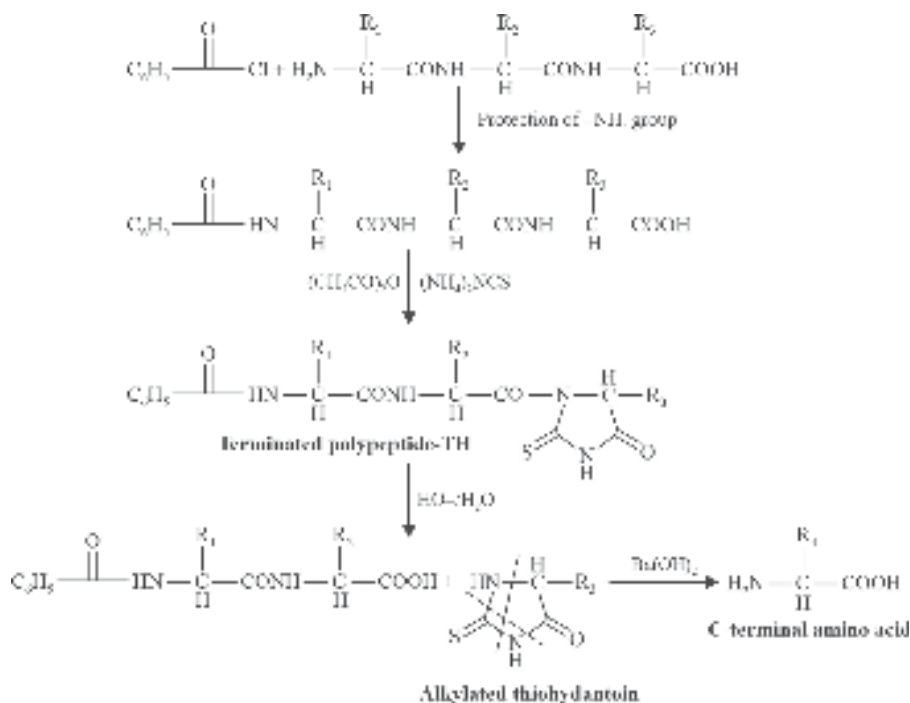
- (iv) **Enzymatic method:** Due to limitation of the Edman degradation technique, technique longer than around 50 residues cannot be sequenced completely. The ability to obtain

peptides of this length, from proteins of greater length is facilitated by the enzyme, endopeptidases. The enzyme, leucine amino peptidase attacks peptides only at the end which contains the free amino group. The resultant smaller peptides can be chromatographically and subjected to Edman's degradation technique.

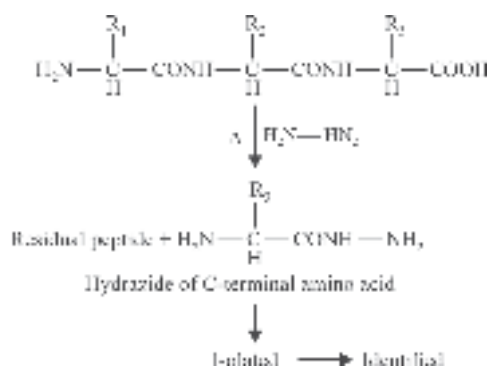
B. C-terminal Analysis

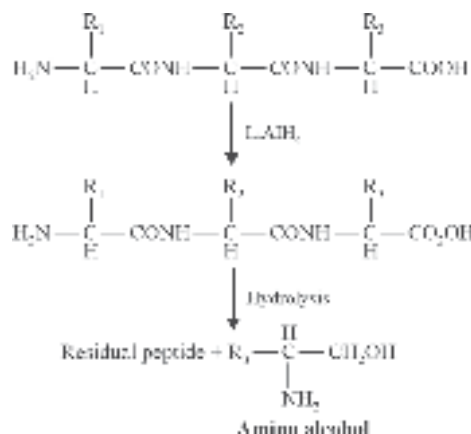
The number of methods for analysis of C-terminal amino acid is limited, however, some methods which can be considered, are given here.

- (i) **Schloack and Kump's method:** In this method, the amino group is protected by treatment with benzoyl chloride. The resultant product is reacted with ammonium thiocyanate to get thiohydantoin derivative of polypeptide. It is hydrolysed and the resulting alkylated thiohydantoin (TH) is hydrolysed to get the C-terminal amino acid which is identified by chromatographic technique.



- (ii) **Hydrazinolysis:** It involves the formation of hydrazide by the reaction of C-terminal amino acid and hydrazine. The hydrazide derivative of C-terminal amino acid is separated and identified.
- (iii) **Reduction:** The C-terminal carboxylic group is reduced with LiAlH_4 to form the primary alcoholic derivative. The tagged C-terminal amino acid is separated by hydrolysis from the residual peptide chain and identified by chromatographic technique.





- (iv) **Enzymatic method:** Carboxypeptidase attacks peptides only at the end which contains the free α -carboxyl group. Suppose there is a peptide ...x, y, z after attacking by enzyme, a number of successive terminal amino acids will be liberated from this peptide in amounts ... $Z > Y > X$. These amino acids can be identified and quantitatively estimated and sequence can be established.

Selective Peptide Cleavage

Cyanogen Bromide method: Cyanogen bromide is a specific reagent to cleave the peptide chain at the peptide bond formed by $-\text{COOH}$ groups of methionine (Fig. 6.5).

The C-terminal side of the methionine is obtained as a smaller peptide, which can be examined by any of the preceding techniques. Mass spectrometry may also be used to determine the amino acid sequence in the protein or in the various fragments obtained by partial hydrolysis.

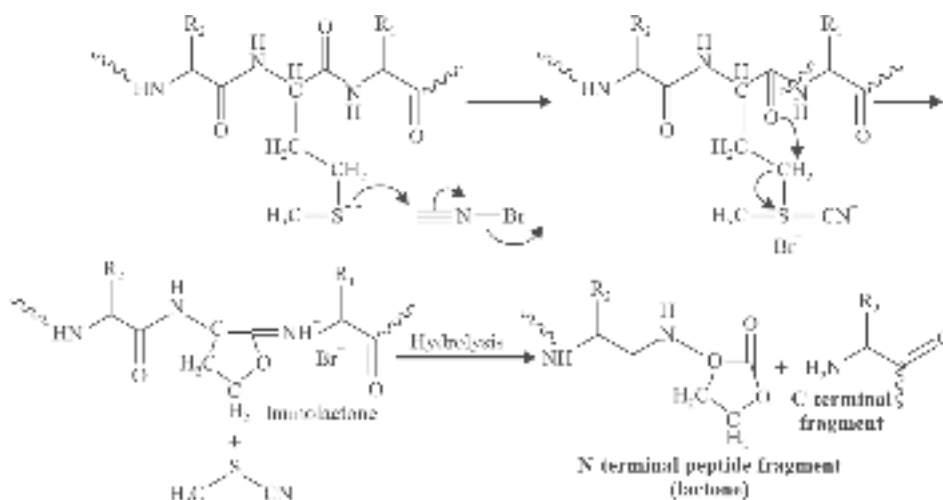


Fig. 6.5: Reaction mechanism for cleavage of methionine peptides with cyanogen bromide

Secondary structure of protein: The secondary protein structure is the specific geometric shape caused by intramolecular and intermolecular hydrogen bonding of amide groups or peptide bonds. In other way, secondary structure is concerned with three dimensional arrangement of the polypeptide chain, i.e. conformation of polypeptide

chains which arise as a result of hydrogen bonding. There are two types of structures: α -Helix and β -pleated sheet structures.

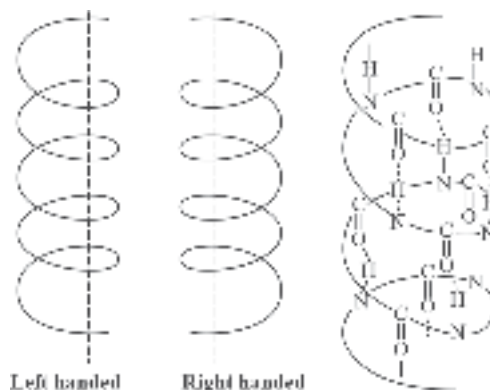


Fig. 6.6: α -Helix structure of protein

In the α -helix, the polypeptide chain is coiled tightly in the fashion of a spring (Fig. 6.6). The “backbone” of the peptide forms the inner part of the coil while the side chains, the amino acid R-groups protrude out from the helically coiled polypeptide backbone. The helix is stabilized by hydrogen bonds between the -N-H of one amino acid and the >C=O on the 4th amino acid away from it.

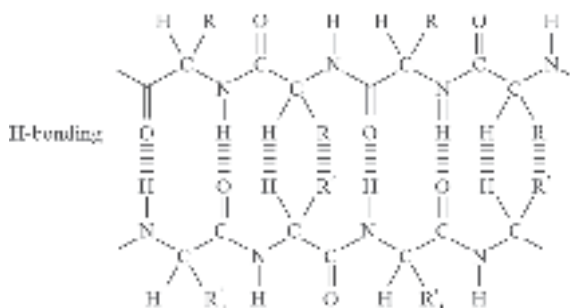


Fig. 6.7: β -Pleated sheet structure of protein

One “turn” of the coil requires 3.6 amino acid units. The helix can be either right-handed or left-handed in the sense of threads on a screw. The naturally occurring alpha helices found in proteins are all right-handed.

In **β -Pleated sheet structure**, polypeptide chains are extended in a linear or zig-zag manner (Fig. 6.7). Neighboring chains are bonded together by reciprocal inter chain hydrogen bonding. This results in a structure resembling pleated sheet.

In β -conformation, there are two types of pleated sheets, in which the alignment of the peptide chains may be parallel to one another (Fig. 6.8) or anti-parallel (Fig. 6.9).

Parallel: Chains run in the same direction.

Tertiary structure of proteins: Dividing line between secondary and tertiary structure is not very clear. It refers to the three dimensional structure of the polypeptide chain that results from interaction between amino acid residue relatively far apart in the sequence. These may include:

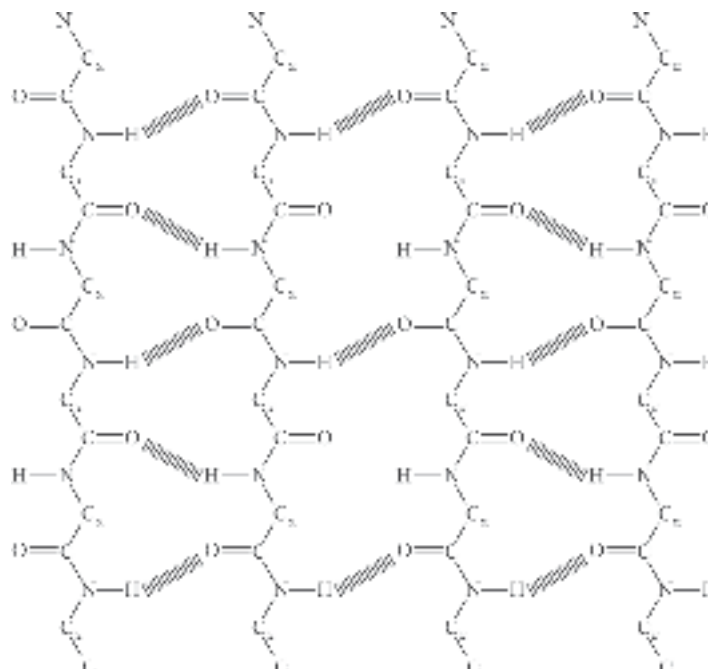


Fig. 6.8: Parallel β -pleated sheet structure of protein

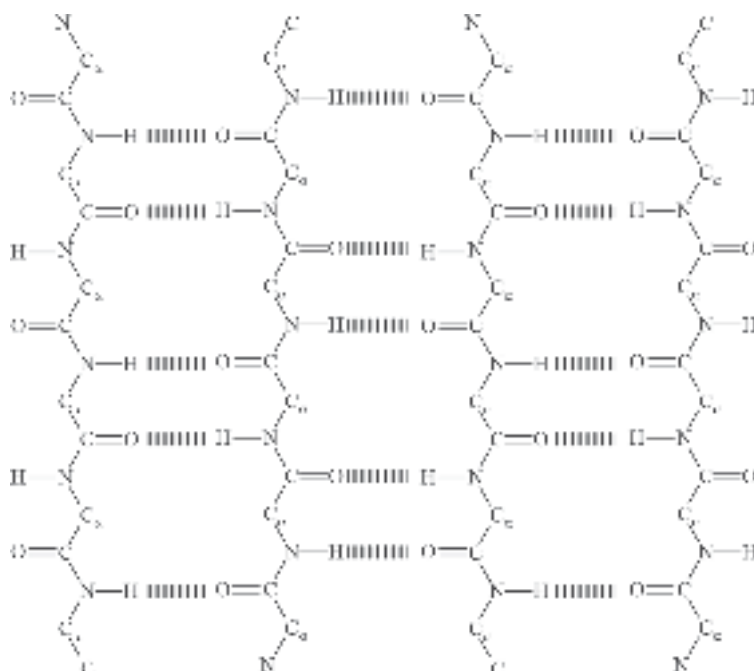
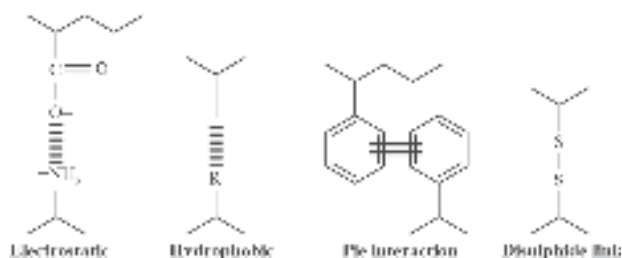


Fig. 6.9: Antiparallel β -pleated sheet structure of protein

- Weak interactions such as hydrogen bonds and van der Waals interaction.
- Ionic bonds involving negatively charged and positively charged amino acid side chain groups.

- Disulphide bonds, covalent linkages that may form as the thiol groups of two cysteine residues are oxidized to a disulphide.



Quaternary structure of proteins: “Gross folding pattern and arrangement of two or more protein chain, means the relation of one protein fold with other. Quaternary structure also results from the non bonding interactions. Proteins such as hemoglobin, which consists of more than one polypeptide chain, are said to possess quaternary structure. These proteins having this type of structure are said to be oligomeric and the individual polypeptide chains are known as promoters or sub-units. The unambiguous determination of quaternary structure is possible only by crystallographic methods.

Insulin

The **hormone insulin** is a protein consisting of two polypeptide chains. In the *beta* cells within islets of Langerhans of the pancreas, insulin is originally produced as a single molecule (preproinsulin) composed of 110 amino acids. On passing through the endoplasmic reticulum, 24 amino acids (the signal peptide) are removed by enzymatic action from one end of the chain, leaving another form (pro-insulin). It is folded and bonded to give the molecule much of the final structure. This passes into vesicles budded off from the Golgi body. Here, a middle section (the C-chain) of 33 amino-acids is removed by the action of the enzymes prohormone convertase 1 and 2, converting it into the final structure with 2 chains, A and B, and 2 amino acids are then removed by another enzyme carboxypeptidase.

Primary structure (amino acid sequence)

Insulin is composed of two peptide chains referred to as the A-chain and B-chain. In most of the species, the A-chain consists of 21 amino acids and the B-chain of 30 amino acids. The A- and B-chains are linked together by two disulphide bonds, and an additional disulphide is formed within the A chain (Fig. 6.10).

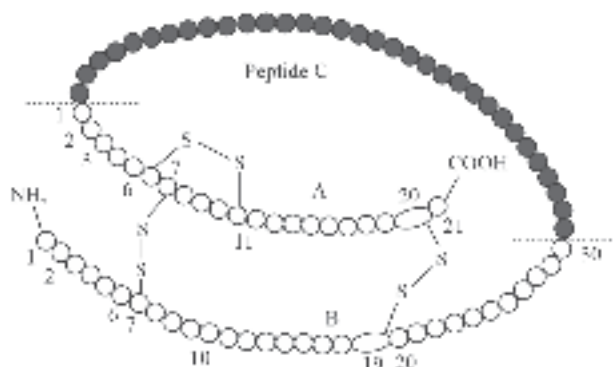


Fig. 6.10: Proinsulin, insulin, chains A and B linked by two disulphide bonds, and C peptide

A-chain (21 Amino Acid Residues)

Amino acid residue number

N-terminal end (free -NH_2 group)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
GLY	ILE	VAL	GLU	GLN	CYS	CYS	THR	SER	ILE	CYS	SER	LEU	TYR
15	16	17	18	19	20	21							
GLN	LEU	GLU	ASN	TYR	CYS	ASN							

C-terminal

B-chain (30 Amino Acid Residues)

N-terminal

1	2	3	4	5	6	7	8	9	10	11	12	13	14
PHE	VAL	ASN	GLN	HIS	LEU	CYS	GLY	ASP	HIS	LEU	VAL	GLU	ALA
15	16	17	18	19	20	21	22	23	24	25	26	27	28
LEU	TYR	LEU	VAL	CYS	GLY	GLU	ARG	GLY	PHE	PHE	TYR	THR	PRO
29	30												
LYS	THR												

C-terminal

These sequences are written using standard 3-letter codes for the 20 amino acids. There is a peptide bond between each amino acid, so they are called residues because H is removed from each intervening amino group, and -OH from the next -COOH group. At one end of each chain (the N-terminal end) is an amino group, and at the other end (the C-terminal end) is a carboxylic acid group.

Insulin produced in other organisms may have a slightly different amino acid sequence, or extra amino acids, but the next levels of structure are not greatly altered by these variations.

Secondary Structure

Some of the joined amino acid residues coil to form short sections of alpha helix, due to hydrogen bonds between $>\text{N-H}$ and $>\text{C=O}$ groups (projecting from peptide bonds of amino acids 3 or 4 residues further along), which stabilizes the structure. Other amino acids give a turn to the amino acid chains so the overall structure is fairly compact. The A-chain, which is fairly compact, contains 2 sections of α -helix (A2 Ile–A8 Thr and A13 Leu–A19 Tyr). In between these sections is a fairly flat ribbon which enables these helices to lie alongside one another, and also brings the side chains of A2 Ile and A19 Tyr into van der Waals contact. The B-chain appears to wrap around the A-chain. It consists of a larger section of α -helix (B9 Ser - B 19 Cys) and the smaller glycine residues at 20 and 23 allow it to fold into V-shape. This brings the C-terminal residues B24 Phe and B26 Tyr into van der Waals contact with B 15 Leu and B 11 Leu of the α -helix.

Tertiary Structure

The three-dimensional structure of insulin is further stabilised by disulphide bridges. These form between thiol groups (-SH) on cysteine residues (Fig. 6.11). There are 6 cysteines, so 3 disulphide bridges are formed: 2 between the A and B chains (between A7 and B7, and A20 and B 19), and 1 within the A chain (A6 and A11).

Quaternary Structure

Insulin can form granules consisting of hexamers (6 insulin molecules grouped around 2 zinc ions) due to interactions between hydrophobic surfaces. This toroidal (doughnut-

shaped) form is the one in which insulin is stored in the beta cells and secreted into the bloodstream.

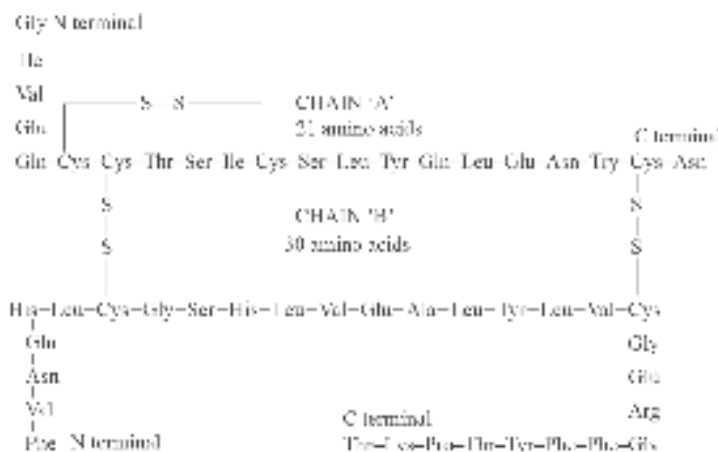


Fig. 6.11: Human insulin. The amino acid diagram of human insulin, showing the A and B chains and 3 disulphide bonds

Biosynthesis of Insulin

Insulin is produced in beta cells, which constitute 75% of the islets of Langerhans of the pancreas. Alpha cells secrete glucagon, and delta cells somatostatin.

Insulin is synthesized in the form of a single polypeptide chain of 110 amino acids, preproinsulin, which is transformed into proinsulin. Proinsulin is cleaved by trypsin-like enzyme to release the mature insulin hormone and free C-peptide (which lacks the dibasic residues). The C peptide exists for connecting the two chains A and B). Although, the amino acid sequence of insulin varies among species, certain segments of the molecule are highly conserved, including the positions of the three disulphide bonds; both ends of the A-chain and the C-terminal residues of the B-chain. These similarities in the amino acid sequence of insulin lead to a three dimensional conformation of insulin that is very similar among species, and insulin from one animal is very likely biologically active in other species. Indeed pig insulin has been widely used to treat human patients.

Insulin molecules have a tendency to form dimers in solution due to hydrogen-bonding between the C-terminal of B-chains. Additionally, in the presence of zinc ions, insulin dimers associates into hexamers.

These interactions have important clinical ramifications. Monomers and dimers readily diffuse into blood, whereas hexamers diffuse poorly. Hence, absorption of insulin preparations containing a high proportion of hexamers is delayed and somewhat slow. This phenomenon, among others, has stimulated development of a number of recombinant insulin analogs. The first of these molecules to be marketed—called **insulin lispro**—is engineered such that lysine and proline residues on the C-terminal end of the B-chain are inverted; this modification does not alter receptor binding, but minimizes the tendency to form dimers and hexamers.

Human insulin is closest in structure and function with cow (bovine) or pig (porcine) insulin. Bovine and porcine insulins differ from human in only 3 and 1 amino acid residues, respectively.

Oxytocin

Oxytocin is a mammalian hormone that also acts as a neurotransmitter in the brain. In females, it is released mainly after distension of the cervix and vagina during labor for facilitating birth, and also after stimulation of the nipples for helping breastfeeding. Oxytocin is released during orgasm in both sexes. In the brain, oxytocin is involved in social recognition and bonding, and might be involved in the formation of trust between people.

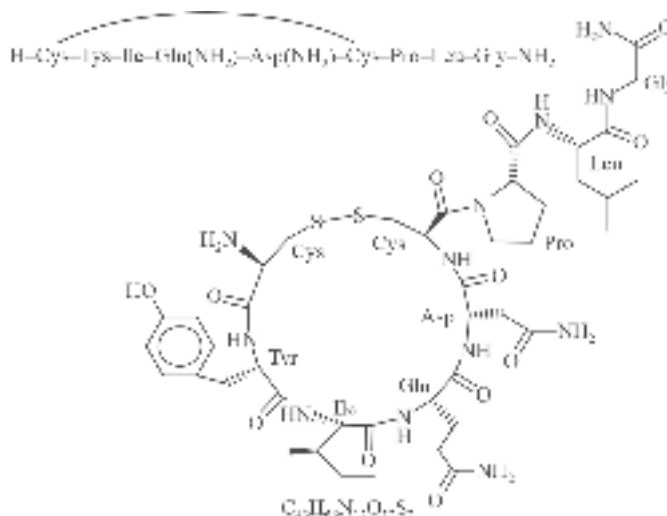


Fig. 6.12: The structure of oxytocin

Oxytocin is a peptide of nine amino acids (a nonpeptide). Its systematic name is cysteine-tyrosine-isoleucine-glutamine-asparagine-cysteine-proline-leucine-glycine-amine (cys-tyr-Ile-gln-asn-cys-pro-leu-gly-NH₂; or (CYIQNCPLG-NH₂). The cysteine residues form a disulphide bond. Oxytocin has a molecular mass of 1007 daltons. One international unit (TU) of oxytocin is equivalent of about 2 micrograms of pure peptide. The biologically active form of oxytocin, commonly measured by RIA and/or HPLC techniques, is also known as the octapeptide “oxytocin disulphide” (oxidized form), but oxytocin also exists as a reduced dithiol nonpeptide called oxytocine. It has been theorized that open chain oxytocine (the reduced form of oxytocin) may also act as a free radical scavenger (by donating an electron to a free radical); oxytocine may then be oxidized back to oxytocin via the redox potential of dehydroascorbate ascorbate. The structure of oxytocin is very similar to that of vasopressin (cys-tyr-phe-gln-asn-cys-pro-arg-gly-NH₂), also a nonapeptide with a sulphur bridge, whose sequence differs from oxytocin by 2 amino acids. At position 3 and 8, isoleucine and leucine in oxytocin are replaced by phenylalanine and arginine in vasopressin, respectively (Fig. 6.12). Oxytocin and vasopressin were discovered, isolated and synthesized by Vincent du Vigneaud in 1953, work for which he received the Nobel Prize in Chemistry in 1955.

Oxytocin and vasopressin are the only known hormones released by the human posterior pituitary gland to act at a distance. However, oxytocin neurons make other peptides, including corticotropin releasing hormone (CRH) and dynorphin, for example, that act locally. The magnocellular neurons that make oxytocin are adjacent to magnocellular neurons that make vasopressin, are similar in many respects.

SHORT AND LONG QUESTIONS

1. Write the four reactions/tests of proteins.
2. Classify the amino acids with examples.
3. Discuss the end group analysis in proteins.
4. What is isoelectric point? Discuss the chemical properties of amino acids.
5. Write a brief note on solid phase synthesis of polypeptide.
6. Give the chemistry and biological activity of oxytocin
7. What do you understand by the term essential and nonessential amino acids? Give two examples of each type. Give the reactions of alanine with (a) Formaldehyde (b) $\text{NaNO}_2 + \text{HCl}$
8. a. Describe the color reactions for proteins.
b. How the primary structure for protein is determined. Explain with appropriate reactions.
9. Discuss the following method, used to synthesize the amino acids with examples.
a. Gabriel's Phthalimide Synthesis
b. Malonic ester synthesis
10. Discuss the primary and secondary structure of insulin.
11. Give the Strecker's, Curtius and Drapsky synthesis for amino acids.

MULTIPLE CHOICE QUESTIONS (TICK MARK THE APPROPRIATE CHOICE)

1. Choose a nano peptide out of the followings:
a. Oxytocin
b. Vasopressin
c. Bradykinin
d. All of the above
2. Which one of these amino acids is a precursor for a mediator of allergies and inflammation?
a. Histidine
b. Tyrosine
c. Phenyl alanine
d. Tryptophan
3. Which out of the followings is not a fibrous protein?
a. Carbonic anhydrase
b. Collagen
c. Fibrinogen
d. Keratin
4. Lysine and tryptophan are:
a. protein
b. essential
c. non-essential
d. none of above
5. Which is included in the primary structure of protein?
a. Linear sequence of amino acids joined by peptide bond
b. 3-dimensional structure of protein
c. Helical structure of protein
d. Subunit structure of protein

Answers

1. (d) 2. (a) 3. (a) 4. (b) 5. (a)

Chemistry of Flavonoids—An Overview

Flavonoids are polyphenolic compounds that are ubiquitous in nature. These are categorized, according to the chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, most of them occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). The flavonoids have got considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, antiallergic, antiplatelet, antiinflammatory, antitumor and antioxidant activities. These are responsible for many of the plant colors that dazzle us with their brilliant shades of yellow, orange, and red.

Classified as plant pigments, flavonoids were discovered in 1938, when a Hungarian scientist, **Albert Szent-Gyorgyi** used the term “vitamin P” to describe them.

The chemistry of flavonoids is complicated. These are secondary metabolites, which mainly consists of benzopyrone ring, bearing a phenolic or poly-phenolic groups at different positions. These are naturally synthesized through the phenyl propanoid pathway with bioactivity dependent on its absorption mechanism and bioavailability. For example, well known flavonols include quercetin, rutin, and hesperidin, while apigenin and luteolin are examples of flavones.

Properties of Flavonoids

- These are crystalline substances with certain melting point.
- These are soluble in water, dilute mineral acid, alkali, alcohol and ether.
- Because of the two free electrons at the 1-oxygen of pyrone ring, flavonoids exhibit weak alkalinity and can form oxonium salt with strong acids like HCl and H₂SO₄. However, the obtained salt is extremely unstable and hydrolyzes immediately with the addition of water.
- Flavones, flavonols and their glycosides are generally greyish yellow to yellow. Chalcones are yellow to orange yellow; flavanones and flavanonols have no conjugation system and thus are generally colorless.
- These absorb wavelength near UV-region, and this color is perceived by insects for pollination.
- Flavones show 2 UV absorption bands: (a) 330–390 nm; (b) 250–270 nm. Therefore, can be distinguished from anthocyanins on the basis of UV spectra. Similarly, flavanols have 2 absorption bands: (a) 350–390 nm (due to absorption of cinnamoyl system in ring B); (b) 250–270 nm (due to absorption of benzoyl system in ring A) (see page 304).

Tests for Identification

- Flavonoids give precipitation reaction with lead acetate. Generally, flavonoids give yellow precipitate on mixing with lead acetate solution.
- Flavonoids give dull green or red brown precipitate with ferric chloride.
- **Shinoda test:** To the solution of flavonoids ethanol, add few magnesium chips and then acidify with 2 drops of cone. HCl After about 30 seconds, appearance of pink color confirms the presence of flavonoids.

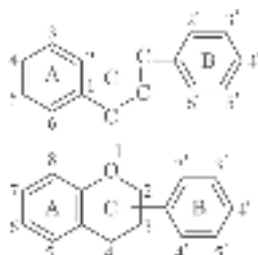
Occurrence

Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognised as flower pigments in most of the angiosperm families (flowering plants). The 30 flavanoids are only present in Asteraceae family. However, their occurrence is not restricted to flowers but include all parts of the plant. No flavanoids are found in algae.

These are most common in bryophytes (mosses), mainly represented by 'O' and C-glycosides of flavones and O-uronic derivatives. Flavonoids are present in vacuoles in water soluble glycosidic form. Depending on the species, these are either concentrated in leaf epidemis or spread on epidermis or mesophyll. These are also present in leaf cuticle without aglycone part.

Classification of Flavonoids

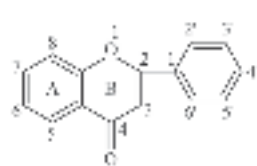
Chemically, the flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear; three carbon chain, as shown here:



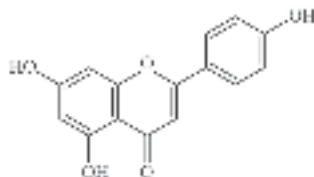
The skeleton above, can be represented as the $C_6-C_3-C_6$ system. The chemical structure of flavonoids are based on a C15 skeleton with a Chromane ring bearing a second aromatic ring B in position 2, 3 or 4.

Various flavonoids are classified on the basis of chemical structure as follows:

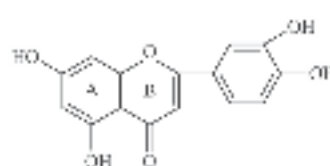
1. **Flavone:** It contain benzo- γ -pyrone ring with phenyl substitution at C-2 position of the pyrone ring." Apigenin (*Apium graveolens*, *Petroselinum crispum*), Luteolin (*Equisetum arvense*) are the examples of flavone.



Flavone (Generalised structure)
(2 Phenyl chromen 4-one)

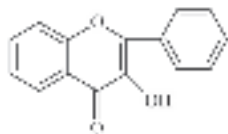


Apigenin

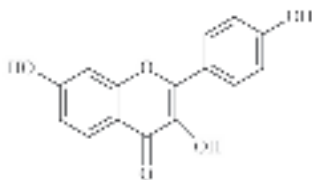


Luteolin

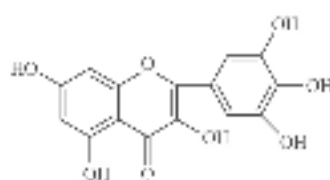
2. **Flavonol:** It is 3-hydroxy derivative of flavone. The presence of 3-OH group affect the UV absorption and color reactions. Quercitol (*Ruta graveolens*, *Fagopyrum esculentum*, *Sambucus nigra*) Kaempferol (*Sambucus nigra*, *Cassia senna*, *Equisetum arvense*, *Lamium album*, *Polygonum bistorta*) and Myricetin are the examples of flavonol.



Flavonol (Generalised structure)
(3-Hydroxy-2-Phenyl chromen-4-one)

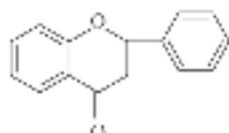


Kaempferol

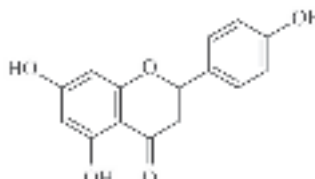


Myricetin

3. **Flavanone:** Flavanones are colorless compound and are rather neglected group of flavonoids. They are characterized by the absence of double bond at 2,3-position. It means that they are 2,3-dihydroderivative of flavone. Naringenin isolated from citrus peel is an example of flavanone.



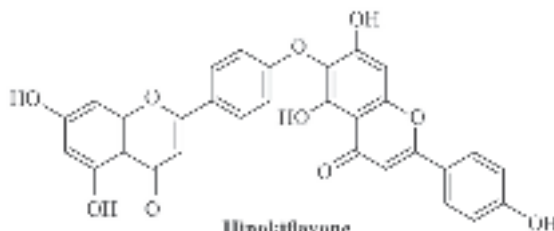
Flavanone (Generalised structure)
(2-Phenyl chromen-4-one)



Naringenin

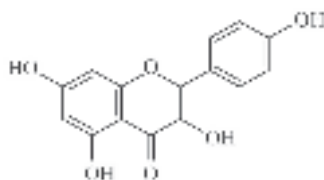
4. **Biflavonoids:** These are dimers of the various flavonoids. For example: hinokiflavone.

It is a characteristic of gymnosperms. Link is formed between very reactive C-6 and C-8 positions by a CC or COC bond.



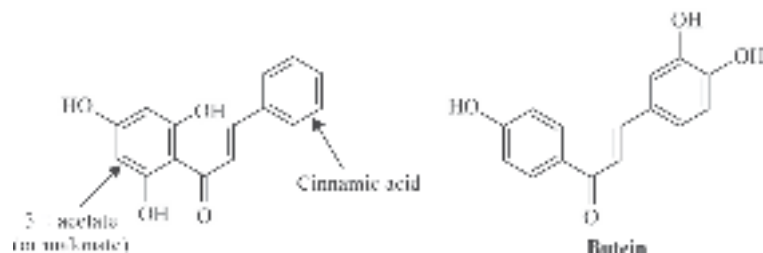
Hinokiflavone

5. **Dihydroflavonols:** It is 3-hydroxy derivative of flavanone. Dihydroxykaempferol is an example of dihydroflavonols.



Dihydrokaempferol

6. **Chalcones:** These do not contain γ -pyrone ring and hence are open chain flavanoids, in which two aromatic rings are joined by three carbon, α,β -unsaturated carbonyl system. Chalcone is derived from three acetates and cinnamic acid as shown below. Butein is an example of chalcone, found in free state in the heartwood of acacia.



7. **Anthocyanins:** These are water soluble vacuolar pigments that may appear red, purple or blue in color depending to pH. These consist of flavylium ion, the basic structure of which is given here. As shown, there are 7 different side groups on the flavylium ion. These side groups can be hydrogen atom, a hydroxide or methoxy group. The most frequent combination of side groups and their names are shown in the Table 7.1:

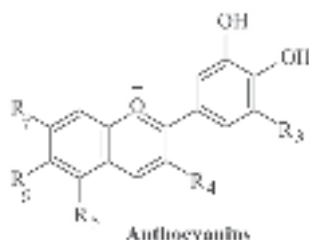
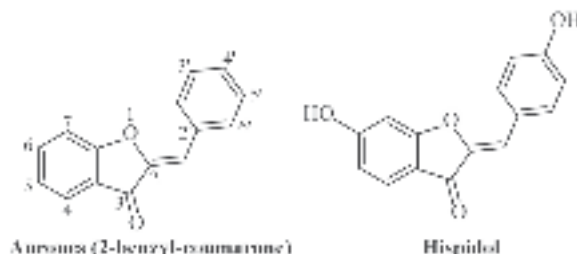


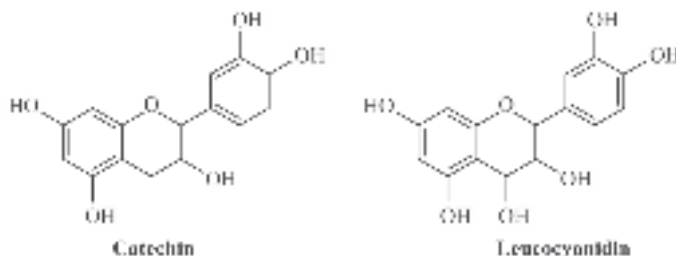
Table 7.1: Anthocyanidins along with their substituents and respective color

Anthocyanidin	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Main color
Apigeninidin	H	OH	H	H	OH	H	OH	Orange
Aurantidin	H	OH	H	OH	OH	OH	OH	Orange
Cyanidin	OH	OH	H	OH	OH	H	OH	Magenta
Delphinidin	OH	OH	OH	OH	OH	H	OH	Purple-blue
Hirsutidin	OCH ₃	OH	OCH ₃	OH	OH	H	OCH ₃	Bluish-red

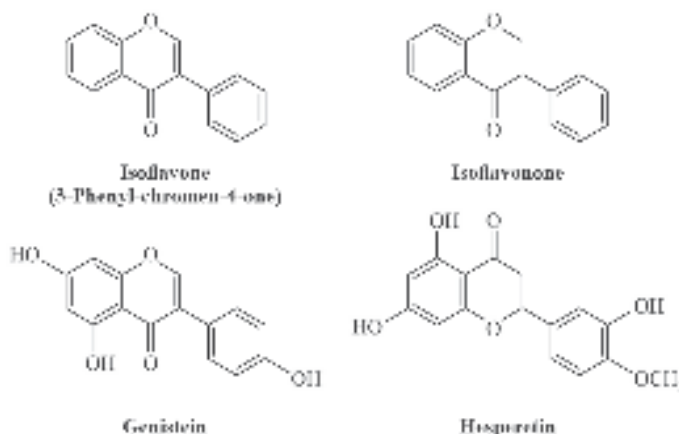
8. **Aurones:** In a few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered furanose ring. These are formed from chalcones by aerial or enzymatic oxidation and are deep yellow in color. Hispidol is an example of aurones.



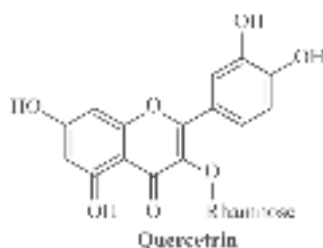
9. **Flavan-3-ol, flavan-3,4-diol:** They contain hydroxyl substitution at C-3 position (flavan-3-ol) or C-3 and 4 positions (flavan-3,4-diol). Catechin and Leucocyanidin are their examples, respectively.



10. **Isoflavones:** These contain benzo- γ -pyrone ring with phenyl substituent at C-3 position of the pyrone ring and isoflavonone is a dihydroderivative of isoflavone. Genistein and hesperetin are examples of isoflavone and isoflavanone, respectively.



11. **Glycosyl flavonoids:** These are present as glycosides. For example, quercetin.



Biosynthesis of Phenolic Compounds

The biosynthesis and accumulation of phytochemicals is an endogenously controlled process during developmental differentiation, and it is regulated by exogenous factors like light, temperature and wounding. Phenylalanine produced in plants via the shikimate pathways is a common precursor of most phenolic compounds in higher plants (Fig. 7.1). The B-ring with the C-3 unit is derived from shikimic acid, while the A-ring originates by the head-to-tail condensation of two malonyl CoA units and acetyl CoA, from the



acetate-malonate pathway. The four main enzymes involved are: CA4H (cinnamic acid 4-hydroxylase); CHS (chalcone synthase); 4-CL(4-coumarate coenzyme A ligase) and PAL (phenylalanine ammonialyase). The other enzymes are CNL (cinnamoyl-CoA ligase), FeH (flavonone-3-hydroxylase), FLS (flavonol synthase), CHI (chalcone isomerase), etc.

Isolation and Separation

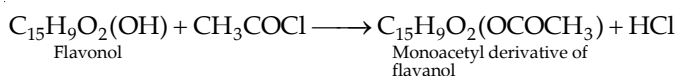
The specific part of the plant is dried in shade and then powdered. The powdered material is extracted with less polar organic solvents like benzene, chloroform and diethylether to obtain flavonoid aglycones, namely flavones, dihydroflavonols, flavonones, 'isoflavones and methoxylated derivatives of aglycone. More polar aglycones such as hydroxylated flavones, flavones, flavonols, biflavonols, aurones, chalcones are normally extracted by using acetone, ethanol, methanol and water or by the combination of these solvents. The methanol-water mixture in different proportion is one of the preferred solvent systems for the extraction of the above polar compounds. Boiling water find its use as an ideal solvent for the extraction of leucocyanidins, catechins and flavonoid glycosides. Acidic methanol is normally used for the extraction of anthocyanidins. The individual flavonoids is separated by chromatographic technique, like column chromatography, TLC, HPLC etc.

General Methods for the Elucidation of the Structure of Flavonols

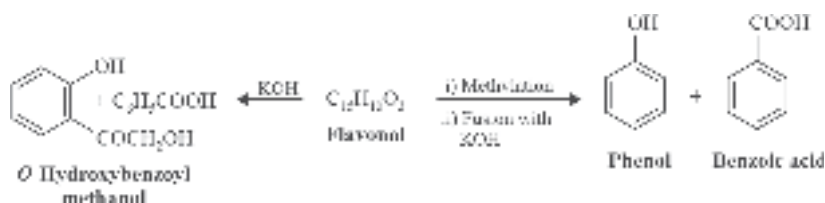
Flavonol (3-Hydroxy Flavone)

The structure of flavonol is elucidated on the basis of the following analytical and synthetic evidences:

- I. The molecular formula: of flavones was found to be $C_{15}H_{10}O_3$.
- II. The number of hydroxyl groups can be estimated by acetylation method. On acetylation, flavonol yield monoacetyl derivative, indicating the presence of one $-OH$ group.

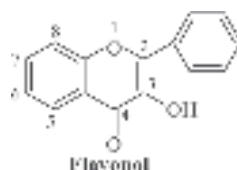


- III. Methylated flavonol yields phenol and benzoic acid when fused with KOH.

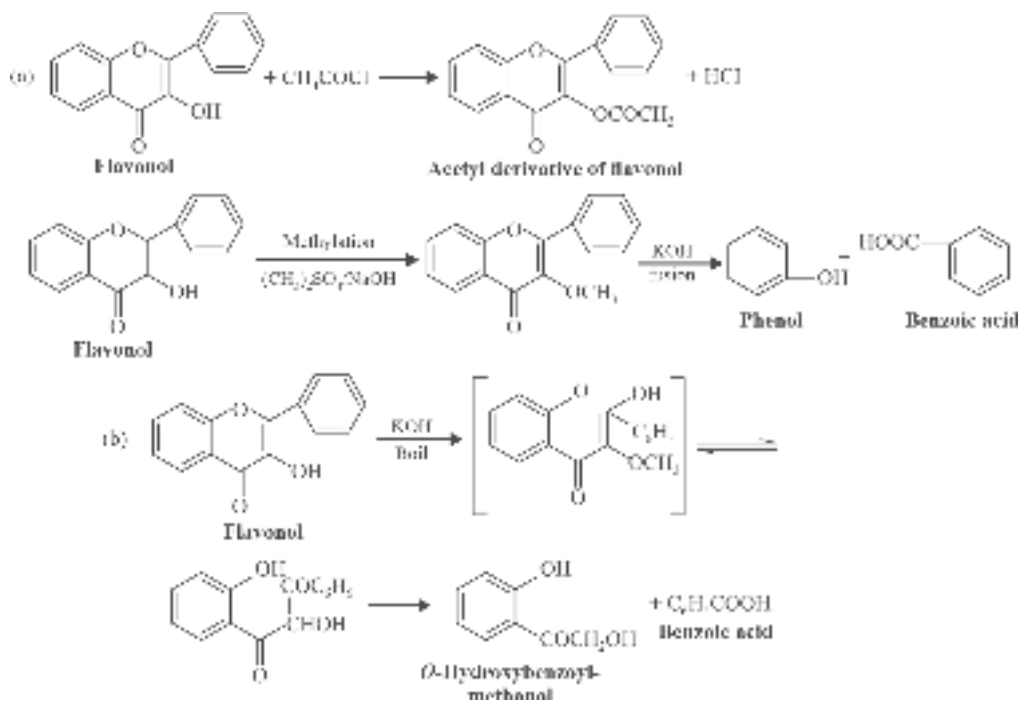


This shows that the methoxy group must be present at C-3 position which must have been lost in KOH fusion.

- IV. On boiling with alc KOH solution flavonol yields a mixture of *o*-hydroxybenzoylmethanol and benzoic acid. The formation of these product reveals that flavonol contains a hydroxyl group at C3 position. Hence, flavonol must be 3-hydroxyflavone (3-hydroxy-2-phenyl- γ -chromane).



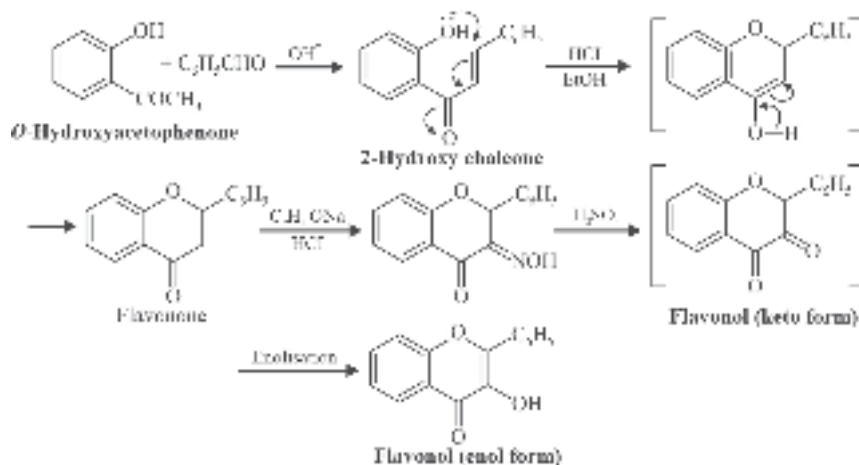
On the basis of the above structure of flavonol, the foregoing reaction can be explained as follows:



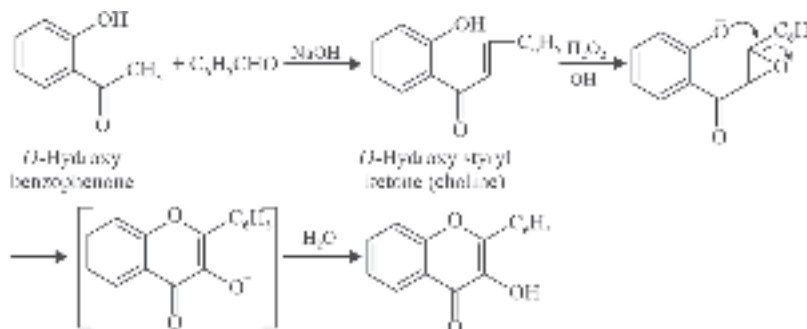
V. Synthesis

Finally, the structure for flavonol has been established by its various synthesis:

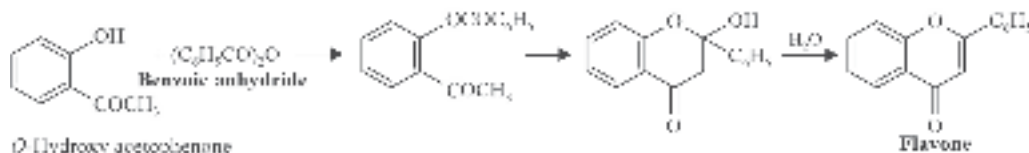
- (a) *Kostanecki's synthesis*: It involves **Claisen reaction** between *o*-hydroxyacetophenone and benzaldehyde.



- (b) *Algar-Flynn Oyamada's synthesis*: In this reaction, 2-hydroxychalcone is treated with hydrogen peroxide to give 3-hydroxyflavanone and then flavonol. Chalcone undergoes an oxidative cyclization to form flavonol.



- (c) *Robinson's synthesis*: In this reaction, *o*-hydroxyacetophenone is condensed with benzoic anhydride (veratric anhydride) in the presence of its potassium benzoate salt.

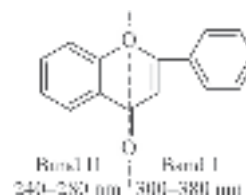


UV-VISIBLE SPECTRA OF FLAVONES AND FLAVONOLS IN METHANOL

The methanol, spectra of flavones and flavonols, exhibits two major absorption peaks in the region, 240–400 nm.

Band I at 300–380 nm appears due to cinnamoyl function and Band II at 240–280 nm for benzoyl function present in these structure.

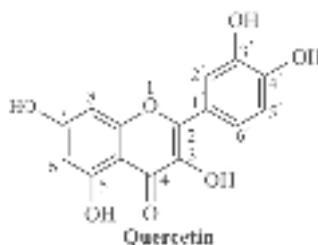
The position of hydroxyl groups at different position can be identified with the help of shift reagents like, CH_3ONa , CH_3COONa , AlCl_3 , H_3BO_3 , etc. as shown in structural elucidation of rutin. These reagents cause shift in λ_{max} .



Examples of Flavonoids

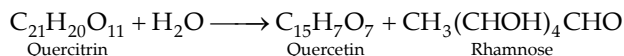
1. Quercetin

Quercetin is 2-(3',4'-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one with molecular weight of 302.24 and melting point of 316°C . The common names of quercetin are quercetine, sophoretin, meletin.



It occurs as the glycoside, quercitrin in the bark of *Quercus tinctoria* (American oak).

On hydrolysis with HCl, quercitrin available yields one molecule of rhamnose and aglycone, quercetin.



Good sources of quercetin include apples, onions, teas, nuts, and red wines. It is also found in leafy vegetables, berries, cauliflower, cabbage and in herbs such as ginkgo. Quercetin has demonstrated significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation. For example, it inhibits both the manufacture and release of histamine and other allergic/inflammatory mediators. In addition, it exerts potent antioxidant activity and vitamin C-sparing action. Quercetin exerts antiviral activity against reverse transcriptase of HIV and other retroviruses, and is shown to reduce the infectivity and cellular replication of herpes simplex virus etc.

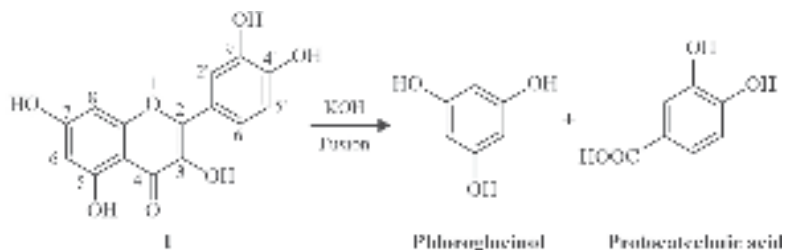
Quercetin is contraindicated with antibiotics; it may interact with fluoroquinolones (a type of medicinal antibiotic), as quercetin competitively binds to bacterial DNA gyrase. It inhibits or enhances the effect of fluoroquinolones. Quercetin is also a potent inhibitor of CYP_{3A4}, an enzyme that breaks down the most drugs in the body. As such, quercetin would be expected to increase serum levels, and therefore effects of drugs metabolized by this enzyme.

Isolation of Quercetin

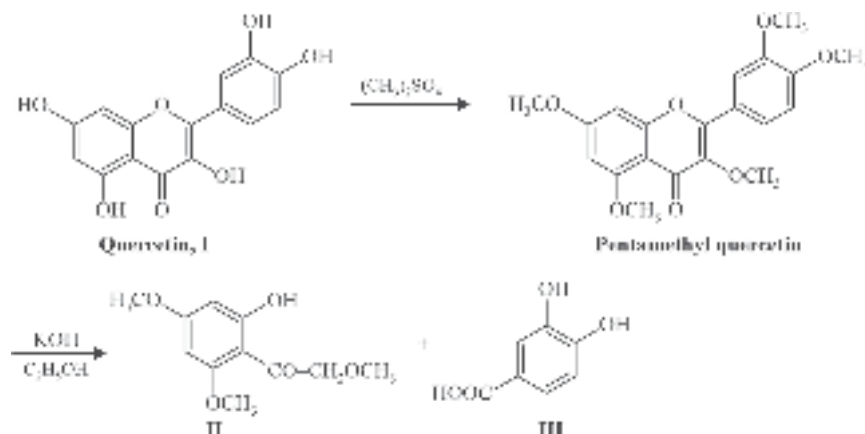
- (i) The plant material is digested with boiling water.
- (ii) The aqueous extract is diluted and added lead acetate to precipitate the tannins.
- (iii) The contents are filtered, filtrate diluted with water, acidified with HCl and boiled.
- (iv) The precipitate is extracted with alcohol and purified by fractional crystallization from organic solvent like benzene, CS₂, etc.

Structural Elucidation of Quercetin

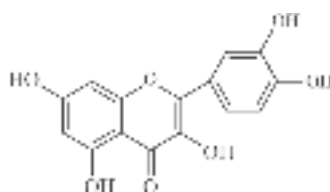
- I. Its molecular formula is found to be C₁₅H₁₀O₇.
- II. It is found to contain five hydroxyl groups as it forms pentaacetyl and pentamethyl derivatives with acetic anhydride and dimethyl sulphate, respectively.
- III. On fusion with KOH, it gives phloroglucinol and protocatechuic acid indicating that quercetin is 5,7,3',4'-tetrahydroxyflavanol or 3,5,7,3',4'-pentahydroxyflavone, I.



- IV. The structure for quercetin I is further proved by the fact that on boiling with alcoholic potash, pentamethylquercetin gives 6-hydroxy- ω , 2,4-trimethoxyacetophenone (II) and veratric acid (III).

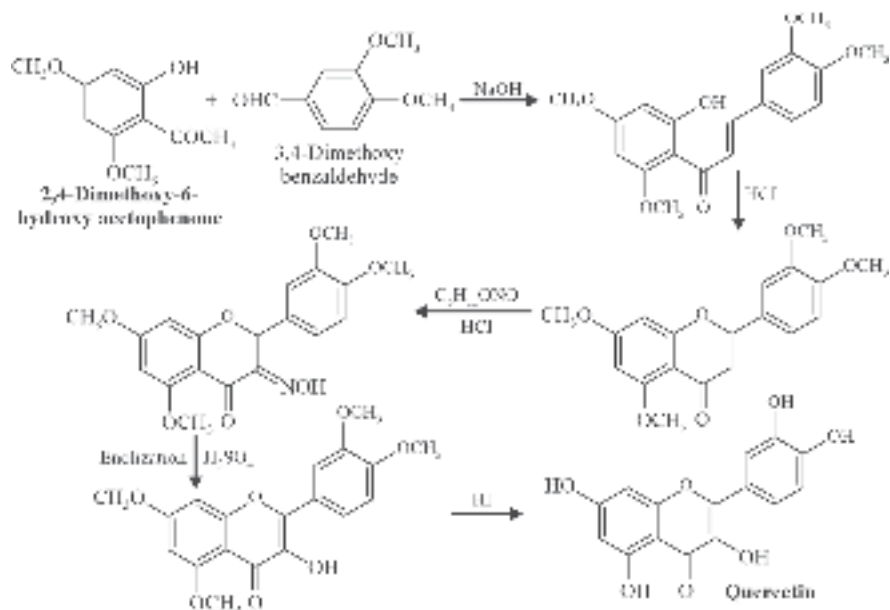


This result suggest that quercetrin is 3,3',4',5,7-pentahydroxy flavones.

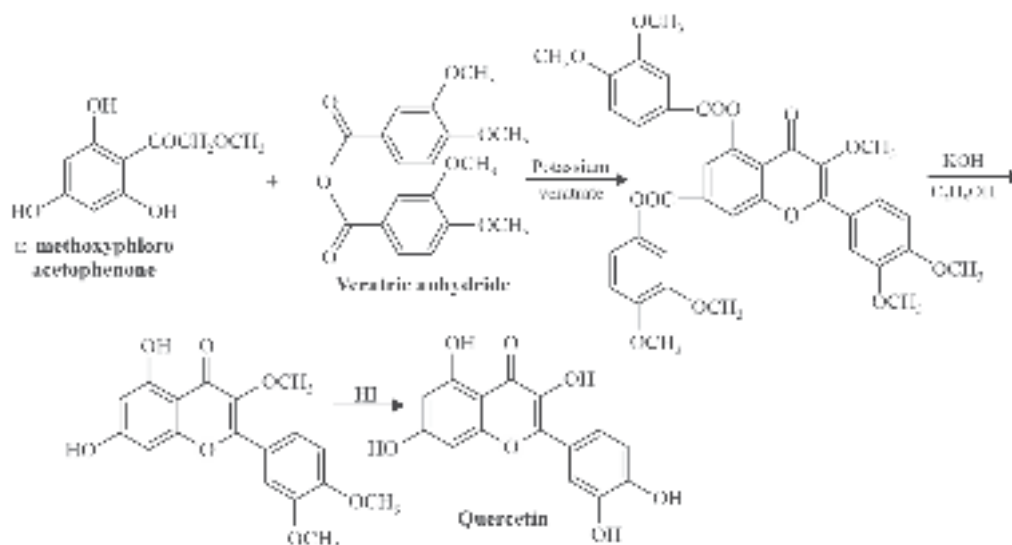


Finally, the structure for quercetin is proved by the synthesis:

- (a) **Kostanecki's synthesis (1904):** In this synthesis, quercetin is obtained by condensation of 2,4-dimethoxy-6-hydroxy acetophenone with 3,4-dimethoxy benzaldehyde in presense of NaOH.



- (b) **Robinson synthesis:** In this synthesis, quercetin is obtained by the condensation of ω -methoxyphloroacetophenone with veratric anhydride in the presence of the potassium salt of veratric acid as follows:



In quercitrin, rhamnose is found to be at C-3 position.

Spectral Data

UV: It is illustrated in Table 7.2 along with bathochromic shift caused by various reagents.

Table 7.2: UV spectra of the quercetin along with shift in λ_{max}

Solvent-reagent	λ_{max} (nm)		λ_{max} shift (nm)		Interpretation
	Band I	Band II	Band I	Band II	
MeOH	374	257	–	–	Flavonol (free 3–OH)
MeOH–NaOH	413	269	+39	+12	Flavonol 2–OH
MeOH–AlCl ₃	431	269	+57	+12	The presence of 3–OH (with or without 5–OH)
MeOH–AlCl ₃ /HCl	416	266	+42	+9	The presence of 5–OH
MeOH–NaOAc	389	275	+15	+18	The presence of 7–OH
MeOH–NaOAc/H ₃ BO ₃	386	260	+12	+3	o-di–OH in the B ring

Note: λ_{max} value increases with increase in number of hydroxyl group.

For example: Myricetin (C₆–OH): 378 nm

Quercetin (C₅–OH): 370 nm.

Kaempferol (C₄–OH): 368 nm.

IR (cm^{–1}): 3330 (OH), 2920 (–CH), 1660 (C = O), 1520 (C = C), 1360 (C–O–C).

¹H–NMR (δ) (DMSO–d₆) (600 Hz): 6.20 (1H, d, J = 2 Hz, C₆–H), 6.42 (1H, d, J = 2 Hz, C₈–H), 6.90 (1H, d, J = 8.5 Hz, C₅–H), 7.69 (1H, d₂, J = 2.2 Hz, C₂' H), 7.56 (1H, dd, J = 8.5, 2.2 Hz, C₆'–H), 9.41 (1H, s, C₄' –OH), 9.35 (1H, s, C₃'–OH), 9.63 (1H, s, C₃–OH), 10.82 (1H, s, C₇–OH), 12.52 (1H, s, C₅–OH), **¹³C NMR (δ)** 147.6, (C-2), 136.6 (C-3), 176.7 (C-4), 157.0 (C-5), 99.0 (C-6), 164.7 (C-7), 94.2 (C-8), 161.6 (C-9), 103.9 (C-10), 122.8 (C-1'), 116.5 (C-2'), 145.9 (C-3'), 148.6 (C-4'), 115.9 (C-5'), 120.8 (C-6').

MS (m/e): 302 (M⁺), 274 (M⁺–CO), 165, 153, 149, 137 (Fig. 7.2).

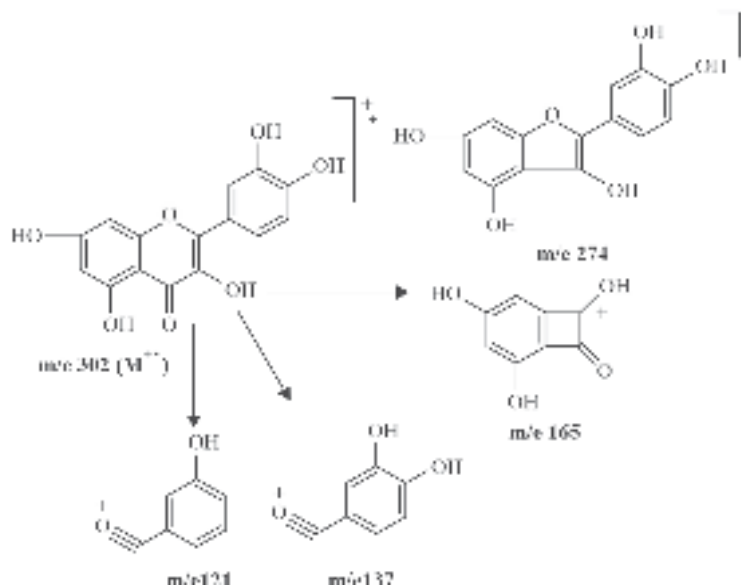


Fig. 7.2: Mass fragmentation pattern of quercetin

2. Rutin

It is a plants originated flavonoid, discovered in 1842, and found as quercetin-3-rutinoside (m.p. 212–214°C). It is a biosynthesized shikimic acid pathway. It is obtained from Buck Wheat (*Fagopyrum esculentum*). It also known as vitamin P. It is yellow crystalline powder found in many plants, cotton seeds, tobacco, eucalyptus etc. It has needle like crystals that darkens on exposure to light. It contains 3 moles of water, becomes anhydrous after heating for 12 hours at 112°C. It is soluble in pyridine, alkaline solution and formamide, methanol, ethanol. It is insoluble in chloroform, ether, benzene. Rutin is composed of one molecule of quercetin as aglycone and rutinose or isoquercetin (quercetin-3-o-glycoside) as secondary glycoside and rhamnose. As shown above, there is structural similarity because the three active components (quercitrin, isoquercitrin and rutin) have identical molecules of quercetin (Fig. 7.3).

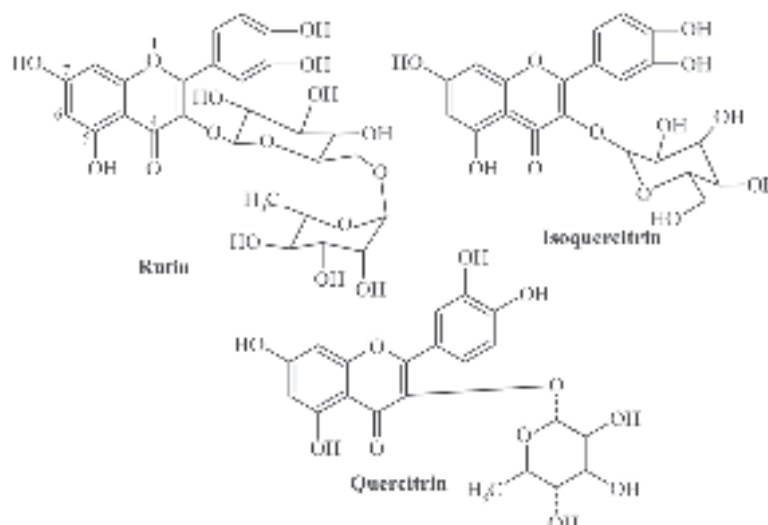
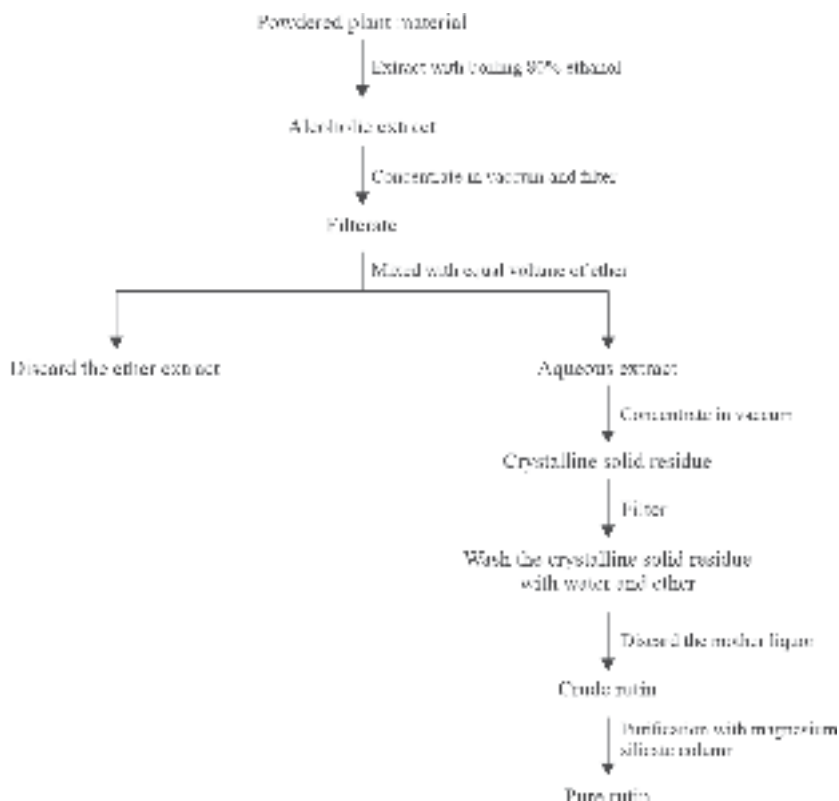


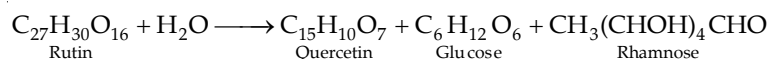
Fig. 7.3: Molecular structure of rutin, isoquercitrin and quercitrin

Isolation of Rutin



Structural elucidation of Rutin

- (i) Its molecular formula was found to be $C_{27}H_{30}O_{16}$.
- (ii) Rutin on hydrolysis with water give quercetin, glucose and rhamnose.

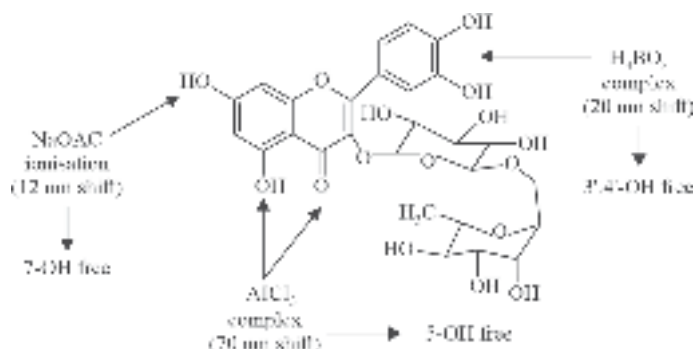


- (iii) On enzymatic hydrolysis by α -L-Rhamnosidase, rutin give rhamnose, isoquercetrin and with β -D-glucosidase, it gives rutinose and quercetin.
- (iv) On complete methylation with Me_2SO_4 , it gives 3',4',5,7-tetramethyl derivative. indicating that the sugar ts attached to C_3 -OH.

Spectral Data

UV: The UV-spectrum of rutin shows two major absorption band at 359 nm (I) and 257 nm (II) which showed the presence of flavonol structure. Bathochromic shift with the CH_3ONa supports the presence of 4'-hydroxyl group and with $AcONa$ indicates the 7-hydroxyl function. The $AlCl_3$ and $AlCl_3/HCl$ spectrum of rutin (70 nm shift) showed the presence of 5-hydroxyl and the UV spectrum of rutin with addition of boric acid (20 nm shift) confirms the presence of ortho-dihydroxy in ring B. .

IR (cm^{-1}): 3330 (OH) (bonded), 2920 ($-CH$ str.), 1660 (C = O), 1600 (aromatic structure), 1520 (C = C), 1360 (C-O-C), 810 (substituted aromatics).



^1H NMR (δ): 6.21 (1H, d, $J = 2$, $\text{C}_6\text{-H}$), 6.40 (1H, d, $J = 2$, $\text{C}_8\text{-H}$), 7.55 (1H, d, $J = 2.1$, $\text{C}_2\text{-H}$), 6.86 (1H, d $J = 9$, $\text{C}_5\text{-H}$), 7.56 (1H, dd, $J = 9, 2.1$, $\text{C}_6\text{-H}$), 9.71 (1H, s, $\text{C}_4\text{-OH}$), 9.21 (1H, s, Cr-OH), 12.62 (1H, s, $\text{C}_5\text{-OH}$), 10.86 (1H, s, $\text{C}_7\text{-OH}$), 5.35 (1H, d, $J = 7.4$, $\text{H}_1\text{-G}$), 5.12 (1H, d, $J = 1.9$, $\text{H}_1\text{-R}$), 1.00 (3H, d, $J = 6.1$, $\text{CH}_3\text{-R}$); ^{13}C NMR (δ) 157.3 (C-2), 134.1 (C-3), 178.2 (C-4), 157.5 (C-5), 99.5 (C-6), 164.9 (C-7), 94.5 (C-8), 162.1 (C-9), 104.8 (C-10), 122.5 (C-1'), 116.1 (C-2'), 145.6 (C-3'), 149.3 (C-4'), 117.1 (C-5'), 122.0 (C-6'), 101.6 (C1-G), 74.9 (C2-G), 77.3 (C3-G), 72.7 (C4-G), 76.7 (C5-G), 67.9 (C6-G), 102.2 (C1-R), 70.8 (C2-R), 71.2 (C3-R), 71.4 (C4-R), 69.1 (C5-R), 18.6 (C6-R). * R and G represent signals from rhamnose and glucose moieties, respectively.

MS (m/e): 611($\text{M}^+ + \text{H}$) (28.88%), 465 (33.33%), 303(100%), 180 (66.6%).

Uses

- Rutin is a strong antioxidant activity.
- It also has anti-inflammatory activity.
- It helps in stoppage of venous edema, which is early sign of chronic renal failure.
- It also strengthens capillaries and help people, who easily bruise or bleed.
- It chelate with iron, therefore reduce fenton reaction.
- It also stabilizes vitamin C, i.e. ascorbic acid action is intensified if taken along with rutin.

SHORT AND LONG QUESTIONS

1. What are flavonoids? Classify them with examples. Mention their chemical test.
2. Discuss the biosynthetic pathway for flavonoids.
3. Give the isolation procedure for flavonoids.
4. Discuss the generalized procedure used for structural elucidation of flavones.
5. Discuss the UV spectrum of flavonoids.
6. What are the uses of quercetin? Discuss its constitution.
7. How the rutin is isolated. Discuss the UV spectrum of rutin along with bathochromic shift caused by various reagents.

MULTIPLE CHOICE QUESTIONS

1. From which, Ring B in flavonoid structure is derived?
 - a. Shikimate pathway
 - b. Acylpolymalonate

- c. Cinnamic acid
 - d. Mevalonic acid
2. The hydrolysis of rutin yields
- a. Quercitrin
 - b. Quercetin
 - c. Genistin
 - d. Cyanidin
3. Which flavonoids are able to decrease the permeability of the capillaries?
- a. Liquiritin and isoliquiritin
 - b. Apigenin and luteolin
 - c. Genistein and ononin
 - d. Hesperidin and rutin

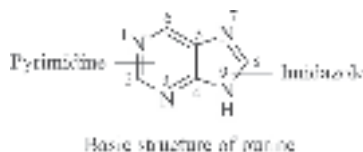
Answer

1. (a) 2. (b) 3. (b)

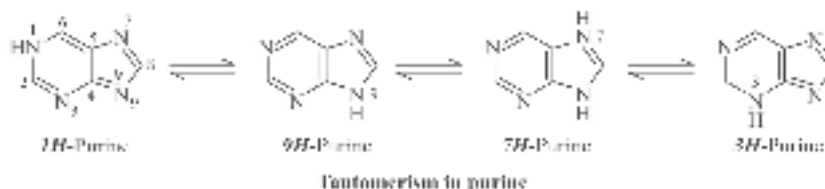
Chapter 8

Purines—Chemistry and Medicinal Aspects

Purines are the cyclic diureides, consisting of a pyrimidine ring fused with an imidazole ring. Purine, m.p. 212°–213°C is a synthetic compound. Although purine is not found naturally, but its derivatives, adenine and guanine are found in nature. These are the building blocks of nucleic acids-RNA and DNA.



Purine can exist in four tautomeric forms in which the hydrogen atom is attached to different nitrogen atom, N-1, N-3, N-7 and N-9.



Purine (pKa 2.4) is a stronger base than pyrimidine (pKa 1.3) but weaker than imidazole (pKa 7.2).

Classification of purines

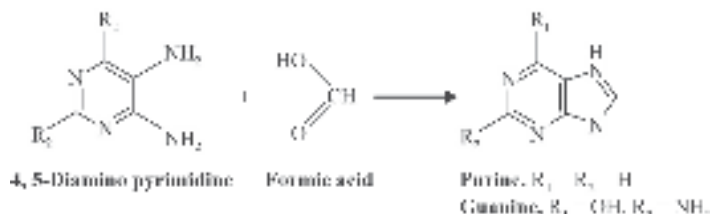
Purines, may be categorised into two groups:

- **Hydroxy purines:** These consist of hydroxy group in their structure, e.g. uric acid, xanthine and its bases.
- **Amino purines:** These consist of amino group in their structure, e.g. adenine and guanine (see Chapter 9).

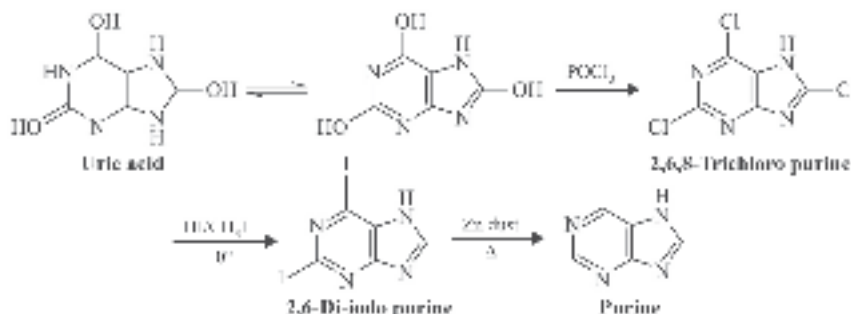
Synthesis of purines

Purine is obtained either by heating 4,5-diamino pyrimidine with formic acid or from uric acid through sequence of reaction.

- From 4,5-diamino pyrimidine:** On heating with formic acid, 4,5-diamino pyrimidine yields purine base.



- (ii) **From uric acid:** Uric acid is converted into 2,6,8-trichloropurine by reacting with phosphorus oxychloride. This on reaction with HI/CH₃I, followed by zinc dust distillation yield the purine base.



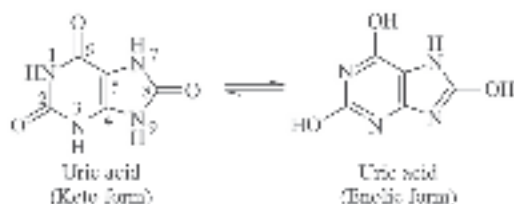
URIC ACID OR 2,6,8-TRICHLOROPURINE

Uric acid is the most important purine and is first discovered by Schesle in 1776 in urine and gallstones, in the human body. It is found as metabolic product of purines. It occurs in human urine in small quantity (about 0.2%). The excessive amount of uric acid is accumulated in the bladder, kidney or joints to form stones and thus the urine of such patients (gouty patients) contains excessive amount of the uric acid. It is also found in the excreta of birds, reptiles and snakes and the particularly large quantities (about 25%) in the guano (the faecal matter of certain birds like cormorants, pelicans and gannets, called guano) of the south sea islands. Snake excreta contain 90% ammonium urate.

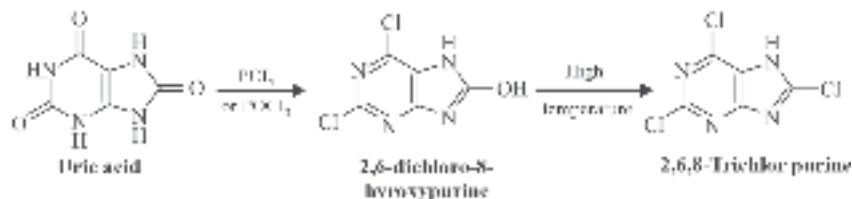
Properties

Uric acid is a white crystalline powder, very sparingly soluble in hot water, and particularly insoluble in cold water. It does not melt but decomposes above 400°C. It forms salts with one or two molecules of a base such as sodium, potassium or lithium hydroxides. The mono-lithium salt is only slightly more soluble than either the corresponding sodium or potassium salt and hence, lithiated water is used in the treatment of gout.

Uric acid exists in two tautomeric forms and the absorption spectra observation shows that the carbonyl form predominates.

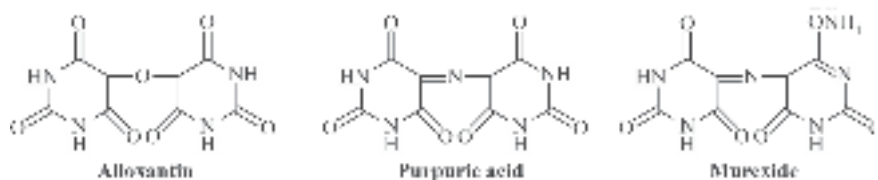


Although uric acid as shown above is a Trienol, it behaves as a weak dibasic acid, e.g. it forms mono- and di-alkali urates (as mentioned above). The dienolic form may be 2,6-, 2,8, or 6,8-, but according to Fischer, it is the 2,6 form that predominates since in this case, the pyrimidine ring becomes aromatic and hence, stabilized by resonance. The existence of 2,6-dienolic form is confirmed by the fact that with phosphorus oxychloride or PCl_5 , uric acid first gives 2,6-dichloro-8-hydroxypurine and then 2,6,8-trichloropurine at higher temperature.



Murexide Color Reaction

Uric acid on evaporation in the presence of a little concentrated nitric acid followed by the addition of a base gives either a purple color (when base is ammonia) or a blue color (when base is caustic soda). In this, alloxantin is first obtained which on reaction with ammonia gives the ammonium salt of purpuric acid (Murexide). This salt is soluble in water and gives purple color to the solution.

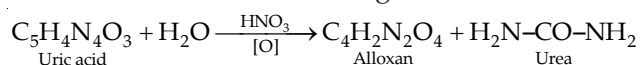


Isolation of Uric Acid

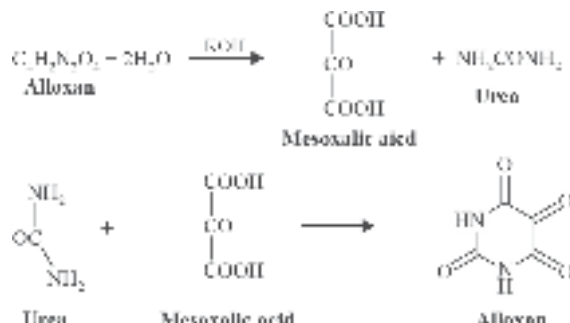
- **From excreta of guano:** It is a commercial source of uric acid. The dry excreta is powdered and then boiled with concentrated caustic soda solution at 80°–100°C for about seven hours for complete evolution of ammonia gases. The contents are filtered and the filtrate is then added in a vessel having hydrochloric acid where the uric acid is crystallized out on cooling. It is purified by dissolving in 5% caustic soda solution, which is concentrated and then cooled to get sodium urate crystals. These are re-dissolved in dilute caustic soda and finally precipitated by acidifying with hydrochloric acid.
- **From human urine:** About 50 ml of concentrated HCl solution is added to 500 ml of human urine and set aside in a cool place for an hour. The liquid is poured off from the crystals of uric acid, which adhere to the side of vessel. The crystals are dissolved in the smallest possible amount of boiling water, boiled with a bone-black and filtered the solution in hot condition. On cooling, colorless crystals of uric acid are obtained.

Structural Elucidation of Uric Acid

1. Its molecular formula is found to be $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$.
2. Mild oxidation of uric acid with nitric acid gives alloxan and urea.

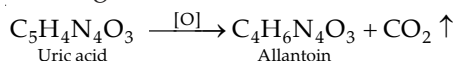


The alloxan, $C_4H_2N_2O_4$, does not possess any free amino or carboxyl group and gives urea and mesoxalic acid on hydrolysis. Formation of these products indicates the presence of mesoxalyl urea structure, which is again confirmed by its synthesis from the condensation reaction of urea with mesoxalic acid.



The formation of alloxan from uric acid indicates that the uric acid has a pyrimidine nucleus.

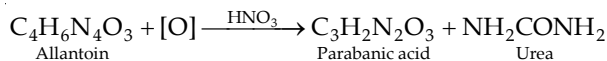
3. Vigorous oxidation of uric acid with neutral or alkaline potassium permanganate or with aqueous lead oxide gives allantoin and carbon dioxide.



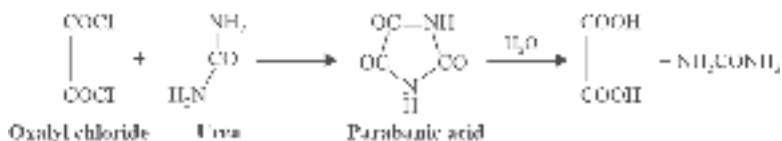
To know the structure of uric acid, it is required to know the structure of allantoin also.

Structure of Allantoin

- (a) Allantoin, $C_4H_6N_4O_3$ on oxidation with nitric acid, gives parabanic acid and urea.



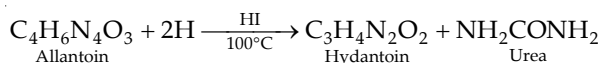
- (b) The parabanic acid does not possess any free amino or carbonyl group and gives urea and oxalic acid on hydrolysis. This reaction indicates that parabanic acid must be oxalyl urea, which is confirmed by its synthesis from oxalyl chloride, and urea.



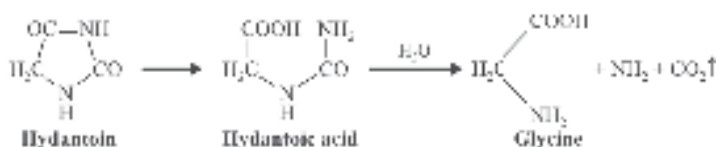
The formation of parabanic acid indicates the presence of imidazole nucleus in allantoin and so in uric acid

- (c) Now the only problem in assigning the complete structure to allantoin is to know which CO of the parabanic acid is linked with urea residue to form allantoin molecule. It is established by the following observations.

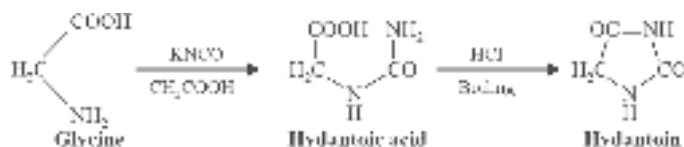
Allantoin on reduction with hydroiodic acid, forms urea and hydantoin.



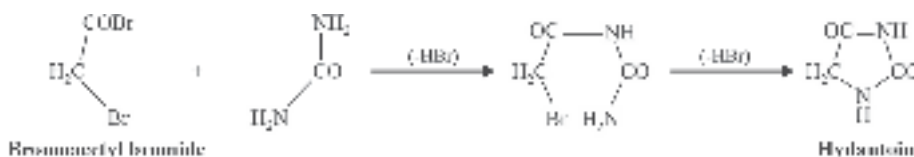
The hydantoin on controlled hydrolysis gives glycine, ammonia and carbon dioxide via hydantoin acid (ureidoacetic acid). Hence, hydantoin must possess the following structure to explain the hydrolytic products.



The above structure for hydantoin is confirmed by its synthesis.



Hydantoin may also be obtained by the electrolytic reduction of parabanic acid, or by the action of bromoacetyl bromide on urea.

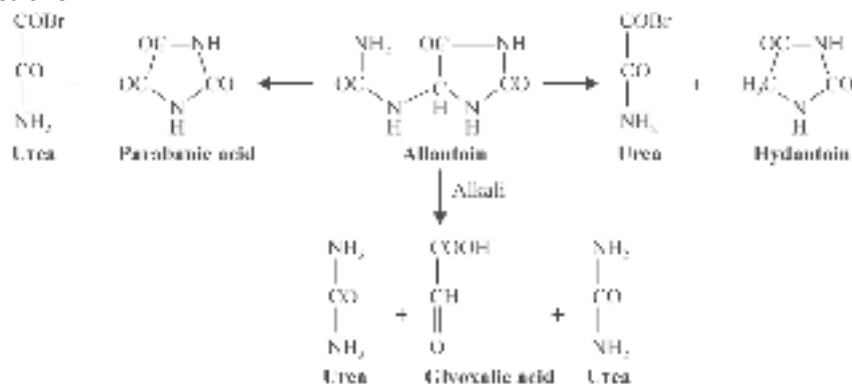


Thus, two products from allantoin are obtained:

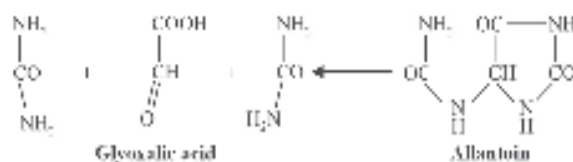
- (i) Parabanic acid.
- (ii) Hydantoin, under different conditions.



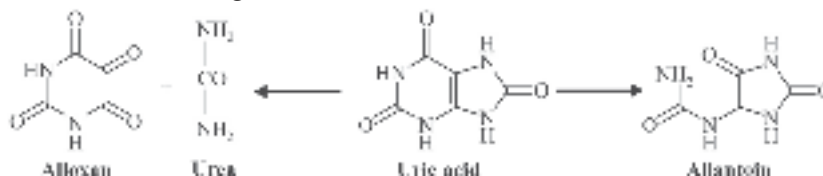
The above two structures, namely parabanic and hydantoin differ only in one position (marked by asterik) which must constitute the point of attachment. of urea residue in allantoin molecule. Hence, allantoin may be assigned the following structure which explains all the reactions.



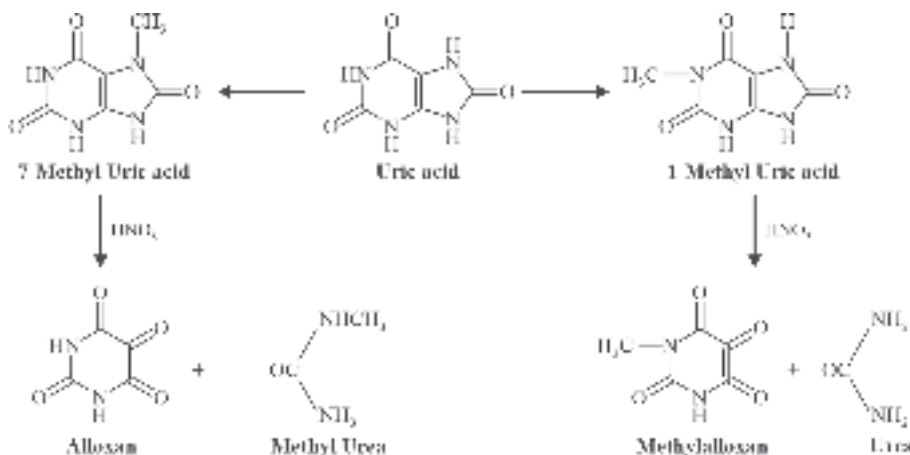
Finally, the assigned structure of allantoin is confirmed by its synthesis. It is condensation of two moles of urea with one mole of glyoxalic acid.



According to the point 3, uric acid on vigorous oxidation gives allantoin with the loss of one carbon atom as carbon dioxide. Secondly, it gives alloxan on oxidation with nitric acid. The formation of these two products is possible if following structure is assigned to uric acid. This structure can give the formation of alloxan and allantoin as well.



The structure is confirmed by Fischer by the synthesis and reaction of two isomeric monomethyl uric acids. On oxidation with nitric acid, 1-methyl uric acid gives methyl alloxan and urea while 7-methyl uric acid gives alloxan and methyl urea.



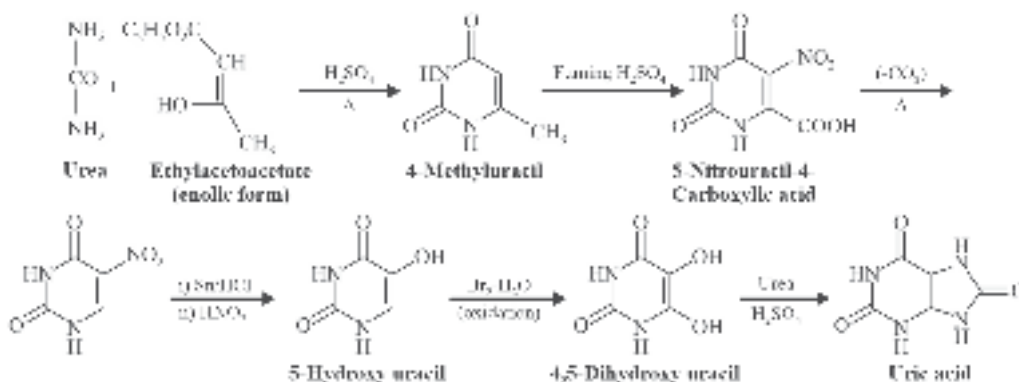
In addition to the above monomethyluric acids, uric acid also forms:

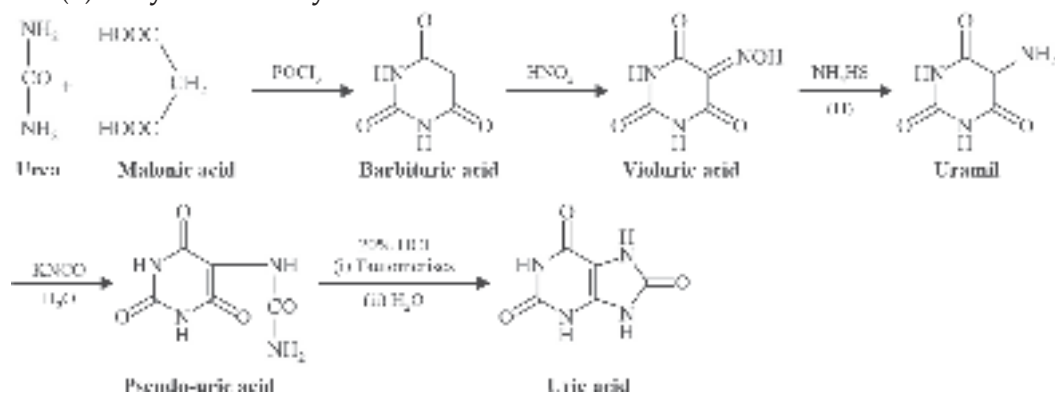
- (i) two other monomethyluric acids, 3- and 9-,
- (ii) six dimethyl, and
- (iii) four trimethyl uric acids.

All these are possible with this assigned structure only.

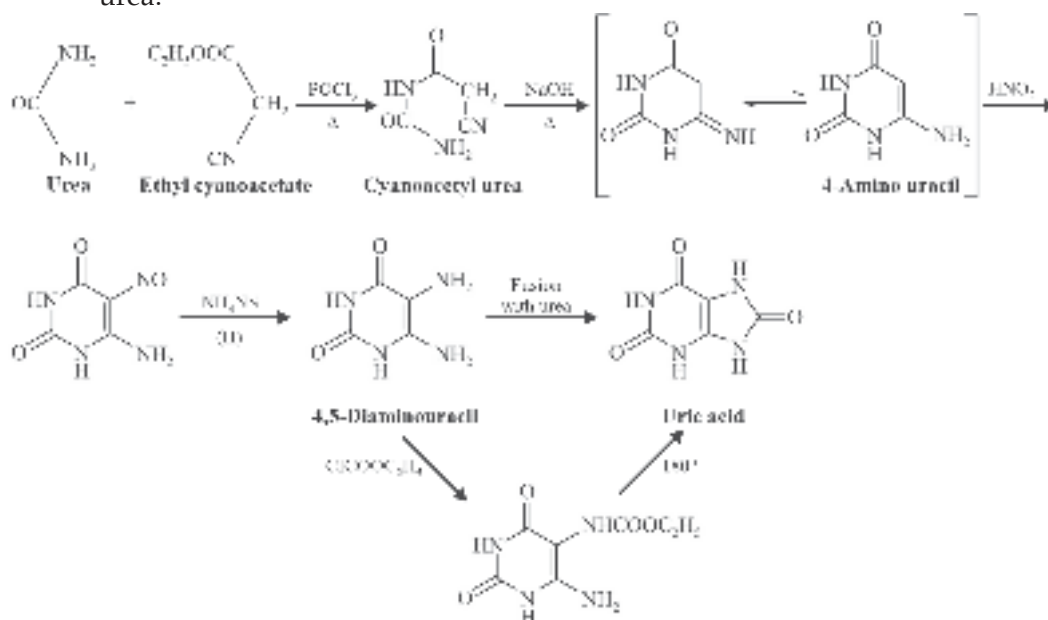
Finally, the assigned structure is confirmed on the basis of synthesis.

(a) Behrend and Roosen synthesis:



(b) **Baeyer-Fischer synthesis:**

(c) **Traube's synthesis:** It is generalised method for synthesis of purines. It involves use of cyanoacetyl urea, formed by the condensation of ethyl cyanoacetate with urea.

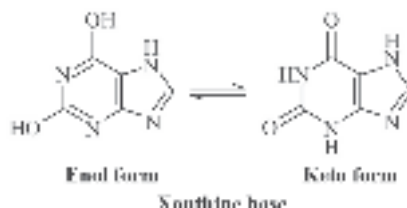
**Spectral Data**

IR (cm^{-1}): 3050, 1680, 750.

MS (m/e): 168 (M^+), 125, 97, 69, 54, 43.

XANTHINE (2,6-DIHYDROXY PURINE)

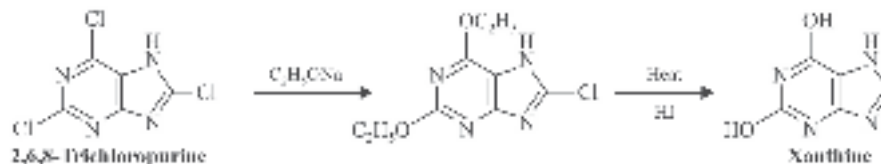
The caffeine, theobromine and theophylline are the examples of xanthine bases. These also contain pyrimidine nucleus, fused with imidazole ring. There occurs in tea extract and animal tissues. On oxidation with potassium chlorate in hydrochloric acid solution, xanthine forms alloxan and urea, and hence, it is related to uric acid which also forms the same products on mild oxidation. Thus, on the basis of its relation with uric acid and synthesis the given structure is assigned to xanthine base. The two tautomeric structures (keto and enol forms) are possible in xanthine bases.



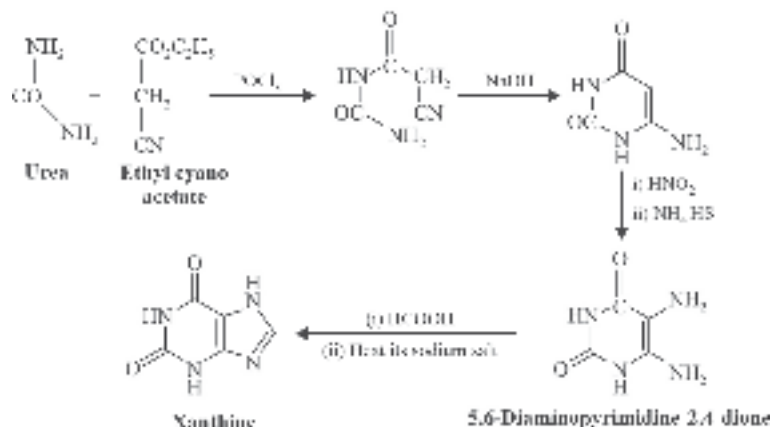
Synthesis of Xanthine

There are two general method of synthesis for xanthine bases namely, Fischer and Traube syntheses.

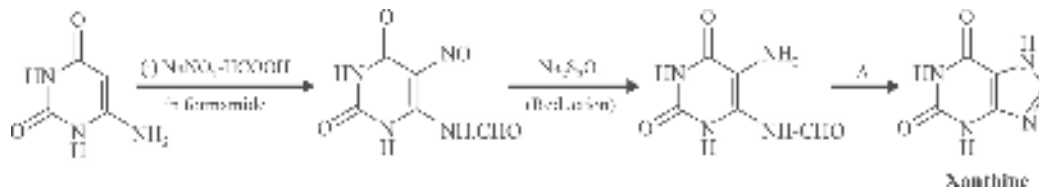
(i) Fischer's synthesis:



(ii) Traube's synthesis:



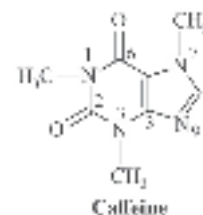
Traube's synthesis is further modified by Bredereck et al.:



1. CAFFEINE (1,3,7-TRIMETHYLXANTHINE)

Caffeine is the most important naturally occurring xanthine derivative. It is the component of the tea leaves (5%), coffee (1–2%) and colanuts (1–2%).

The purified caffeine is a colorless and intensely bitter white powder, m.p. 135°C. It is added to colas and other soft drinks to impart a pleasing bitter taste. However, caffeine is also an addictive stimulant. In humans, it stimulates the central nervous system, heart rate and respiration; has psychotropic (mood altering) properties, and act as mild diuretic. A normal dose of caffeine is generally considered to be 100 mg, which is roughly the amount found in cup of coffee. However, more than half of all American adults consume more than 300 mg of caffeine everyday, which makes it America's most popular drug. Caffeine is generally consumed in coffee, cola, chocolate and tea, although it is also available over the counter as a stimulant.



Caffeine is believed to work by blocking adenosine receptors in the brain and other organs. This reduces the ability of adenosine to bind to the receptors, which would slow down cellular activity. The stimulated nerve cells release the epinephrine (adrenaline) which increases heart rate, blood pressure, decreases blood flow to the skin and organs, and causes the liver to release glucose. Caffeine also increases level of the neurotransmitter dopamine.

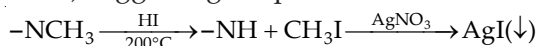
It is sparingly soluble in water and alcohol. It is a weak base and forms salts with strong acids, which are easily decomposed by water. Medicinally, caffeine is used in the form of its citrate and hydrochloride.

Isolation of Caffeine

- (i) Boil tea leaves extract (50 g) with 250 ml solvent (methanol/water-methanol mixture). Strain it.
- (ii) Treat the filtrate with excess amount of lead acetate solution for complete precipitation of tannins. Filter off the precipitate to get light yellow colored filtrate.
- (iii) If color is light brown then again treat with more amount of lead acetate to get light yellow colored filtrate.
- (iv) Treat with excess amount of dil. H_2SO_4 for removal of excess of lead acetate as lead sulphate. Filter off the precipitate.
- (v) Add dilute ammonia solution to filtrate to impart the pH at 7.0.
- (vi) Extract neutralized filtrate with chloroform in separating funnel (3 times extracted with chloroform, 50 ml each time).
- (vii) Mix the CHCl_3 extract and evaporate off the solvent on water bath (avoid charring of caffeine by excess evaporation).
- (viii) Crystallize the crude caffeine by methanol to get needle shaped crystals.

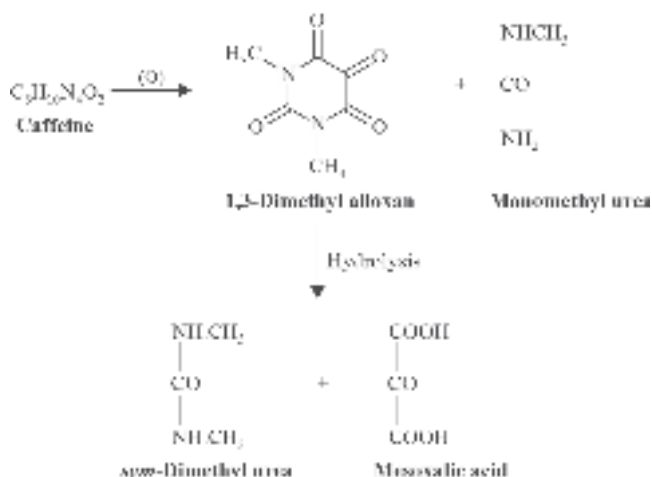
Structural elucidation of caffeine

1. Its molecular composition is $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$.
2. On heating with hydroiodic acid at about 200°C , caffeine gives three moles of methyl iodide (gravimetrically estimated via formation of insoluble silver iodide—Herzig-meyer method) suggesting the presence of three N-CH_3 groups.

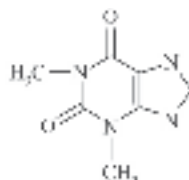


3. Caffeine on oxidation with potassium chlorate in hydrochloric acid solution gives 1,3-Dimethyl alloxan and monomethyl urea. 1,3-Dimethyl alloxan is identified as mesoxalyl-sym-dimethyl urea due to formation dimethyl urea and mesoxalic acid on hydrolysis.

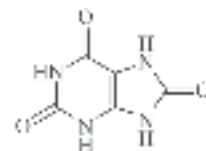
- (i) The formation of 1,3-dimethyl alloxan and mono methyl urea suggests that the caffeine has uric acid skeleton, which on oxidation also uses the same products-alloxan and urea.



(ii) The formation of 1,3-dimethylalloxan so establishes the position of two of the three methyl groups. Hence, the probable partial structure of caffeine may be drawn as given here:

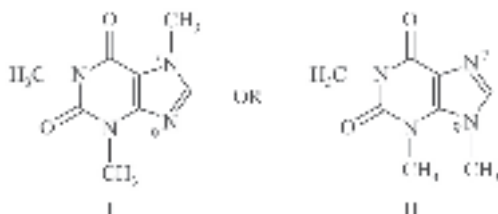


Partial structure of caffeine

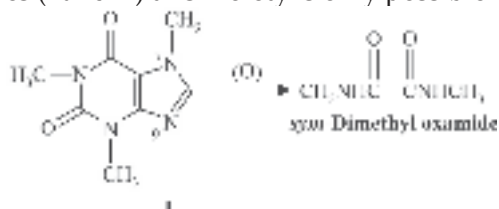


Uric acid

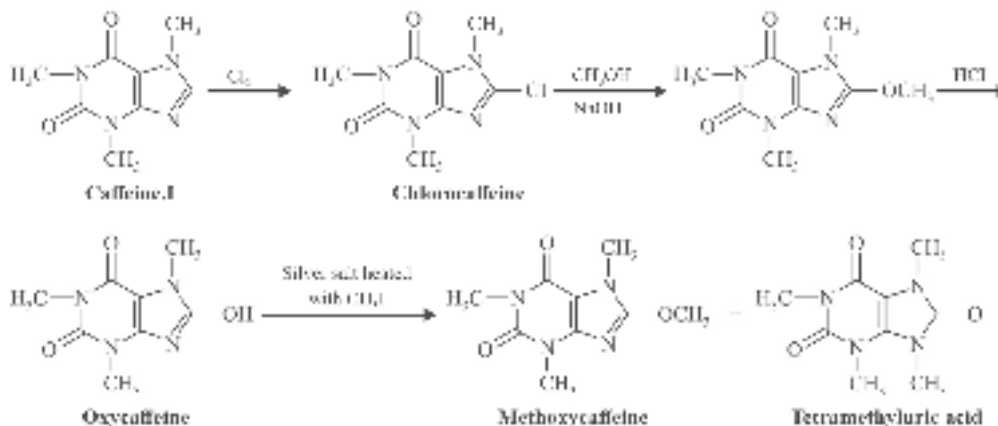
4. **Position of third methyl group:** Now the problem is to assign the position of the third N-methyl group. From the above, it can be predicted that 'the third N-methyl group may either attach to N-7 or N-9 position and so two structures are possible for caffeine as given here.



5. On vigorous oxidation, caffeine gives *sym*-dimethyl oxamide ($\text{CH}_3\text{NH}\cdot\text{CO}\cdot\text{CO}\cdot\text{NHCH}_3$), which indicates the presence of $\text{CH}_3\text{N}-\text{C}-\text{C}-\text{N}-\text{CH}_3$ moiety in its structure. Out of above two structures (I and II) this moiety is only possible in structure, I.



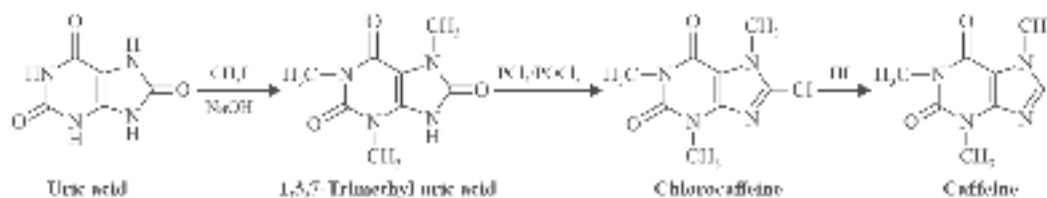
6. The structure I, for caffeine also explains Fischer observation. The caffeine on chlorination gives a 8-chlorocaffeine. This on treatment with methanolic NaOH followed by treatment with HCl gives oxycaffeine. The silver salt of oxycaffeine on treatment with methyl iodide gives a mixture of tetramethyl uric acid (containing four N-methyl groups) and methoxy caffeine (containing three N-methyl and one O-methyl group).



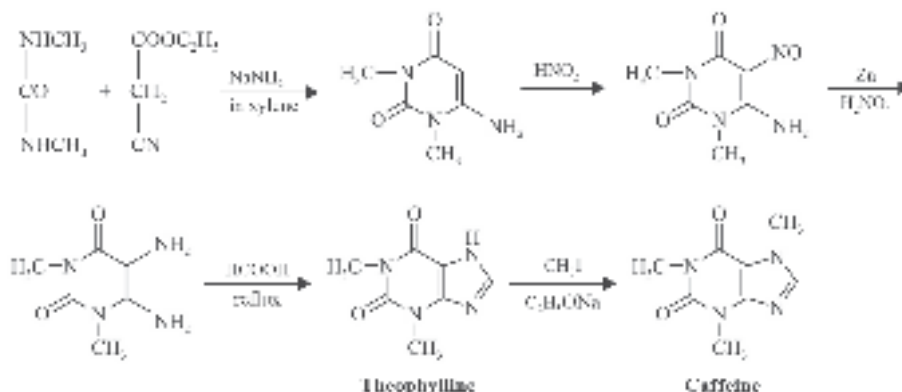
7. Finally, the above structure, I for caffeine is proved by the synthesis.

Synthesis of Caffeine

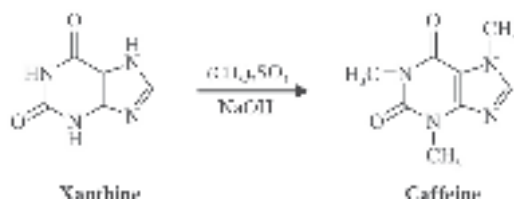
(i) **Fischer synthesis:** Caffeine is synthesized from uric acid.



(ii) **Traube's synthesis:** Caffeine is synthesized from *sym*-dimethyl urea and ethyl cyanoacetate.



(iii) From xanthine:



The method (ii) and (iii) are the commercial methods for synthesis of caffeine.

Spectral Data

UV: λ_{max} 274.

IR (cm^{-1}): 3134, 2850, 1705, 1660, 1604, 1230, 740.

$^1\text{H-NMR}$ (δ) 3.35 ($\text{N}_1\text{-CH}_3$), 3.55 ($\text{N}_3\text{-CH}_3$), 3.95 ($\text{N}_7\text{-CH}_3$), 7.60 (H-8).

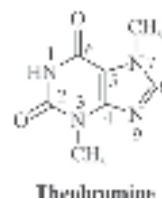
$^{13}\text{C-NMR}$: 27.5 (C-1), 29.3 (C-3), 33.2 (C-7), 107.1 (C-5), 141.2 (C-8), 148.3 (C-4), 151.3 (C-2).

MS (m/e): 194 (M^+ , 100%, Base peak), 165 ($\text{M}^+ - \text{CO}$), 109 ($\text{C}_5\text{H}_{17}\text{N}_3$, 66%), 82 (37%), 67 (54%), 55 (80%), 42.

2. THEOBROMINE (3,7-DIMETHYLXANTHINE)

Theobromine is found in cocoa beans (1 to 2%) tea leaves, etc. It is used as a diuretic. Its name is derived from the greek words '*theos*', meaning god and '*brosi*', meaning food of god. It is a white crystalline compound, soluble in hot water. Theobromine's effects on

human body are similar to those of caffeine, but about 10 times weaker. In addition, caffeine is metabolised more quickly, is addictive and increases alertness and emotional stress. By contrast, theobromine produces feelings of well being, is not addictive, has no effect on CNS, and provides only gentle stimulation to kidneys. Its effect on the body is much longer lasting than caffeine.

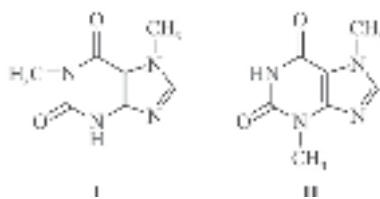


Isolation of Theobromine

- (i) Prepare the mixture of cocoa powder (10 g), MgO (3 g) in water (20 ml) and methanol 10 ml) in a round bottom flask (250 ml). The mixture is stirred with a glass rod and heated on heating mantle to dryness. It takes approximately 1 hour. Add 170 ml of methylene chloride to the dry substance obtained and heat under reflux for 30 min.
- (ii) Next, filter the contents on a Buchner funnel. Dry the solution over anhydrous MgSO_4 . Crush the solid substance and once more put it into a round bottom flask, add 170 ml of methylene chloride. Heat the mixture under reflux for additional 30 min and once more filter on the Blichner funnel.
- (iii) Dry the extract over anhydrous MgSO_4 , then filter on the funnel with cotton to remove MgSO_4 .
- (iv) Transfer the combined fractions into a clean and dry round-bottom flask and concentrate the solution to 50 ml. Transfer the solution to a beaker (100 ml), wash carefully with chloroform and transfer also to the beaker. Add 45 ml of ether and leave to crystallization to obtain micro-crystals then wash them on a Buchner funnel 5 times with 10 ml of ether, yield 0.15 g, mp. 351°C .

Structural Elucidation of Theobromine

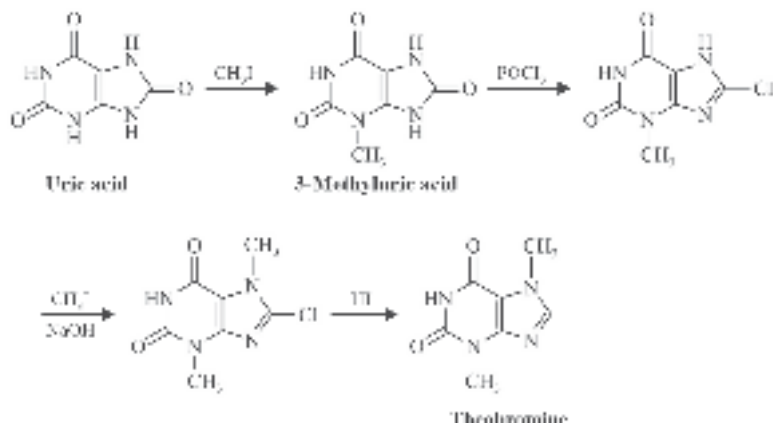
1. Its molecular composition is $\text{C}_7\text{H}_8\text{N}_4\text{O}_2$.
2. By the Herzig Meyer method, theobromine is found to contain two $\text{N}-\text{CH}_3$ groups.
3. By oxidation with potassium chlorate in hydrochloric acid, theobromine gives monomethyl alloxan and monomethyl urea indicating that theobromine has uric acid skeleton. The oxidation reaction also confirms the presence of one methyl group in pyrimidine acid (alloxan) and another in imidazole ring (urea).
4. Silver salt of theobromine gives caffeine (1,3,7-trimethylxanthine) on methylation, indicating that the methyl group of imidazole ring its present at C-7 position while the methyl group of pyrimidine ring may be at C-1 or C-3 position. On the basis, two structures are assigned to theobromine **I** or **II**.



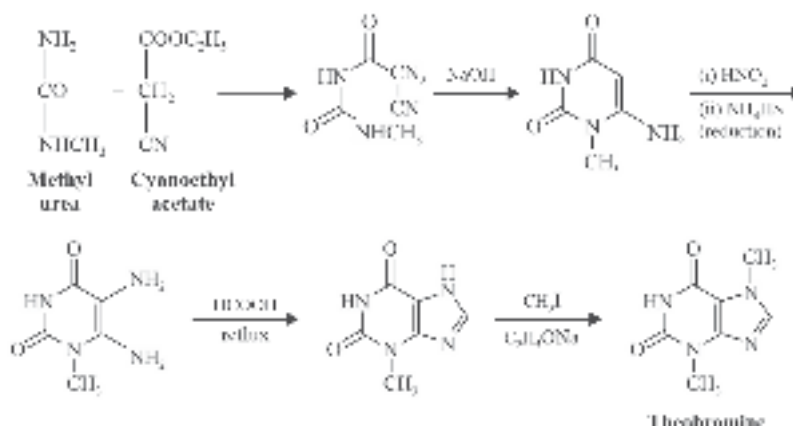
5. Out of the above two structures, structure II is found to be structure of theobromine, since it may be prepared from 3-methyluric acid as well as from 7-methyl uric acid but not from 1-methyluric acid.

Synthesis of Theobromine

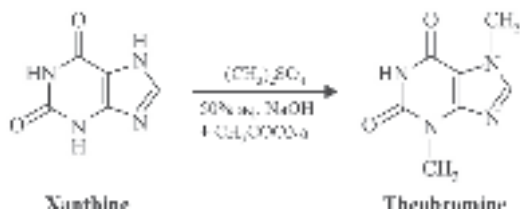
(i) Fischer's synthesis:



(ii) Traube's synthesis of theobromine:



(iii) From xanthine:



Spectral Data

UV: λ_{max} 272 nm (aqueous acid), 274 nm (aqueous alkali).

IR (KBr) (cm^{-1}): 1690, 1665, 1595, 1550, 1221, 680.

$^1\text{H-NMR}$ (δ): 3.5 ($\text{N}_3\text{-CH}_3$), 3.8 ($\text{N}_7\text{-CH}_3$), 8.00 (= C-H) and 10.9 (OC-NH-CO).

MS (m/e): 180, 137, 109, 82, 67, 55, 42.

3. THEOPHYLLINE (1,3-DIMETHYLXANTHINE)

Theophylline, an isomer of theobromine, found in tea leaves and prepared synthetically. It is used as a diuretic and anti-asthmatic drug.

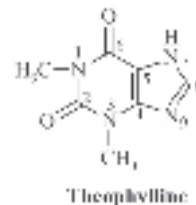
Theophylline, m.p. 274°C, is a white crystalline powder with bitter taste. It is soluble in hot water.

Theophylline is a phosphodiesterase (PDE) inhibitor and so is widely used in therapy for respiratory diseases. The main actions of theophylline are:

- Relaxation of bronchial smooth muscle.
- Positive inotropic effect (increases heart muscle contractivity).
- Positive chronotropic (increases heart rate).
- Increases renal blood flow.

Main derivatives of theophylline which are clinically used include:

- Aminophylline
- Chloine theophyllinate
- Theophylline calcium salicylate
- Theophylline sodium glycinate

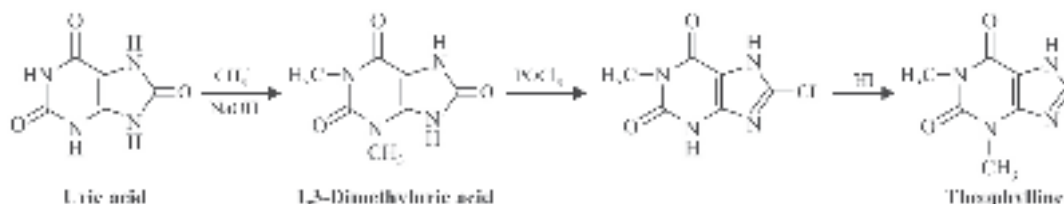


Structural Elucidation of Theophylline

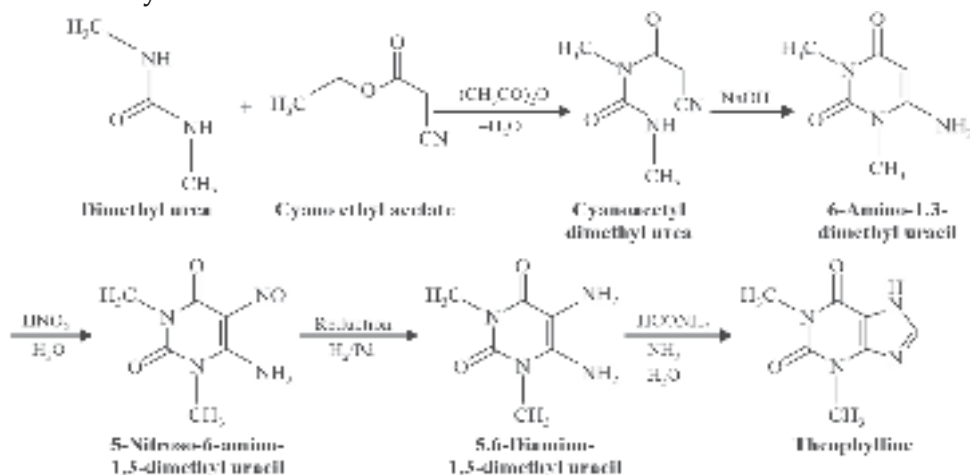
1. Its molecular formula is found to be $C_7H_8N_4O_2$.
2. It consists of two $N-CH_3$ groups. On oxidation it gives dimethyl alloxan and urea, while on methylation it is converted into caffeine.
3. The reaction confirms the presence of both the methyl groups in the pyrimidine nucleus. On this basis, the given structure is assigned to the theophylline.
4. The proposed structure is confirmed by the synthesis.

Synthesis of Theophylline

(i) Fischer's synthesis:



(ii) Traube's synthesis:



Spectral Data

UV: $\alpha_{\max}$ 271 (aqueous acid).

IR (cm^{-1}): 3121 (C–H aromatic stretching) 2922 (C–H).

$^1\text{H-NMR}$: (δ): 3.24 (N–CH₃), 3.43 (N–CH₃), and 7.89 (= C–H).

Relationship of Uric Acid and Xanthine Bases

The prime derivative, uric acid and xanthine bases have similar basic skeleton, because on oxidation, they form similar products, alloxan and urea or its methyl derivative and urea or its methyl derivative.

Uric acid $\rightarrow$ Alloxan + Urea

Xanthine $\rightarrow$ Alloxan + Urea

Caffeine $\rightarrow$ Dimethylalloxan + Methylurea

Theobromine $\rightarrow$ Methylalloxan + Methylurea

Theophylline $\rightarrow$ Dimethylalloxan + Urea

SHORT AND LONG QUESTIONS

1. Write the structure and inter-relationship of uric acid, theobromine and caffeine. Write the therapeutic uses of theophylline.
2. Explain the chemistry of caffeine or uric acid.
3. Illustrate the synthesis of following:
 - a. Uric acid
 - b. Theophylline
4. What are xanthine bases? How these are related to purines. Give the isolation procedure for caffeine.
5. Illustrate the murexide test for caffeine

MULTIPLE CHOICE QUESTIONS

1. Caffeine is:
 - a. Trimethyl xanthine
 - b. Dimethyl xanthine
 - c. Monomethyl xanthine
 - d. None of above
2. Caffeine on vigorous oxidation with KClO_3/HCl gives:
 - a. Trimethyl alloxan and urea
 - b. Methyl alloxan and dimethyl urea
 - c. Dimethyl alloxan and methyl urea
 - d. None of the above
3. Uric acid on vigorous oxidation gives mainly:
 - a. Allantoin
 - b. Alloxan
 - c. Urea
 - d. All of above

Answer

1. (a) 2. (c) 3. (a)

Introduction to Nucleic Acids: Structural Properties of Nucleic Acid Building Blocks

There are two major types of nucleic acids—DNA and RNA. The name ‘nucleic acids’ is given due to simple reason that they are made up of structures, called **nucleotides**. DNA and RNA are chain-like macromolecules that function in the storage and transfer of genetic information. They are major components of all cells ~15% of the cells dry weight. Just as the amino acids are building blocks of proteins, the nucleotides are the monomeric unit of nucleic acids. These building blocks were discovered in 1869 by **Friedrich Miesher**, Swiss biochemist. In this chapter, the structure and chemistry of the nucleotides and polynucleotides will be discussed.

Components of Nucleic Acids—Sugars and Bases

The primary structure of a polynucleotide has some analogy with the primary structure of proteins. Like proteins, nucleic acids are the polymers, in which nucleotides are monomer units. In both RNA and DNA, each nucleotide repeating unit consists of three characteristic major components:

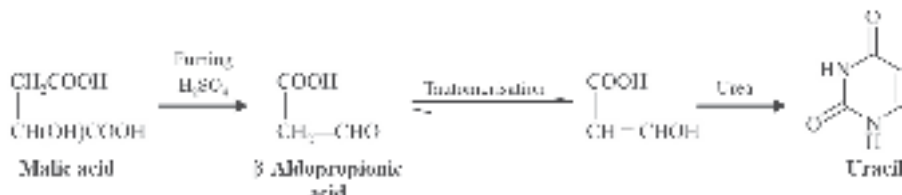
- (a) Nitrogenous heterocyclic base, which is either the derivative of a pyrimidine or a purine.
- (b) Pentose sugar (the type differs between RNA and DNA).
- (c) Molecule of phosphoric acid.

NUCLEIC ACID BASES

The common nucleotides come in five different bases: Adenine, guanine, cytosine and thymine in DNA, with uracil substituting for thymine in RNA. In strict analogy with the amino acids, nucleotide bases have different functional groups and these differences determine the polynucleotide’s structure and function. The bases are either pyrimidines or purines.

Pyrimidines are six membered heterocyclic aromatic rings (Fig. 9.1). The 2 nitrogen atoms are connected to the four carbon atoms by conjugated double bonds, thus giving the base substantial aromatic character. All the common pyrimidines in DNA and RNA have a keto group at C-2, but these differ in the C-4 substituent at the “top” of the ring. As we will see later, the substituent at C-4 as well as N-3 of the ring are involved in H-bonding to complementary bases in the secondary structure of nucleic acids. Cytosine is referred to as the “amino” pyrimidine base, because of its exocyclic amino group at C-4. The “keto” bases are uracil and thymine, again named because of their keto groups

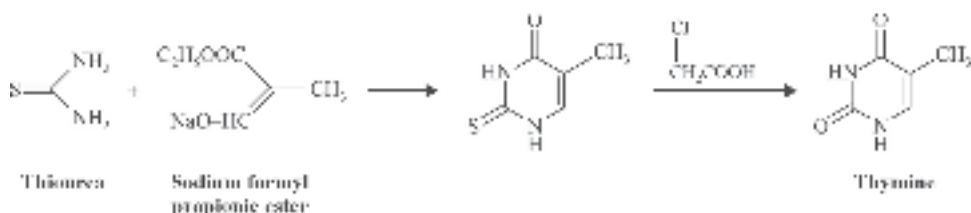
3. **Davidson-Baudisch synthesis:** The Davidson-Baudisch synthesis of uracil is a simple one-pot method, involving the treatment of urea and malic acid with fuming sulphuric acid. The Davidson-Baudisch synthesis is facile and can afford various uracils in moderate yield.



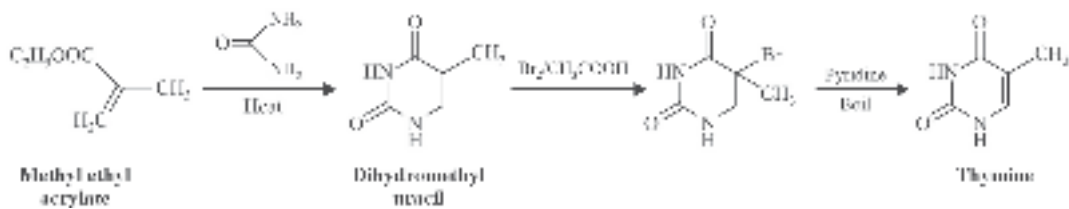
Thymine

It has been synthesized by similar methods, which are used for uracil.

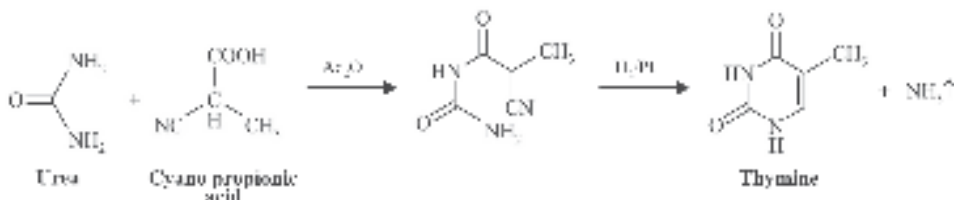
1. **Wheeler-Liddle method:**



2. **Fischer-Roeder method:**

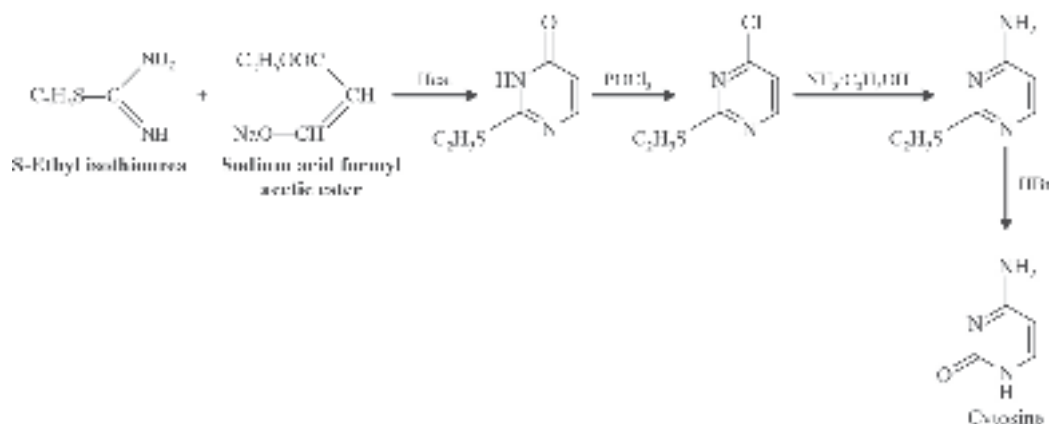


3. **Bergmann synthesis.** In the Bergmann synthesis, a substituted cyanoacetic acid is condensed with urea in the presence of acetic anhydride and subsequently reduced by catalytic hydrogenation.

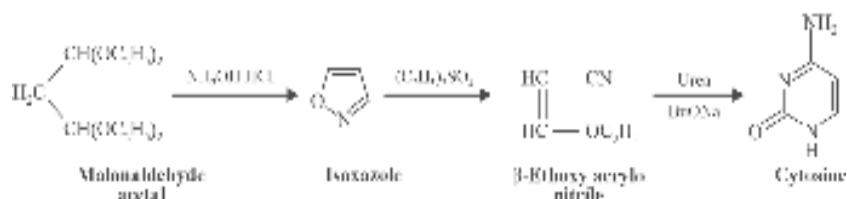


Cytosine

1. **Wheeler-Johnson method:** It is obtained from S-ethyl isothioureia and sodium formyl ester.



2. **Tariso method:** Cytosine is obtained from malonaldehyde acetal by reaction with hydroxylamine. The formed ethoxy acrylonitrile is reacted with urea in presence of base to give cytosine.



Purines have two heterocyclic rings, a 6-membered pyrimidine ring, fused to a 5-membered imidazole ring. Unfortunately, the convention for numbering the ring atoms in purines differs from those of pyrimidines.

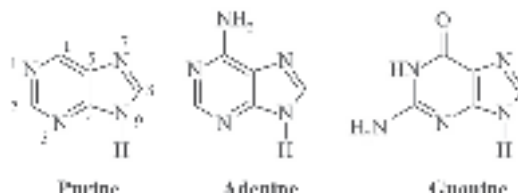
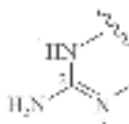


Fig. 9.3: Common purine bases present in nucleic acid

- (i) The substituents present at the “top” of the 6-member ring of the 2-ring system (i.e. at C-6) are major determinants of the H-bonding (or base pairing) capacity of the purines. The “amino” base for purines is adenine, which is 6-aminopurine. This amino group serves as the H-bond donor in base pairs with the C-2 keto group of thymine or uracil. Using similar conventions, the “keto” base for purines is guanine; note the keto group at C-6.
- (ii) The C-2 of guanine is bonded to two nitrogens within the ring (as is true for all purines) and also to an exocyclic amino group. Thus atoms 1, 2, and 3 of guanine form a guanidino group:



This is the same as the functional group in arginine, but it is not protonated at neutral pH because of the electron-withdrawing, properties of the aromatic ring system. The “guan” part of the name of the guanidino group and of guanine comes from *guano*, or bat droppings. These excretions are rich sources of purines. Purines also undergo keto-enol tautomerization, and again the keto tautomer is the more prevalent in nucleic acids (Fig. 9.4).

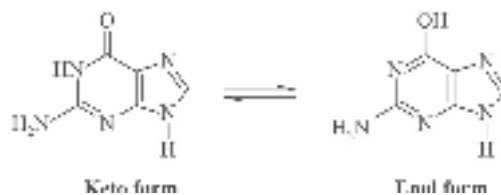


Fig. 9.4: Tautomers of guanine

These common bases can and often are derivatized by the cell for various structural and functional uses. The most common derivatives are those which are methylated (Fig. 9.5). A list of some other types of modified bases is given in the Table 9.1. Most of these bizarre bases are found in tRNA.

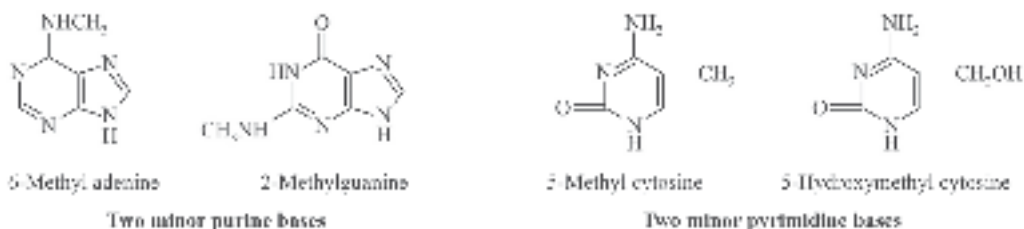


Fig. 9.5: Derivatized nucleic acid bases

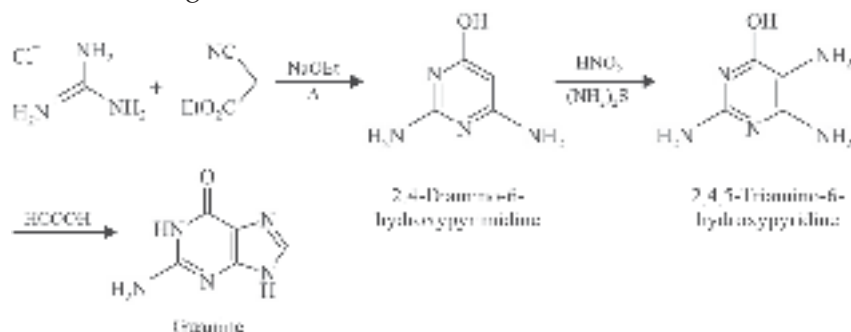
Table 9.1: Other rare bases found in nucleic acids

5,6-Dihydrouracil	1-Methyl uracil	2-Methyl uracil	5-Hydroxymethyl uracil
2-Thiouracil	N-Acetyl uracil	3-Methyl cytosine	5-Methyl cytosine
5-Hydroxymethyl cystosine	1-Methyladenine	2-Methyladenine	7-Methyl adenine
N,N-Dimethyl adenine	N-(Isopentenyl) adenine	1-Methyladenine	7-Methyl guanine
N-Methylguanine	N,N-Dimethyl guanine		

Synthesis of Purine Bases

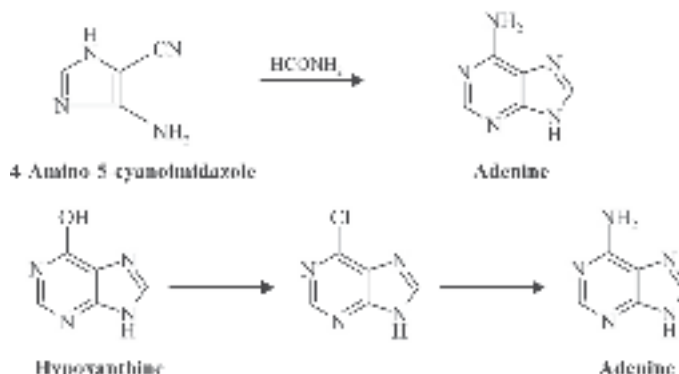
Guanine (Traube's synthesis)

Traube's synthesis involves heating 2,4,5-triamino-6-hydroxypyrimidine (as the sulphate) with formic acid for long hours.



Adenine

It can be prepared either from 4-amino-5-cyano imidazole or hypoxanthine.



Also see Chapter 8 for synthesis of purine bases.

All these bases have substantial **aromatic character**. Delocalized π -electrons are shared around the ring. Because of this, the bases **absorb in the UV region** in the range from 250–280 nm (Fig. 9.6). The extinction coefficient of DNA/DNA allows the determination of concentration of nucleic acids in a sample as UV absorption is directly proportional to nucleic acid molecules in the sample. The extinction coefficient of DNA and RNA depends on nucleotide composition and wavelength of UV light.

The knowledge of the ratio of the average extinction coefficients of the bases at two UV wavelengths, commonly 260 *vs* 280 nm allows one to assess the purity of the nucleic acid solution. Additionally, the OD of a particular concentration of nucleic acid bases depends on the structure into which they are assembled. Hence, absorbance can be used as a structural probe of nucleic acids.

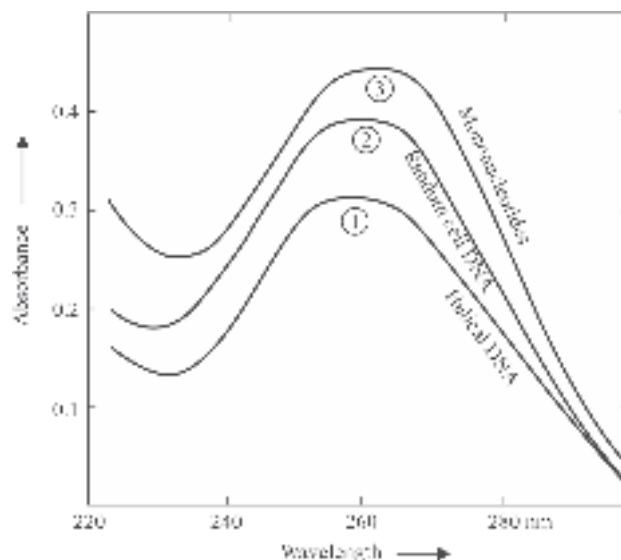


Fig. 9.6: UV spectrum of DNA

NUCLEOSIDES

Nucleosides are purine or pyrimidine bases attached to a pentose sugar. The ribose (β -D-ribofuranose) is present in RNA, hence nucleosides formed from this sugar are known as ribonucleosides while those with 2-deoxyribose (β -D-2-deoxyribofuranose in DNA) are referred to as deoxynucleosides. The sugar present in nucleoside is in furanose form (Fig. 9.7).

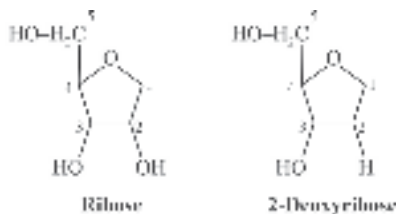


Fig. 9.7: Pentose sugar present in nucleic acid

The purine or pyrimidine base is connected to the (deoxy) ribose via an N-glycosidic bond between the N-1 of the pyrimidine, or N-9 of the purine, and C-1 of the sugar (Fig. 9.8). (Nucleosides are nonreducing in nature, indicating the aldehydic group is not free. It means that C-1 position is involved in the glycosidic linkage). Note that the sugar is the β -anomer at C-1' (the bond points "up" relative to the sugar ring) and the base is above the sugar ring in the nucleoside.

The purine or pyrimidine ring can rotate freely around the N-glycosidic bond. In the *syn*-N-conformation, the purine ring is 'over' the pentose ring and in the *anti*-conformation, it is away from the pentose ring.

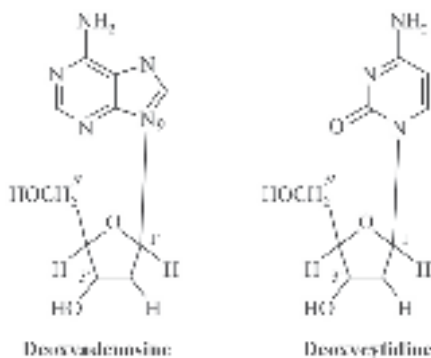


Fig. 9.8: Deoxyadenosine and deoxycytidine

NUCLEOTIDE

A nucleotide is a base attached to a sugar attached to a phosphate; it is a nucleoside, esterified to a phosphate (Fig. 9.9). The phosphate is attached by an ester linkage to a hydroxy group on the sugar, usually present at C-5' or C-3' position. Note that the atoms in the (deoxy) ribose ring are numbered 1', 2', 3', etc. when in nucleotides or nucleic acids to avoid confusion with the numbering system of the bases (Fig. 9.8). Deoxyadenosine and deoxycytidine.

Sometimes the connection with phosphate is at the 2' position, in RNA, as seen in splicing. 1, 2 or 3 phosphates (or more) can be attached to 5' or 3' position. Starting at the 5'-OH, these phosphates are called α , β , γ .

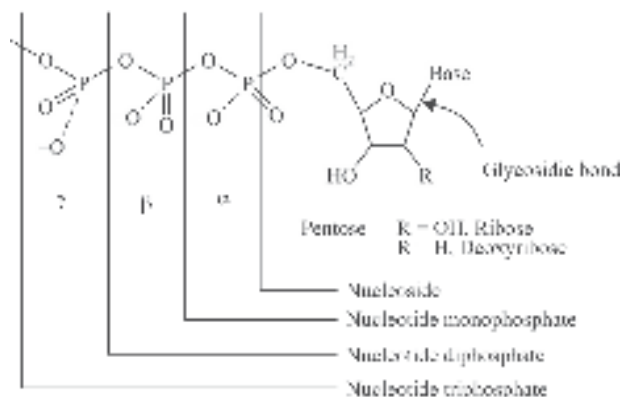


Fig. 9.9: Nucleoside and nucleotides

The **nomenclature** for the five types of bases, nucleosides and nucleotides is as follows:

Base	Nucleoside	Nucleotide	Abbreviation
Adenine	Adenosine	Adenosine-5'-monophosphate = adenylic acid	AMP, dAMP
Guanine	Guanosine	Guanosine-5'-monophosphate = guanylic acid	GMP, dGMP
Cytosine	Cytidine	Cytidine-5'-monophosphate = cytidylic acid	CMP, dCMP
Uracil	Uridine	Uridine-5'-monophosphate ~ uridylic acid	UMP
Thymine	Thymidine	Thymidine-5'-monophosphate = thymidylic acid	(d) TMP

Nucleotides join together through phosphodiester linkages between the 5' and 3' carbon atoms to form nucleic acids. The 3'-OH of the sugar group forms a bond with one of the negatively charged oxygen of the phosphate group attached to the 5' carbon of another sugar. When many of these nucleotide subunits combine, the result is the large single-stranded polynucleotide or nucleic acid.

The close look of the Fig. 9.10 shows that at one end of the large molecule, the carbon group is unbound and at the other end, the -OH is unbound. These different ends are called the 5'- and 3'-ends, respectively.

NUCLEIC ACIDS

There are two types of nucleic acid. In deoxyribonucleic acid (DNA), the monomers are deoxynucleotides and the main bases are adenine (A), guanine (G), cytosine (C) and thymine (T). In RNA (ribonucleic acid), the monomers are ribonucleotides and chief bases are A, G, C and uracil.

The basic differences between DNA and RNA are:

- Ribose is sugar moiety of the RNA molecules whereas it is deoxyribose in DNA. RNA has a hydroxyl group on the 2' carbon of the sugar.
- The pyrimidines base present in RNA is uracil whereas it is thymine in DNA (Fig. 9.11).

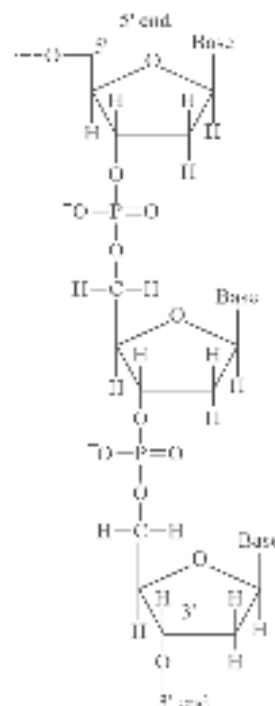


Fig. 9.10: The structural view of nucleotide (of DNA)

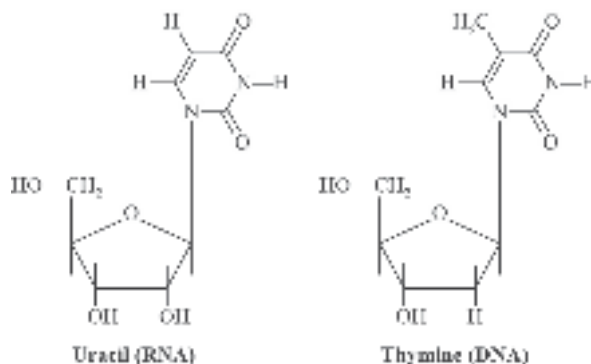


Fig. 9.11: Pyrimidine Bases

(iii) RNA exists as a single strand, whereas DNA exists as double strand.

Ribonucleic Acid (RNA)

Ribonucleic acids consist of:

- (i) Ribose (a pentose = sugar with 5 carbons).
- (ii) Phosphoric acid.
- (iii) Purine bases (adenine and guanine) and pyrimidine bases (cytosine and uracil).

An RNA molecule is a **linear polymer** in which the monomers (nucleotides) are linked together by means of **phosphodiester bridges**, or bonds. These bonds link the 3' carbon in the ribose of one nucleotide to the 5' carbon in the ribose of the adjacent nucleotide. This is illustrated in Fig. 9.12.

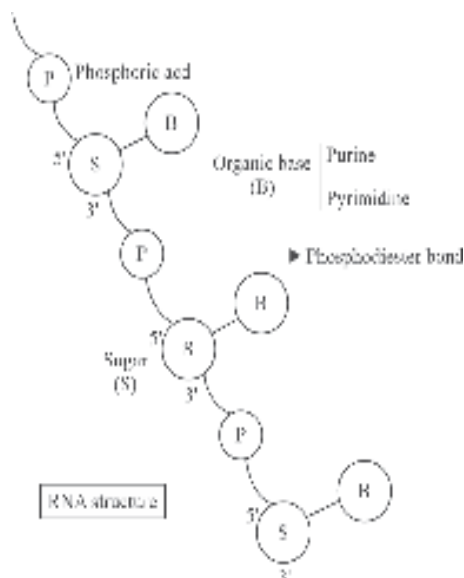


Fig. 9.12: A segment of a single nucleic acid chain

RNA is found in the nucleus, where it is synthesised, and in the cytoplasm, as messenger RNA, transfer RNA or ribosomal RNA. All these forms of RNA are involved in the protein synthesis.

The rule $A + C = U + G$ cannot be applied here.

Most RNA in the cell is single stranded and does not form a double helix.

Although each RNA molecule has only a single polynucleotide chain, it is not a smooth linear structure. It has extensive regions of complementary AU, or GC pairs. Therefore, the molecule folds on itself forming structures called hairpin loops. In the base paired region, the RNA molecule adopts a helical structure as in DNA.

Different Types of RNA

Messenger RNA (mRNA)

This is a copy of a gene. It acts as a *photocopy* by having a sequence complementary to one strand of the DNA and identical to the other strand. The mRNA acts as a bus-boy (busybody) to carry the information stored in the DNA in the nucleus to the cytoplasm where the ribosomes can make it into protein.

Transfer RNA (tRNA)

This is a small RNA. It has a very specific secondary and tertiary structure such that it can bind an amino acid at one end, and mRNA at the other end. It acts as an adaptor to carry the amino acid elements of a protein to the appropriate place coded by the mRNA.

Ribosomal RNA (rRNA)

This is one of the structural components of the ribosome. It has sequence complementary to regions of the mRNA so that the ribosome knows where to bind to an mRNA it needs to make protein from.

Small nuclear RNA (snRNA)

This is involved in the machinery that processes RNAs. They travel between the nucleus and the cytoplasm. It acts as a power source to run the whole RNA cell and power the other RNA strands around the cell.

Deoxyribonucleic Acid (DNA)

A portion of the DNA chain is shown in Fig. 9.13. It differs from RNA chain only in the fact that here the sugar is 2'-deoxyribose rather than ribose. The bases from the 5'-terminus are thymine, guanine, cytosine and adenine. Each base forms β -glycosidic bond with 2'-deoxyribose.

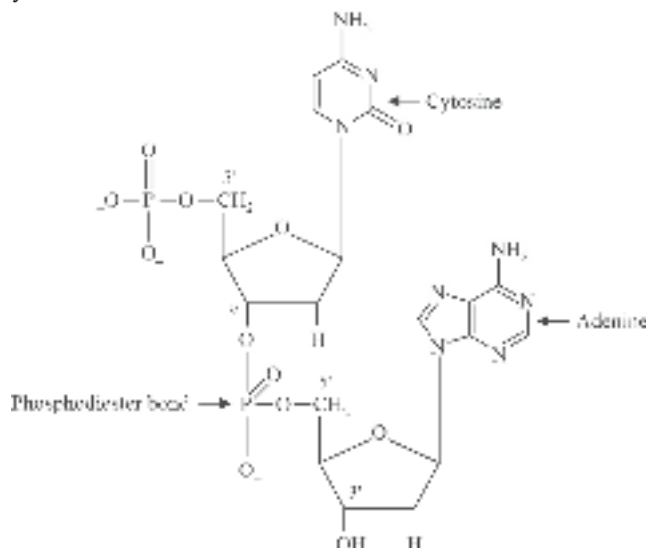


Fig. 9.13: A portion of DNA chain (5-cytidylyladenylate)

Helical Structure of DNA

Figure 9.14 shows a single strand of DNA. However, as stated earlier, DNA exists as a double-helix, meaning two strands of DNA bind together.

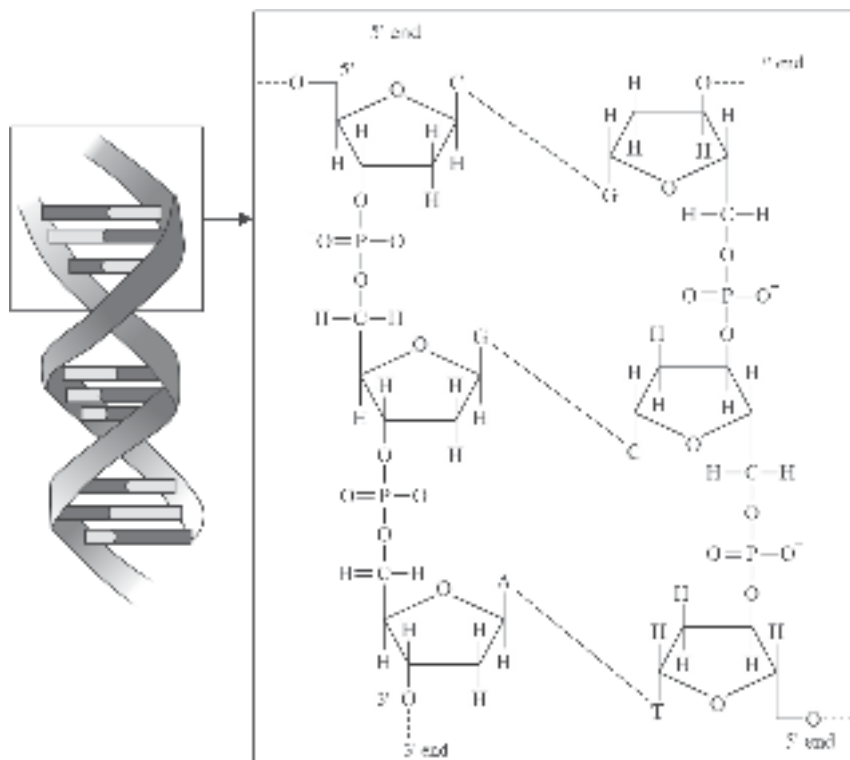


Fig. 9.14: Double helical DNAs

The double helix structure was proposed by James Watson and Francis Crick in 1953. It was based on model building pattern and X-ray diffraction pattern.

As seen in Fig. 9.14, one strand is oriented in the 5' to 3' direction while the complementary strand runs in the 3' to 5' direction. Because the two strands are oppositely oriented, they are said to be anti-parallel to each other. The two strands bond through their nitrogen bases (marked A, C, G, or T for adenine, cytosine, guanine or thymine). Note that adenine only bonds with thymine, and cytosine only bonds with guanine. The nitrogen bases are held together by hydrogen bonds: adenine and thymine form two hydrogen bonds; cytosine and guanine form three hydrogen bonds.

An important thing to remember about the structure of the DNA helix is that as a result of anti-parallel pairing, the nitrogen base groups face the inside of the helix while the sugar and phosphate groups face outward. The sugar and phosphate groups in the helix, therefore, make up the phosphate backbone of DNA. The backbone is highly negatively charged as a result of the phosphate groups.

Importance of the Hydrogen Bond

Hydrogen bonding is essential to the three-dimensional structure of DNA. These bonds do not, however, contribute largely to the stability of the double helix. Hydrogen bonds

are very weak interactions and the orientation of the bases must be just right for the interactions to take place. While the large number of hydrogen bonds present in a double helix of DNA leads to a cumulative effect of stability, *it is the interactions gained through the stacking of the base pairs that leads to most of the helical stability.*

Hydrogen bonding is most important for the specificity of the helix (Fig 9.15). Since the hydrogen bonds rely on strict patterns of hydrogen bond donors and acceptors, and because these structures must be in just the right spots, hydrogen bonding allows for only complementary strands to come together: A-T (two hydrogen bonds), and C-G (3 hydrogen bonds). This complementary nature allows DNA to carry the information that it does.

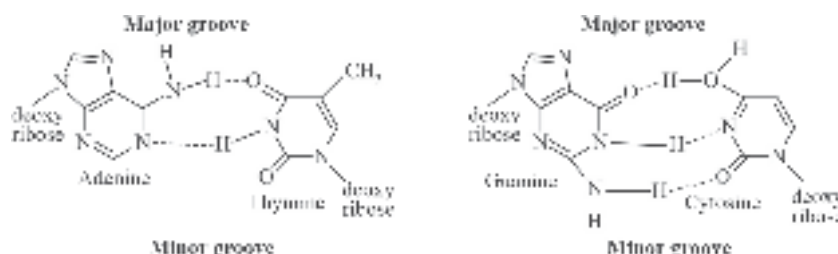


Fig. 9.15. A:T base pair and G:C base pair

Chargaff's Rule

Chargaff's rule states that the molar ratio of A to T and of G to C is almost always approximately equal in a DNA molecule. Chargaff's rule is true as a result of the strict hydrogen bond forming rules in base pairing. For every G in a double-strand of DNA, there must be an accompanying complementary C. Similarly, for each A, there is a complementary paired T.

DNA is a Right-handed Helix

The two strands are not in a simple side-by-side arrangement, which would be called a paranemic joint (Fig. 9. 16). Rather the two strands are coiled around the same helical axis and are intertwined with themselves (which is referred to as a plectonemic coil). One consequence of this intertwining is that the two strands cannot be separated without the DNA rotating, one turn of the DNA for every "untwisting" of the two strands.

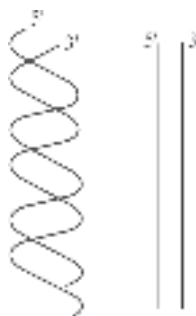


Fig. 9.16: Duplex DNA has the two strands wrapped around each other in a plectonemic coil (left), not a paranemic duplex (right)

Major and Minor Grooves

As a result of the double helical nature of DNA, the molecule has two asymmetric grooves. One groove is smaller than the other. This asymmetry is a result of the geometrical configuration of the bonds between the phosphate, sugar, and base groups that forces the base groups to attach at 120° angles instead of 180° . The larger groove is called the major groove while the smaller one is called the minor groove (Fig. 9.15).

Since the major and minor grooves expose the edges of the bases, the grooves can be used to tell the base sequence of a specific DNA molecule to make specific contacts with amino acids in DNA-binding proteins. The possibility for such recognition is critical, since proteins must be able to recognize specific DNA sequences on which to bind in order for the proper functions of the body and cell to be carried out. As you might expect, the major groove is more informative than the minor groove. This fact makes the minor groove less ideal for protein binding.

Characteristics of the DNA Double-Helix

DNA adapts two different forms of helices under different conditions- the B- and A-forms. These two forms differ in their helical twist, rise, pitch and number of base pairs per turn.

The twist of a helix refers to the number of degrees of angular rotation needed to get from one base unit to another. In the B-form of helix, this is 36° while in the A-form it is 33° .

Rise refers to the height change from one base pair to the next and is 3.4 angstroms in the B-form and 2.6 angstroms in the A-form.

The pitch is the height change to get one full rotation (360°) of the helix. This value is 34 angstroms in the B-form since there are ten base pairs per turn. In the A-form, this value is 28 angstroms since there are eleven base pairs per full turn.

Of the two forms, the B-form is far more common, existing under most physiological conditions. The A-form is only adopted by DNA under conditions of low humidity. RNA, however, generally adopts the A-form in situations where the major and minor grooves are closer to the same size and the base pairs are a bit tilted with respect to the helical axis.

Another DNA structure is called the Z form, because its bases seem to zigzag. Z DNA is left-handed. One turn spans $46\text{\AA}$, comprising 12 base pairs. The DNA molecule with alternating G-C sequences in alcohol or high salt solution tends to have such structure.

Denaturation and Renaturation: Thermodynamics

All the bases of DNA (adenine, guanine, cytosine) have strong absorbance at 260 nm due to aromatic rings. Double stranded DNA absorbs less light at 260 nm compared to single stranded DNA. Hence, stacking interactions between adjacent nucleotide pairs in duplex nucleic acids decrease the UV absorption per nucleotides. Thus, the absorbance increases when the duplex is denatured, meaning the two strands separated out. This increase in absorbance is called hyperchromicity. Denaturation is also referred to as melting, since this transition can be caused by heating. Renaturation is also referred to as annealing; this is favored by cooling to about $20\text{--}25^\circ\text{C}$ below the melting temperature and by keeping the salt concentration fairly high. The melting temperature is the temperature at which

the absorbance has increased by half the final amount. For instance, if the hyperchromic shift is from 1.0 to 1.4, the midpoint of the transition is 1.2, and the temperature at which the absorbance reaches 1.2 is the melting temperature, or T_m (Fig. 9.17).

A related process to renaturation or annealing is hybridization, although this properly refers to the combining of complementary DNA strands from different sources. For example, one could hybridize a mouse globin gene to a human globin gene; they will form a duplex in the regions where the sequences are quite similar. This is a powerful, simple assay for related DNA or RNA sequences.

Only complementary strands of quite similar sequences hybridize. The higher the similarity, the stronger the duplex and the higher is the T_m of the heteroduplex.

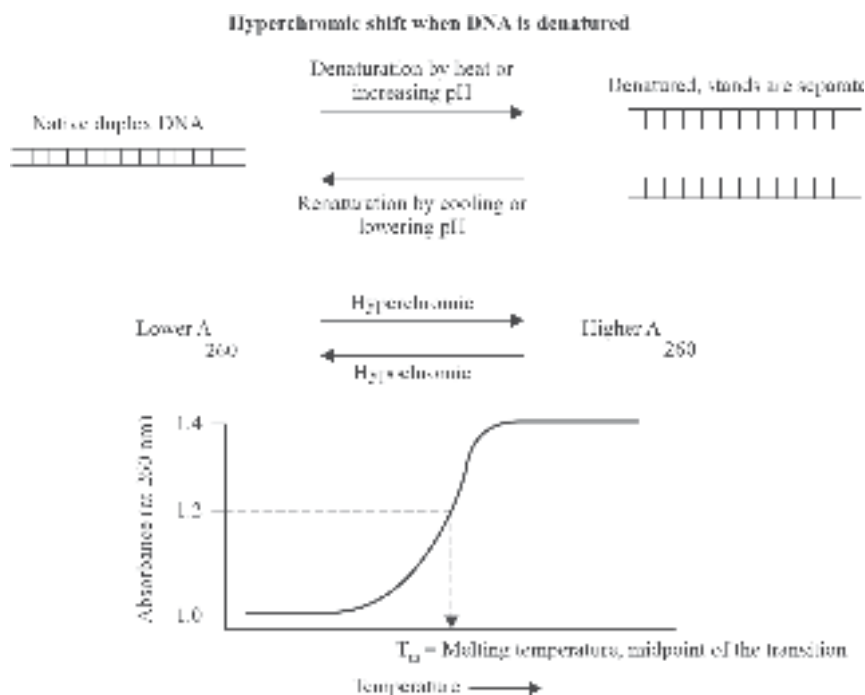


Fig. 9.17: Denaturation and renaturation process

Factors that affect the melting temperature

- (a) **G + C content:** The higher the G + C content, the higher the T_m . G:C base pairs have 3 Hydrogen bonds whereas A:T base pairs have only 2, and the base-stacking interactions between G:C base pairs are considerably stronger than those between A:T base pairs.
- (b) **Ionic strength (m):** The T_m increases as the cation concentration increases. The phosphodiester backbone has a negative charge at every nucleotide (every phosphate) so DNA and RNA are polyanions. These negative charges tend to repel each other but that repulsion is greatly decreased when each phosphate is surrounded by a cloud of small cations.
- (c) **Agents** that disrupt H-bonds or interfere with base stacking, such as formamide or urea, decrease the T_m .

SHORT AND LONG QUESTIONS

1. Write the structure and functions of DNA.
2. Write a note on components of nucleic acids.
3. What are nucleosides and nucleotides? Mention the various purine and pyrimidine bases present in nucleic acid.
4. Discuss the attachment of sugar and phosphate in the nucleotides.
5. What is Chargaff rule? Explain.
6. Write the basic structure of nucleic acid.

MULTIPLE CHOICE QUESTIONS

1. Which of the following compounds are considered the building blocks of nucleic acids?
 - a. Nucleotides
 - b. Nucleosides
 - c. Purines
 - d. Monosaccharides
2. The sugar molecule in a nucleotide is:
 - a. Pentose
 - b. Hexose
 - c. Tetrose
 - d. Triose
3. Group of adjacent nucleotides are joined by:
 - a. Phosphodiester bond
 - b. Peptide bond
 - c. Ionic bond
 - d. Covalent bond
4. What is the composition of nucleotide?
 - a. A sugar + a phosphate
 - b. A base + a sugar
 - c. A base + a phosphate
 - d. A base + a sugar + phosphate
5. Which of the following are not the components of RNA?
 - a. Thymine
 - b. Adenine
 - c. Guanine
 - d. Cytosine
6. The sugar in RNA is, the sugar in DNA is
 - a. Deoxyribose, Ribose
 - b. Ribose, Deoxyribose
 - c. Ribose, Phosphate
 - d. Ribose, Uracil
7. The length of one turn of DNA is
 - a. 3.4 Å
 - b. 34 Å
 - c. 20 Å
 - d. 3.04 Å

Answers

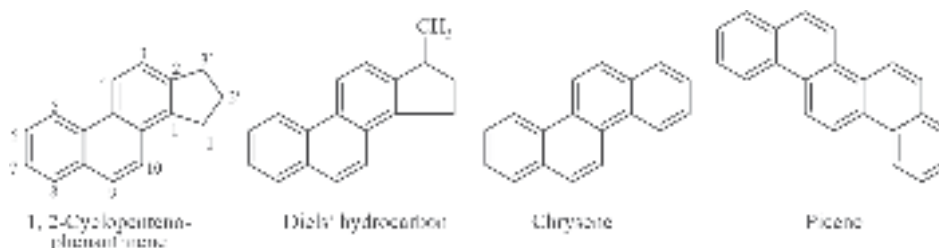
1. (a) 2. (a) 3. (a) 4. (d) 5. (a) 6. (b) 7. (a)

Chapter 10

Steroids—Chemistry and Medicinal Importance

Introduction

The term 'steroids' referred to a group of structurally related compounds, which are widely distributed in animals and plants. These include sterols (from which the name 'steroid' is derived), vitamin D, the bile acids, sex hormones, the adrenal cortex hormone, some carcinogenic hydrocarbons, sapogenins, etc. The structures of all these steroidal substances are based on the 1,2-cyclopenteno-phenanthrene skeleton.



All of these steroids give Diel's hydrocarbon, $C_{18}H_{16}$ on dehydrogenation with selenium at 360°C among other products. In contrast, the same reaction with selenium at 420°C temperature, gives mainly chrysene and a small amount of picene. Diel's hydrocarbon is a solid and its structure is confirmed on the basis of synthesis starting from 2-(1-naphthyl)-ethyl magnesium bromide and 2,5-dimethylcyclopentenanone (Scheme 10.1).

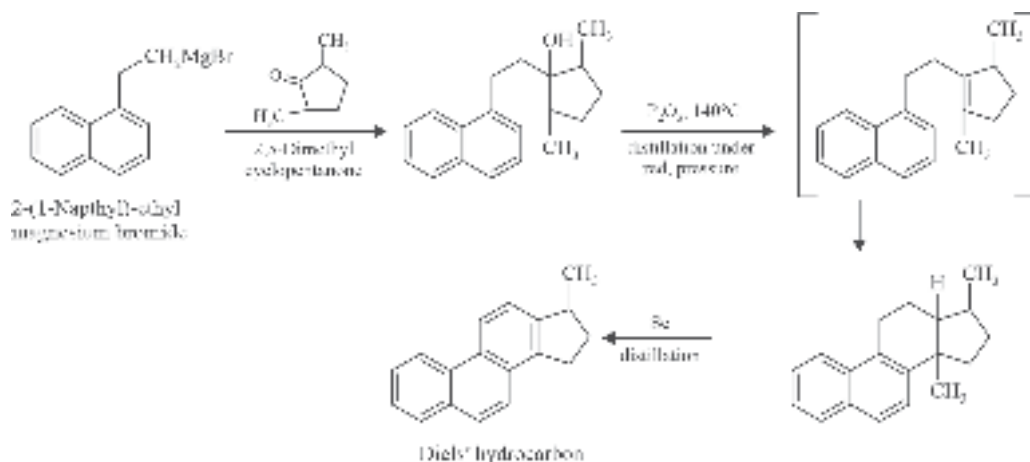
Color Tests for Steroids

- (i) **Salkowski test:** Add concentrated sulphuric acid to a solution of cholesterol in chloroform, red color is developed in the chloroform layer.

The action of concentrated sulphuric acid results in dehydration of cholesterol molecule with a formation of a red colored compound, bicholestadien disulphonate.

- (ii) **Liebermann-Burchard test:** Transfer 1.0 ml of cholesterol solutions in chloroform in test tube. Add 5–6 drops of acetic anhydride to it with gentle mixing. Cautiously, add 0.5 ml of concentrated sulphuric acid down the side of the tube without mixing. The appearance of a blue-green color is a positive test for cholesterol. Ergosterol quickly develops a red color.

This test detects the presence of steroid, used not only as qualitative test but also used for quantitative estimation in blood, etc. Acetic anhydride reacts with the C-3 hydroxyl group of cholesterol and related steroid in the presence of strong acids to form a blue-green complex. The reaction must be carried out in the absence of water.

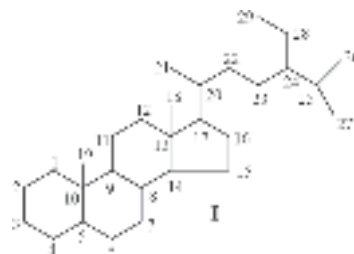


Scheme 10.1: Synthesis of Diels' hydrocarbon

- (iii) On adding an ethanolic solution of digitonin (saponin) to an alcoholic solution of cholesterol, a white precipitate of cholesterol digitonide is formed. This is a molecular complex containing one molecule of cholesterol and one of digitonin, from which the components may be recovered by dissolving the complex in pyridine (which brings about complete dissociation and then adding ether (the cholesterol remains in solution and the digitonin is precipitated). An alternative method is to dissolve the digitonide in dimethyl sulphoxide and heat on a steam bath. Dissociation occurs, and on cooling only the sterol is precipitated. Digitonide formation is used for the quantitative estimation of cholesterol. An interesting point in this connection is that 3β -hydroxy steroids usually form complexes with digitonin, whereas the corresponding 3α -compounds do not.
- (iv) **Rosenheim test:** The sterol in chloroform solution gives blue or pink color with trichloroacetic acid.
- (v) **Lifschutz test:** The sterol and perbenzoic acid are heated in glacial acetic acid and then added conc. sulphuric acid. The appearance of blue or green color indicates the presence of sterol.
- (vi) **Feiser test:** The sterol is treated with selenium dioxide in benzene at 20°C . The 5α -series gives yellow color while 5β -series does not give any color.

Nomenclature System in Steroids

Numbering system for nomenclature purpose in steroids is as shown here in the given structure, (I). The numbering system remains unchanged, even some of the carbon atoms in (I) are missing. Solid lines (preferably thickened) denote groups above the plane of the nucleus (β -configuration), and dotted or broken lines denote groups below the plane (α -configuration), if the configuration of the substituent is unknown, its bond to the nucleus is drawn as a wavy line and this is indicated by ϵ in the name. Wherever possible, the name of the steroid should specify stereochemical configuration.



The structural formula given in Fig. 10.1 represents the more important parent steroidal hydrocarbons.

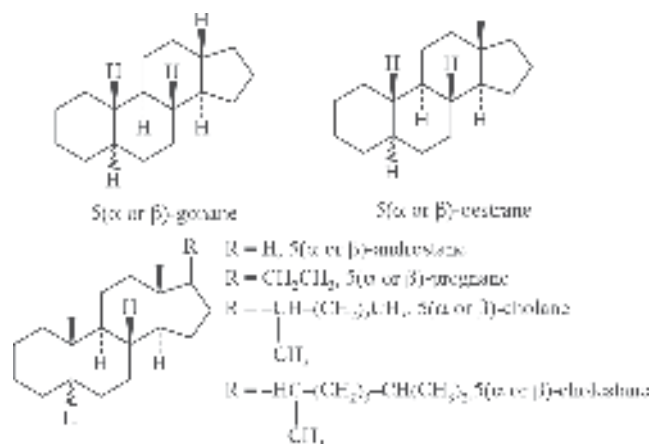


Fig. 10.1: Some important steroidal hydrocarbons

When a methylene group is missing from the side-chain, the same is indicated by the prefix 'nor' preceded by the number of the carbon atom, from where has disappeared. When a ring has been contracted or enlarged, this is indicated by prefixes 'nor' and 'homo' respectively, preceded by an italic capital letter indicating the ring affected. The prefix 'nor' is also used to indicate the loss of an angular methyl group, and in this case is preceded by the number designating that methyl group: 18-nor and 19-nor. When ring-fission has occurred with the addition of a hydrogen $\lambda\delta\beta\beta$ atom to each new terminal group, this is indicated by the numbers showing the position of the bond broken, followed by the prefix 'seco'. The prefix 'cyclo', preceded by the numbers of the positions concerned, is used to indicate a three-membered ring (Fig. 10.2).

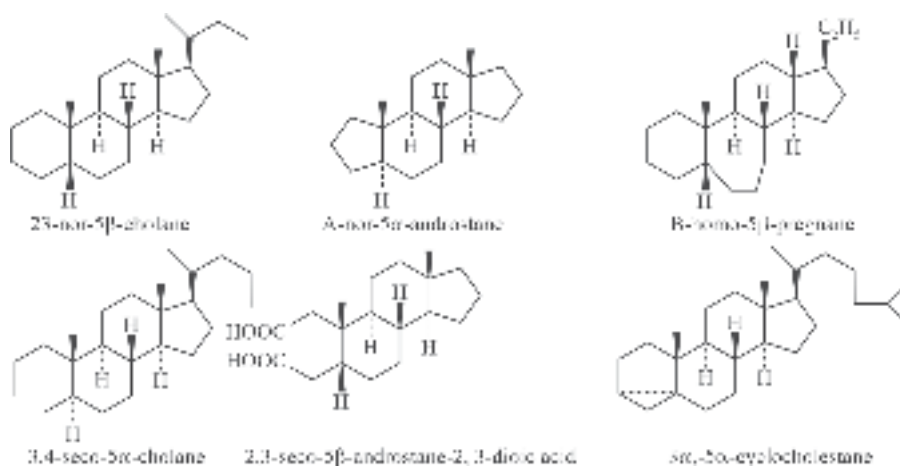
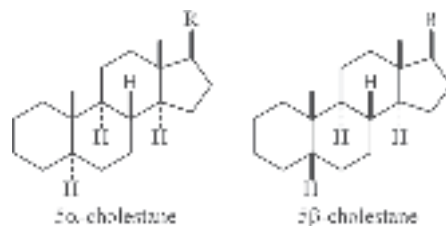


Fig. 10.2: Nomenclature system in steroids

Trivial names have been retained for steroid hormones and closely related compounds. Because of the introduction of these rules of nomenclature, some names used in the earlier literature are now discarded, e.g. coprostan is now named as 5β-cholestan-3-one; iso-compounds (i-compounds) are now called cyclo-compounds.

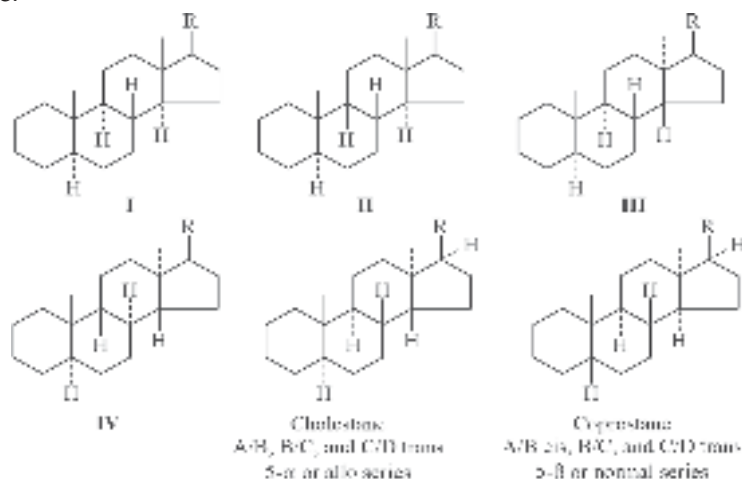
Compounds derived from 5 α -cholestane belong to the allo-series, the prefix 'allo' being reserved to indicate this configuration (i.e. Sex.). Compounds derived from 5 β -cholestane (coprostane) belong to the normal-series. It is not customary to prefix compounds of the latter series by the word 'normal', e.g. cholic acid can be derived from 5 β -cholestane (coprostane). Although this scheme has been discarded, many of the compounds named as allo-compounds have retained this prefix. Some examples of these rules are cited in Fig. 10.2.



Stereochemistry of Steroids

In a fully saturated sterol, there are eight asymmetric carbon atoms in the nucleus (C-3, 5, 8, 9, 10, 13, 14 and 17 positions). Thus, there are $2^8 = 256$ optical isomers possible. The side chain also contains one asymmetric carbon atom (C-20 position); (hence with this inclusion, the total number of isomers becomes 512 (2^9)). The stereoisomerism of steroids includes: (a) Configuration of nucleus and (b) Configuration of substituent groups.

- (a) **Configuration of nucleus:** There are six asymmetric carbon atoms (C-5, 8, 9, 10, 13, and 14 positions) in the nucleus and so $2^6 = 64$ isomers are possible. All the steroids except heart poison belong either to the cholestane series or to the coprostane series. In both type, the ring B and C are fused in a *trans* manner. The rings A/B could be *cis* or *trans*. The rings A and B are *trans* in cholestane series while in coprostane, they are *cis*. The ring C/D is *trans* in both the series. By convention, complete line represents group above the plane and dotted line indicates groups below the plane. Furthermore by convention, angular methyl group at C-10 position in cholestane series has been shown above the plane and therefore, there are four possible isomers, I to IV. As per X-ray analysis, the 'H' atom at C-9 position is *trans* to angular methyl group at C-10 position. On this basis, there are two possible isomers for cholestane, either I or III. Furthermore, it is found that methyl groups at C-10 position and C-13 are *cis* in position and so cholestane is I. Consequently, coprostane is also I, except in this case, the hydrogen atom at C-5 position is above the plane.



- (b) **Configuration of substituent groups:** The side chain is found to be above the plane of the molecule (*cis* with respect to two angular methyl groups. The C-20 methyl group has the β -configuration with respect to C-17 position).

The hydroxyl group at C-3 position in cholestanol (or cholesterol) is present above the plane of the ring (*cis* with respect to methyl group at C-10 position). This configuration is found in every natural sterol and gives rise to beta series. When hydroxyl group is present below the plane, it is referred to α or epi series

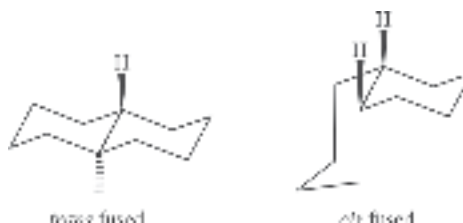
Conformation of Steroids

The term 'conformation' denotes the different possible arrangements of atoms in space. These arrangements can be transformed into one another by mere rotation of bonds without breaking bonds. In a completely saturated steroid, rings A, B, C are cyclohexane rings and ring D is a cyclopentane ring. Cyclohexane ring can exist in two conformations, namely chair and boat forms. A cyclohexane ring prefers to assume the least energy geometry of a chair (Figs 10.3a and 10.3c) rather than a boat conformation (Fig. 10.3b).



Fig. 10.3: Conformation of steroids

When two rings are fused together, the geometry at the point of fusion has to be specified. The two rings could be fused in two different ways. In one form, the two substituents at the ring junction are oriented in opposite direction and are said to be '*trans*' to each other. These rings are '*trans*' fused. In the other form, the two substituents at the ring junction are oriented in the same direction and are said to be '*cis*' to each other. The rings are '*cis* fused'.



A six membered ring can invert from one chair form to another if the other rings fused to it are connected by '*cis* junction' but not if they are attached by '*trans* junction'.

A/B rings

Fusion of rings A and B may be either *cis* or *trans*. Hence, variation in stereochemistry at C-5 is more common than those at other junctions to give two isomeric 5α and 5β hydrocarbons. The typical saturated sterols like cholesterol are A/B-*trans* or 5α compounds. The bile acids are A/B-*cis* or 5β compounds. Formerly, the term 'allo' was used to indicate 5α configuration.

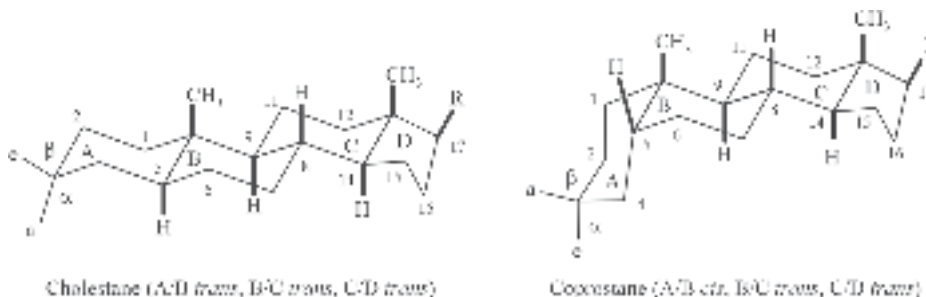
B/C rings

X-ray crystallographic studies by **Bernal** have shown that in a vast majority of steroids, the B/C ring junction is *trans*. The *trans* B/C ring junction confers conformational stability

to steroids, wherein ring A is expected to be conformationally mobile due to the A/B *cis* junction.

C/D rings

The C/D ringjunction is *trans* in sterols, bile acids and related compounds. However, C/D ring junction is *cis* fused in cardiac glycosides and toad poisons. The interlocking of four rings provides certain rigidity to the steroid structure, which is more or less unchanging. This overall structural rigidity makes the difference between the axial and equatorial positions, which assumes great significance in chemical reactions.



All the cyclohexane rings in the steroid nucleus are in chair forms. The structure of cholestane and coprostanane series are given above in chair form. Equatorial groups are more stable than axial.

STEROLS

Sterols occur in oils and fats of both animal and plant origin. They are crystalline compound and contain an alcoholic group; they occur free or as esters of the higher fatty acids, and are isolated from the unsaponifiable portion of oils and fats. Based on source, the sterols are classified as follows:

- (i) **Zoosterols:** These are obtained from animal sources, e.g. cholesterol.
- (ii) **Phytosterol:** These are obtained from plant sources, e.g. stigmasterol, ergosterol, etc.
- (iii) **Mycosterol:** These occur in yeast and fungi, e.g. mycosterol.
- (iv) **Marine sterol:** These occur in marine organism, e.g. stellasterol.

1. Cholesterol

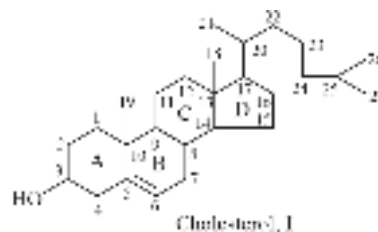
Cholesterol, $C_{27}H_{46}O$ is representative of animal sterol, found in free form or as fatty esters in all animal cells, particularly in the brain and spinal cord. Furthermore, cholesterol is a precursor of bile acids, cholecalciferol (a precursor of vitamin D_3) and steroidal hormones (sex hormones and adrenocortical hormones). Cholesterol is first isolated from human gallstones (these consist almost entirely of cholesterol). The main sources of cholesterol are the fish-liver oils, and the brain and spinal cord of cattle. It is obtained by extraction with solvent and purified by crystallization of its dibromo derivative. Then, debromination is carried by zinc and acetic acid or sodium iodide and ethanol.

Lanolin, the fat from wool, is a mixture of cholesteryl palmitate, stearate and oleate.

Cholesterol is a white crystalline solid, m.p. 149°C which is optically active, ($[\alpha]_D^{39}$).

Structural elucidation of cholesterol

The structure of cholesterol is elucidated only after a tremendous amount of work done particularly by Wieland, Windaus and their coworkers (1903–1932). Only a brief outline is given here under different headings. In order to better understanding of structural elucidation, it is necessary to have the established



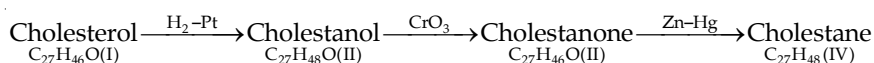
structure of cholesterol, **I** at the beginning. The numbering system is also shown in the structure. The cholesterol molecule consists of a *side-chain* and a *nucleus* which is composed of four rings; usually designated as A, B, C and D, beginning from the six membered ring on the left. It should be noted the nucleus contains two angular methyl groups, one at C-10 and the other at C-13 position. In view of these facts, the elucidation of structure of cholesterol is presented into four parts:

- (A) Nature of the ring system.
- (B) Positions of the hydroxyl group and double bond.
- (C) Nature and position of side chain.
- (D) Position of two angular methyl groups.

(A) Nature of the Ring System

This includes the determination of the nature of the ring system present in cholesterol molecule.

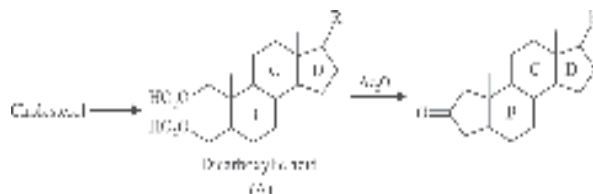
- (a) **Presence of double bond:** The hydrogenation reaction indicates the presence of one double bond.
- (b) **Presence of hydroxyl bond:** The acetylation reaction shows the presence of one hydroxyl group, respectively.
- (c) To know the nature of hydroxyl group as well as ring system, the following sequence of reactions is carried out.



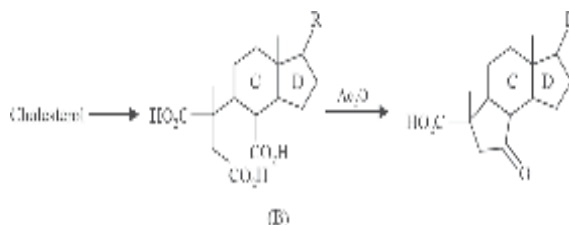
Conclusion:

- (i) The conversion of cholesterol into saturated structure, cholestanol (**II**) shows the presence of one double bond in cholesterol (**I**);
- (ii) The oxidation of (**II**) to the ketone cholestanone (**III**) shows that hydroxyl group in cholesterol structure is secondary in nature.
- (iii) Cholestane (**IV**) is a saturated hydrocarbon, formed from reduction of keto group. Its molecular formula, $\text{C}_{27}\text{H}_{48}$ corresponds to the general formula $\text{C}_n\text{H}_{2n-6}$, and consequently is tetracyclic; thus cholesterol has tetracyclic structure.
- (d) The cholesterol on distillation with selenium at 360°C gives Diels' hydrocarbon along with chrysene. The formation of these compound indicates the presence of steroid nucleus (means four rings, A, B, C and D) in cholesterol. Thus, on the basis of this dehydrogenation, the presence of the cyclopentenophenanthrene nucleus is predicted.
- (e) The same structure consisting of four rings A, B, C and D has been obtained from a detailed investigation of the oxidation products of the sterols and bile acids.

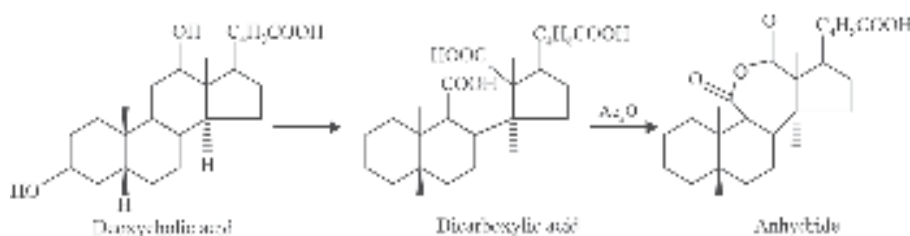
Ring A: Cholesterol is converted into the compound (dicarboxylic acid, A), which gives a cyclopentanone, and so ring, A is six-membered (R is the appropriate side-chain).



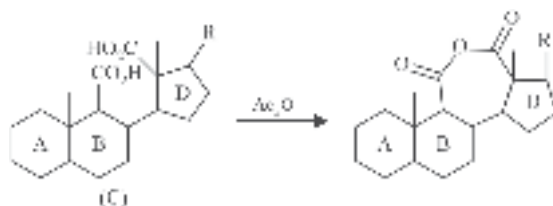
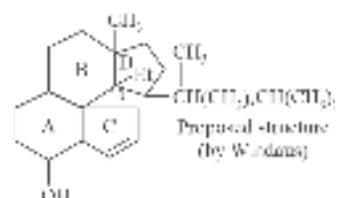
Ring B: Cholesterol is converted into the tricarboxylic acid (B), which gives the cyclopentanone derivative as shown below. Hence, the ring B is also six-membered.



Ring C: Deoxycholic acid is converted into a dicarboxylic acid, which forms cyclic anhydride on dehydration with acetic anhydride. On the basis of anhydride formation, five membered ring structure is suggested for ring, C.

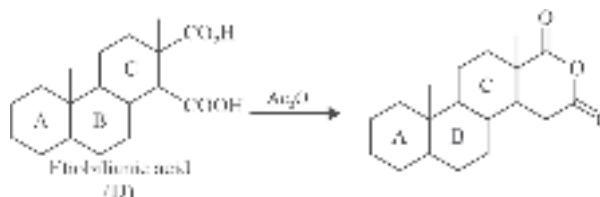


On this basis, Windaus and Wieland (1928) proposed the below given structure for cholesterol, and the uncertain point (at that time) was the nature of the two extra carbon atoms. These were assumed to be present as an ethyl group at position 10, but Wieland et al. (1930) finally proved that there was no ethyl group at this position. These two 'homeless' carbon atoms were not placed until Rosenheim and King first proposed that steroids contained the chrysene nucleus and then proposed the cyclopentanophenanthrene nucleus. It means, the ring C is also six membered.



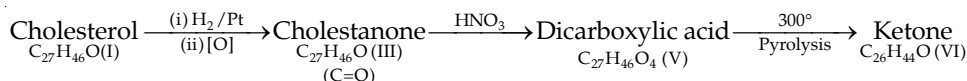
Note: Using the correct structure (C) of cholesterol, the oxidation followed cyclisation reaction results in the formation of seven membered cyclic anhydride. Thus, in this case (and in some others), the Blanc rule fails and leads to erroneous conclusions.

Ring D: The etiobilanic acid (see below section C, iii) obtained from 5 β -cholestane (coprostone) on treatment with acetic anhydride gives a cyclic anhydride. Hence, ring D is five-membered.



(B) Positions of the Hydroxyl Group and Double Bond

(a) To assign the position of hydroxyl group, the following sequence of reactions is carried out:

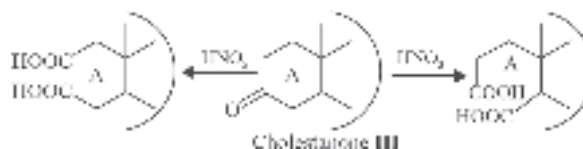


Conclusion:

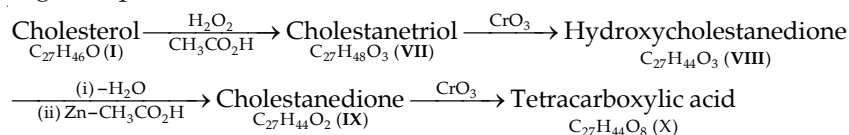
- (i) The cholestanone on oxidation gives the dicarboxylic acid (V) containing the same number of carbon atoms as the ketone (II I) from which it is derived, the keto group in (III) must therefore be in a ring.
- (ii) The pyrolysis of the dicarboxylic acid (V) produces a ketone with the loss of one carbon atom, it therefore follows from Blanc's rule that (V) is either a 1,6- or 1,7-dicarboxylic acid. Now, we have seen that the nucleus contains three six-membered rings and one five-membered ring. Thus, the dicarboxylic acid (V) must be obtained by the opening of ring A, B or C, and consequently it follows that the hydroxyl group in cholesterol (which was converted into the keto group in cholestanone; see (i) above) is in ring A, B or C.

Blanc rule: The effect of heating on various dicarboxylic acids is summarized in the form of **blanc rule**. According to this, dicarboxylic acid having two carboxylic groups on the same carbon atoms gives monocarboxylic acid on heating; 1,4 and 1,5-dicarboxylic acids on heating with acetic anhydrides form cyclic anhydride while 1,6 and 1,7-dicarboxylic acids on heating with acetic anhydrides form cyclic ketones.

- (iii) Actually, two isomeric dicarboxylic acids are obtained when cholestanone is oxidized. The formation of these two acids indicates that the keto group in cholestanone is flanked on either side by a methylene group, i.e. the grouping $-\text{CH}_2\text{COCH}_2-$ is present in cholestanone. The closed look of preferred structure (I) of cholesterol shows that such an arrangement is possible only if the hydroxyl group is in ring, A.

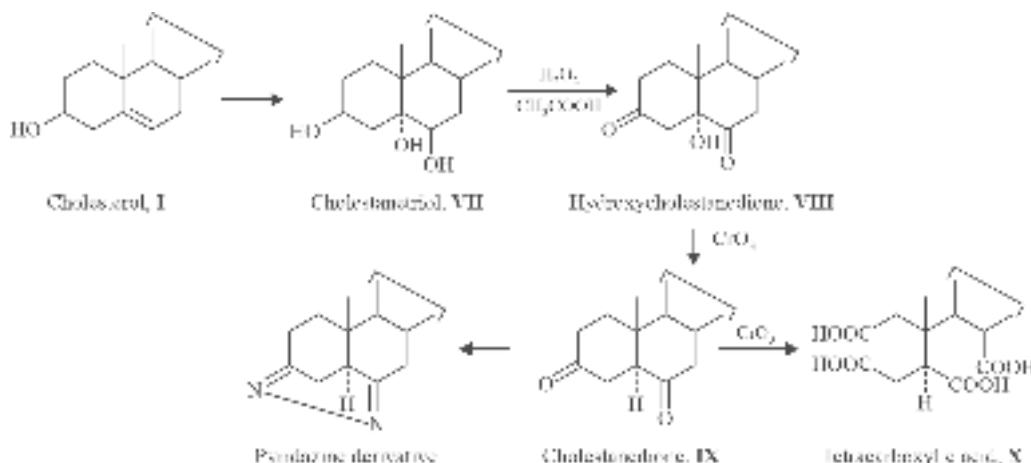


(b) To assign the position of double bond, let us consider the further set of reactions:

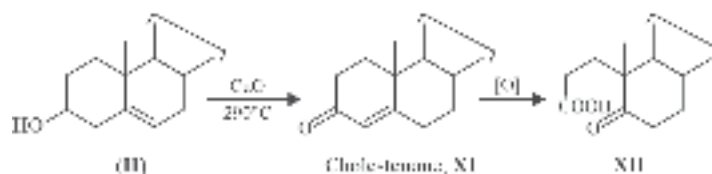


Conclusion:

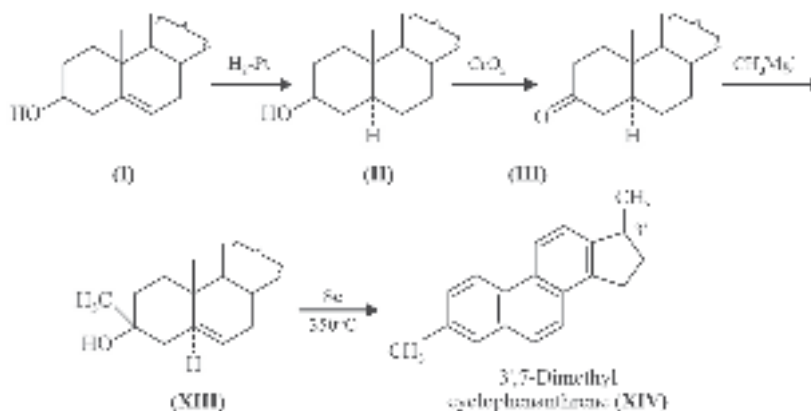
- (i) In the conversion of (I) into (VII), the double bond in (I) is hydroxylated. Since only two of the three hydroxyl groups in (VII) are oxidized to produce (VIII), these two groups are secondary alcoholic groups (one of these being the secondary alcoholic group in cholesterol), and the third, being resistant to oxidation, is probably a tertiary alcoholic group.
- (ii) Dehydration of (VIII) (by heating in vacuo) and subsequent reduction of the double bond forms (IX), and this, on oxidation, gives a tetracarboxylic acid without loss of carbon atoms. Thus, the two keto groups in (IX) must be in different rings; had they been in the same ring, then carbon would have been lost and (X) not obtained. It therefore follows that the hydroxyl group and double bond in cholesterol must be in different rings.
- (iii) Furthermore, since (IX) forms a pyridazine derivative with hydrazine, (IX) is a γ -diketone.
- (iv) Since we have already tentatively placed the hydroxyl group in ring A, the above reactions can be readily explained if we place the hydroxyl group at C-3 position, and the double bond between 5 and 6. In the following equations only rings A and B are drawn to focus in steroid molecule that is under consideration.



Supporting evidence, however, for the above interpretation is obtained by the fact when cholesterol is heated with copper oxide at 290°C , cholestenone (XI) is produced and this on oxidation with permanganate forms a keto-acid (XII) with the loss of one carbon atom. The formation of (XII) indicates that the keto group and the double bond in cholestenone are in the same ring. The ultraviolet absorption spectrum of cholestenone, λ_{max} 240 nm shows the keto group and the double bond are conjugated. These results can be explained if we assume that the double bond in cholesterol migrates in the formation of cholestenone, the simplest explanation being that the hydroxyl group is at C-3 position and the double bond between 5 and 6, position 5 being common to both rings A and B. On this basis, the above sequence of reactions can be organized as:

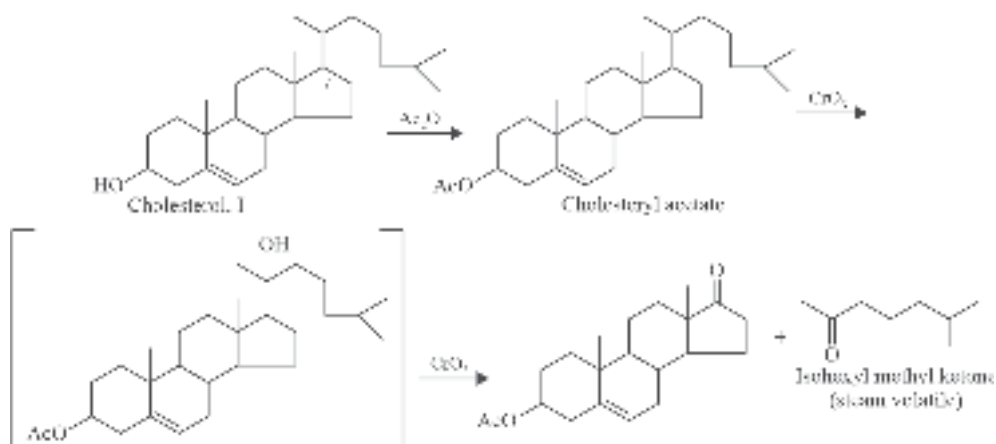


The position of the hydroxyl group at C-3 position is definitely proved by the Kon et al. These authors reduced cholestanol (II) to cholestanol (II), oxidized cholestanol to cholestanone (III), treated this with methylmagnesium iodide to get a tertiary alcohol (XIII). This on dehydrogenation with selenium gives the 3',7-dimethylcyclopentenophenanthrene (XIV). The structure of compound (XIV) is proved by synthesis, and so the complete sequence of reactions may be formulated as with the hydroxyl at C-3 position.



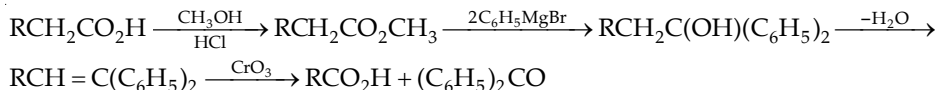
(C) Nature and Position of the Side-chain

Acetylation of cholesterol produces cholesteryl acetate, which on oxidation with chromium trioxide, forms a steam volatile ketone and the acetate of a hydroxyketone (non steam volatile). The ketone was shown to be isohexyl methyl ketone, $\text{CH}_3\text{CO}(\text{CH}_2)_3\text{CH}(\text{CH}_3)_2$. Thus, this ketone is present as the side chain of cholesterol, carbon atom bearing the keto group. This reaction do not show where the side chain is attached to the nucleus of cholesterol, but if we assume that side chain is attached at C-17 position, the complete sequence of reactions can be formulated as follows:

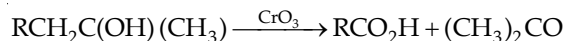


The nature of the side-chain can also be shown with the application of the **Barbier-Wieland degradation**. Since, this method also provide the evidence for the point of attachment to the side-chain, it is worth to consider the problem of the nature of the side-chain again.

The Barbier-Wieland degradation offers a means of 'stepping down' an acid one carbon atom at a time as follows:

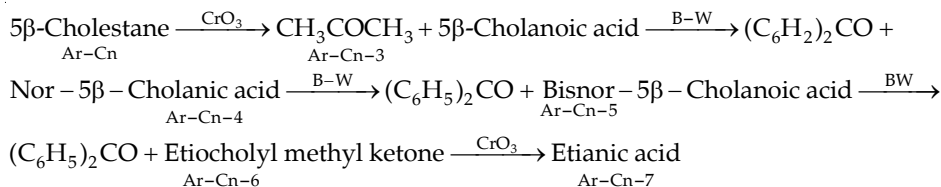


Methyl magnesium bromide may be used instead of phenyl magnesium bromide, and the alcohol obtained may be directly oxidized:



The same sequence of reactions using phenyl magnesium bromide can be applied to cholesterol for determination of nature of side chain.

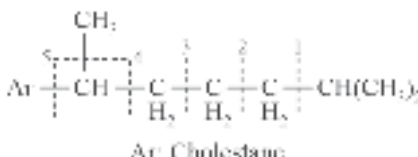
Cholesterol is first converted into 5 β -cholestane (coprostan). If we represent the nucleus of cholestane, as Ar and the side-chain as C_n then we may formulate the Barbier-Wieland degradation (B-W) of 5 β -cholestane as follows:



From the above sequence of reactions, following conclusion can be drawn:

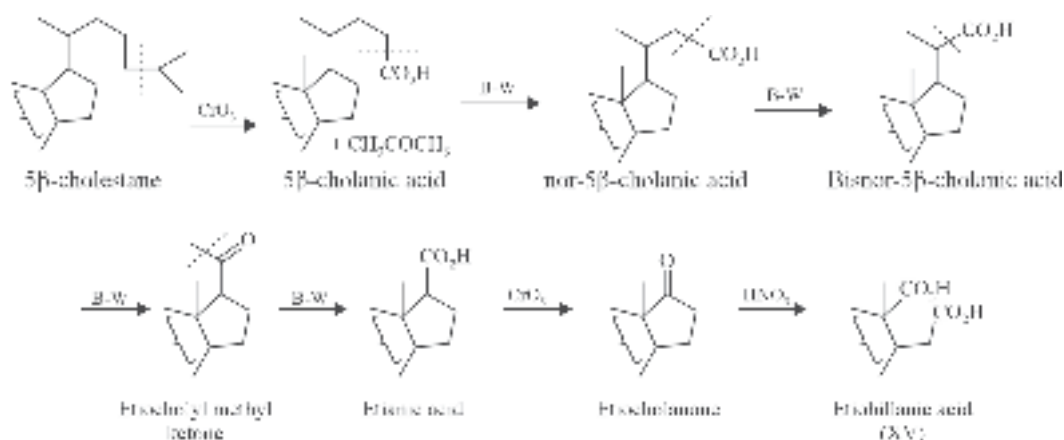
- (i) The formation of acetone from 5 β -cholestanic indicates that the side chain terminates in an isopropyl group.
- (ii) The conversion of bisnor- β -cholanoic acid into a ketone shows that there is an alkyl group on the α -carbon atom in the former compound.
- (iii) Furthermore, since the ketone is oxidized to 5 β -etianic acid (formerly known as a etiocholanoic acid) with the loss of one carbon atom, the ketone must be a methyl ketone, and so the alkyl group on the α -carbon atom in bisnor-5 β -cholanoic acid is a methyl group.

Now the carboxyl group in etianic acid is directly attached to the nucleus; this is shown by the following fact. When etianic acid is subjected to one more Barbier-Wieland degradation, a ketone, **etiocholanone** is obtained and this, on oxidation with nitric acid, gives a dicarboxylic acid, etioibilianic acid without loss of any carbon atom. Thus, etiocholanone must be a cyclic ketone, and so it follows that there are eight carbon atoms in the side-chain, which must have the following structure in order to account for the foregoing degradations {see also the end of this section (C):



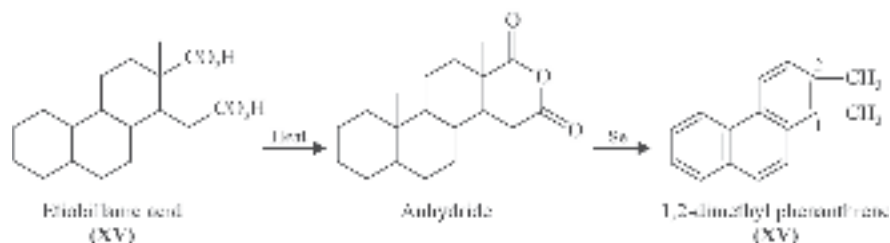
The problem now is “what is the position of this side-chain”? This is partly answered by the following observation. The dicarboxylic acid, **etiobilanic acid**, forms an anhydride when heated with acetic anhydride. Thus, the ketone (etiocholanone) is probably a five-membered ring ketone (in accordance with Blanc’s rule), and therefore the side-chain is attached to the five membered ring, **D**. The actual point of attachment to this ring, however, is not shown by this work. The formation of Diel’s hydrocarbon from cholesterol suggests that the side-chain is at C-17 position, since selenium dehydrogenatrons may degrade a side-chain to a methyl group. The side chain attachment via C-17 position also supported by evidence obtained from X-ray photographs.

On the basis of assignment of the nature and position of the side-chain, the conversion of 5 β -cholestane into etiobilanic acid (**XV**) can be represented as follows in Scheme 10.2:



Scheme 10.2: Barbier Wieland degradation

The point of interest in this connection, is that when the anhydride of etiobilanic acid is distilled with selenium, 1,2-dimethylphenanthrene is obtained. This also provides proof for the presence of the phenanthrene nucleus in cholesterol, and also evidence for the position in the C-13 angular methyl group.



(D) Positions of the two Angular Methyl Groups

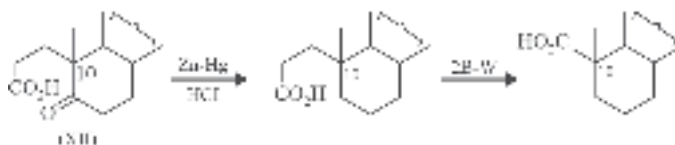
The cyclopentanophenanthrene nucleus of cholesterol accounts for seventeen carbon atoms, and the side-chain for eight. Thus twenty-five carbon atoms in all have been accounted for, but since the molecular formula of cholesterol is $C_{27}H_{46}O$, two more carbon atoms must be fitted into the structure. These two carbon atoms has been shown to be angular methyl groups.

C-10 methyl group

In elucidating the positions of the hydroxyl group and double bond, one of the compounds obtained was the keto-acid (XII). This compound on **Clemmensen reduction**, followed by two Barbier-Wieland degradations, gives an acid which is very difficult to esterify and evolves carbon monoxide when warmed with concentrated sulphuric acid. Since these reactions are characteristic of an acid containing a carboxyl group attached to a tertiary carbon atom, the side chain in (XII) must be of the type.



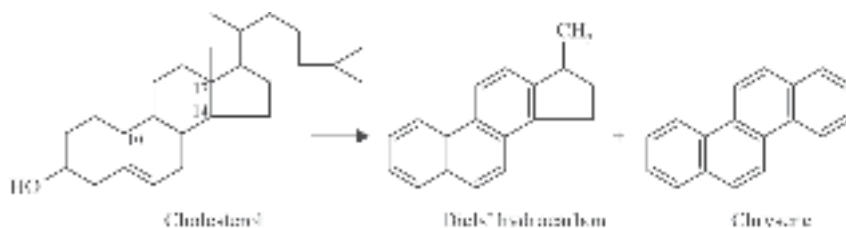
Thus, there must be an alkyl group at C-10 position in (XII). This could be an ethyl group (as originally believed by Windaus-and-Wieland) or a methyl group, provided that in the later case the second 'missing' carbon atom can be accounted for. As we shall see later, there is also a methyl group at C-13 position, and so the alkyl group at C-10 position must be a methyl group. On this basis, the degradation of (XII) may be formulated:



C-13 methyl group

The position of the other angular methyl group is indicated by the following evidences:

- (i) The formation of 1,2-dimethylphenanthrene from anhydride of etiobilianic acid on distillation indicates the presence of C-13 methyl group.
- (ii) When cholesterol is distilled with selenium, chrysene is obtained along with Diels' hydrocarbon. One possible explanation is that there is an angular methyl group at C-13 position, and on selenium dehydrogenation, this methyl group enters the five membered ring, **D** to form a six-membered ring as shown in below given reaction:



This evidence, however, is not conclusive, since ring expansion could have taken place, had the angular methyl group been at C-14 position.

Deoxycholic acid $\xrightarrow{O_2}$ Dihydrodeoxycholic acid $\xrightarrow{HNO_3}$ Deoxybiliary acid $\xrightarrow{\Delta, -CO_2}$ Deoxybilanic acid

Deoxybilanic acid $\xrightarrow{KMnO_4}$ Pyrodeoxybilanic acid $\xrightarrow{CH_3OH}$ Methyl dihydro-dicarboxylate $\xrightarrow{HNO_3}$ XIX

XVII + XVIII $\xrightarrow{Heat}$ XIX $\xrightarrow{HNO_3}$ XX

The compound, **XVII** is shown to be butane-2,2,4-tricarboxylic acid. This indicates the presence of methyl group at C-10 position. The compound **XVIII** is shown to be tetracarboxylic acid containing a cyclopentane ring with a side chain $-\text{CH}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-\text{COOH}$. This compound is derived from ring, **D**. The compound, **XX** is also shown to be a tricarboxylic acid containing cyclopentane ring. Furthermore, one carboxyl group in (**XX**) is shown to be attached to a tertiary carbon atom, and so it follows that there is a methyl group at C-13 or C-14 position. The compound, **XX** is then shown to have *trans* configuration, means the two carboxylic groups are *trans*. Then its precursor, **XIX** must have its two rings in the *trans* configuration (the methyl group and hydrogen atom at the junction of the rings are thus *trans*). Theoretical consideration of the strain involved in the *cis*- and *trans* forms of (**XIX**) suggest that the *cis*-form of (**XIX**) would have been obtained, had the methyl been at C-14 position. Thus, the position of this angular methyl group appears (from this evidence) to be at 13, and this is supported by the fact that etiobilianic acid (**XV**), section (iii)) gives 1, 2-dimethylphenanthrene (**XVI**) on dehydrogenation with selenium. Had the angular methyl group been at position 14, 1-methylphenanthrene would most likely have been obtained.

Synthesis of Cholesterol

Two groups of workers, **Sir R Robinson** and **Woodward *et al*** have synthesized cholesterol. The stereochemical problem is the main problem in the synthesis of

[illegible]

¹H NMR (δ) (CdCl₂): 1.13 (1H, C₁-H), 1.38 (1H, C₁-H), 1.31 (1H, C₂-H), 1.56 (1H, C₂-H), 3.25 (C₃-H), 3.58 (1H, OH), 2.33 (1H, C₂-H), 1.56 (1H, C₄-H), 5.38 (1H, C₅-H), 2.04 (1H, C₆-H), 1.79 (1H, C₆-H), 1.80 (1H, C₈-H), 1.44 (1H, C₉-H), 1.21 (1H, C₁₁-H), 1.50 (1H, C₁₁-H), 1.31 (1H, C₁₂-H), 1.44 (1H, C₉-H), 1.21 (1H, C₁₁-H), 1.50 (1H, C₁₁-H), 1.31 (1H, C₁₂-H), 1.50 (1H, C₁₂-H), 1.40 (1H, C₁₄-H), 1.40 (1H, C₁₄-H), 1.60 (1H, C₁₅-H), 1.31 (1H, C₁₂-H), 1.50 (1H, C₁₂-H), 1.40 (1H, C₁₄-H), 1.60 (1H, C₁₅-H), 1.35 (1H, C₁₅-H), 1.61

(1H, C₁₆-H), 1.36 (1H, C₂₀-H), 1.64 (1H, C₁₅H), 1.25 (6H, C₂₂₋₂₅), 1.62 (1H, C₂₅, H), 0.91 (6H, C₂₆₋₂₇-CH₃), 1.04 (3H, C₁₆-CH₃), 1.30 (3H, C₁₄-CH₃), 0.96 (3H, C₂₁-CH₃), 1.30 (3H, C₁₉-CH₃), 0.96 (3H, C₂₁-CH₃), ¹³C-NMR (δ): 16(C₁₈), 18(C₁₉), 24(C₂₆₋₂₇), 34 (C₂), 36 (C₂₀), 38 (C₁), 35 (C₈), 44 (C₅, C₁₄), 345 (C₁₃), 52 (C₉), 55 (C₁₇), 74 (C₃), 122 (C₆), 142 (C₅).

MS (m/e): 386, 368, 301, 275, 273, 255, 231, 178.

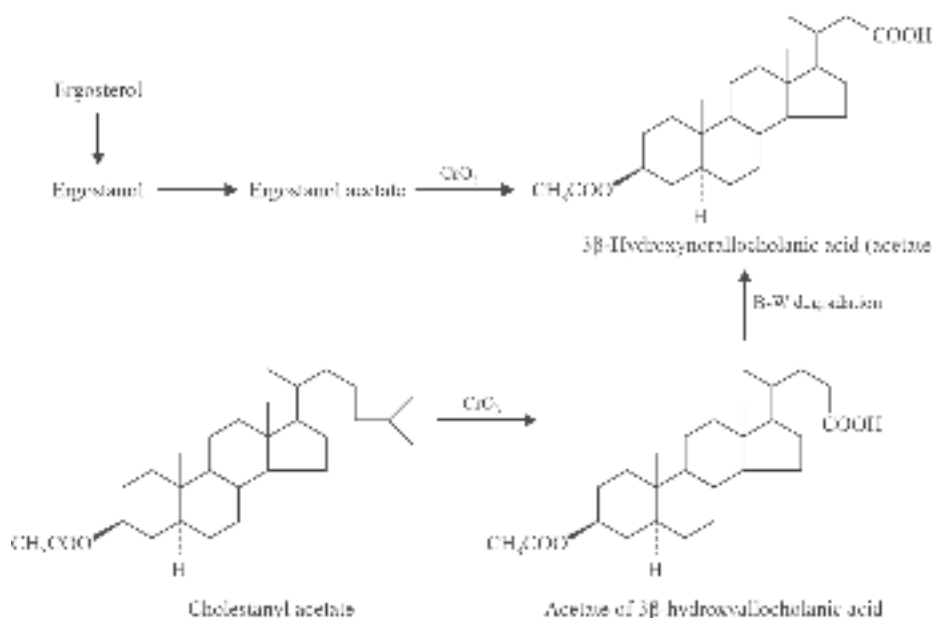
Fragmentation pattern is shown in Fig. 10.4.

2. Ergosterol

It is a provitamin D₂ and found in both plants and animals.

Structural Elucidation of Ergosterol

- (i) **Molecular formula:** It is found to be C₂₈H₄₄O.
- (ii) **Presence of hydroxyl group:** Ergosterol forms monoacetate on acetylation, indicating the presence of one hydroxyl group.
- (iii) **Presence of double bonds:** On catalytic hydrogenation, ergosterol forms ergostanol, C₂₈H₅₀O with the consumption of six moles of hydrogen atoms, indicating the presence of 3 double bonds.
- (iv) Catalytic hydrogenation of ergosterol products ergostanol, C₂₈H₅₀O. This is acetylated to form ergostanol acetate. This on oxidation gives the acetate of 3β-hydroxynorallocholic acid. The identity of 3β-hydroxy nor allocholic acid is established by its formation from cholesteryl acetate on oxidation followed by one Barbier-Wieland degradation.



- (v) **Nature of side chain:** The above reaction indicates that ergosterol and cholesterol have identical basic skeleton, same position of side chain and same position of hydroxyl group. The only difference is nature of side chain as cholesterol contains molecular formula, C₂₇H₄₆O while ergosterol has C₂₈H₄₄O. Ozonolysis of ergosterol

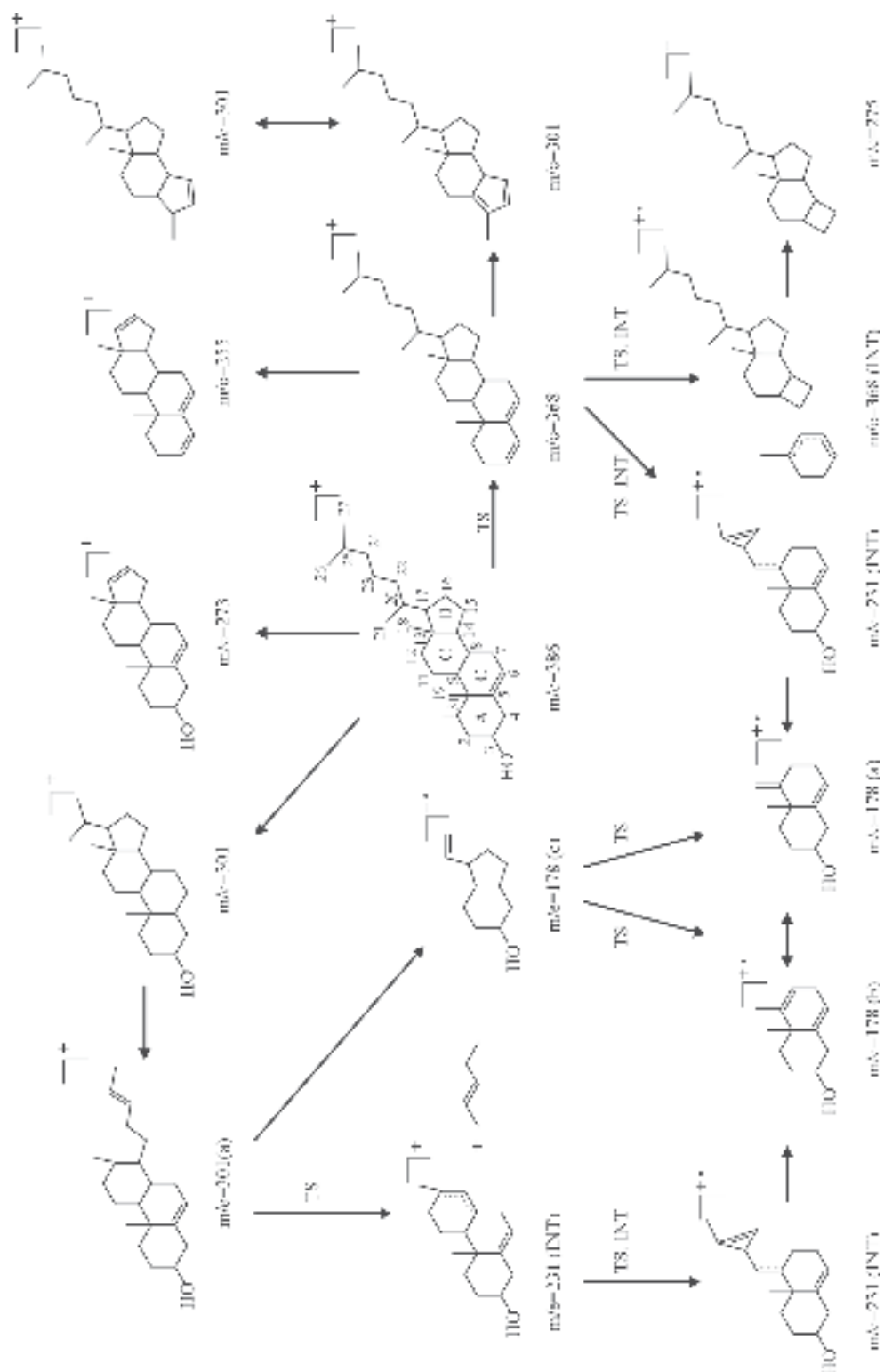
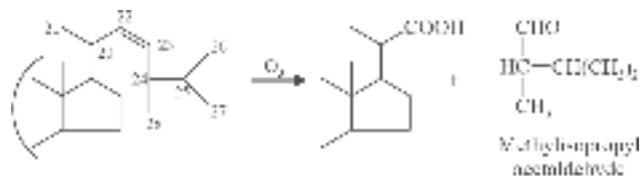
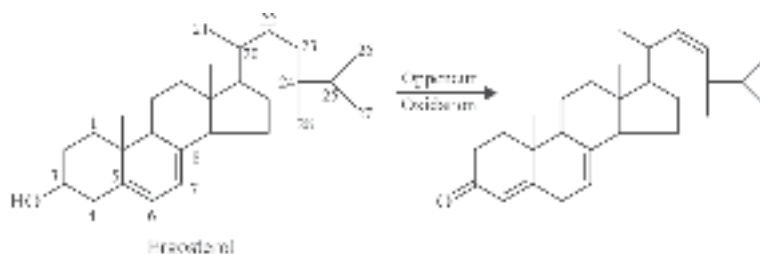


Fig. 10.4: Mass fragmentation pattern of cholesterol

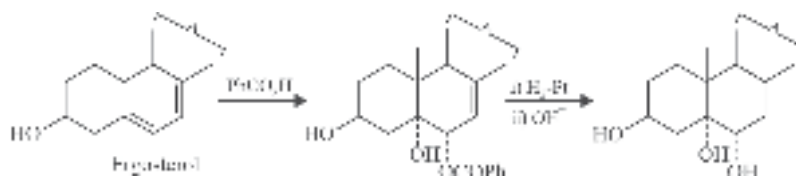
gives methyl isopropyl acetaldehyde along with other products. This product is only formed if following structure is assigned to the side chain.



- (vi) Ergosterol contains three double bonds, one of them is accounted in side chain. Now, the position of two double bonds present in steroid nucleus has to be assigned to give the structure to ergosterol. Ergosterol forms adduct on heating with maleic anhydride at 135°C temperature. This reaction indicates that double bonds are conjugated. Conjugated acyclic dienes shows the $\lambda_{\max}$ at 220–250 nm but if the diene is in ring system, the absorption maximum is shifted to 260–290 nm. On the basis of $\lambda_{\max}$ it is predicted that both the double bonds are present in the one ring. When ergosterol is subjected to oppeneur oxidation (by heating with aluminium *t*-butoxide and acetone), the product is a, α - β -unsaturated ketone ($\lambda_{\max}$ 235 nm). This can only be explained if one double bond is present in 5:6 position, which moves to the 4:5 position during Oppenauer oxidation. Thus, to provide conjugation system, the other double bond should be present at 7:8 position. Thus, conjugation system is present in only one ring that is ring B.
- (vii) To explain the above reaction, the following structure is assigned to ergosterol.



This is supported by the oxidation of ergosterol with per benzoic acid to give the mono benzoate of a triol. This, on catalytic hydrogenation followed by hydrolysis, gave a saturated triol that underwent fission when treated with lead tetra-acetate. Hence, two hydroxyl groups must be in the vicinal position and also, since the 'triol forms only a diacetate, one hydroxyl group is therefore tertiary. These results are readily explained on the basis that one double bond is in the 5,6-position.



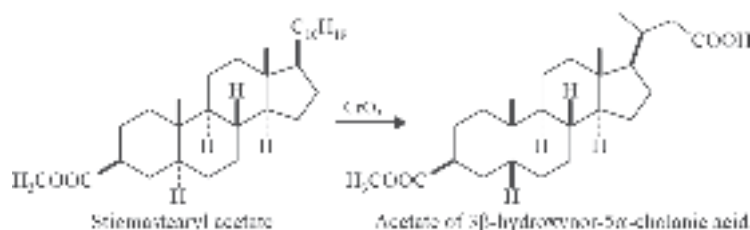
3. Stigmasterol

This is best obtained from soya bean oil. The soyabean oil is saponified and then extracted with ether to separate the unsaponifiable fraction. The ether is distilled off and replaced by petroleum ether saturated with steam, where upon, the crude sterol fraction is precipitated. It is then acetylated and brominated. The resulting stigmasteryl acetate

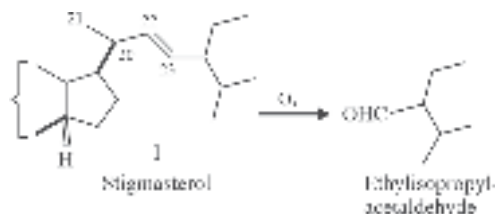
tetrabromide is sparingly soluble in acetic acid, acetone and ether and can be separated from far more soluble sitosteryl acetate dibromide. The bromine is then removed by treatment with zinc dust and the acetate is hydrolyzed with aq. KOH solution. The stigmasterol is obtained by crystallization from 95% alcohol, m.p. 170°C.

Structural Elucidation of Stigmasterol

- (i) **Molecular formula:** It is found to be $C_{29}H_{48}O$.
- (ii) **Presence of hydroxyl group:** On the basis of acetate formation, presence of hydroxyl group is confirmed.
- (iii) **Presence of double bonds:** Stigmasterol also forms a tetrabromide; thus it contains two double bonds.
- (iv) **Presence of steroid nucleus:** It gives Diel's hydrocarbon on dehydrogenation with selenium, indicating the presence of steroid nucleus.
- (v) Hydrogenation of stigmasterol produces stigmasteranol, $C_{29}H_{52}O$, and since the acetate of this gives the same acetate of 3β -hydroxynor-5 α -cholan-3-ol on oxidation with chromium trioxide as obtained from cholesterol, it follows that stigmasteranol differs from 5 α -cholestan-3 β -ol only in the nature of the side chain.



- (vi) Ozonolysis of stigmasterol gives, among other products, ethyl isopropyl acetaldehyde. This suggests that the side-chain is as shown in (I), with a double bond at 22, 23 as well as the presence of C-24 ethyl group.



- (vii) Thus, the final problem is to ascertain the position of the second double bond in stigmasterol. This has been shown to be 5, 6 by the method used for cholesterol. Stigmasterol, on hydroxylation with hydrogen peroxide in acetic acid, gives a trio!, which, on oxidation with chromium trioxide, forms a hydroxyl diketone. This, on dehydration followed by reduction, forms a dione which combines with hydrazine to form a pyridazine derivative. These reactions can be explained only if double bond is present at 5,6 position as shown in Fig. 10.5. This position for the nuclear double bond is supported by other evidence. Also, the infrared spectrum of stigmasterol showed a band at 970 cm^{-1} . Hence, the 22 (23)-double bond has the trans-configuration.

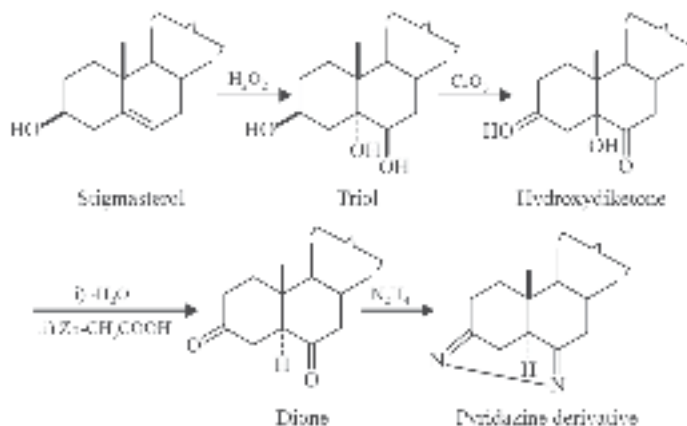
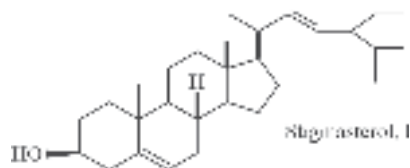


Fig. 10.5: Reactions related to position of double bond

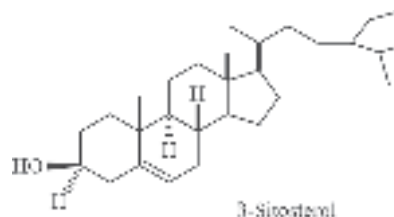
(vii) On the basis of above evidences, the following structure is assigned to the stigmasterol.



4. β -Sitosterol (22,23-dihydrostigmasterol)

β -Sitosterol lowers plasma concentration of low density lipoprotein (LDL), has no effect on very low density lipoprotein (VLDL). The sterol is believed to lower plasma levels of cholesterol by interfering with its absorption. The unwanted effects include laxative nausea and vomiting.

It has a double bond at 5(6)-position, but no double bond HO at 22 (23)-positions. Thus, it is a 22(23)-dihydrostigmasterol. The position of double bond at C-5 position can be ascertained in the same way as in case of stigmasterol described above.

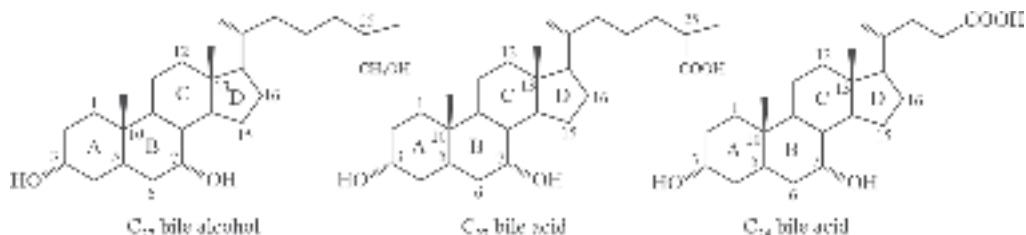


BILE ACIDS

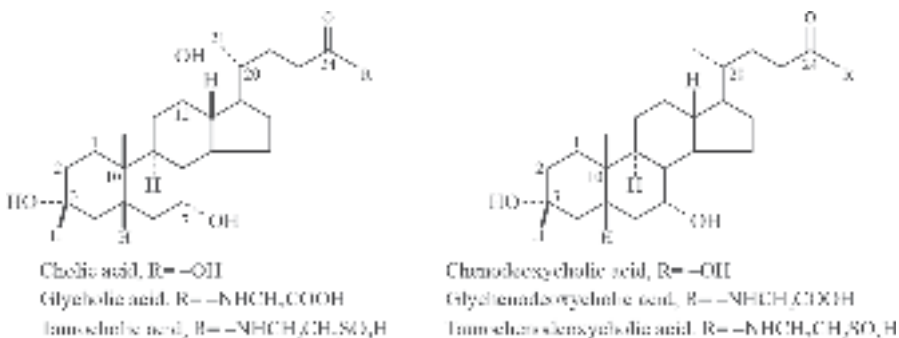
The bile acids are the end products of cholesterol catabolism in animals. These act as powerful detergents or emulsifying agents in the intestines to aid the digestion and absorption of fatty acids, monoacylglycerols and other fatty products and to prevent the precipitation of cholesterol in bile.

All bile acids consist of two connecting units—a rigid steroid nucleus and a short aliphatic side chain. The steroid nucleus of bile acids has the saturated tetracyclic hydrocarbon, cyclopentanophenanthrene, containing three six membered rings (A, B and C) and a five membered ring, D. In addition, there are angular methyl groups at C-10 and C-13 positions. In higher vertebrates, the bile acid nucleus is curved (beaked) because the A and the B rings are in a *cis*-fused configuration. Some bile acids in lower vertebrates, known as *allo*-bile acids, are flat because of an A/B *trans*-fusion (5 α -stereochemistry). The side chain structure determines the class of the compound (bile acids or bile alcohols). There are four different types of bile alcohols (C_{27} , C_{26} , C_{25} and C_{24}), and they occur in the less evolved forms of life.

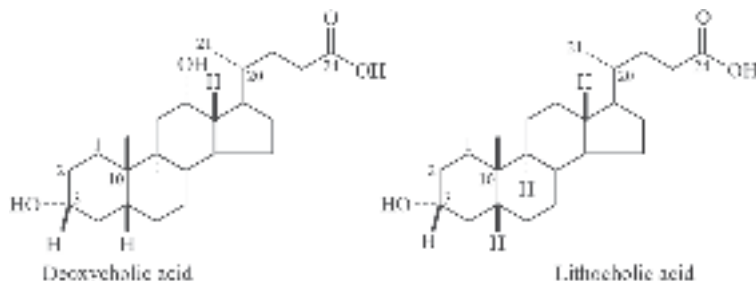
There are two major classes of bile acids depending on the length of the side chain: C_{27} and C_{24} bile acids. In mammals, C_{24} bile acids predominate and they are major components of bile amounting to about 12% of the total (with roughly 4% phospholipids and 1% cholesterol). Bile acids are facially amphipathic, i.e. they contain both hydrophobic (lipid soluble) and hydrophilic (polar) faces.



The principal primary bile acids in human bile are cholic acid ($3\alpha, 7\alpha, 12\alpha$ -trihydroxy- 5β cholan-24-oic acid), and chenodeoxycholic acid ($3\alpha, 7\alpha$, dihydroxy- 5β -cholan-24-oic acid). In the liver, they are conjugated with glycine (H_2NCH_2COOH) or taurine ($H_2NCH_2CH_2SO_3H$) to give the glycholic acid, taurocholic acid, glychenodeoxycholic acid and taurochenodeoxycholic acid; before secreted in the bile.

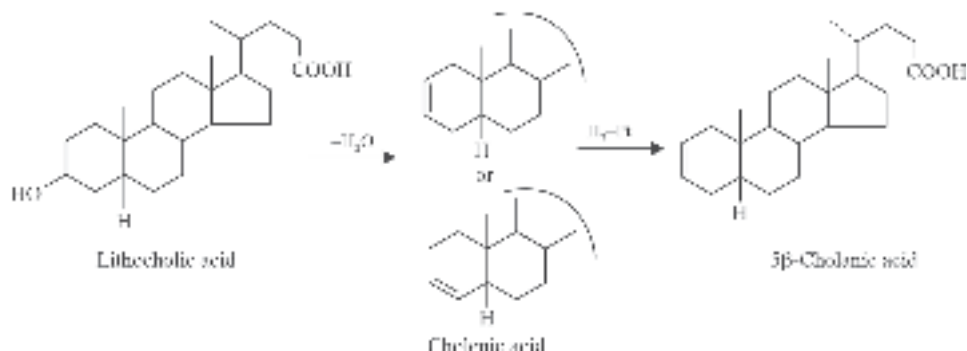


Deoxycholic acid and lithocholic acid are secondary bile acids, and formed in the colon by bacterial deconjugation and by 7α -dehydroxylation of cholic acid and chenodeoxycholic acid, respectively.



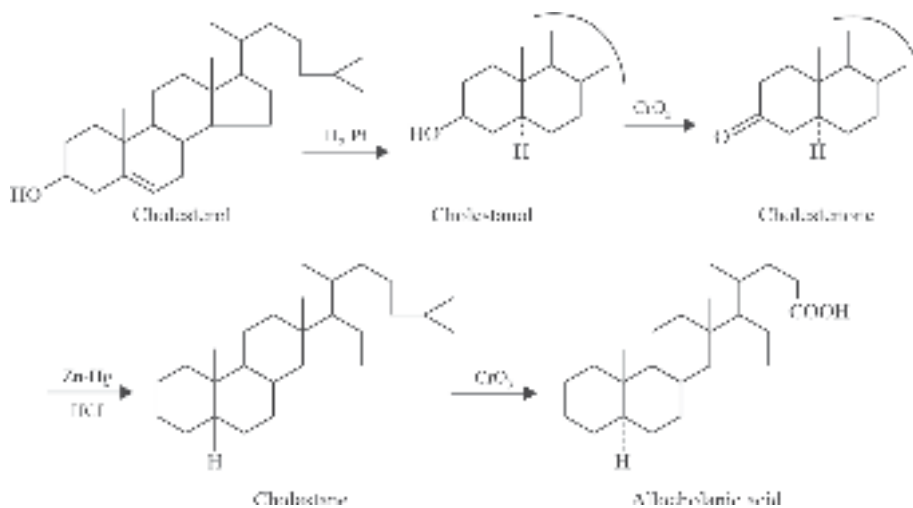
Structural Features of Bile Acids

The bile acids are hydroxyl derivatives of either cholanic acid or allocholanic acids. Dehydration of a bile acid by heating in vacuum followed by catalytic reduction gives either cholanic acid or allocholanic acid. For example, lithocholic acid can be converted into cholanic acid as follows:

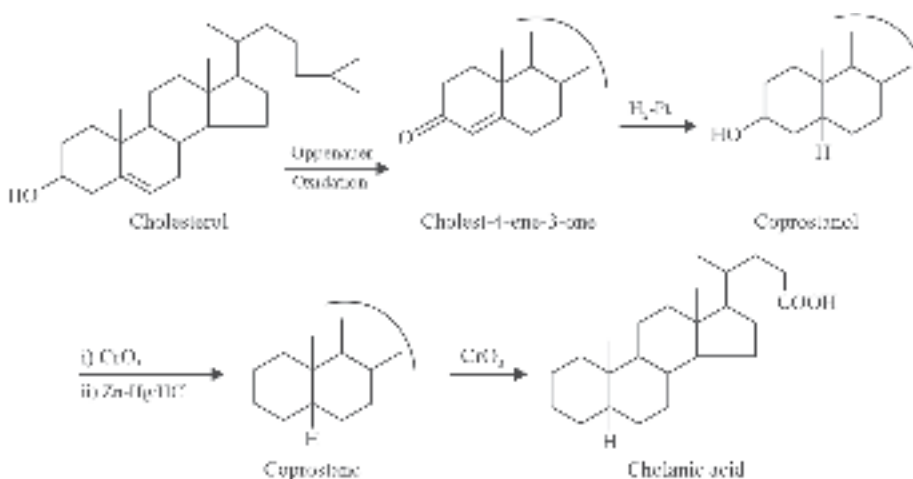


The cholanic acid and allocholanic acid are derived from coprostanol and cholestane respectively as given below. These reactions show the relationship between the bile acids and sterols.

Allocholanic Acid (5α-Cholanic Acid)



Cholanic Acid (5β-Cholanic Acid)



Detection of Bile Acids

a. Hay test

Prepare 2 test tubes each containing 3 ml of distilled water. Supplement one of them with a drop of bile.

Introduce a few grains of powdered sulphur to each sample. The sulphur grains float on the surface of water, whereas in a sample containing the bile, sulphur grains fall down to the bottom of the test tube.

b. Emulsifying action of bile acids

Prepare 2 test tubes each containing 3 ml of distilled water and add 3 drops of olive oil to each of them. Supplement one of the samples with a few drops of bile. Shake intensively both tubes. The oil does not dissolve in water. The water-oil mixture separates into two phases. The oil locates on the top and water drops down to the bottom. A phase border is visible. In contrast to that in the sample containing water, olive oil and bile (bile acids) form a stable emulsion.

Biosynthesis of Bile Acids

Bile acids are synthesized from cholesterol involving a number of complex steps in both the steroid nucleus and the side chain (Scheme 10.4). This biosynthesis involves at least five steps each on the nucleus and the side chain. Since these steps may in principle occur in any order, elucidation of individual steps of bile acid biosynthesis is not straightforward. In the first step, believed to be the rate limiting step, cholesterol is oxidized to 7α -hydroxycholesterol by cholesterol 7α -hydroxylase. 7α -Hydroxy cholesterol is then converted to the key intermediate cholest- 7α -hydroxy- Δ^4 -3-one through the action of an isomerase and a reductase. This unsaturated oxo derivative is the branching point for cholic and chenodeoxycholic acid biosynthesis.

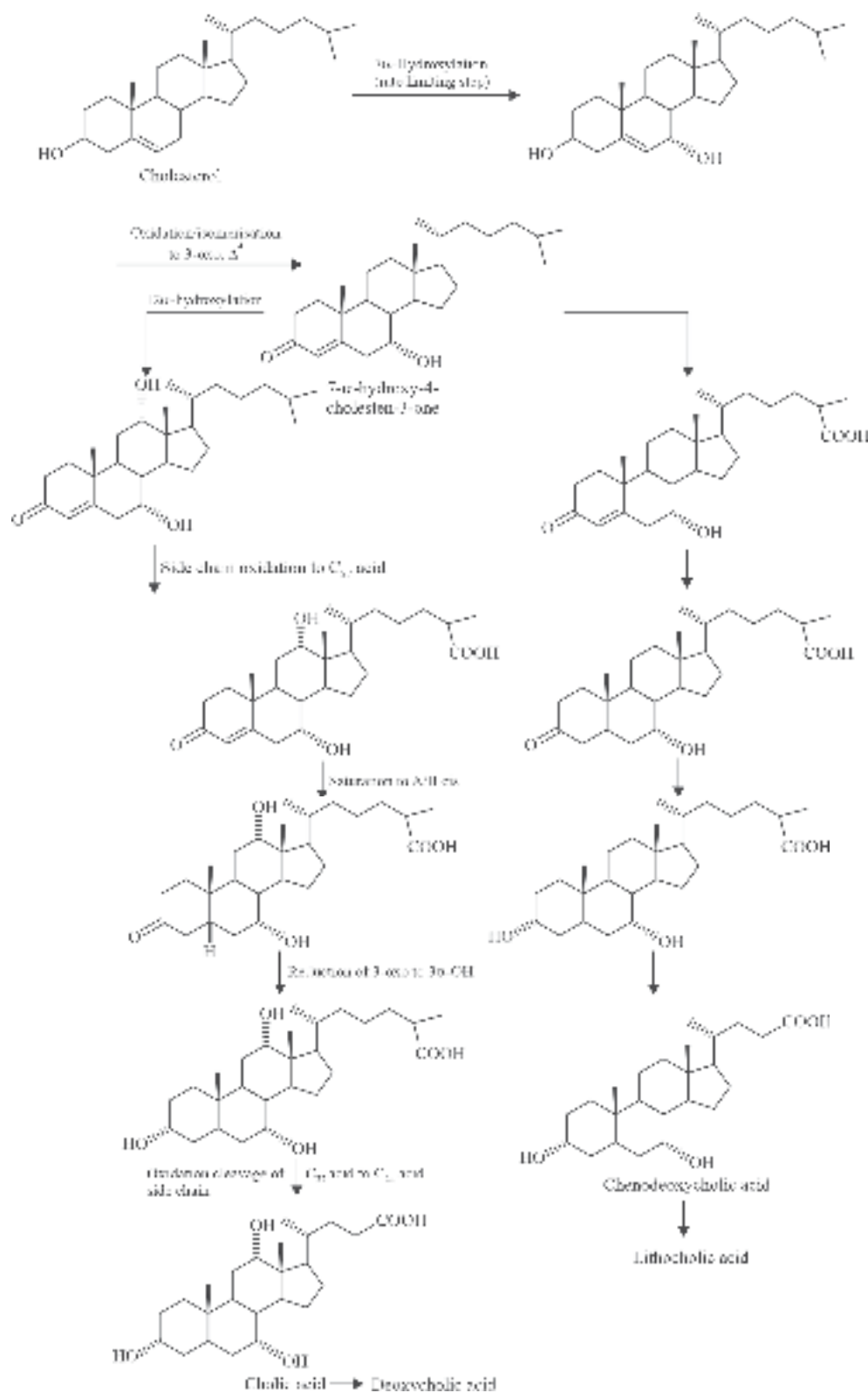
STEROIDAL HORMONES

Steroidal hormones are crucial substances for the proper function of the body. They mediate a wide variety of vital physiological functions ranging from anti-inflammatory agents to regulating events during pregnancy. They are synthesized and secreted into the bloodstream by endocrine glands such as the adrenal cortex and the gonads (ovary and testis). Steroidal hormones are all characterized by the steroid nucleus which is composed of three six member rings and one five member ring, labelled as A, B, C, and D, respectively.

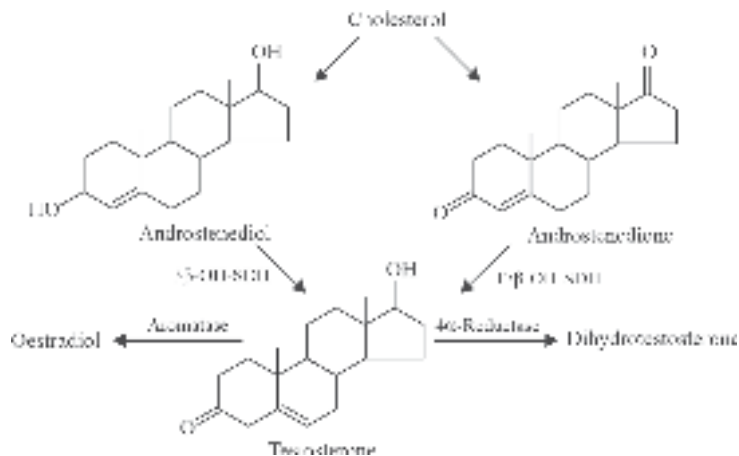
Five classes of steroid hormones: 1. Androgens; 2. Oestrogens, 3. Progestins, 4. Mineralocorticoids, 5. Glucocorticoids.

1. ANDROGENS

Naturally occurring androgens stimulate and/or control the sex of males, including the male sex organs and other characteristics. Discovered in 1936, androgens are also called androgenic hormones or testoids. Androgens are the precursor of estrogens, female sex hormones. Chemically, these are characterized by the presence of androstane nucleus having 19 carbon atoms.

**Scheme 10.4:** Biosynthesis of bile acids from cholesterol

Testosterone: Testosterone is the main androgen. It is the main male sex hormone and an anabolic steroid. It plays key roles in health and well-being including enhanced libido, energy, immune function and protection against osteoporosis. A healthy adult male produces eight to ten times more testosterone than an healthy adult female. Testosterone is first identified in 1935. In the body, it is derived from cholesterol via androstenedione by the action of 17β -hydroxy steroid dehydrogenase (17β -OH-SDH) or from androstenediol by 3β -hydroxysteroid dehydrogenase (3β -OH-SDH) (Scheme 10.5).

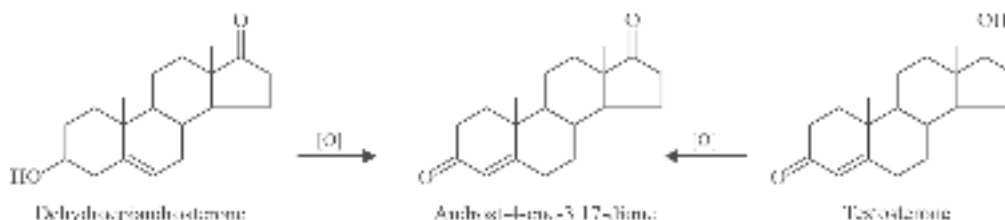


Scheme 10.5: Biosynthesis of testosterone from cholesterol

Testosterone is not orally active due to extensive first-pass hepatic metabolism. The ester preparations are widely used as intramuscular injections in an oily vehicle. The testosterone esters are released slowly from the depot, and then the ester groups are rapidly removed, liberating free testosterone. The testosterone esters include *testosterone enanthate*, *testosterone propionate* and *testosterone cypionate*. The enanthate compound is most commonly used.

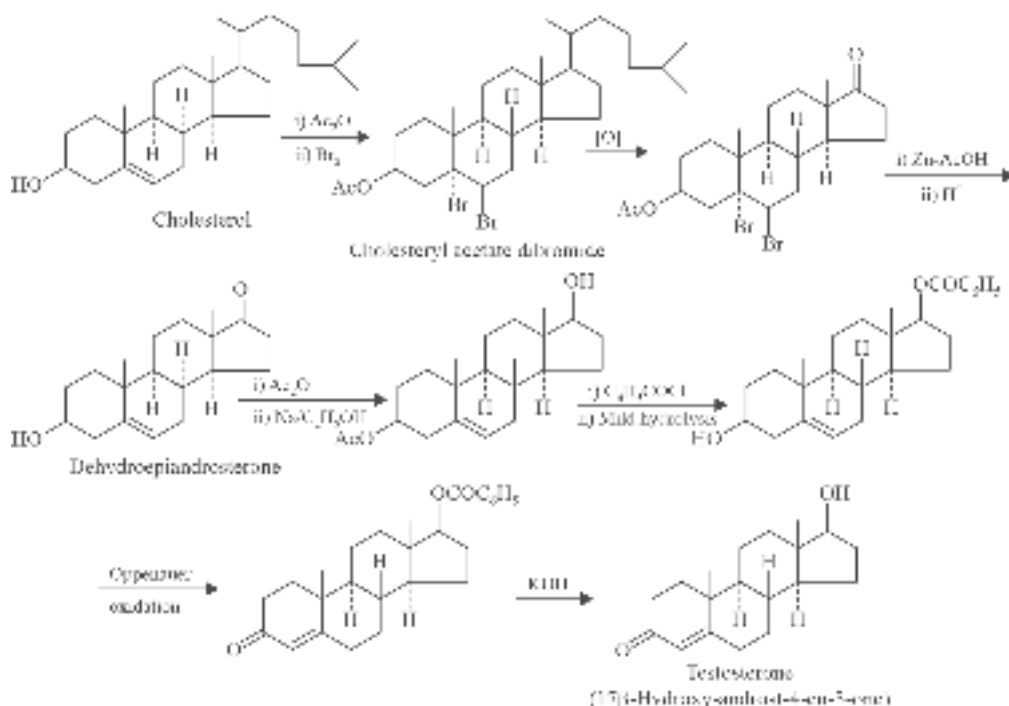
Structural Elucidation of Testosterone

- (i) **Molecular formula:** It is found to be $C_{19}H_{28}O_2$.
- (ii) **Presence of double bond:** Hydrogenation reaction indicates the presence of one double bond.
- (iii) The usual reaction shows that two oxygen atoms are present as keto group and secondary alcoholic group.
- (iv) Its saturated molecular corresponds to C_nH_{2n-6} indicating the tetracyclic structure.
- (v) Its UV spectrum suggests the presence of α,β -unsaturated ketonic group.
- (vi) On oxidation, it gives the diketone which is also formed from oxidation of dehydroepiandrosterone. The diketone can only be formed if following structure is assigned to the testosterone.



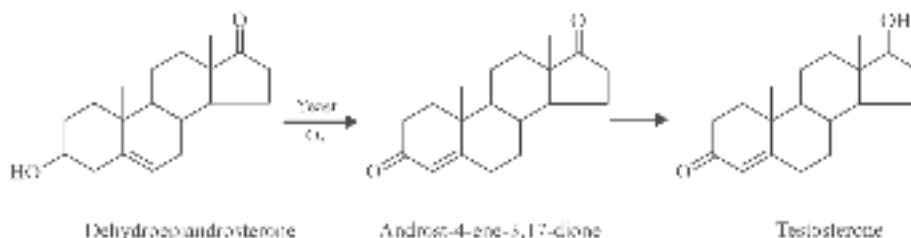
(vii) Finally the proposed structure is confirmed by synthesis.

(a) **From cholesterol:** Testosterone can be synthesized from cholesterol as shown in Scheme 10.6.



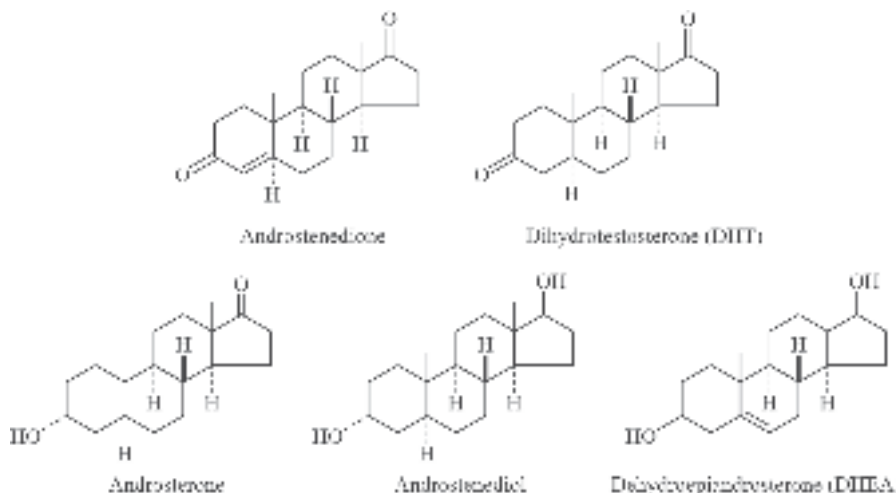
Scheme 10.6: Synthesis of testosterone from cholesterol

(b) **Microbial synthesis:** Testosterone can also be prepared from dehydroepiandrosterone by means of microorganism. The first step makes use of yeast in the presence of oxygen while the second step uses the fermenting yeast.



Other androgens hormones are:

- **Androstenedione (Andro):** It is produced by the testes, adrenal cortex, and ovaries. Manmade androstenedione has been banned by the IOC (International Olympic Committee) and other athletic organizations.
- **Androstenediol.** It is a weak androgen and oestrogen.
- **Androsterone, $C_{19}H_{30}O_2$.** It is weak androgen that is 1/7th that of testosterone.
- **Dehydroepiandrosterone (DHEA), $C_{19}H_{28}O_2$:** It is produced in the adrenal cortex from cholesterol.
- **Dihydrotestosterone (DHT).**



All the above androgens are structurally elucidated in a similar way as testosterone.

Spectral Data of Testosterone

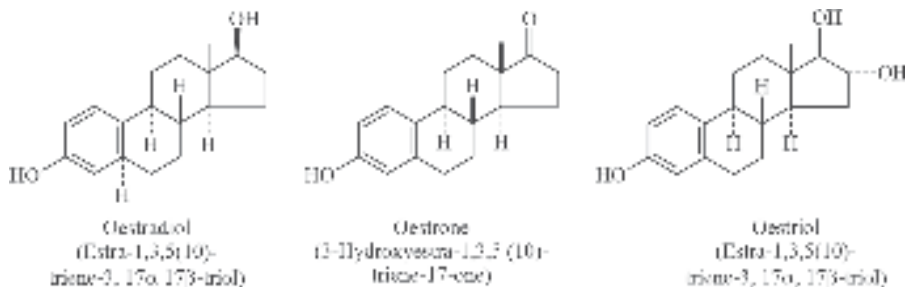
UV (nm) (methanol): $\lambda_{\max}$ 279.4.

IR (cm⁻¹) (KBr): 3628 (OH), 2954 (–CH), 1663 (C = O), 1613 (C = C).

¹H-NMR: (CDCl₃) (δ): 1.702 (C-1 α H), 2.034 (C-1 β H), 2.347 (C-2 α H), 2.424 (C-2 β H), 5.731 (C-4H), 2.278 (C-6 α H), 2.391 (C-6 β H), 1.007 (C-7 α H), 1.848 (C-7 β H), 1.579 (C-8 β H), 0.932 (C-9 α H), 1.603 (C-11 α H), 1.436 (C-11 β H), 1.093 (C-12 α H), 1.862 (C-12 β H), 0.978 (C-14 α H), 1.628 (C-15 α H), 1.313 (C-15 β H), 1.466 (C-16 α H), 2.084 (C-16 β H), 3.655 (C-17 α H), 0.795 (C-18-CH₃), 1.198 (C-19-CH₃), 1.6 (C-17, OH), **¹³C-NMR** (δ): 35.73 (C-1), 33.94 (C-2), 199.80 (C-3), 123 (C-4), 171 (C-5), 32.8 (C-6), 31.55 (C-7), 36.67 (C-8), 53.93 (C-9), 38.67 (C-10), 20.65 (C-11), 36.55 (C-12 β), 42.82 (C-13 α), 50.49 (C-14 β), 23.34 (C-15 β), 30.41 (C-16 α), 81.56 (C-17), 11.56 (C-18 α), 17.42 (C-19).

2. OESTROGENS

Oestrogens originate in the adrenal cortex and gonads. These primarily affect maturation and function of secondary sex organs (female sexual determination). There are three naturally occurring estrogens, oestradiol, oestrone and oestriol. Oestradiol is the most potent and oestriol is the least potent among three. All these contain oestrane nucleus having 18 carbon atoms.

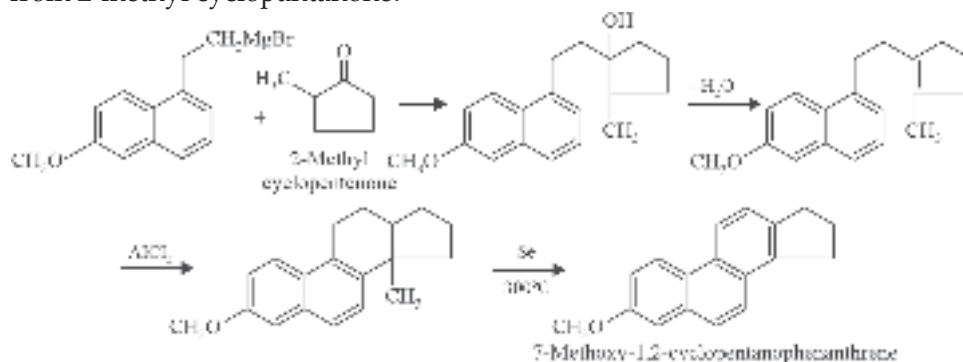


Oestrone

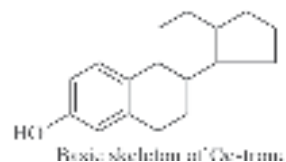
Oestrone is a hormone, produced by the ovaries, adrenal glands and fat. It is one of the major oestrogens in postmenopausal women.

Structural Elucidation of Oestrone

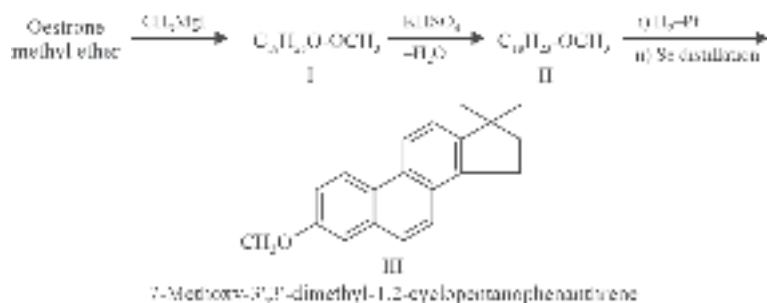
- (i) Oestrone, m.p. 259°C has molecular formula $C_{18}H_{22}O_2$.
- (ii) Nature of oxygen atoms: It forms an oxime on reaction with hydroxylamine and monoacetate on acetylation reaction. These reactions indicate the presence of keto and hydroxyl groups respectively. The hydroxylic group is found to be phenolic in nature as it reacts with diazonium salt in alkaline solution. The presence of phenolic group also suggests the presence of at least one benzene ring. The presence of benzene ring is also by its $\lambda_{\max}$ 280 nm in UV spectrum.
- (iii) On catalytic hydrogenation, it produces the octahydrooestrone. Two hydrogen atoms are consumed in the conversion of keto group into hydroxyl group and six hydrogen atoms consumed by three double bonds of benzene rings. Its saturated molecular formula, $C_{18}H_{30}O_2$ corresponds to general formula, C_nH_{2n-6} , indicating the tetracyclic structure. The same is confirmed by dehydrogenation reaction with zinc dust. On dehydrogenation with zinc dust, it yields chrysene, indicating the presence of steroid nucleus.
- (iv) When methyl ether of oestrone is subjected to the Wolf-Kishner reduction, and the product so obtained distilled with selenium, the compound, 7-methoxy-1,2-cyclopentanophenanthrene is formed. Its structure is confirmed by the synthesis from 2-methyl cyclopentanone.



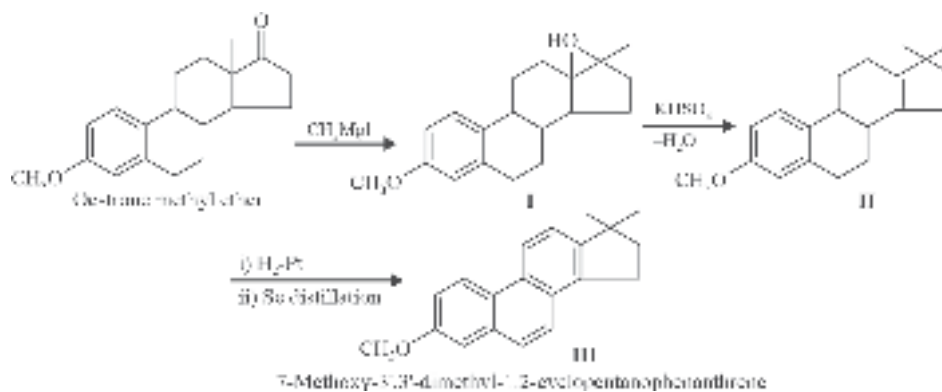
The above reaction also suggests that in oestrone, the benzene ring is ring A with hydroxyl group at C-3 position. Hence, basic skeleton to the oestrone can be assigned.



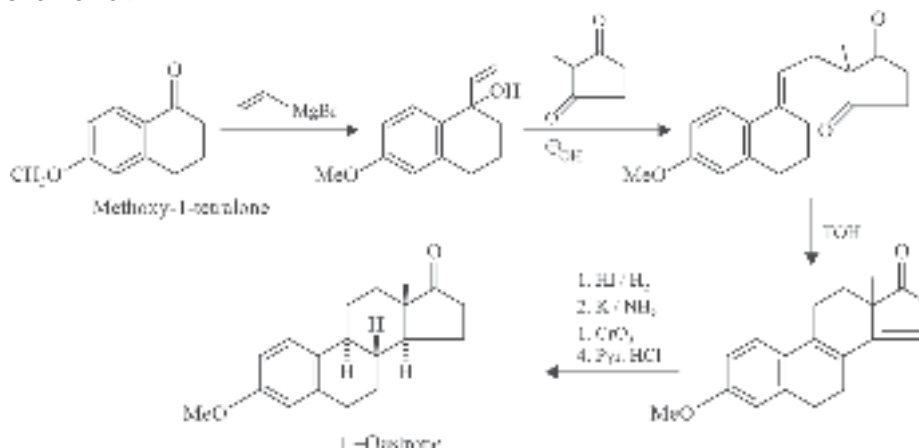
- (v) In the above structure, keto group has to be accommodated. Further, the above ring skeleton contains the 17 carbon atoms and so extra carbon atom is shown to be angular methyl group. To assign the position, the following sequence of reaction is carried out.



The sequence of reaction includes: treatment of oestrone methyl ether with CH_3MgI to get compound, **I**. This compound on dehydration, catalytic reduction followed by distillation with selenium gives 7-methoxy-3',3'-dimethyl-1,2-cyclopentanophenanthrene (**III**). This product can only be formed if there is a keto group at C-17 position and an angular methyl group at C-13 position in oestrone methyl ether or oestrone.



(vi) The proposed structure is finally confirmed on the basis of synthesis. It is synthesized as per Torgov et al. synthesis from methoxy-1-tetralone as shown in Scheme 10.7.



Scheme 10.7: Synthesis of oestrone

Spectral Data

IR (cm^{-1}) (KBr): 3657 (OH), 3022 (CH), 1710 (C = O).

$^1\text{H-NMR}$ (δ) (CDCl_3): 1.30 (3H, C-18- CH_3), 1.63 (1H, C-14-H), 1.57 (1H, C-9-H), 1.44, 1.69 (2H, C-7- CH_2), 1.86, 1.61 (2H, C-11- CH_2), 2.02, 1.77 (2H, C-12- CH_2), 2.11, 2.01 (2H, C-16- CH_2), 2.83 (H, C-9-H), 5.35 (1H, OH), 6.40, 6.73, 6.75 (3H, Ar-H), 27.2 (C-7), 26.2 (C-11), 21.7 (C-14), 32.7 (C-12), 35.7 (C-16), 37.56 (C-16), 37.6 (C-8), 43.5 (C-9), 48.1 (C-13), 115.3 (C-4), 112.8 (C-2), 126.8 (C-1), 132.0 (C-10), 138.1 (C-5), 154.0 (C-3), 220.0 (C-17).

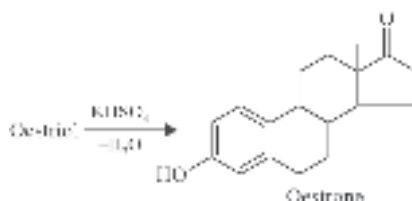
Oestriol

It is a hydroxylated metabolite of oestradiol or oestrone, with hydroxyl group at C-3 β , 16- α , and 17- β position. Oestriol is a major urinary estrogen. During pregnancy, large

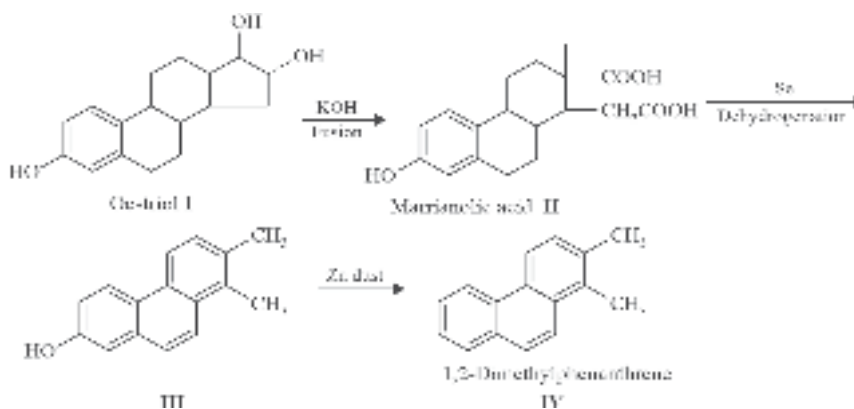
amount of oestriol is produced by the placenta. Isomers with inversion of the hydroxyl group or groups are called epiestriol. Oestriol is more soluble in water than oestrone and also more potent than either oestrone or oestradiol, when taken orally.

Structural Elucidation of Oestriol

- (i) **Molecular formulae:** It is found to be $C_{18}H_{24}O_3$.
- (ii) **Presence of hydroxyl groups:** On acetylation, it forms triacetate, indicating the presence of three hydroxyl groups. One hydroxyl group is phenolic in nature since it reacts with diazonium salt in alkaline media. Two hydroxyl groups are secondary alcoholic groups since on oxidation oestriol yields diketone. Furthermore, X-ray analysis indicates that two alcoholic groups are present in vicinal position.
- (iii) On heating oestriol with $KHSO_4$, oestrone is formed with the removal of one molecule of water. It indicates that oestriol has same carbon skeleton as oestrone and two alcoholic groups are present at C-16 and 17 positions.



- (iv) On the basis of above evidences, the structure, **I** is assigned to oestriol, which is supported by following sequence of reaction.

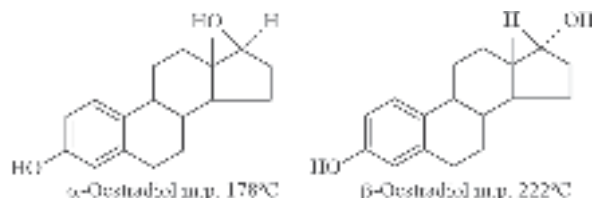


On fusion with KOH, oestriol forms maarrianolic acid, **II**. The compound, **II** on dehydrogenation with selenium gives hydroxydimethyl phenanthrene, **III** which on distillation with zinc dust gives dimethyl phenanthrene. Since maarrianolic acid forms an anhydride on heating with acetic anhydride, it, therefore, suggest that oestriol contains a phenanthrene nucleus and a five membered ring, the position of the latter being 1:2 (where the two methyl groups on compound **IV**). Finally, the compound, **III** is shown to be 7-hydroxy-1,2-dimethylphenanthrene by synthesis.

- (v) Since oestriol does not react with acetone to form isopropylidene derivative, its vicinal OH groups are *trans* in position. The hydroxyl group at C-17 position is beta due to its formation from oestrone.

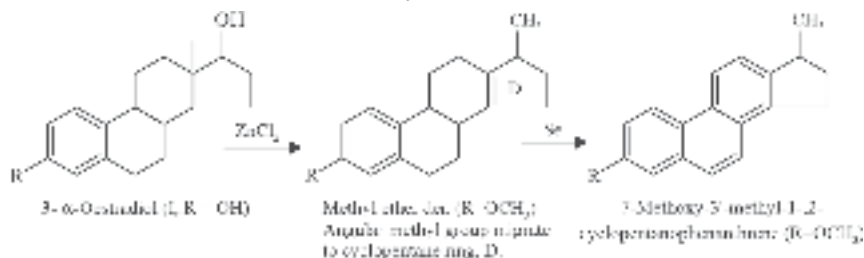
Oestradiol

There are two isomers of oestradiol, α and β . The α -isomer is more active than β -isomer.



Structural Elucidation of Oestradiol

- (i) **Molecular formula:** It is found to be $C_{18}H_{24}O_2$.
- (ii) **Presence of hydroxyl groups:** It contains two hydroxyl groups, one phenolic and another is secondary alcoholic group.
- (iii) α -Oestradiol can be formed from reduction of oestrone, suggesting the same skeleton as oestrone.
- (iv) On the basis of these evidences, the structure, I is assigned to oestradiol. This structure is supported by the formation of 7-methoxy-3'-methyl-1,2-cyclopentanophenanthrene from its methyl ether derivative (I, R = OCH_3).



The rearrangement takes place when phenolic methyl ether is heated with zinc chloride, the angular methyl group migrates to cyclopentane D ring. This compound on dehydrogenation with selenium produces the 7-methoxy-3'-methyl-1,2-cyclopentenophenanthrene.

Spectral Data

UV (nm): λ_{max} 221.

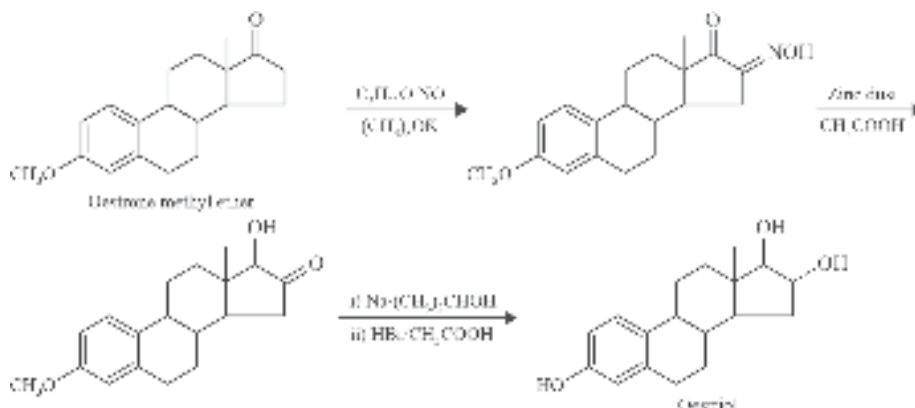
IR (cm^{-1}): 3417 (OH), 2000 (CH), 2963, 2907, 2809 (CH, CH_2 , CH_3 respectively), 1500 (C = C), 1500, 1400, 1300, 1200.

^1H -NMR (δ): 1.04 (C-18, CH_3), 1.31, 1.56 (2H, C-12), 1.35, 1.60 (2H, C-14-H), 1.53, 1.78 (2H, C-16-H), 1.61, 1.86 (2H, C-11-H), 1.57 (1H, C-8-H), 1.40 (1H, C-14-H), 1.69, 1.44 (2H, C-7-H), 2.83 (1H, C-9-H), 2.90, 2.80 (2H, C-6-H), 5.35 (1H, OH), 6.40 (1H, C-2-H), 6.73 (1H, C-4-H), 6.75 (1H, C-1-H), **^{13}C -NMR (δ)** 12 (C-18), 23.8 (C-16), 27.8 (C-7), 29.7 (C-6), 30.9 (C-16), 36.4 (C-12), 38.6 (C-8), 43.1 (C-13), 44.1 (C-9), 81.7 (C-17), 112.8 (C-2), 126.7 (C-1), 132.8 (C-10), 138.1 (C-5), 154 (C-3).

Relationship of Oestrone, Oestriol and Oestradiol

- (i) Oestrone may be converted to oestradiol by reduction by either catalytic hydrogenation or treatment with lithium aluminium hydride.

- (ii) Oestrone may be produced from oestriol by dehydration process using potassium hydrogen sulphate and at the same time, oestrone may be converted into oestriol by following sequence of reactions.

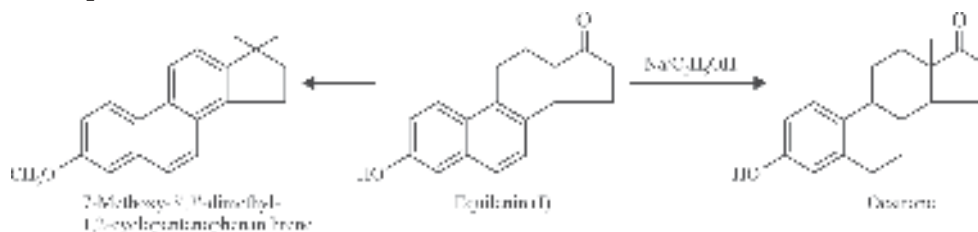


Equilenin

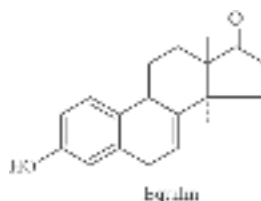
It is not a potent steroidal oestrogen, isolated from the urine of pregnant mares.

Structural Elucidation

- (i) Its molecular formula is found to be $\text{C}_{18}\text{H}_{18}\text{O}_2$.
- (ii) The usual tests show the presence of keto and phenolic groups.
- (iii) On reaction of its methyl ether derivative with methylmagnesium iodide, it gives alcohol. This alcohol on catalytic reduction, followed by dehydrogenation with selenium produces the 7-methoxy-3',3'-dimethyl-1,2-cyclopentanophenanthrene (same as oestrone methyl ether). This reaction indicates that equilenin has similar structural feature to oestrone.
- (iv) The UV spectrum of equilenin suggest the presence of naphthalene nucleus. As in the oestrone, the ring A is benzenoid so in equilenin, the ring, **B** is also benzenoid to justify the presence of naphthalene moiety.
- (v) The equilenin gives oestrone on reduction method.
- (vi) The above reactions can only be explained if below given structure, **I** is assigned to the equilenin.



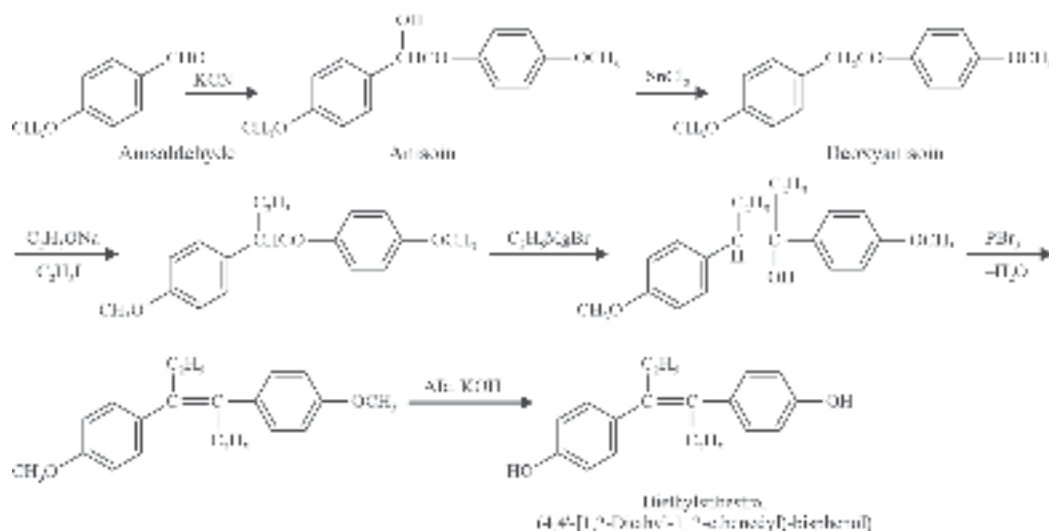
Equilin, $\text{C}_{18}\text{H}_{20}\text{O}_2$, m.p. $238-240^\circ\text{C}$ is also found to be present in urine of pregnant mares.



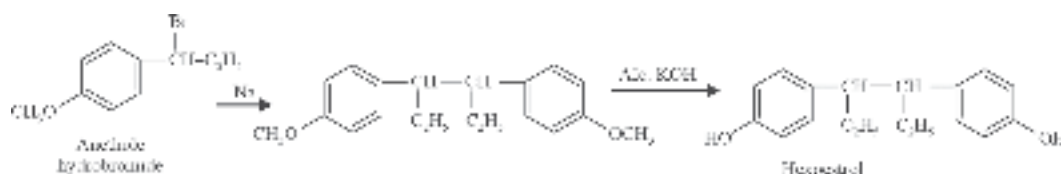
Synthetic Non-steroidal Oestrogens

Many compounds, in spite of not having steroid nucleus, possess the oestrogenic activity.

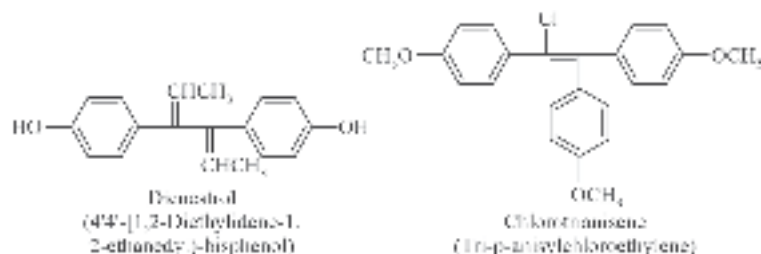
Diethylstilbestrol (DES): It is a synthetic nonsteroidal oestrogen that is developed to supplement a woman's natural oestrogen production. There are two geometrical isomers. Its *cis* isomer has only one-tenth the activity of the *trans*. This is mainly due to improper positioning and distance (about 7 Å) between the two hydroxyl groups in the *cis* isomer. The *trans* isomer is well adsorbed orally. Diethylstilbestrol is more active than oestrone when administered subcutaneously. It can also be used orally. The synthesis of DES is as follows:



Hexoestrol: It is saturated version of diethylstilbestrol, prepared from anethole hydrobromide. Its meso isomer is more active.



Dienestrol: Dienestrol is a synthetic, non-steroidal estrogen. It is an estrogen receptor agonist. Estrogens work partly by increasing a normal clear discharge from the vagina and making the vulva and urethra healthy. It is orally active but is only currently available as a topical cream for the treatment of atrophic vaginitis.



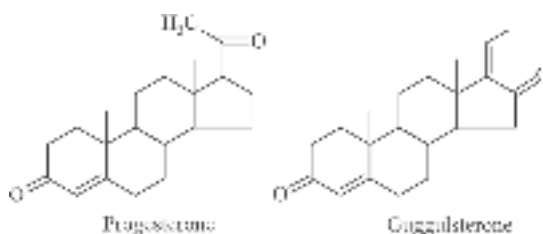
Chlorotrianisene (Trace): It is also related with stilbesterol, nonsteroidal synthetic oestrogen. After menopause, when the body no longer produces oestrogen, chlorotrianisene is used as a simple replacement of oestrogen. It is considered to be stored in adipose tissue, liberated slowly for extended period and then metabolized to a more potent estrogen than the parent substance.

3. PROGESTINS

Progestins originate from both ovaries and placenta, and mediate menstrual cycle and maintain pregnancy. **Progesterone** is prime naturally occurring progestins. All other endogenous steroids are devoid of gestational activity. It is mainly produced in the ovaries, testes and adrenal glands. It is responsible for modification of uterine tissue, implantation and development of fertilized eggs. It also prevents the ovulation in pregnant women.

Progesterone

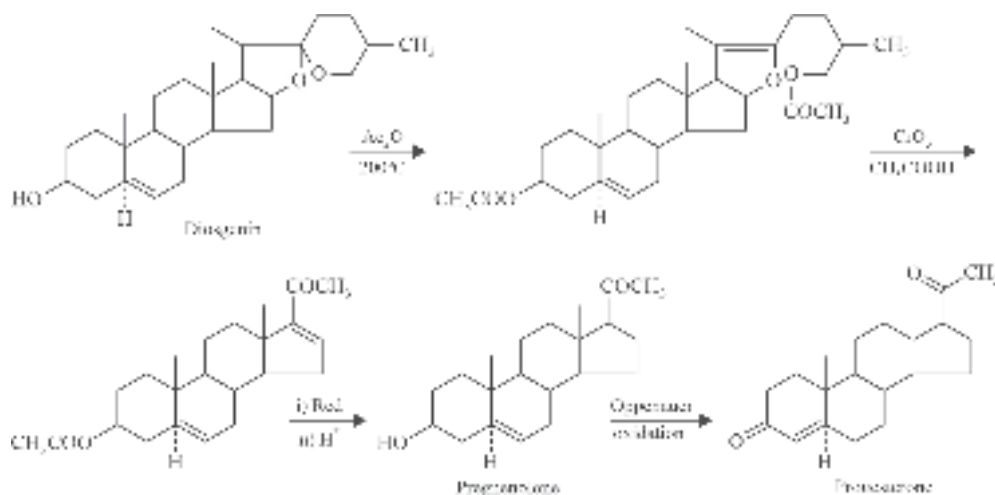
Progesterone is one of the steroid hormones, having pregnane nucleus with 21 carbon atoms. It is secreted by the **corpus luteum** and by the **placenta** and is responsible for preparing the body for pregnancy and, if pregnancy occurs, maintaining it until birth.



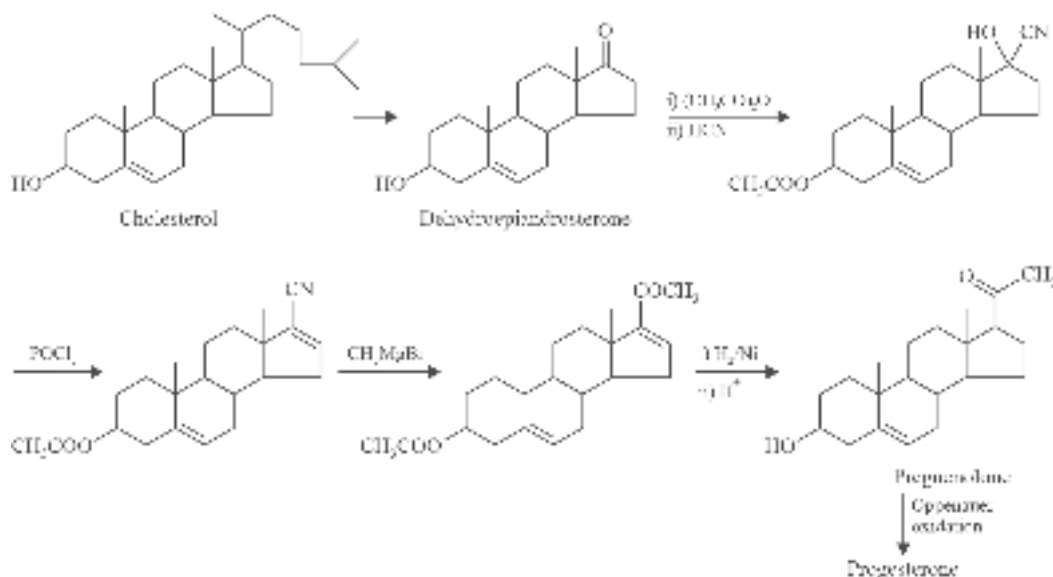
Guggulsterone is an analogue of progesterone. That sterol is found in the resin of the guggul tree (*Commiphora mukul*). Guggul tree extract has been suggested to lower low-density lipoprotein levels in animal models.

Structural Elucidation of Progesterone

- (i) **Molecular formula:** It is found to be $C_{21}H_{30}O_2$.
- (ii) **Presence of keto groups:** The usual tests indicate the presence of two keto groups.
- (iii) **Presence of double bond:** It contains one double bond.
- (iv) Its saturated molecular formulae, $C_{21}H_{36}$ corresponds to C_nH_{2n-2} , suggesting the tetracyclic structure. As progesterone can be prepared from stigmasterol or cholesterol, it contains the same steroid nucleus.
- (v) The UV spectrum suggest the presence of α,β -unsaturated ketonic moiety (λ_{max} 240 nm).
- (vi) The final structure is assigned on the basis of its synthesis.
 - (a) **From diosgenin:** It may be prepared from diosgenin, available as glycoside in the root of *Trillium erectum* (Scheme 10.8).
 - (b) **From cholesterol:** The progesterone is synthesized from cholesterol via dehydroepiandrosterone (Scheme 10.9).
 - (c) **From ergosterol** (Scheme 10.10).



Scheme 10.8: Synthesis of progesterone from diosgenin



Scheme 10.9: Synthesis of progesterone from cholesterol

(d) **From stigmasterol** (Scheme 10.11).

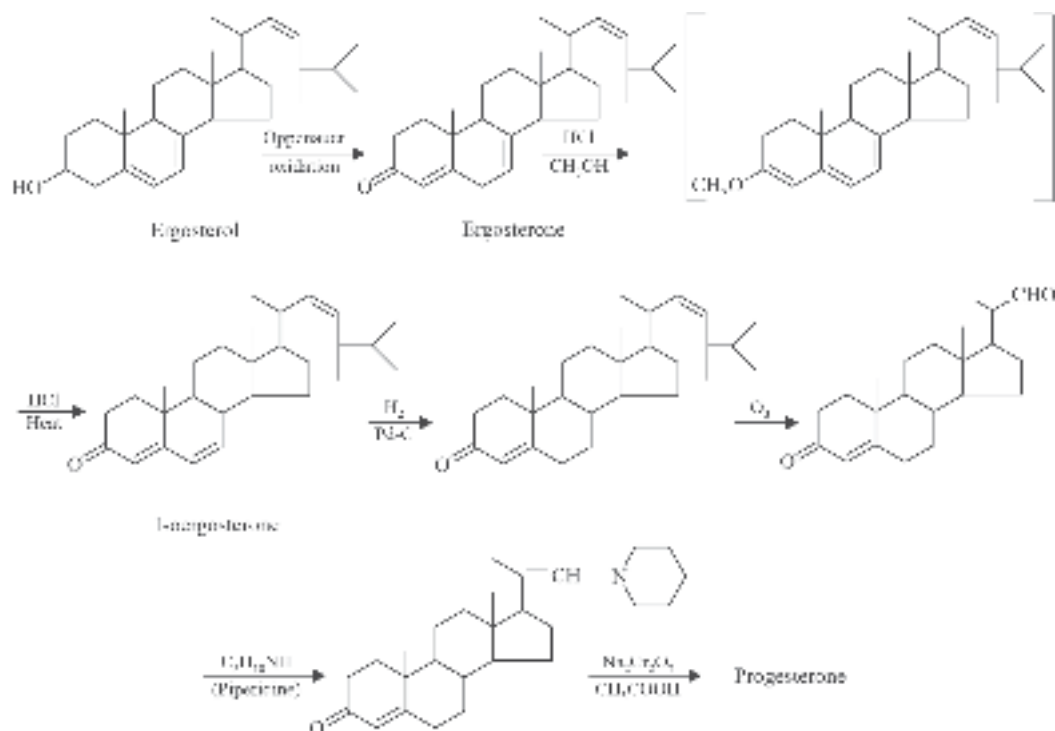
(e) **From pregnanediol** (Scheme 10.12).

Spectral Data

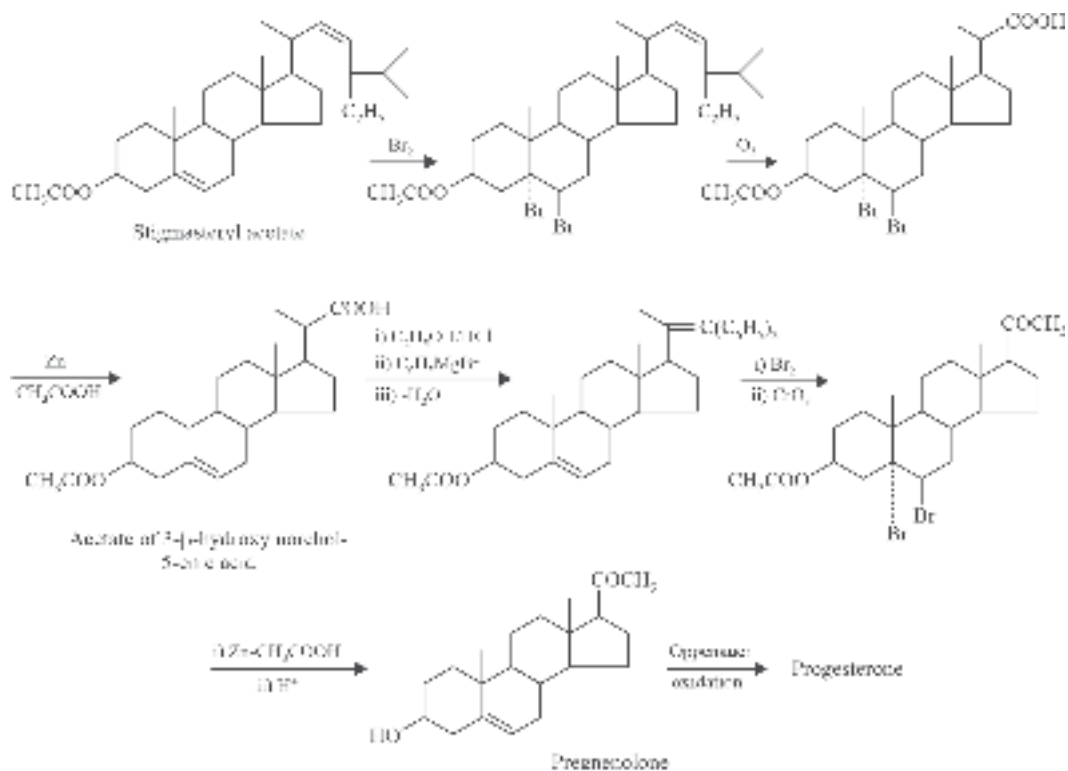
UV (nm): λ_{max} 241 (in methanol).

IR (cm⁻¹) (KBr): 2930 (antisymmetric stret), 2850 (symmetric stret), 1700, 1614 1232, 1209, 872.

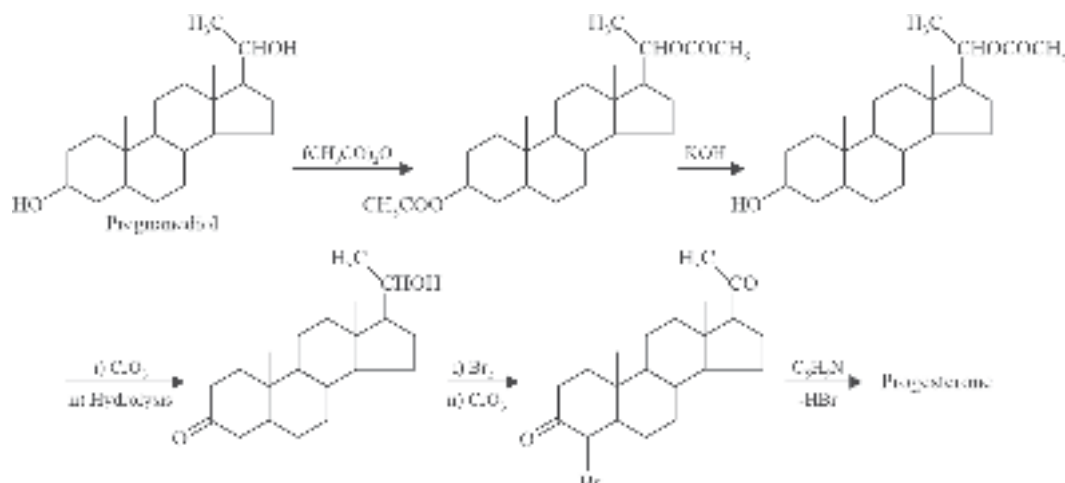
¹H-NMR (δ) (CDCl₃): 0.670 (3H, C₁₈-CH₃), 0.992 (3H, C₁₉-CH₃), 1.057 (1H, C₇-H), 1.19 (1H, C₁₄-H), 1.192 (1H, C₈-H), 1.28 (1H, C₁₅-H), 1.45 (1H, C₁₁-H), 1.46 (1H, C₁₆-H), 1.565 (1H, C₈-H), 1.65 (1H, C₁₁-H), 1.69 (1H, C₁₆-H), 1.1 (1H, C₅-H), 1.87 (1H, C₇-H), 2.05 (1H, C₁-H), 2.07 (1H, C₁₂-H), 2.13 (1H), 2.187 (2H, C₁₆-H), 2.289 (1H, C₆-H), 2.37-2.47 (2H, C₂-H), 2.40 (1H, C₅-H), 2.54 (1H, C₁₇-H), 5.337 (1H, C₄-H).



Scheme 10.10: Synthesis of progesterone from ergosterol



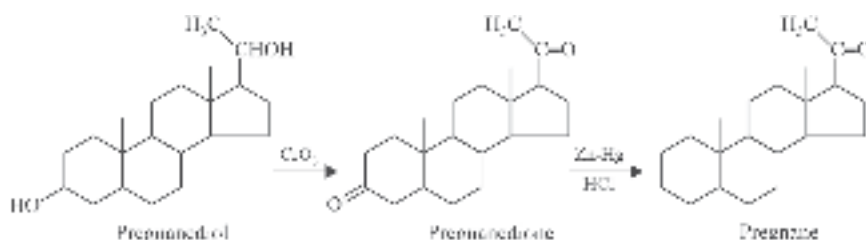
Scheme 10.11: Synthesis of progesterone from stigmasterol



Scheme 10.12: Synthesis of progesterone from pregnanediol

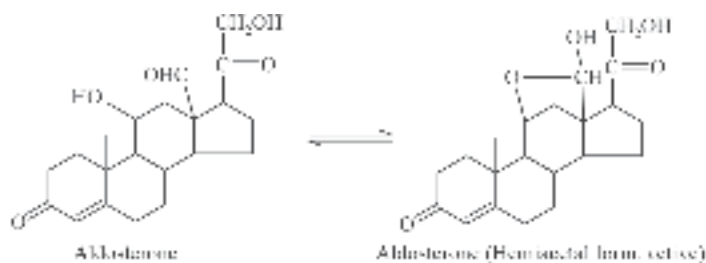
β -Pregnane-3, 20 α -diol, $C_{21}H_{36}O_2$

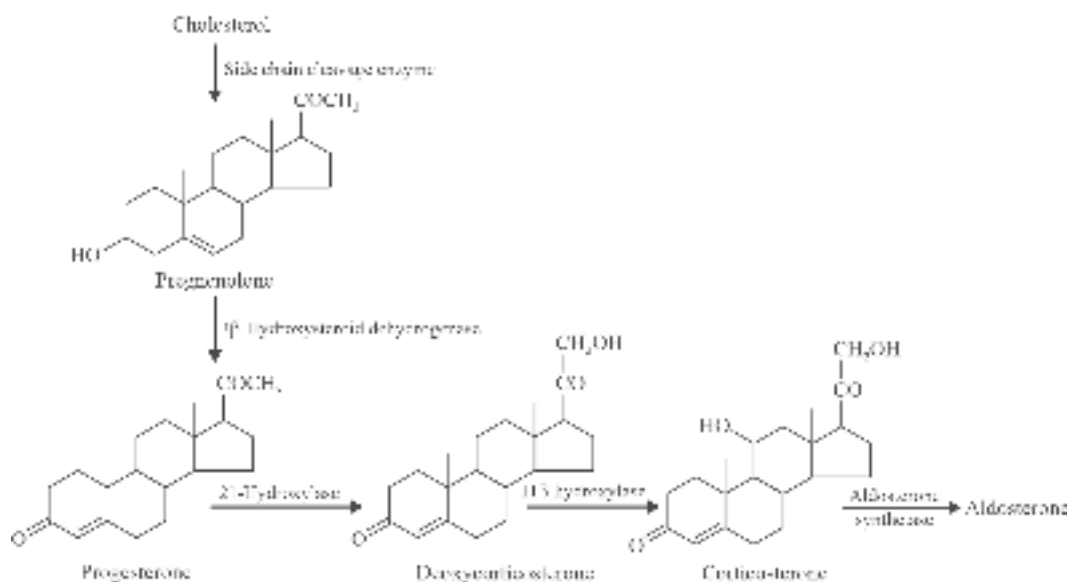
It is isolated from urine of pregnant women. It is inactive metabolite of progesterone. It contains two secondary alcoholic groups. On oxidation, it is converted into diketone, pregnandione which on elemenson reduction forms, pregnane ($C_{21}H_{36}$), saturated compound. This suggest the presence of steroid nucleus and side chain at C-17 position. Hence, it is similar to progesterone. The progesterone contains keto group at C-3 and 20 position in place of hydroxyl group. The double bond is absent in Pregnanediol.



4. MINERALOCORTICOIDS

These control salt and water balance and reduce levels of K^+ (increases its excretion). Aldosterone (21 carbons) is the main naturally occurring mineralocorticoids. It has aldehyde group at C-18 position and biosynthesized from cholesterol (Scheme 10.13). Aldosterone is spontaneously converted into its hemiacetal form (active).





Scheme 10.13: Biosynthesis of aldosterone from cholesterol

5. GLUCOCORTICOIDS

These increase gluconeogenesis (formation of glucose from protein) and exert a lesser effect on salt and water balance. Hence, these also reduce the inflammation. Glucocorticoids also stimulate and promote fat synthesis.

Structural Features

These contains pregnane nucleus. Many glucocorticoids have been isolated from the adrenal cortex; structures of most physiologically active glucocorticoids are given in Fig 10.6.

Due to presence of α -hydroxy-keto moiety, these are strong-reducing agents. The keto group at C-21 position behaves in a usual way but keto group at C-11 position does not react with hydroxylamine to form oxime. The keto group at C-11 position is resistant to hydrolysis but can be reduced in acidic condition.

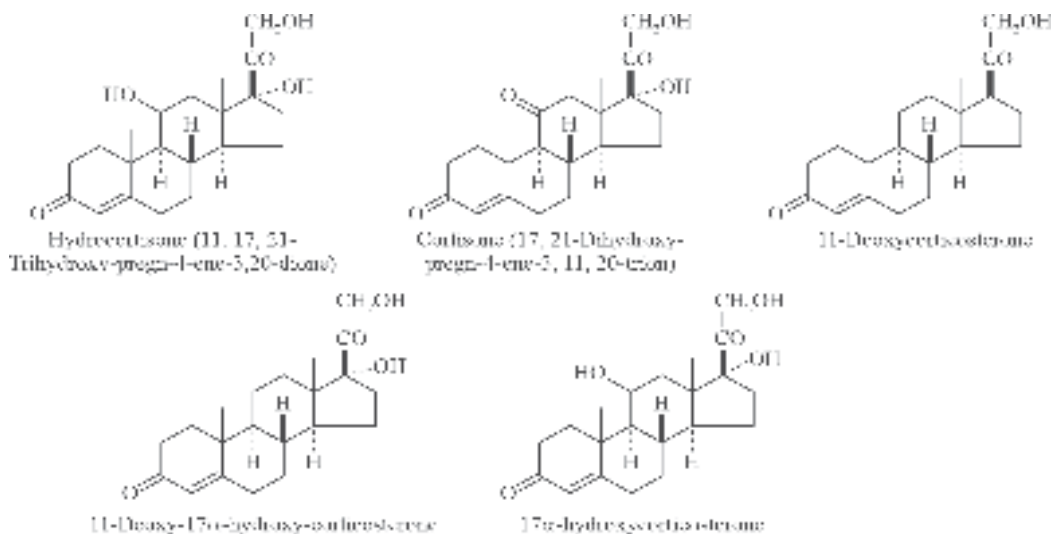
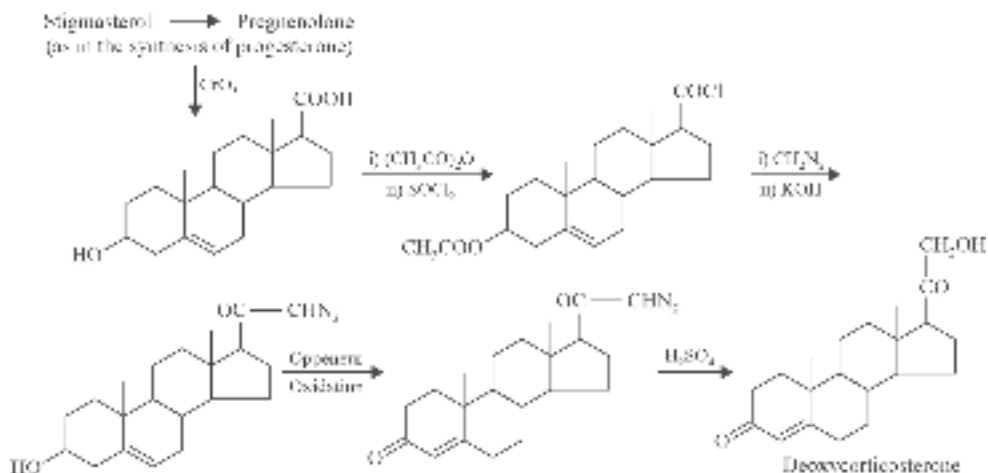


Fig 10.6: Examples of glucocorticoids

The structures of glucocorticoids are elucidated on the basis of partial degradation and synthesis from sterols of known structures. For example, deoxycorticosterone is synthesized from stigmaterol (Scheme 10.14).

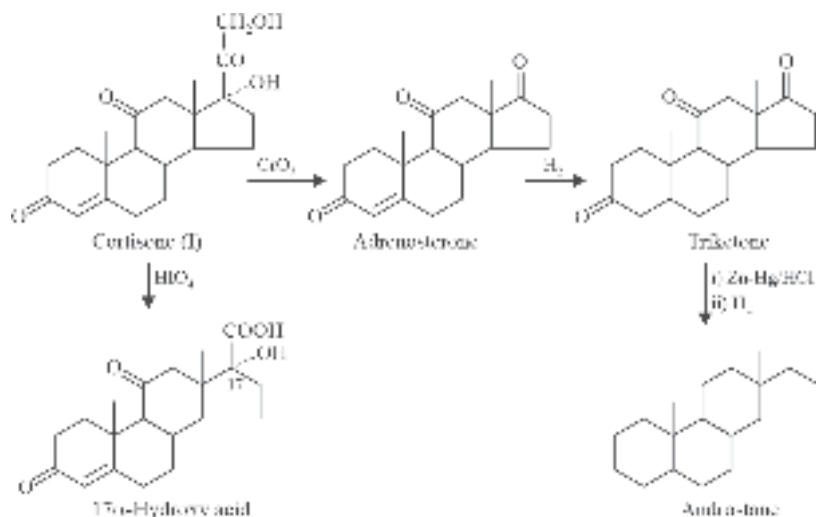


Scheme 10.14: Synthesis of deoxycorticosterone from stigmaterol

Cortisone: It is the naturally occurring cortisone with good systemic anti-inflammatory activity. It shows its activity *in vivo* after conversion to hydrocortisone, mediated by 11α -hydroxy steroid dehydrogenase. It is used for the entire spectrum of clinical uses, e.g. arthritis, blood/hormone/immune system disorders, allergic reactions, certain skin and eye conditions, breathing problems, and certain cancers.

Structural Elucidation of Cortisone

- (i) Its molecular formulae is found to be $C_{21}H_{29}O_5$.
- (ii) It contains an α,β -unsaturated group as it is sensitive to alkali.
- (iii) Usual test shows the presence of double bond, two keto groups, one primary alcoholic group, and one tertiary alcoholic group.
- (iv) It contains alpha keto ($-\text{COCH}_2\text{OH}$) group, since it reduces the silver nitrate and Fehling solution.

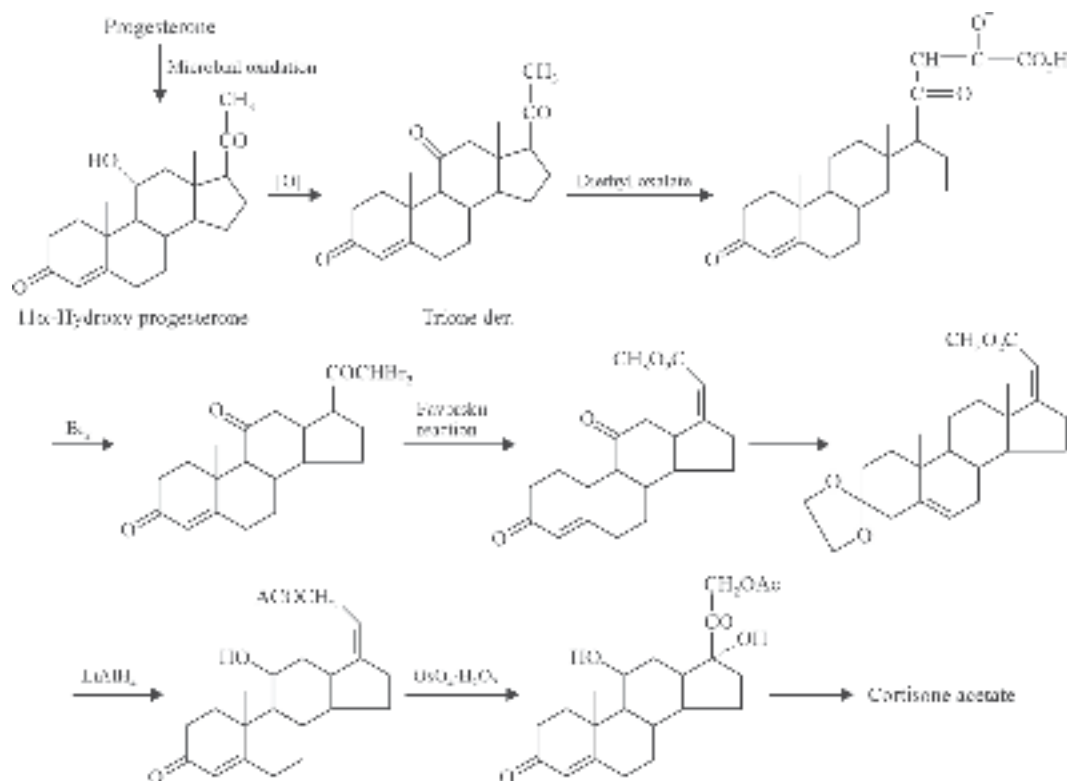


- (v) Oxidation of cortisone with chromic acid gives adrenosterone. Both adrenosterone and cortisone are found to contain keto group at C-3 position. Both are found to have α,β -unsaturated ketones by the comparison of UV spectra with that of testosterone and progesterone. This also confirms the presence of double bond between C-4 and C-5 positions.
- (vi) Hydrogenation of adrenosterone gives the triketone of known structure, m.p. 178°C which is found to be a derivative of 3,17-diketone. This is further confirmed by the formation of androstane by reduction of triketone first by Clemmensen reduction followed by hydrogen. Thus, cortisone is related to androstane.
- (vii) Oxidation of cortisone with HIO_4 gives 17α -hydroxy acid which proves the presence of second hydroxyl group.

All the above reactions can be explained by assigning the structure, I to cortisone which is further confirmed from synthesis.

Synthesis

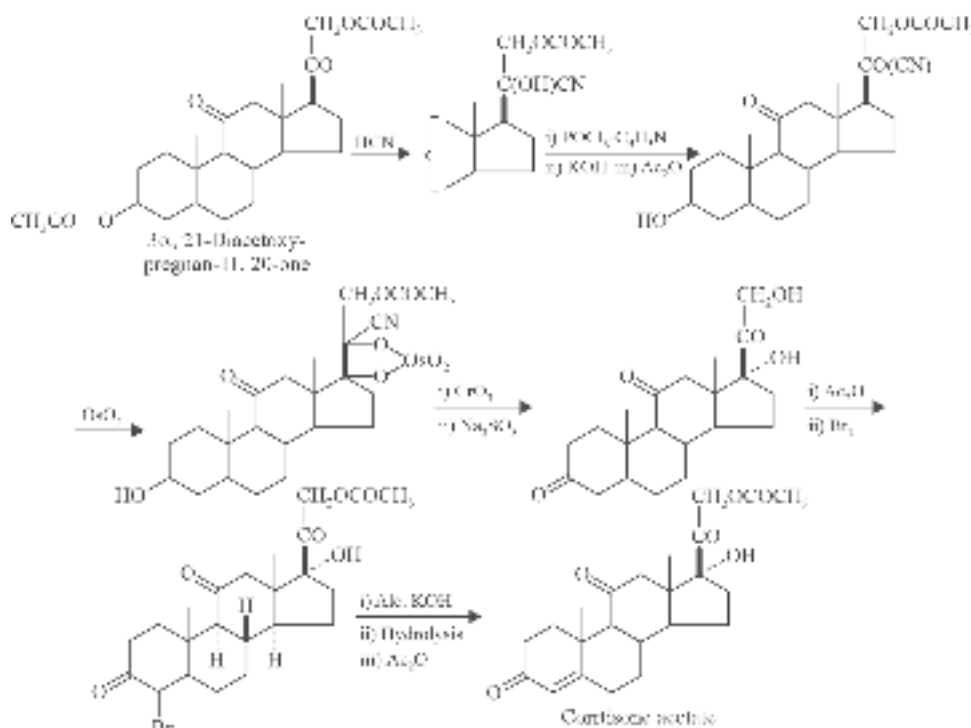
- (a) Its large-scale production is done from progesterone *via* microbiological oxidation with a soil organism, **Rhizopus arrhizus** (Scheme 10.15) to convert progesterone into 11α -hydroxy progesterone leading to formation of the trione. Condensation with diethyl oxalate activates the appropriate carbon toward selective bromination to form the dibromide. Rearrangement followed by dehydrohalogenation is the next step, and the ketone at C-3 position is protected as its ketal. Reaction with lithium aluminum hydride reduces the ester and the C-11 ketone to the alcohol.



Scheme 10.15: Microbial synthesis of cortisone from progesterone

Acetylation of one of the alcohol groups (the less-hindered primary alcohol) and removal of the protecting group at C-3 position then gives the unsaturated acetate, and osmium tetroxide and hydrogen peroxide oxidize the double bond to give hydrocortisone acetate, after which oxidation of the alcohol group and hydrolysis of the acetate gives cortisone.

(b) Second method: In this 3,20-diacetoxy pregnan-11,20-dione is used as starting material for the complete synthesis of cortisone (Scheme 10.16).



Scheme 10.16: Synthesis of cortisone from 3,20-diacetoxy pregnan-11,20-dione

Spectral Data of Cortisone

IR (cm⁻¹): 3425 (OH), 2950 (CH), 1750 (C = O), 1200, 1050, 863.

¹H-NMR (δ) (DMSO-d₆): 0.47 (C-19-CH₃), 1.32 (C-18-CH₃), 1.94 (C₁₂-H), 2.87 (C₁₂-H), 4.087 (C₂₁-H), 4.447 (C₂₁-H), 4.70 (C₂₁-OH), 5.6 (C₄-H), 5.64 (C₁₇-H); ¹³C-NMR (δ): 35.6 (C1), 33.9 (C2), 199.4 (C-3), 123.8 (C-4), 171 (C-5), 32.7 (C-6), 31.6 (C-7), 36.4 (C-8), 53.7 (C-9), 38.6 (C-10), 20.6 (C-11), 31.3 (C-12), 45.2 (C-13), 50.1 (C-14), 38.8 (C-15), 81.4 (C-17), 13.8 (C-18), 17.3 (C-19).

SHORT AND LONG QUESTIONS

1. What are steroids? Write a note on nomenclature and configuration of steroids.
2. How the structure of cholesterol is elucidated.
3. What are bile acids? Discuss their structural features.
4. Explain the biosynthesis of sex hormones from cholesterol.
5. Write a note on synthetic estrogens.
6. What are androgens? Give the chemistry of testosterone.

7. Give the synthesis of following:
 - a. Progesterone from diosgenin
 - b. Progesterone from cholesterol
 - c. Testosterone from diosgenin
8. Briefly discuss the UV absorption spectra of steroids.

MULTIPLE CHOICE QUESTIONS

1. Testosterone may be synthesized from the following starting material
 - a. Chloesterol
 - b. Dehydroepiandrosterone
 - c. Both (a) and (b)
 - d. None of the above
2. What do all steroids have in common?
 - a. They contain four rings of carbon atoms
 - b. They contain at least one fatty acid group
 - c. They are manufactured by the liver
 - d. They are water-soluble
3. What product is formed due to the degradation of cholesterol in the body?
 - a. Bile acid
 - b. Steroid hormones
 - c. Vitamin D
 - d. All of the above
4. The hormone cortisone contains the steroid core as:
 - a. Estrane
 - b. Andrsotane
 - c. Cholestane
 - d. Pregnane
5. In hyperplasia, what is produced in more quantities by adrenal glands?
 - a. Androgens
 - b. Estrogens
 - c. Cortisone
 - d. Adrenaline

Answers

1. (c) 2. (a) 3. (d) 4. (d) 5. (a)

Vitamins—Chemistry and Biological Significance

Vitamins are organic compounds that the body requires in order to properly function. These are essential for continuance of life (utilized in small quantities). Most of the vitamins are not synthesized inside the body. Due to this reason, **Funk**, in 1911, named these substances 'vitamine' to indicate that these food factors were necessary for life, therefore, a vital amine. The final "e" was dropped when chemical analysis of these factors showed that not all of them were amines.

Contrary to popular belief, vitamins cannot be metabolized in the body to produce energy; however, some (the B complex vitamins) are necessary for energy production. Vitamins are utilized in a regulatory manner rather than in a structural function. Vitamins are separated into two major groupings, **fat-soluble** and **water-soluble vitamins**. The fat soluble group contains vitamins **A, D, E, and K**, and **β -Carotene**). They are stored in the liver and used up by the body very slowly. The rest of the vitamin family, **B-Complexes** and **Vitamin C** comprise of the water soluble family. The body uses these vitamins very quickly. Excess amounts are eliminated in urine. The chemical entities Choline and inositol are possible vitamins, though they are under doubtful condition for their actual status.

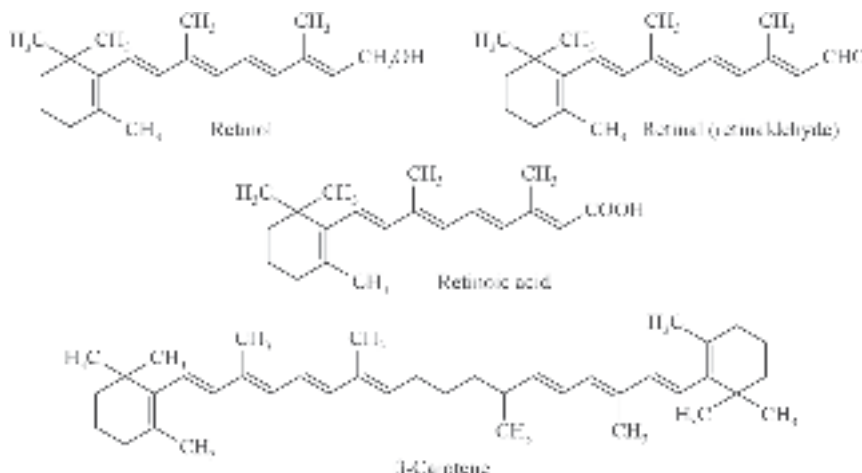
FAT SOLUBLE VITAMINS

The fat soluble vitamins include vitamins A, D, E and K since they are soluble in fat and are absorbed by the body from the intestinal tract. The human body has to use bile acids to absorb fat soluble vitamins. Once these vitamins are absorbed, the body stores them in body fat.

Vitamin A

Vitamin A is a subclass of a family of fat soluble compounds, referred to as retinoic acids. These consist of four isoprene units, joined in a head to tail fashion. These are two main forms of vitamin A: provitamin A carotenoids (β -carotene and others), and performed vitamin A.

- Provitamin A carotenoids are found in plants. There are many forms of provitamin A, but β -carotene is only one that is metabolized by mammals into vitamin A.
- Preformed vitamin A (retinol, retinal retinoic acid, and retinyl esters) is the most active form of vitamin A. Most of these are found in animal sources of food and is also the form supplied by most supplements. Some supplements provide a combination of provitamin A (β -carotene) and preformed vitamin A.



Retinol is a vitamin A alcohol containing β -ionone ring. The side chain has two isoprene units, four double bonds and one hydroxyl group. It is present in animal tissues as retinyl ester with long chain fatty acids.

Retinal is an aldehyde form, obtained by the oxidation of retinol.

Retinoic acid is produced from the oxidation of retinal.

Sources of Vitamin A

Top sources of vitamin A include:

- Beef liver • Egg yolk • Cheddar cheese • Fortified milk

Top sources of β -carotene include:

- Sweet potato • Carrots • Pumpkin • Cantaloupe
- Broccoli • Apricots • Spinach and collard greens

Functions of Vitamin A

Vitamin A is essential for numerous intrinsic processes. The most well known and understood process is that of vision. The 11-*cis* retinal form of vitamin A is essential for the neural transmission of light into vision. Epithelial cells are highly dependent on retinoic acid and are commonly used to treat a variety of skin diseases. This vitamin also participates in physiological activities related to the immune system, maintenance of epithelial and mucosal tissues, growth, reproduction, and bone development.

Vision support

The human retina contains four kinds of photopigments that store vitamin A compounds. One of these pigments, called rhodopsin, is located in the rod cells of the retina. Rhodopsin allows the rod cells to detect small amounts of light, and thus, plays a fundamental role in the adaptation of the eye to low light conditions and night vision.

Retinal, the aldehydic form of the vitamin, participates in the synthesis of rhodopsin. and in the series of chemical reactions that causes visual excitation, which is triggered by light striking the rod cells. The remaining three pigments, collectively known as iodopsins, are found in the cone cells of the retina and are responsible for day vision.

Immune function support

Vitamin A stimulates several immune system activities, possibly by promoting the growth and preventing the stress-induced shrinkage of the thymus gland. Vitamin A is known to enhance the function of white blood cells, increase the response of antibodies to antigens, and to have antiviral activity.

In addition, retinoic acid is needed to maintain the normal structure and function of epithelial and mucosal tissues, which are found in the lungs, trachea, skin, oral cavity, and gastrointestinal tract. These tissues, when healthy and intact, serve as the first line of defense for the immune system, providing a protective barrier for disease-causing microorganisms.

Cell growth support

Vitamin A is also necessary for normal cell growth and development. Although the mechanisms by which vitamin A promotes cell growth and development are not yet fully understood, it is known that retinoic acid is necessary for the synthesis of many glycoproteins, which control cellular adhesion (the ability of cells to attach to one another), cell growth and cell differentiation.

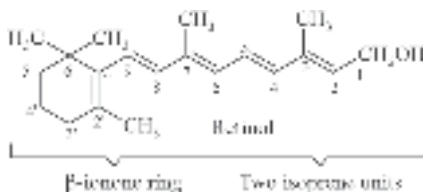
Other roles for Vitamin A

It is also known that vitamin A is essential for reproductive processes in both males and females and plays a role in normal bone metabolism.

Deficiency sign

Due to vitamin A deficiency, one can suffer from problems like night blindness, or conjunctival xerosis. The conjunctiva in the eye becomes dry due to lack of moisture. Skin becomes rough and thick and infections start appearing on the body parts. The lack of vitamin A can also induce gallbladder and kidney stones as well as strange bone growths, and it can also affect the normal nervous system. It also raises the chances of cancer.

Retinol (2E, 4E, 6E, 8E)-3,7-dimethyl-9-(2',6',6'-trimethylcyclohex-1-enyl) nona-2,4,6,8-tetraen-1-ol). It is also referred as vitamin A₁, since a second compound known as vitamin A₂ has also been known.

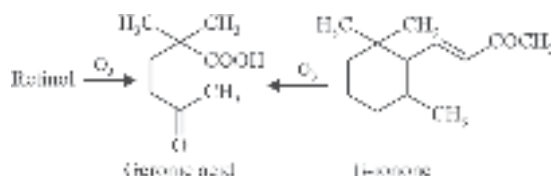


The most useable form of the vitamin is retinol, often called preformed vitamin A as it is the active form in the body. Retinol (an alcohol) can only be found in animal sources and can be converted by the body into retinal (an aldehyde) and retinoic acid (a carboxylic acid), other active forms of vitamin A.

Structural Elucidation of Retinol

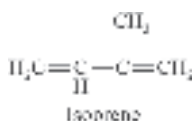
- (i) **Molecular formula:** It is found to be C₂₀H₃₀O.
- (ii) **Presence of alcoholic group:** The formation of esters with *p*-nitrobenzoic acid indicates the adjustment of oxygen atom as alcoholic group. The alcoholic group is primary in nature because it gives an aldehyde on oxidation.

- (iii) **Presence of double bonds:** On catalytic reduction, retinol takes up five molecules of hydrogen and forms perhydroretinol ($C_{20}H_{40}O$), which corresponds to general formula (C_nH_{2n}) for the compounds having acyclic ring. The retinol forms an adduct with maleic anhydride, indicating the presence of conjugated system of double bonds, which is further confirmed by its ultraviolet spectrum.
- (iv) **Presence of β -ionone ring:** On ozonolysis, retinol gives one molecule of geronic acid, so one β -ionone nucleus may be present in the vitamin, since β -ionone also gives geronic acid on oxidation.

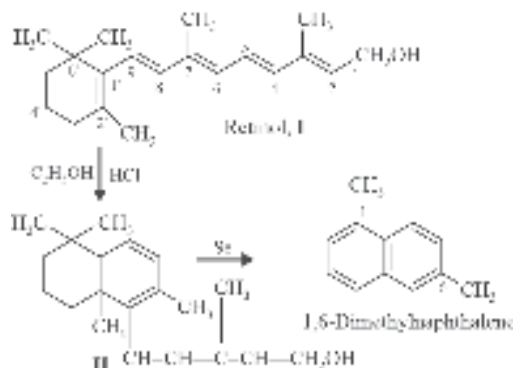


Perhydrovitamin A_1 synthesized from β -ionone is shown identical with the compound obtained from reduction of vitamin A_1 . This again confirms the presence of β -ionone ring.

- (v) **Presence of C-methyl group:** On oxidation with alkaline potassium permanganate, retinol yields two molecules of acetic acid per molecule of the vitamin indicating the presence of two $-C(CH_3)=$ in side chain. The presence of $-C$ -methyl group is further confirmed **Kuhn Roth** method of oxidation by chromic acid, which gives three molecules of acetic acid.
- (vi) The presence of C-methyl group in side chain gives an idea regarding the presence of isoprene unit just like most of carotenoids.



- (vii) **Presence of isoprene units:** It has also been confirmed from the following facts:
- (a) Alkaline *oxidation* of the retinol with $KMnO_4$ yields two molecules of CH_3COOH indicating the presence of two isoprene units.



- (b) Retinol on heating with alcoholic HCl is converted into compound, II which on dehydrogenation with selenium gives the 1,6-dimethylnaphthalene. The

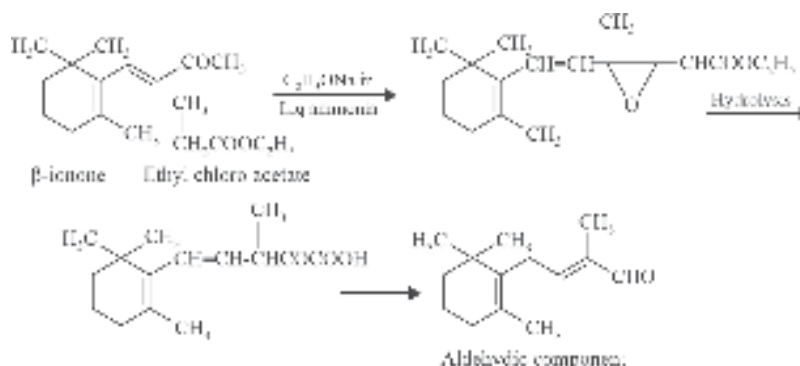
formation of this compound can only take place if there are two isoprene units in the vitamin and so above structure, **I** is assigned to retinol.

(viii) Further the above assigned structure is confirmed by the synthesis.

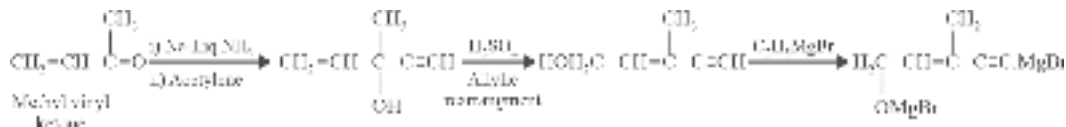
(a) **Isler method:** In this method, formation of an aldehyde and alcohol takes place. Both the compounds are then condensed to get Retinol (vitamin A₁).

Synthesis of aldehydic component

It is synthesized from β -ionone by means of **Darzen glycidic reaction**.

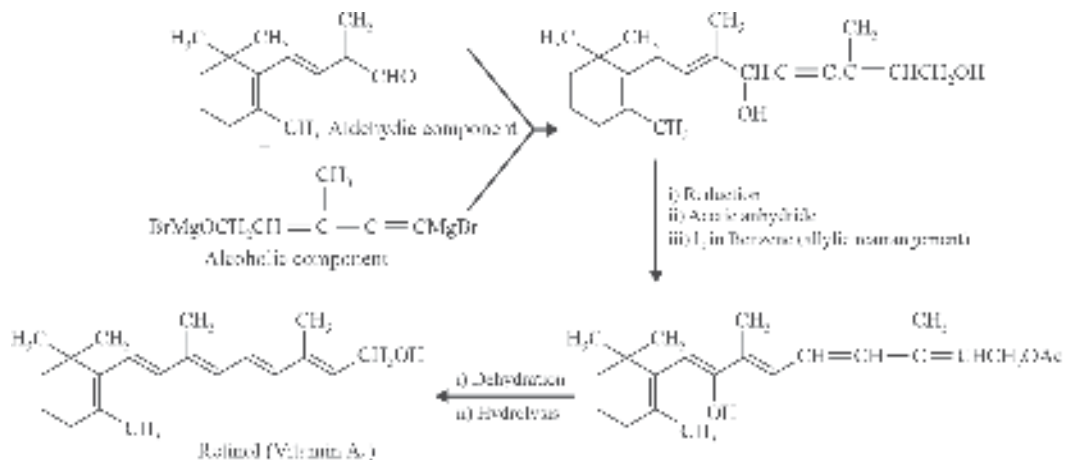


Synthesis of alcoholic component

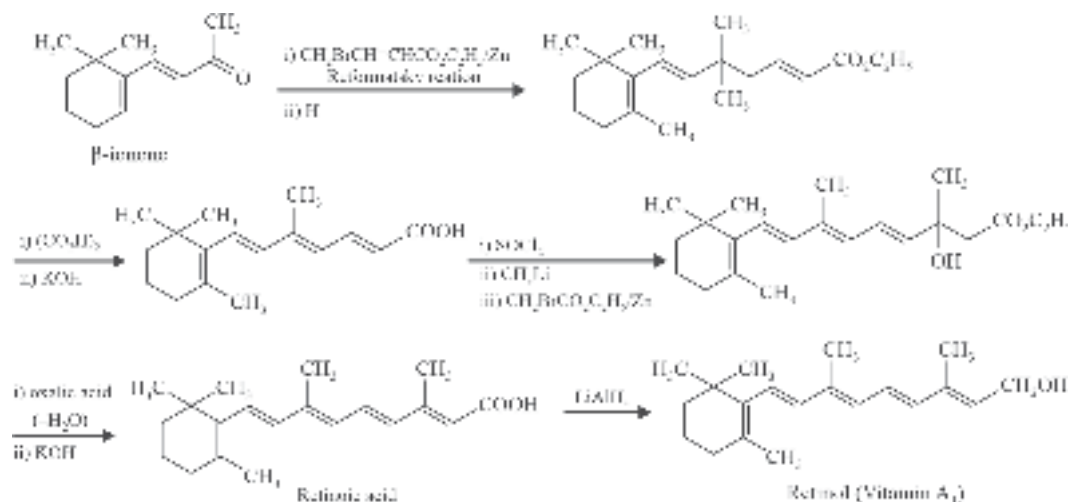


Synthesis of Retinol (Vitamin A₁)

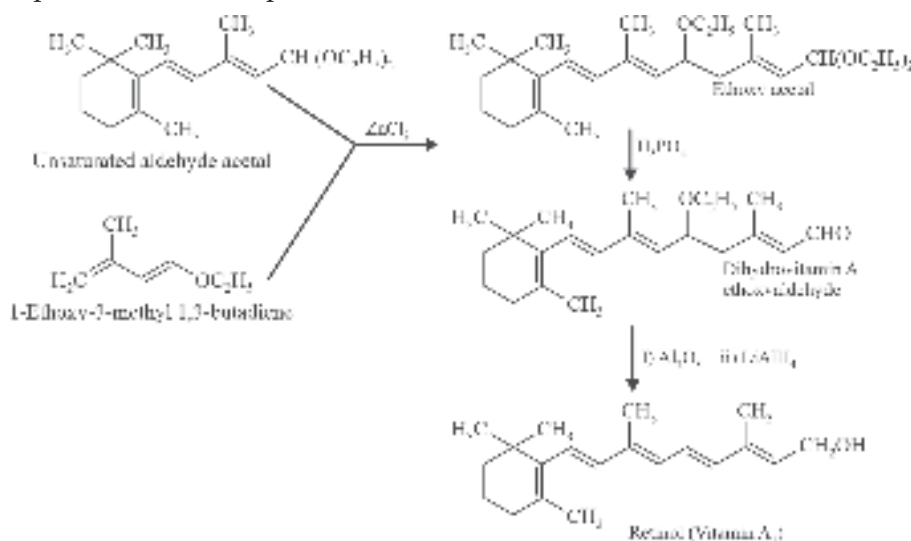
(a) The aldehydic and alcoholic components are condensed to get retinol (vitamin A₁).



(b) **van Drop** (1946) have synthesized retinoic acid by the reaction of β -ionone with ethyl- γ -bromo-crotonate. The resulting retinoic acid gives retinol on reduction with LiAlH_4 .



(c) **Krasnaja et al.** synthesized Retinal by condensation of unsaturated aldehydes acetals with 1-ethoxy-3-methyl 1,3-butadiene. This method gives better yield in comparison to Van drop method.



Spectral Data

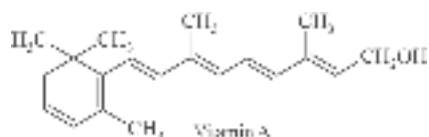
UV (nm): $\lambda_{\max}$ 325.

IR (cm⁻¹): 3369 (bonded OH str.), 2926 (CH, CH₂, str.), 1613 (Phenyl ring str.), 1462 (C–C str.), 1209 (C–O str.).

¹H-NMR (δ): 1.25 (2 $\times$ CH₃ at C-6'), 1.57 (2H, C-5'), 1.74 (2H, C-4'), 1.82 (3H, CH₃, C-2'), 1.96 (2H, C-3'), 2.21 (9H, CH₃, C-2), 2.21 (3H, CH₃, C-7), 3.65 (3H, OH), 4.18 (2H, CH₂), 5.63 (1H, C-2), 6.23 (1H, =CH), 6.51 (4H, 4 $\times$ =CH), **¹³C-NMR (δ):** 13, 21, 28, 33, 34, 40, 59, 127, 128, 129, 130, 136, 137.

Vitamin A₂

It is isolated from natural sources and chemically, it is dehydrovitamin A₁ (3-dehydroretinol).



Vitamin D

Vitamin D is a name given to two fat soluble compounds: ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Vitamin D₃ plays an essential role in the metabolism of calcium and phosphorus in the body and prevents rickets in children. A plentiful supply of 7-dehydrocholesterol, the precursor of vitamin D₃, exists in human skin and needs only to be activated by a moderate amount of ultraviolet light (less than half an hour of sunlight) to become fully potent (Fig. 11.1). Vitamin D₂ is commercially synthesized from ergosterol.

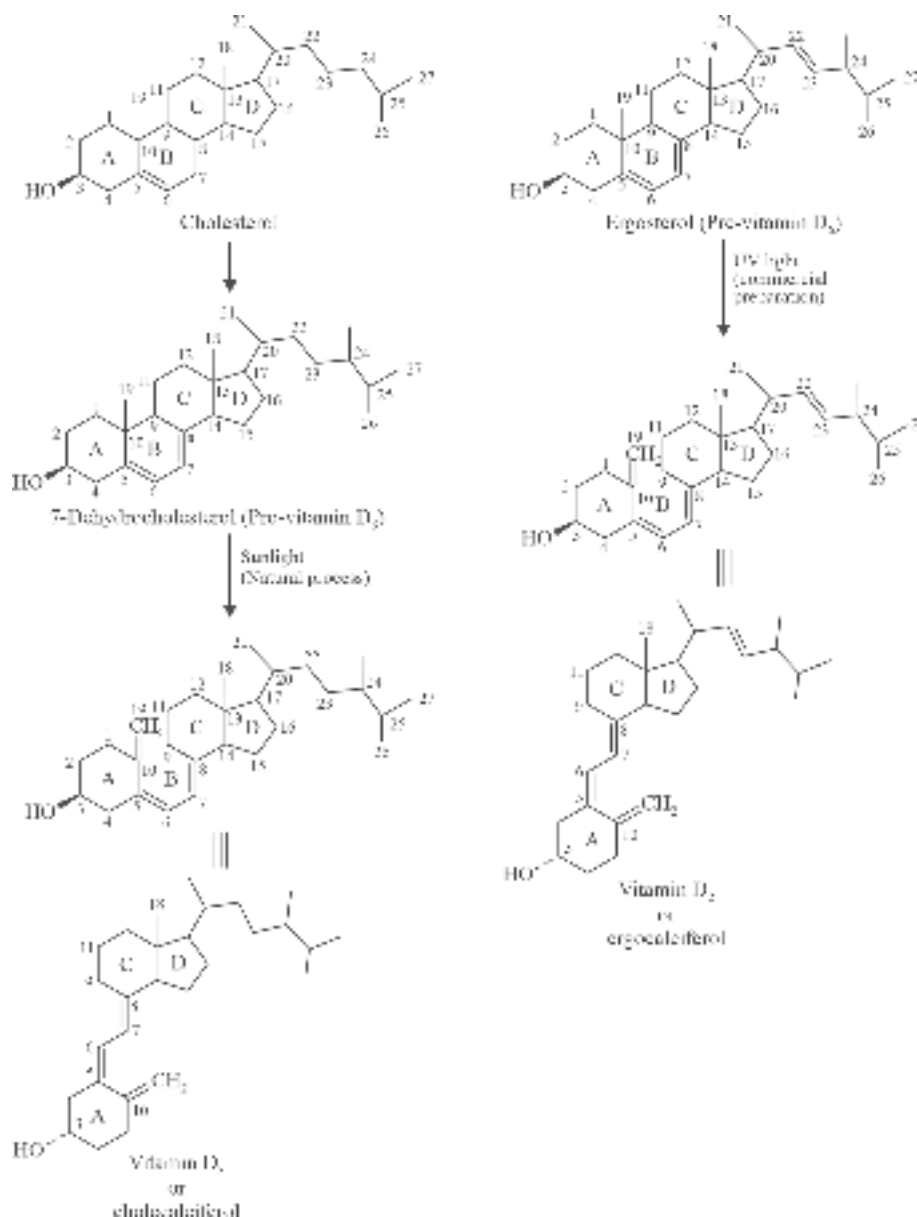


Fig 11.1: Biosynthesis of vitamin D₂ and vitamin D₃

Apart from the regulated exposure to sunlight, other sources that can provide a good amount of vitamin D are herring, mackerel, salmon, oysters, cottage cheese, egg yolk, fortified soy beverages, etc.

Functions of Vitamin D

Some of the functions of vitamin D are:

Vitamin D is essential to maintain strong and healthy bones and joints, since it helps in retaining and absorbing calcium. It has been proven that people, who ensure a sufficient and regular intake of vitamin D are less likely to develop osteoporosis and joint pains.

It is found to be highly beneficial in slowing down and lessening the effects of arthritis and reducing backache.

Research conducted towards the advantages of vitamin D has shown that it can be helpful in preventing certain types of cancers.

The symptoms of vitamin D deficiency include:

Rickets: It is the most frequent vitamin D deficiency symptom in children. As a result of vitamin D deficiency, the bones are weakened, the bone tissue fail to mineralize leading to soft and deformed bones.

Osteoporosis: It is a similar symptom to ricket, but it is found as vitamin D deficiency symptom in adults. Because of the low vitamin D level in the body, there is deficient calcification in bones, which become brittle and soft.

Depression and fatigue: According to some scientists, depression is the result of the lack of vitamin D. They argue that due to urbanization, the sunlight cannot adequately reach to the skin, reducing thus the 25 (OH) level in the body, causing depression. It has been also discovered that a great number of people suffer from seasonal affective disorder during the winter due to insufficient exposure to direct sunlight. The parathyroid hormone is the one causing factor for vitamin D deficiency symptom—depression.

Hyperparathyroidism: Results from hypocalcemia, which is a blood condition with unusually low vitamin D level, may cause the hyperparathyroidism.

Obesity: Vitamin D deficiency is frequently linked to obesity, as the insufficient level of vitamin D holds back the production of hormone, leptin, which regulates the fat in the body. Inadequate exposure to sunlight disrupts the normal function of the body, determining the individual to eat more than it is necessary for the body.

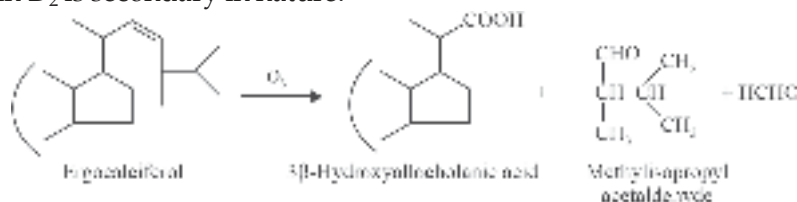
Vitamin D₂ (Ergocalciferol)

It is available as white, colorless crystal, insoluble in water, soluble in organic solvents, and slightly soluble in vegetable oils.

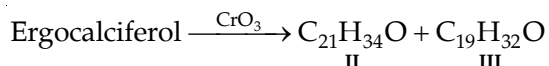
Structural Elucidation of Vitamin D₂

(i) **Molecular formula:** It is found to be C₂₈H₄₄O.

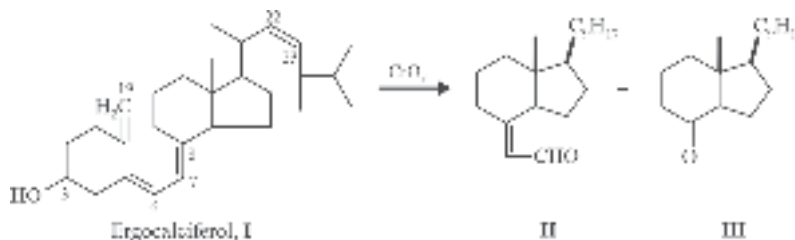
(ii) **Presence of hydroxyl group:** Formation of monoesters indicates the presence of one hydroxyl group. Ergocalciferol on oxidation gives ketone, so hydroxyl group in vitamin D₂ is secondary in nature.



- (iii) **Nature of side chain:** Like ergosterol, ergocalciferol on ozonolysis gives methylisopropyl acetaldehyde. This can be explained only if it has same side chain as present in ergosterol structure.
- (iv) **Number of rings:** On catalytic hydrogenation, ergocalciferol is converted into a saturated compound, octahydroergocalciferol. This reaction indicates the presence of four double bonds. The parent hydrocarbon of ergocalciferol is $C_{28}H_{52}$ which corresponds to general formula C_nH_{2n-4} for tricyclic ring skeleton. Out of four double bonds, one double bond must be present in the side chain (as in ergosterol structure) to give the reason for the formation of methylisopropylacetaldehyde (as side chain) while three of them in the nucleus.
- (v) Further ergocalciferol does not give Diel's hydrocarbon on distillation with selenium. It indicates that ergocalciferol does not contain four rings system and one of the rings of ergosterol is opened during irradiation to form vitamin D_2 .
- (vi) The UV spectrum of the vitamin indicates the presence of conjugated triene system in the nucleus, which is confirmed by the formation of adduct with maleic anhydride.
- (vii) On oxidation, the ergocalciferol gives the two compounds, **II** and **III**.

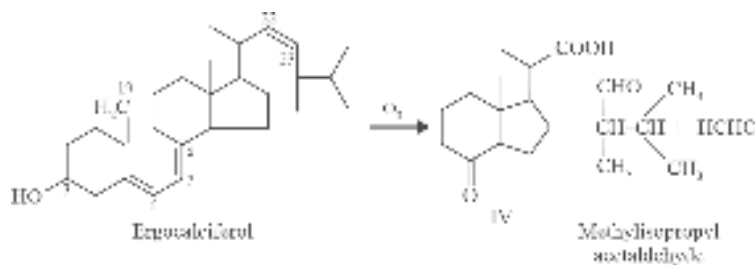


- (a) The absorption spectrum of semicarbazone of **II** ($C_{21}H_{34}O$) is shown to be the characteristic of α,β -unsaturated aldehydes (λ_{max} 275 nm).
- (b) The absence of hydroxyl group and carbon content of **II** indicates the absence of ring A. These suggest that in ergocalciferol ring B is open at C-9, 10 and scission of double bond at C-5, 6 position to produce the compound, **II**.
- (c) The formation of ketone, **III** confirms the presence of double bond at C-7, 8.

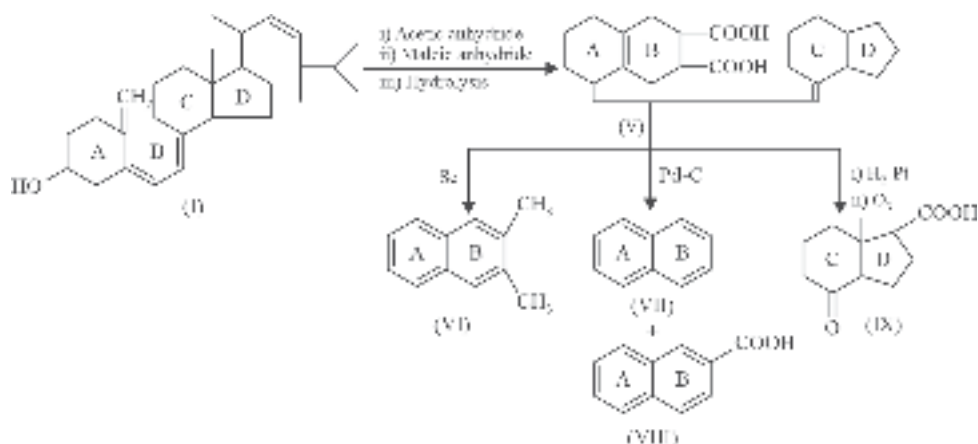


- (d) The isolation of formaldehyde during the ozonolysis shows the presence of exocyclic methylene group at C-10 position (see point iii).
- (e) The formation of a keto acid, **IV** during ozonolysis (see point iii) suggests that ring B is opened at C-9, 10 and two double bonds are present at C-7, 8 and C-22, 23. The position of latter double bond is further confirmed by the formation of methylisopropylacetaldehyde. On the basis of these evidences, the structure, I is assigned to ergocalciferol which can explain the formation of compounds, II and III.

The assigned structure, to ergocalciferol also explains the formation of compounds, II and III. It also justify the formation of methyl isopropyl acetaldehyde along with formaldehyde and carboxy fragment as shown below.



(viii) The above structure is further confirmed by following sequence of reactions (Scheme 11.1). Ergocalciferol acetate forms an adduct with maleic anhydride which on hydrolysis gives the dicarboxylic acid, V. The structure of compound, V is proved with the formation of the products (VI), (VII), (VIII) and (IX).



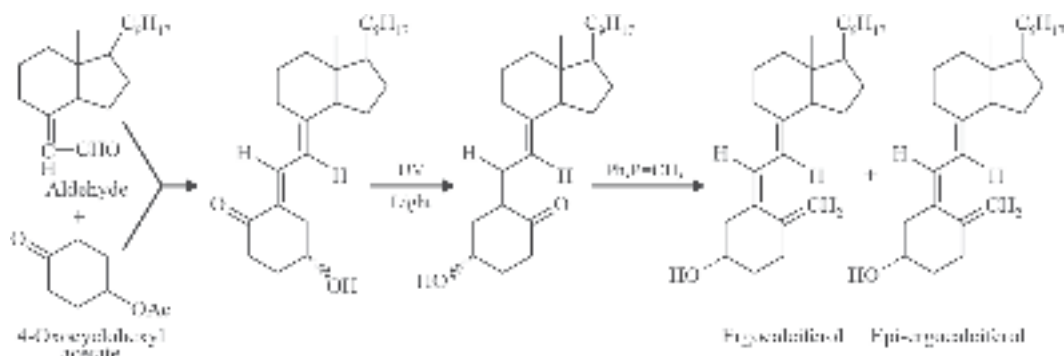
Scheme 11.1: Reactions of ergocalciferol

Formation of 2,3-dimethylnaphthalene, **VI** is justified with the fact that carboxyl groups sometimes give methyl groups on selenium dehydrogenation. Similarly, formation of naphthalene, **VII** and naphthalene-2-carboxylic acid, **VIII** shows the presence of rings A and B in (V).

Catalytic reduction of compound, **V** (which is used to reduce the double bond in the side chain only), followed by ozonolysis, gives (IX).

The above point establishes the structure of (V) and shows the position of double bonds at C-5, 6, C-10, 19 and C-7, 8 positions.

Lastly, the proposed structure is confirmed by synthesis. The complete synthesis of ergocalciferol is not yet established. The partial synthesis of ergocalciferol from aldehyde (II) is as follows:



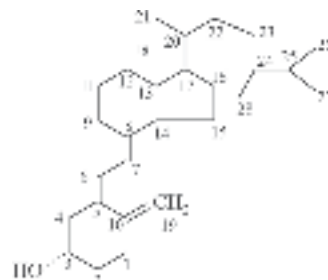
Spectral Data

UV (nm): λ_{max} 265.

IR (cm^{-1}): 3289 (OH str.), 1650–1600 (conjugated C = C).

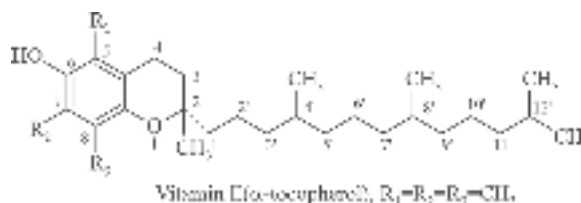
$^1\text{H-NMR}$ (δ) (CDCl_3): 0.54 (s, C-18, CH_3), 0.85 (d, C27 CH_3), 0.88 (δ , C-24 CH_3), 0.92 (d, C-21 CH_3), 2.55 (dd, C-24 H), 2.80 (dd, C-9 βH), 3.94 (m, C-3 αH), 4.83 (d, C-19 EH), 5.05 (dd, C-19 ZH) (gem-vinylic proton), 6.03 (d, C-7H), 6.24 (d, C-6H); $^{13}\text{C-NMR}$ (δ) (D_2O): 31.95 (C-1), 35.15 (C-2), 69.17 (C-3), 45.90 (C-4), 145.05 (C-5), 122.41 (C-6), 117.47 (C-7), 142.26 (C-8), 29.02 (C-9), 135.04 (C-10), 22.25 (C-11), 40.52 (C-12), 45.83 (C-13), 56.33 (C-14), 23.18 (C-15), 27.60 (C-16), 56.56 (C-17), 112.41 (C-19), 36.14 (C-20), 18.84 (C-21), 36.14 (C-22), 23.86 (C-23), 39.49 (C-24), 28.01 (C-25), 22.83 (C-26), 22.57 (C-27).

MS (m/e): 384 (M^+), 351, 271 (loss of alkyl chain).

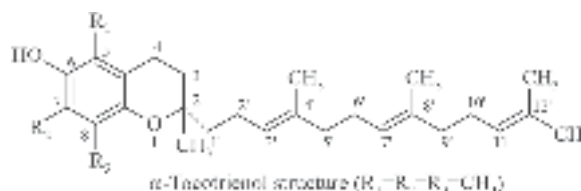


Vitamin E

Vitamin E is a fat soluble antioxidant vitamin that helps to neutralize potentially damaging free radicals in our body. The vitamin can exist as two types of structures: the tocopherol and tocotrienol structures. Both structures are composed of a substituted chromanol ring with a 16 carbon atoms saturated side chain in tocopherols, and with 3 double bonds in tocotrienols. There are many derivatives of these structures due to the different substituents possible on the aromatic ring at C-5, 6, 7, and 8 positions (Table 11.1).



The tocotrienols share the same ring structure, but have an unsaturated tail.



Depending upon the substituent present, naturally occurring vitamin E exists in eight chemical forms (α -, β -, γ - and δ -tocopherol and α -, β -, γ -, and δ -tocotrienol) that have varying levels of biological activity. The α -tocopherol is the only form that is recognized to meet human requirements. Of its eight different forms, α -tocopherol is the most active and one of the most powerful biologically active antioxidants.

In tocopherol, there are three asymmetric carbon atoms (2, 4' and 8') and exist in eight isomeric structures. In contrast in trienol, there is only one asymmetric carbon atom.

Table 11.1: Types of vitamin E

<i>Position of methyl groups in aromatic ring</i>	<i>Tocopherol structure</i>	<i>Tocotrienol structure</i>
5, 7, 8	α -Tocopherol	α -Tocotrienol
5, 8	β -Tocopherol	β -Tocotrienol
7, 8	τ -Tocopherol	τ -Tocotrienol
8	δ -Tocopherol	δ -Tocotrienol

Numerous foods provide vitamin E. Nuts, seeds, and vegetable oils are among the best sources of α -tocopherol, and significant amounts are available in green leafy vegetables and fortified cereals.

Functions of Vitamin E in the Body

Antioxidant: As an antioxidant, vitamin E intercepts free radicals and prevents cell damage. It particularly protects red blood cells.

Vitamin maintenance: Vitamin E also assists in the maintenance of vitamins A and C in the body.

Skin: Vitamin E supports healthy skin and assists in cell production. It protects against scar tissue and skin inflammation.

Immune system: As part of the immune system, vitamin E enhances antibody formation and benefits people suffering from a cold or other viral infection.

Organ functions: Vitamin E is essential for cardiovascular health and for sex organ functioning.

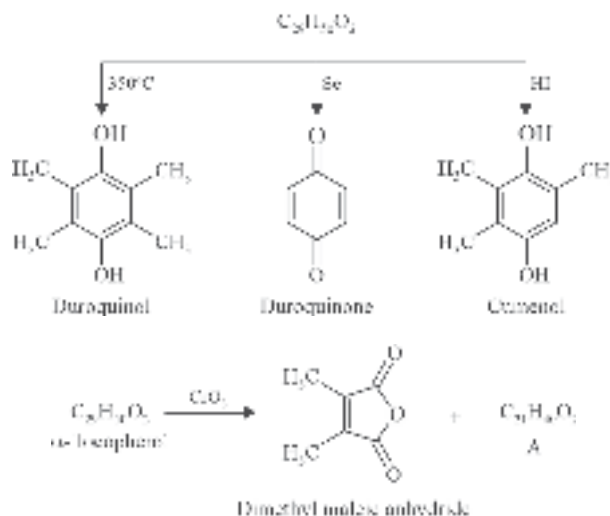
Specific conditions: Vitamin E may provide some relief for menstrual pain, premenstrual syndrome, menopausal symptoms and muscle and joint pain.

Deficiency signs of vitamin E include:

- Mild anemia
- Nonspecific neurological deficits
- Disorders related to reproduction and infertility
- Fragile red blood cells
- Cataracts
- Certain neurological damage
- Decrease in sex drive
- Muscle, liver, bone marrow and brain function abnormalities

Structural Elucidation of α -Tocopherol

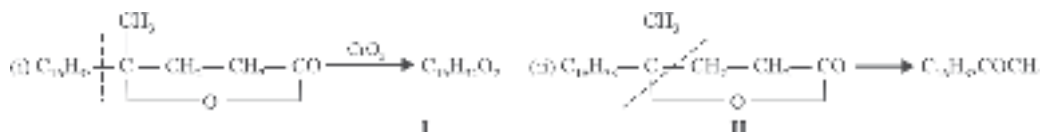
- Molecular formula:** It is found to be $C_{29}H_{50}O_2$.
- Presence of hydroxyl group:** Formation of monoacetate derivative indicates the presence of one hydroxyl group. The UV spectrum or color reaction with ferric chloride solution indicates that hydroxylic group is phenolic in nature.
- Presence of ether group:** The second oxygen atom is found to be in the form of cyclic ether due to its inertness.
- When α -tocopherol is heated at 350°C , duroquinol is obtained which indicates the presence of benzenoid nucleus.
- Oxidation of α -tocopherol with chromic acid forms dimethylmaleic anhydride and a compound, **A** having molecular formula $C_{21}H_{40}O_2$.



The compound, A is found to be optically active lactone. It is shown to be derived from a γ -hydroxy acid in which the hydroxyl group is tertiary due to resistant to oxidation. The salt of this acid lactonises immediately on treatment with acid. Thus, on this basis, the partial structure for lactone can be given as shown here.



- (vi) Now, the oxidation of α -tocopherol acetate with chromic acid produces an acid $C_{16}H_{32}O_2$ (I) and a ketone $C_{18}H_{36}O$ (II). Both of these compounds are produced by the oxidative cleavage of lactone at different points in the chain. According to Fernholz, if in the lactone, $R_1 = C_{16}H_{33}$ and $R_2 = CH_3$, then the formation of products (I) and (II) can be predicted as:



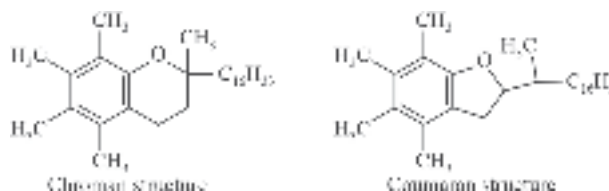
So, then Fernholz showed that the acid, I contained methyl groups and thus proposed a structure to acid, I based on the isoprene unit.



The above evidences indicate the presence of benzenoid nucleus and long side chain in α -tocopherol.

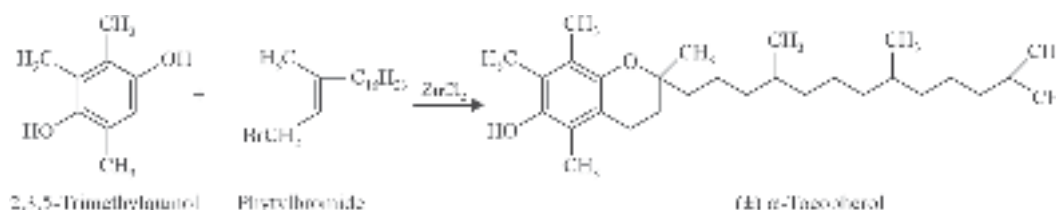
- (vii) The rate of oxidation reaction of monoethers of duroquinol with silver nitrate is compared with α -tocopherol under the same conditions. It is found that duroquinol is oxidized at very slow rate in compared to α -tocopherol. Again duroquinol is oxidized to duroquinone while α -tocopherol into red oil having the same molecular weight. Since duroquinone is not split off during this oxidation, it is predicted that side chain is connected to the aromatic ring by a carbon bond as well as an ether linkage. Thus, α -tocopherol is either a chroman or coumaran derivative. According

to Fernholz, the oxidation products are best explained on the basis of chroman structure. This is supported by ultraviolet absorption spectrum of α -tocopherol.

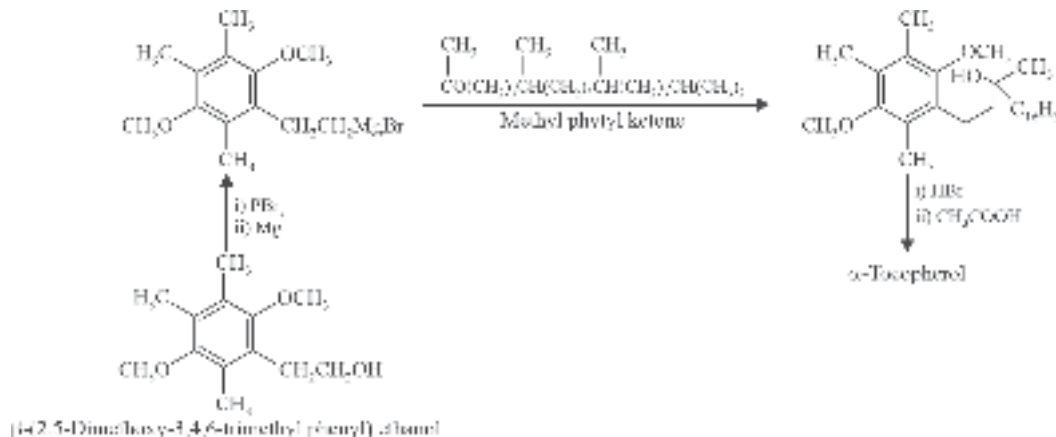


(viii) Lastly, the structure is confirmed by its synthesis:

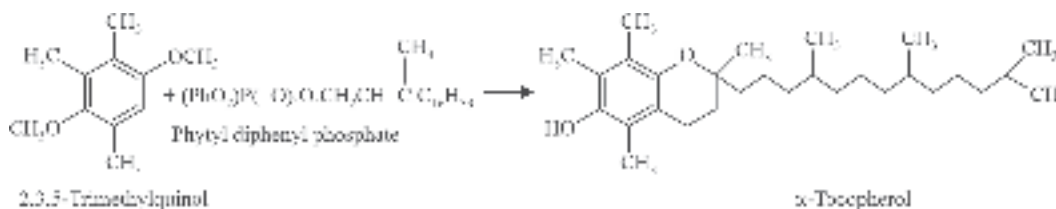
(a) **Karrer et al. synthesis:** The α -tocopherol is synthesized by condensation reaction of trimethylquinol with phytol bromide.



(b) **Smith et al. synthesis:** In this, α -tocopherol is synthesized from methyl phytol ketone.



(c) **Miller et al. synthesis:** In this, the tocopherol is synthesized by heating 2,3,5-trimethylquinol with phytol diphenyl phosphate (3,3-dialkyl allyl diphenyl phosphate ester).



Spectral Data

UV (nm): 295 (C_2H_5OH).

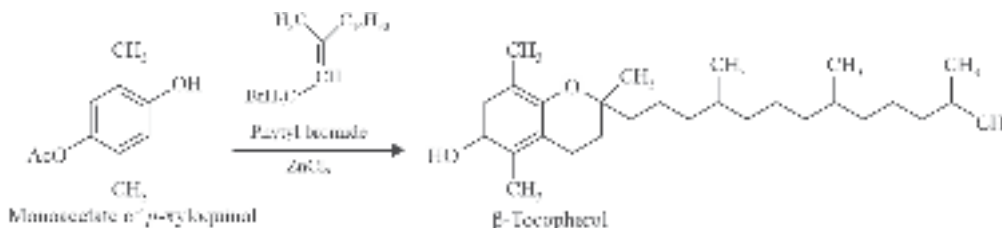
¹H-NMR (δ): 0.90 (12H, m, CH₃), 1.0–1.9 (26H, m, CH₃–2, CH₂, CH), 2.10 and 2.17 (9H, both and CH₃–Ar), 2.61 (2H, t, 4-H) J = 6.7), 4.3 (1H, s OH).

¹³C-NMR (δ): 11.26, 11.75 and 12.19 (CH₃-Ar), 19.67 and 19.75 (4'-CH₃, 8'-CH₃), 20.79 (12'-C), 22.62 and 22.70 (12'-CH₃), 24.46 and 24.81 (2'-C, 6'-C, 10'-C), 74.55 (2-C), 116.38 (5-C), 118.51 (10-C), 121.05 (8-C), 122.64 (7-C), 144.57 (9-C), 145.63 (6-C).

 Trinitol

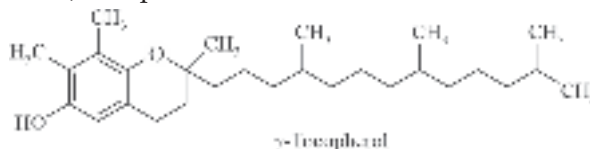
 p-Xylenol

- (i) **Molecular formula:** It is found to be $C_{28}H_{48}O_2$ which differ from α -tocopherol by CH_2 moiety.
- (ii) On thermal decomposition, it gives Trimethylquinol.
- (iii) On heating with hydroiodic acid, it gives *p*-xylenol.
- (iv) When oxidized with chromic acid, it gives the same lactone, $C_{21}H_{40}O_2$ as given by α -tocopherol.
- (v) The above evidences indicates that α -tocopherol differs from β -tocopherol, $C_{21}H_{40}O_2$ in having one more methyl group in benzene ring.
- (vi) Finally, the structure is confirmed by synthesis, starting from the monoacetate of *p*-xyloquinol and phytyl bromide.

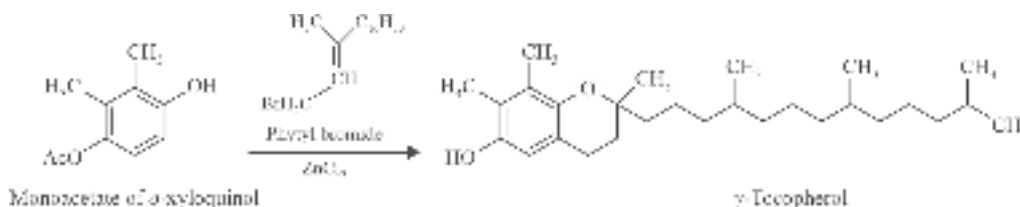


- (i) This is isomeric with β -tocopherol; the only difference is the positions of the two methyl groups in the benzene ring.
- (ii) When heated with HI, it gives *o*-xyloquinol.

Thus, the structure of γ -tocopherol is:

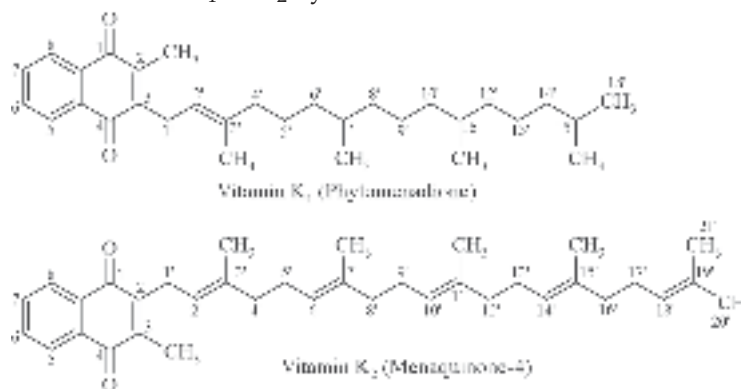


This structure has been confirmed by synthesis, starting from the monoacetate of *o*-xyloquinol and phytol bromide.



Vitamin K

Vitamin K is a group of structurally similar, fat soluble vitamins that are needed not only for blood coagulation, but also involved in metabolic pathways in bone and other tissue. Chemically, these are 2-methyl-1,4-naphthoquinone derivatives. This group of vitamins includes two natural vitamins: vitamin K₁ and vitamin K₂. Vitamin K₁ was isolated from alfalfa by Dam et al. and Doisy et al. in 1939 and K₂ from putrefied fish meal by Doisy et al. in 1939. Vitamin K₁, also known as phyloquinone or phytomenadione, is synthesized by plants and found chiefly in leafy green vegetables such as spinach, cabbage, kale, cauliflower, broccoli, and brussels sprouts; some fruits such as avocado, kiwifruit and grapes are also high in vitamin K. Vitamin K₂ (menaquinone-4) is synthesized in animal tissues and is found in meat, eggs, and dairy products. Vitamin K₂ homologs (menaquinones) are characterized by the number of isoprenoid residues comprising the side chain. Menaquinones are abbreviated MK-n, where 'n' represents the number of isoprenoid side chains. Thus, menaquinone-4 abbreviated MK-4, has 4 isoprene residues in the side chain. Bacteria can produce a range of vitamin K₂ forms, including the conversion of K₁ to K₂ by bacteria in the small intestines.



Physiological Effects of Vitamin K

Vitamin K serves as an essential cofactor for a carboxylase that catalyzes carboxylation of glutamic acid residues on vitamin K-dependent proteins.

It acts as a cofactor to the carboxylase that generates λ -carboxyglutamic acid, vitamin K undergoes a cycle of oxidation and reduction that allows its reuse. The essential details of this cycle are illustrated in Fig. 11.2.



Fig. 11.2

- Vitamin K (usually K₁) is reduced to vitamin KH₂.
- Oxygenation of vitamin KH₂ provides the energy to drive the carboxylation reaction, leading to formation of γ -carboxyglutamic acid residues and vitamin K oxide.

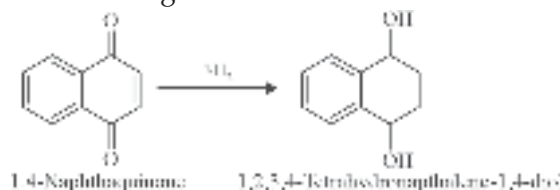
- Vitamin K oxide is reduced by another reductase back to vitamin K, ready to enter another cycle. Anticoagulants such as Warfarin block the reduction of vitamin K oxide to vitamin K, explaining their antagonistic effects on this cycle (Fig. 11.2).

Vitamin K₁

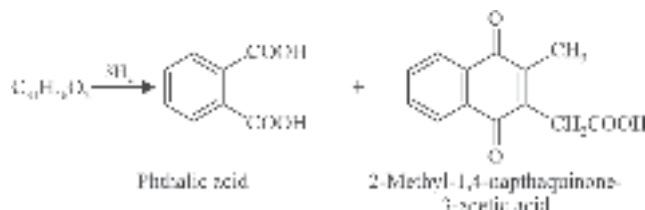
Chemically, it is 2-methyl-3-phytyl-1,4-naphthoquinone, which is available as yellow colored oil.

Structural Elucidation of Vitamin K₁

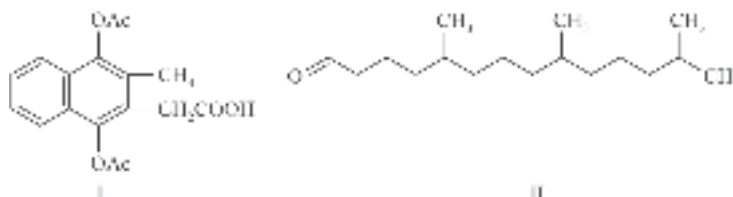
- Molecular formula:** It is found to be C₃₁H₄₆O₂.
- Presence of 1,4-naphthaquinone structure:** The UV spectrum of vitamin K shows absorption maxima at 243, 249, 260, 270 nm (all with $\epsilon \sim 20,000$), and 325 nm ($\epsilon \sim 3,000$). These absorption maxima corresponds to the absorption maxima of 2,3-dimethyl-1,4-naphthaquinone. Thus, vitamin K₁ appears seems to be 1,4-naphthaquinone derivatives. This fact is further supported by the fact that the vitamin is very sensitive to light and alkalis.



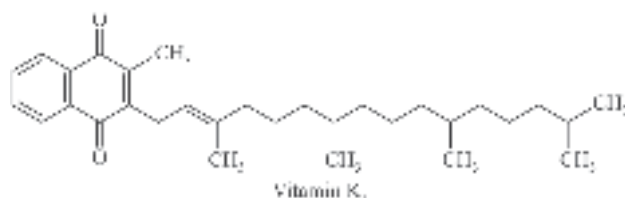
- Presence of ethylenic bond:** On catalytic hydrogenation, this yellow colored oil gets converted into colorless compound with the consumption of four molecules of hydrogen. Since, it can be predicted that 1,4-naphthaquinone is reduced under these conditions by consuming three molecules of hydrogen, the addition of a fourth hydrogen molecule suggests the presence of an ethylenic double bond in a side chain.
- Vitamin K₁ on oxidation with chromic acid gives phthalic acid but when the oxidation is carried out under controlled conditions, it also gives a compound having molecular formula, C₁₀H₁₀O₄. This compound is identified as 2-methyl-1,4-naphthaquinone-3-acetic acid. Formation of unsubstituted phthalic acid indicates that benzene ring is unsubstituted while 2-methyl-1,4-naphthaquinone-3-acetic acid reveals that C-2 position in naphthaquinone ring is substituted with methyl group and other group is present at C-3 position.



- On acetylation under reducing conditions, vitamin K₁ is converted into the diacetate of dihydrovitamin K₁ and this diacetate is difficult to hydrolyse, which is a characteristic property of 2,3-disubstituted 1,4-naphthaquinones. On ozonolysis, the diacetate of dihydrovitamin K₁ gives an acid (I) and a ketone (II). The ketone is found to be identical with the ketone, obtained by the oxidation of phytol.



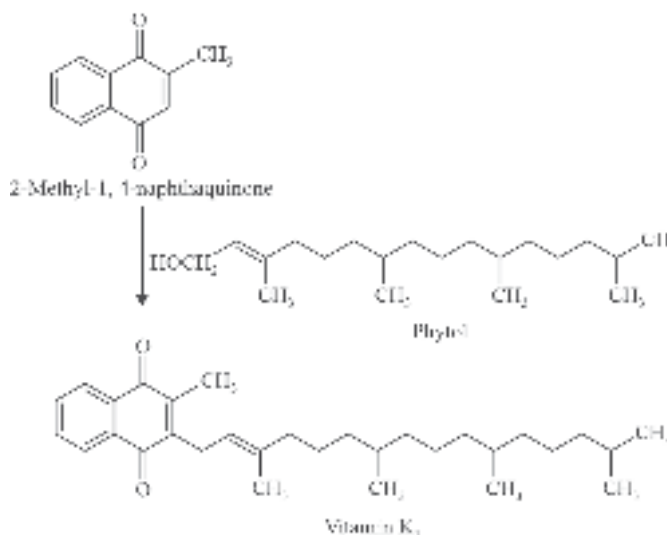
- (vi) The formation of compound, II indicates that the phytyl radical as side chain is attached to C-3 position is phytyl radical. The later is attached is through the carbon atom $>C=O$ in II, to the carbon atom appearing as COOH in compound, I by means of a double bond. On the above basis, under given structure is assigned to vitamin K₁.



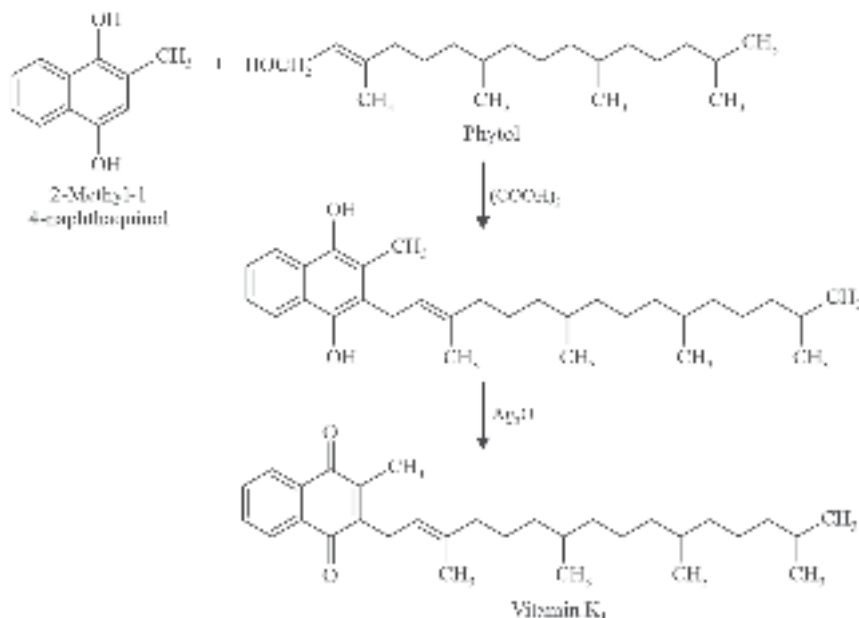
- (vii) The final proposed structure is proved by its synthesis.

Synthesis of Vitamin K₁

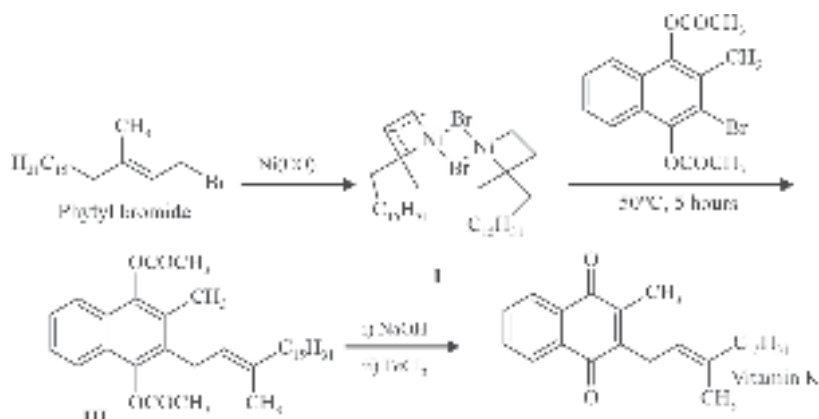
- (a) Almquist et al. obtained vitamin K₁ by condensing 2-methyl-1,4-naphthaquinone with phytol.



- (b) Fieser et al. has obtained a better yield about 25% by heating 2-methyl-1,4-naphthaquinol with phytol in dioxane solution in the presence of anhydrous oxalic acid, then the product, dihydrovitamin K₁ was oxidized with silver oxide in ether. The low yield is due to the formation of by product, 2-3-dihydro-2-methyl-2-phytyl-1,4-naphthaquinone.



- (c) **Wendler synthesis:** In 1954, Wendler et al. has modified the Fieser method by condensing 1-acetyl derivative of 2-methyl-1,4-naphthoquinol with phytol in presence of BF_3 and obtained vitamin K₁ in good yield.
- (d) **Sato synthesis (1972)** synthesized vitamin K₁ using a π -allylic nickel (I) complex. Reaction of phytol bromide with nickel carbonyl in benzene solution under nitrogen gave the π -allylic nickel (I) bromide. Benzene solvent was replaced by hexamethylphosphoramide as solvent and 2-bromo-3-methyl-1,4-diacetoxynaphthalene **II** was added. The product on chromatography on silica gel gave **III**, which was hydrolyzed by alkali to produce **IV**. This compound, **IV** was oxidized with ferric chloride to give vitamin K₁ in high yield.



Spectral Data

UV (nm) (pet. ether): 242, 248, 260, 269, 325.

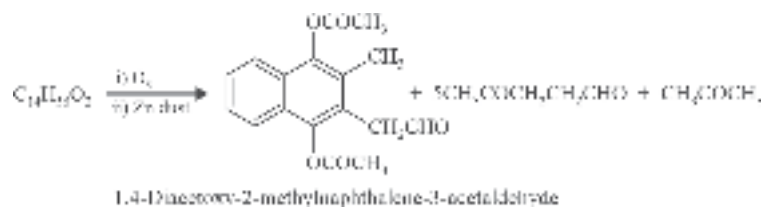
IR (cm^{-1}): 2980, 1690, 1627, 1350.

¹H-NMR (CDCl₃) (δ): 0.82 (C-11' CH₃), 0.82 (C-7' CH₃), 0.860 (C-15', C-16'-CH₃), 0.95–1.45 (m, 8 × CH₂), 1.51 (C-15' H), 1.782 (C-3'-CH₃), 1.94 (C-4' CH₂), 2.18 (C-2-CH₃), 3.36 (C-1'-CH₂), 5.01 (C-2' H), 7.67 (2H, C-6, C-7, J = 6.7 Hz), 8.06 (2H, C-5, C-8, J = 6.7 Hz).

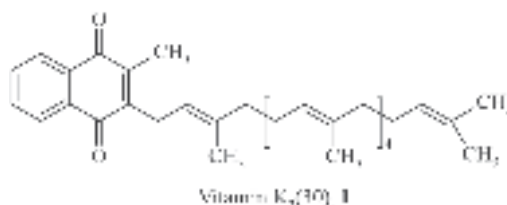
Vitamin K₂

Structural Elucidation of Vitamin K₂

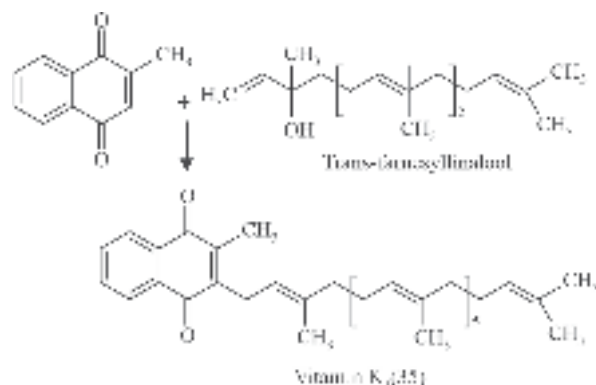
- (i) **Molecular formula:** It is found to be C₄₁H₅₆O₂.
- (ii) It gives the same UV spectrum as vitamin K₁. This gives an indication of presence of same 1,4-naphthaquinone moiety as present in vitamin K₁. Due to presence of this moiety, it is sensitive to light and alkalies.
- (iii) On catalytic reduction, it consumes 9 molecules of hydrogen. The three molecules of hydrogen can be accounted for 1,4-naphthaquinone. The consumption of six hydrogen molecules indicates the presence of six double bonds, may be present in the side chain.
Since, this does not form any adduct with maleic anhydride, so there is no conjugation system. Presence of six double bonds are further shown by the fact that diacetate of dihydrovitamin K₂ (obtained by reductive acetylation of vitamin K₂) absorbs six molecules of bromine.
- (iv) On oxidation with potassium permanganate, it forms phthalic acid, which shows that benzene ring is unsubstituted.
- (v) On ozonolysis of one molecule of vitamin K₂ in acetic acid, followed by treatment with zinc dust, five molecules of levulaldehyde, one molecule of acetone, and one molecule of 1,4-diacetoxy-2-methylnaphthalene-3-acetaldehyde are produced.



- (vi) On the basis of above evidence, the below given structure, **I** is assigned to vitamin K₂.



- (vii) The final structure for vitamin K₂ is given on the basis of its total synthesis. **Isler** et al. carried by the synthesis of vitamin K₂ and observed that it is not identical with assigned structure of vitamin K₂. Then, on further workup by synthesis, it is found that vitamin K₂ contains a C₃₅ side chain and not C₃₀ side chain as proposed above in structure, **I**. The vitamin K₂ is synthesized by condensing methyl-1,4-naphthaquinone with *trans*-farnesyllinalool.



Spectral Data

¹H-NMR (δ): 3.37 (H-1'), 5.06 (m, H-2', 6', 10', 14'), 7.68 (H-6, 7), 8.08 (H-5, 8).

WATER SOLUBLE VITAMINS

Water-soluble vitamins cannot be stored in the body, so these are obtained from diet every day. They are destroyed by overcooking. These are easily absorbed by the body. Human body does not store large amounts of water-soluble vitamins. B-complex vitamins and vitamin C are water-soluble vitamins that are not stored in the body and must be replaced each day.

VITAMIN C

Vitamin C, also known as **ascorbic acid**, is water-soluble, and probably the most famous among the vitamins. Even before its discovery in 1932, physicians recognized that there must be a compound in citrus fruits preventing scurvy, a disease that killed as many as 2 million sailors between 1500 and 1800.

Vitamin C is widely distributed in fruits and vegetables. Citrus fruits, peppers, green vegetables (e.g. broccoli, Brussels sprouts), and fruits like strawberries, guava, mango and kiwi are particularly rich sources.

Functions of Vitamin C

The most prominent role of vitamin C is its immune stimulating effect, which is important for the defence mechanism against infections such as common colds. It also acts as an inhibitor of histamine, a compound that is released during allergic reactions. As a powerful antioxidant, it can neutralize harmful free radicals and aids in neutralizing pollutants and toxins. Thus, it is able to prevent the formation of potentially carcinogenic nitrosamines in the stomach (due to consumption of nitrite-containing foods, such as smoked meat). Importantly, vitamin C is also able to regenerate other antioxidants such as vitamin E. Vitamin C is required for the synthesis of collagen, the intercellular "cement" substance which gives structure to muscles, vascular tissues, bones, tendons and ligaments. Due to these functions, vitamin C especially in combination with zinc, is important for the healing of wounds. Vitamin C contributes to the health of teeth and gums, preventing hemorrhaging and bleeding. It also improves the absorption of iron from the diet, and is needed for the metabolism of bile acids, which may have implications for blood cholesterol levels and gallstones. In addition, vitamin C plays an important

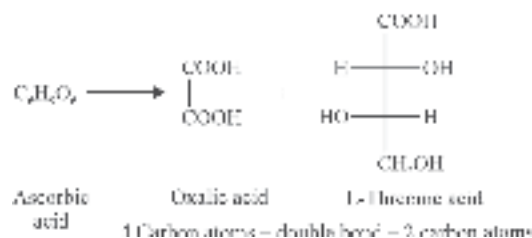
role in the synthesis of several important peptide hormones and neurotransmitters and carnitine. Finally, vitamin C is also a crucial factor in the eye's ability to deal with oxidative stress, and can delay the progression of advanced age-related macular degeneration and vision-loss in combination with other antioxidant vitamins and zinc.

Deficiency sign

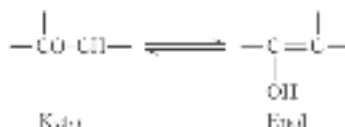
Early symptoms of vitamin C deficiency include fatigue, loss of appetite, drowsiness and insomnia, irritability, low resistance to infections and petechiae (minor capillary bleeding). Severe vitamin C deficiency leads to scurvy, characterized by weakening of collagenous structures, resulting in widespread capillary bleeding. Infantile scurvy causes bone malformations. Bleeding gums and loosening of the teeth are usually the earliest signs of clinical deficiency. Hemorrhages under the skin cause extreme tenderness of extremities and pain during movement. If left untreated, gangrene and death may result.

Structural Elucidation of Vitamin C

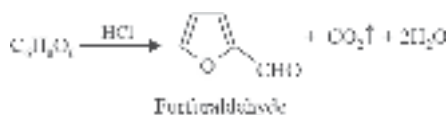
- (i) **Molecular formula:** It is found to be $C_6H_8O_6$.
- (ii) **Presence of double bond:** Formation of monoozonide structure on treatment with ozone shows the presence of one double bond.
- (iii) On oxidation with $KMnO_4$, vitamin C forms oxalic acid and threonic acid, which indicates that 6 carbon atoms of ascorbic acid molecule are adjusted into four carbon atoms system separated from another two carbon atom system by means of double bond.



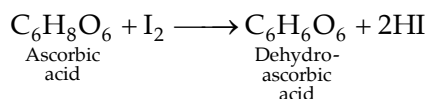
- (iv) **Presence of acidic group:** Formation of monosodium and monopotassium salt indicates the presence of acidic group.
- (v) **Presence of keto-enol system:** Ascorbic acid is an unsaturated compound; acts as a strong reducing agent; also forms a phenyl hydrazone and gives violet color with ferric chloride. All these reactions suggest the presence of keto-enol system in ascorbic acid structure. This is further confirmed by the formation of a dimethyl derivative with diazomethane. This dimethyl derivative does not give CH_3OH on treatment with alkali showing the absence of $-COOH$ group in the vitamin but presence of enolic group.



- (vi) On boiling with hydrochloric acid, vitamin C gives a furfuraldehyde. This reaction indicates the presence of at least five carbon atoms in a straight chain and also presence of numbers of hydroxyl groups.



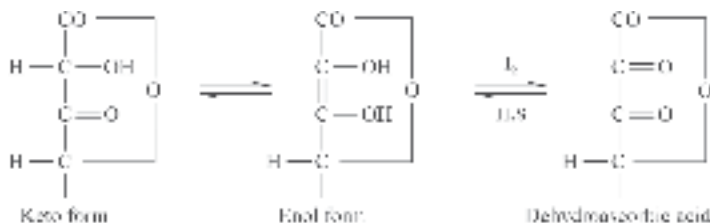
- (vii) On treatment with aqueous iodine solution, ascorbic acid gives dehydroascorbic acid. Two molecules of iodine are consumed and two molecules of hydrogen iodide are produced during the reaction.



Formation of dehydroascorbic acid provides the following conclusions:

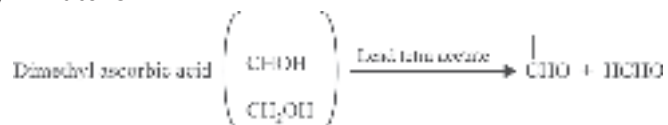
- The oxidation product, dehydroascorbic acid is a lactone and can be reconverted into ascorbic acid by reacting with hydrogen sulphide. Since, this oxidation-reduction process is carried out with the help of mild reagents, lactone structure is not disturbed and so lactone structure is also present in ascorbic acid.
- Dehydroascorbic acid forms osazone with phenylhydrazine, so it must have two carbonyl functional groups in adjacent position.

Thus, on the basis of above evidences, the partial structure indicating the presence of β -hydroxyl ketone grouping in ascorbic acid can be given which explains all the reactions.

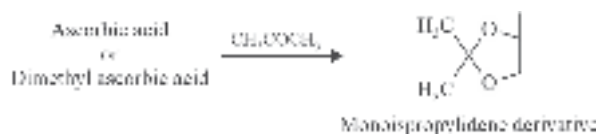


- (viii) **Presence of hydroxyl groups:** On acetylation, the dimethyl derivative of vitamin C forms diacetate indicating the presence of two alcoholic groups. This can be further confirmed by the following reactions:

- The dimethyl ascorbic acid on oxidation with lead tetraacetate gives HCHO. The formation of formaldehyde indicates that one of the alcoholic group is primary in nature.



- Ascorbic acid as well as its dimethyl derivative forms monoisopropylidene derivative, when condensed with acetone.



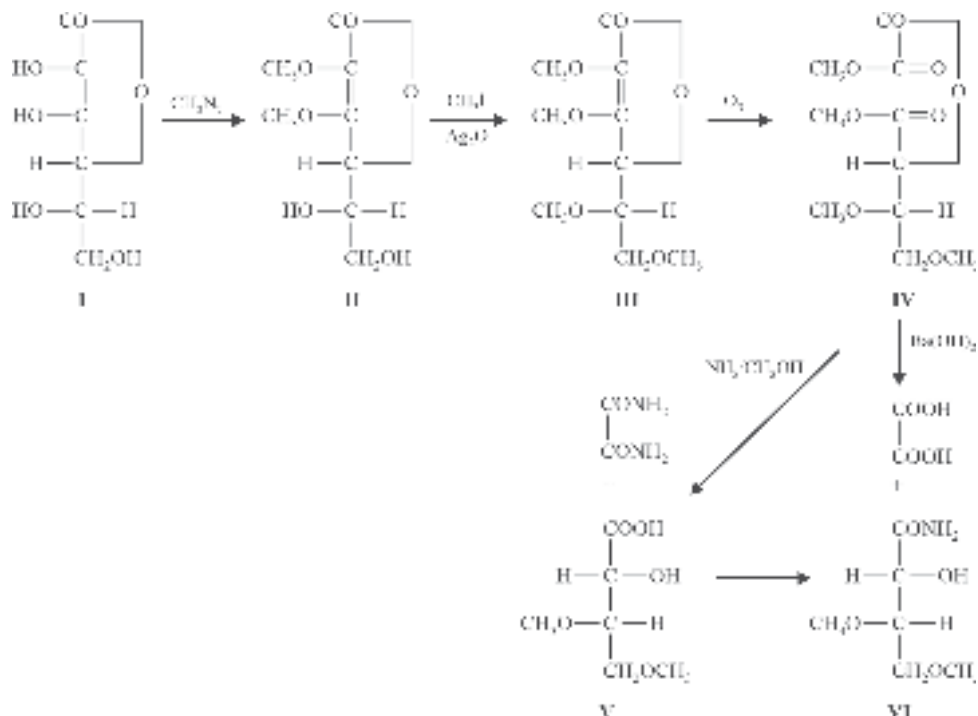
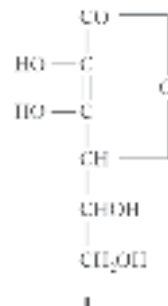
- Also, the presence of four hydroxyl group in the vitamin is proved by the formation of tetraacetate and tetramethyl derivatives on acetylation and methylation (Ag_2O/CH_3I), respectively.

So, the complete structure of ascorbic acid, **I** can be given as below:

Now, in this structure two points need further confirmation:

- (a) Size of the lactone.
 - (b) Configuration of C₄ and C₅.
- (ix) Size of the lactone is shown to be five membered by the following facts.

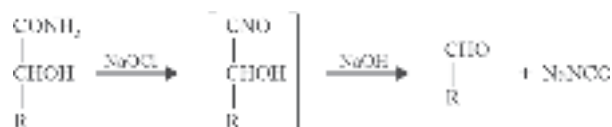
- (a) Ascorbic acid is stable towards alkali.
- (b) The rate of hydrolysis of dehydroascorbic acid corresponds to the rate of hydrolysis of λ -lactone.
- (c) Further the structure of the ascorbic acid is being confirmed by other evidences: Ascorbic acid when reacted with diazomethane forms dimethylascorbic acid, **II**; in this two methoxyl groups are most likely to be present at C-2 and C-3 position, since diazomethane readily methylates acidic hydroxyl group. This dimethyl derivative is neutral and dissolves in sodium hydroxide forming sodium salt without elimination of methyl group, which suggests two enolic hydroxyl groups are present and cannot be carbomethoxyl group.



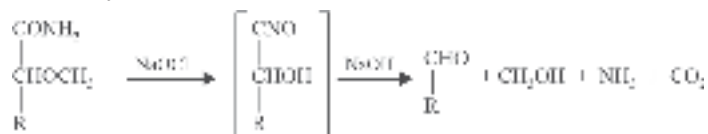
The similarity in both the structures of ascorbic acid as well as of its dimethyl derivative is shown by the fact that the absorption spectra of both are similar. When this dimethyl derivative is treated with methyl iodide in presence of silver oxide two new methyl groups are introduced to give **III**, as all methyl groups behaves as methyl ethers, it therefore follows that two alcoholic groups are present in dimethyl ascorbic acid. Ozonolysis of this tetramethyl derivative gives the neutral substance with the same number of carbon atoms but there is a breakage in carbon-carbon bond, so there must be a ring system present in the tetramethyl compound to hold together the two fragments **IV**. This ozonised

product, on hydrolysis with barium hydroxide, gives oxalic acid and dimethyl-L-threonic acid **V**. These products contain three carboxyl groups in all, and since ozonolysis of a double bond produces only two, the third carboxyl group must be present as a lactone in order that ascorbic acid should be neutral compound.

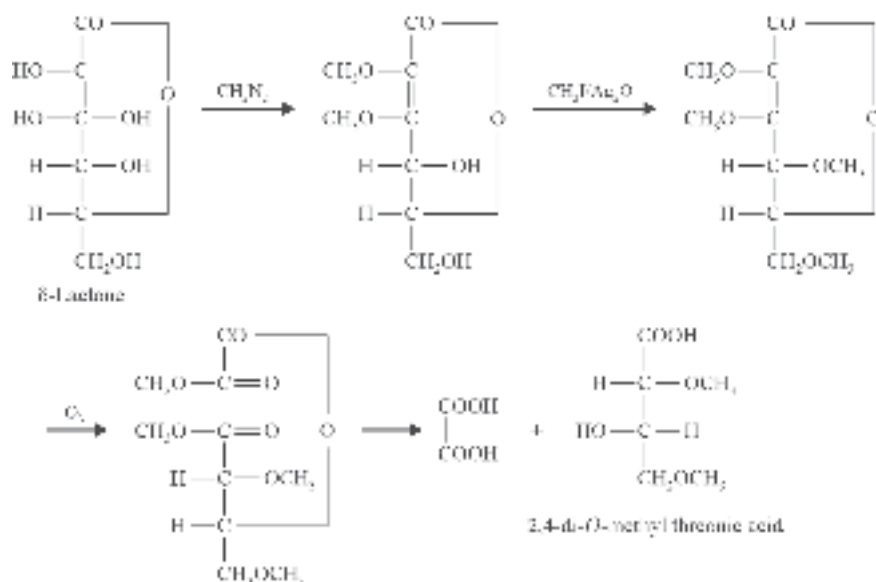
- (d) Dimethyl L-threonic acid, **V** is converted into amide, **VI** which gives **Weerman test** (specific test, used to detect the presence of free hydroxyl group in the α -position to an amide group e.g. an α -hydroxyamide). On treatment of methylated hydroxyamide with alkaline sodium hypochlorite gives an aldehyde and sodium cyanate. This reaction is only possible if there is a free hydroxyl group on the α -carbon atom with respect to amide group.



While, if there is no free OH group on α -carbon atom; for example, methoxy group is present on the α -carbon atom, then treatment with alkaline sodium hypochlorite produces an aldehyde, methanol, ammonia, and carbon dioxide.



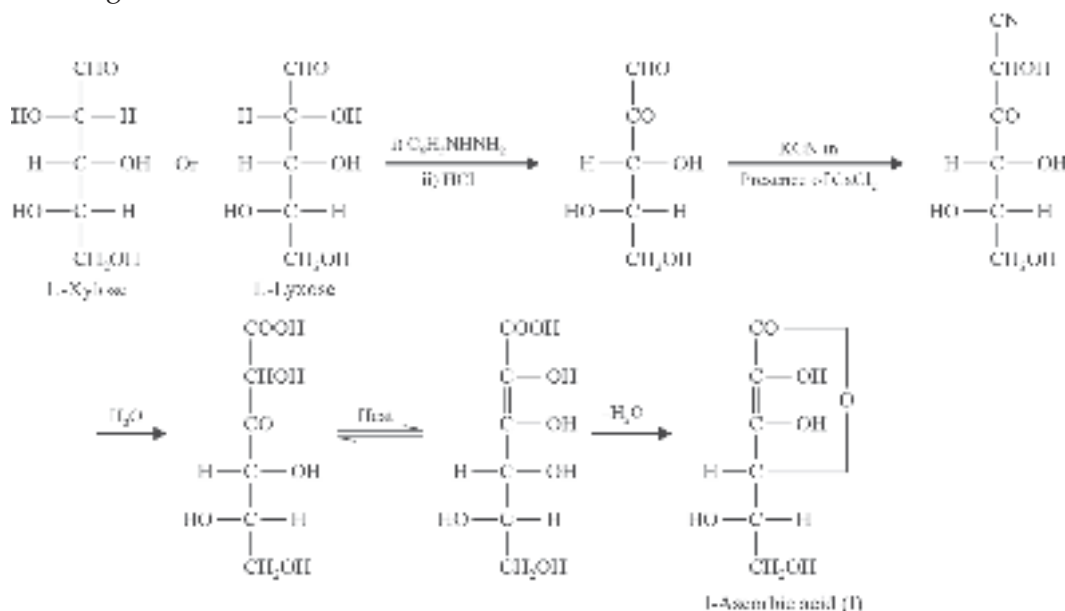
So, the compound, **VI** must contain an α -hydroxy amide group and thus, the compound **V** has a free alpha hydroxyl group. The formation of 3,4-di-O-methyl-L-threonic acid from compound, **IV** can only be possible ring is γ since δ lactone will form 2,4-di-O-methyl-L-threonic acid, which does not give Weerman test. The formation of amide, **VI** together with oxamide is also possible by the action of ammonia in methanol on compound, **IV**.



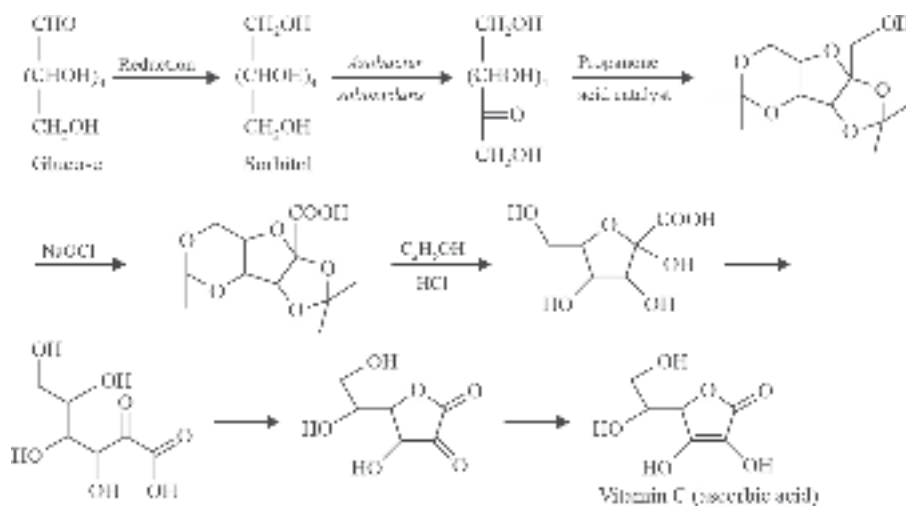
All these reactions are explained on the basis of structure, **I** (point ix) assigned to the ascorbic acid.

(x) Finally, the structure of Vitamin C (ascorbic acid) I is confirmed by its synthesis.

(a) **Haworth and Hirst et al. synthesis:** They have synthesized ascorbic acid from L-lyxose or L-xylose as the starting material. Both of these are obtained from D-glucose.



(b) Commercially, the Vitamin C is synthesized from glucose (**Gorlich's synthesis**) as given in Scheme 11.2.



Scheme 11.2: Synthesis of vitamin C from glucose

(c) A major component of plant ascorbate synthesis is through the 1-galactose pathway, in which GDP-mannose is converted to 1-ascorbate by four successive intermediates, as summarized in Fig. 11.3. Laing et al. identify homologous genes from Arabidopsis (VTC_2) and kiwi fruit encoding 1-galactose guanyltriferase that catalyzes the conversion of GDP-1-galactose to 1-galactose-1-P. This activity represents the second committed step in plant

vitamin C biosynthesis from GDP-D-mannose and the final gene to be cloned for the 1-galactose ascorbate synthesis pathway (Fig. 11.3).

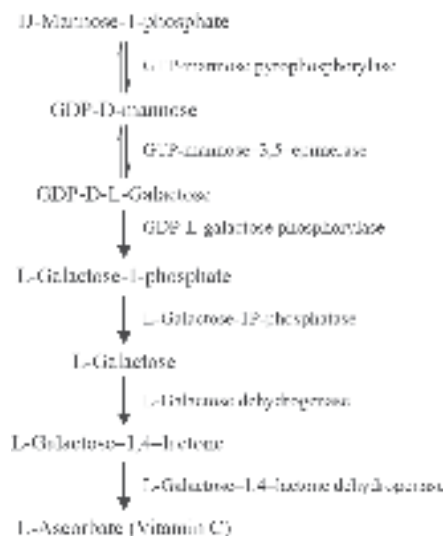


Fig. 11.3: L-galactose pathway

Spectral Data

UV (nm): λ_{248} (in water).

IR (cm^{-1} (KBr)) 3535, 3240, 3330 (O–H str.), 3002, 2978 (C–H str.), 1753 (C = O str.), 1670 (C = C str.), 1138 (C–O str.), 1065 (C–C str.)

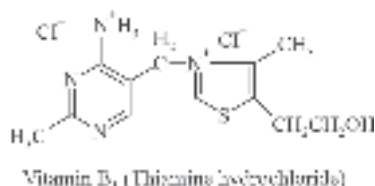
$^1\text{H-NMR}$ (δ): 3.81, 3.93, 5.0; $^{13}\text{C-NMR}$ (δ) 174.0, 156.3, 118.5, 69, 63.

VITAMIN B COMPLEX

Vitamin B complex contains mainly vitamin B₁ (Thiamine), vitamin B₂ (Riboflavin), vitamin B₃ (Niacin or Niacinamide), vitamin B₅ (Pantothenic acid), vitamin B₆ (Pyridoxine), vitamin B₇ (Biotin), vitamin B₉ (Folic acid), vitamin B₁₂ (Cyanocobalamin).

Vitamin B₁

Vitamin B₁ also called thiamine, is one of the 8B complex vitamins. The name B₁ given because it is the first B vitamin to be discovered. Vitamin B₁ is found in brown rice, lentils and peas among other things. A lack of vitamin B₁ causes the deficiency disease, **beriberi**. It occurs in all cells as its pyrophosphate ester. Thiamine is obtained as white crystalline hydrochloride salt, which has slight odor and bitter taste.

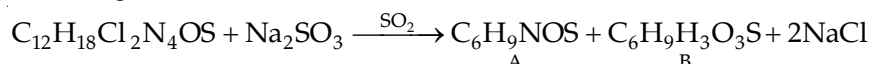


Function of Vitamin B₁

- Essential for converting carbohydrate into energy.
- Helps in maintaining healthy nervous system.
- Necessary for healthy mucous membranes.

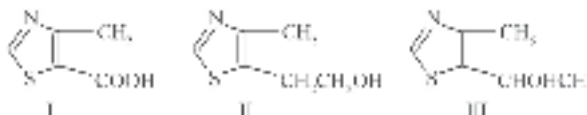
Structure Elucidation of Vitamin B₁ (Thiamine)

1. **Molecular formula:** The molecular formula of thiamine chloride monochloride salt is found to be C₁₂H₁₈Cl₂N₄OS.
2. **Treatment with sodium sulphite solution:** On treatment with a saturated solution of sodium sulphite in the presence of sulphur dioxide at room temperature, thiamine salt is decomposed quantitatively into two compounds, **A** and **B**. Hence, it is necessary to elucidate the structure of compound, A and B to get the structure of Vitamin B₁.

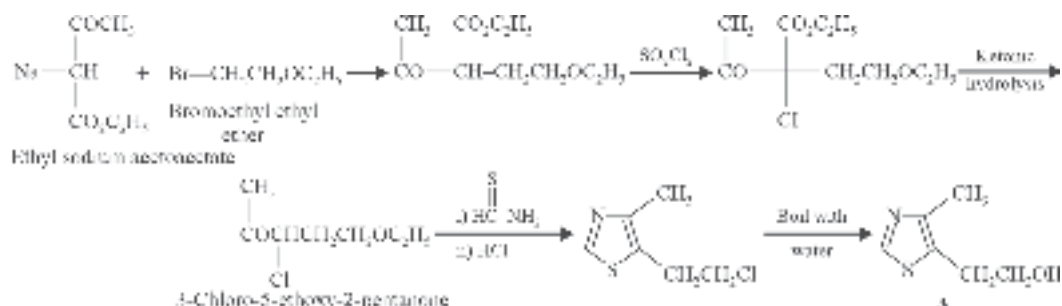


Structural elucidation of Compound, A

- (i) **Molecular formula:** It is found to be C₆H₉NOS.
- (ii) **Nature of nitrogen atom:** This compound shows basic properties. On the basis of its non reactivity with nitrous acid, it is inferred that the nitrogen atom is in the tertiary state. It is further confirmed by the formation of quaternary ammonium salt with the consumption of one molecule of alkyl halide.
- (iii) **Presence of alcoholic group:** The oxygen atom is shown to be present as alcoholic group by usual chemical test. On treatment of the compound 'A' with HCl, it forms chloro derivative which gives almost same UV spectrum as parent hydroxy compound. This indicates that hydroxyl group is primary in nature and present in side chain.
- (iv) **Nature of sulphur atom:** The sulphur did not give the reaction neither of mercapto compound nor of a sulphide. The stability of this sulphur atom indicates the presence of sulphur atom in a heterocyclic ring structure. This conclusion is confirmed by the fact that (A) has an UV spectrum, which is the characteristic of a thiazole nucleus.
- (v) Oxidation of compound A with nitric acid gives the compound C₅H₅O₂NS(I) which can also be obtained by the direct oxidation of thiamine with nitric acid. It is identified as 4-methylthiazole-5-carboxylic acid. This indicates that compound A has a side chain of two carbon atoms in place of the carboxylic group in this compound, I.



- (vi) As indicated in point (iii), alcoholic group is present in the side chain, hence it could be either -CH₂CH₂OH(II) or -CHOHCH₃(III). Either of these could lose a carbon atom to form a carboxyl group directly attached to the thiazole nucleus. The second alternative (III) -CHOHCH₃, was excluded by the fact that compound, A does not give iodoform test and is not optically active. Thus, the structure, II is assigned to the compound, A, which is further confirmed by its synthesis.

Clarke's synthesis**2. Structural Elucidation of Compound, B**

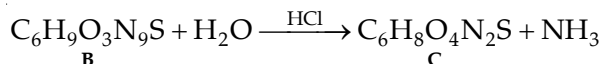
(i) **Molecular formula:** It is found to be $C_6H_9N_3O_3S$.

(ii) **Presence of sulphonic group:** The sulphur atom is found to be present as sulphonic acid as it gives sulphuric acid on heating under reduced pressure at $200^\circ C$ temperature. It is again confirmed by the formation of sodium sulphite on heating with concentrated sodium hydroxide solution.



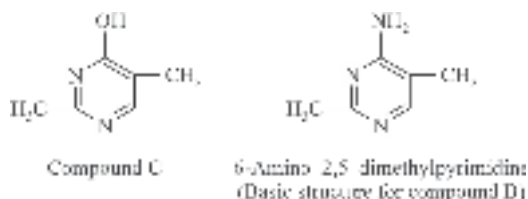
(iii) **Pressure of amino group:** On reaction with nitrous acid, the compound, **B** evolves nitrogen gas, confirming the presence of at least one amino group. Furthermore, since the evolution of N_2 is slow and reaction of compound, **B** with benzoyl chloride is also slow, this suggests that compound, **B** has an amidine structure.

(iv) **Presence of pyrimidine nucleus:** On heating compound, **B** with HCl at $150^\circ C$ temperature, it gives Compound, **C** and ammonia gas. The formation of ammonia indicates the replacement of amino group by hydroxyl group.

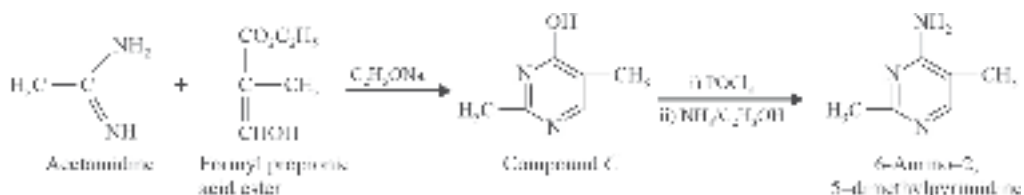


This type of reaction is characteristic of 2- and 6-aminopyrimidines. Hence, presence of pyrimidine nucleus is suggested in compound, **B**. This is further supported by UV spectrum of compound, **C** which is almost similar to 6-hydroxypyrimidines. Thus, the compound, **B** is probably 6-aminopyrimidine derivative.

(v) On reduction of compound, **B** with sodium in liquid ammonia, a sulphonic acid is eliminated with the formation of an amino methyl pyrimidine. On comparison of the UV spectrum of this product with various synthetic compounds, it is observed that compound, **B** is 6-amino-2,5-dimethyl pyrimidine derivative.

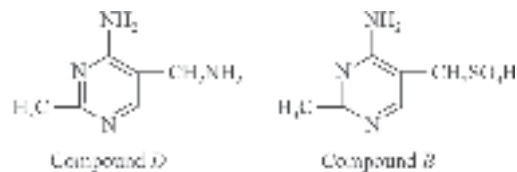


(vi) The proposed structure of compound is confirmed by synthesis.

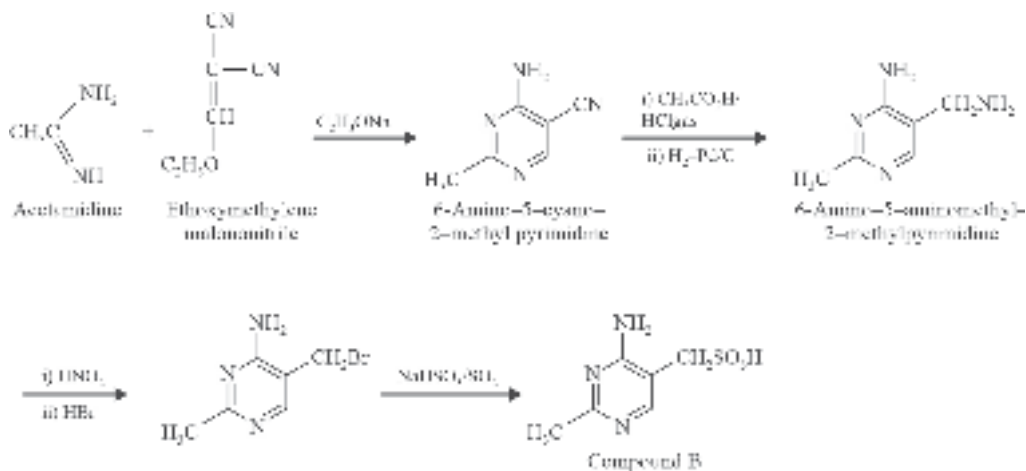


(vii) **Position of sulphonic acid group:**

The compound, **B** is 6-amino-2,5-dimethyl pyrimidine derivative with one hydrogen atom (other than amino group) replaced by a sulphonic acid. The compound, **B** contains two methyl groups and free amino group. Out of these two methyl groups, one methyl group is replaced by sulphonic acid group. To confirm this, thiamine is treated with sodium in liquid ammonia to get diamino derivative, **D** with molecular formula, $\text{C}_6\text{H}_{10}\text{N}_4$. This compound is identified as 6-amino-5-aminomethyl-2-methylpyrimidine by comparison with UV spectra of methylated aminopyrimidine of known structure. On this basis, the structure is assigned to the compound, **B** in which amino group of 5-aminomethyl is replaced by sulphonic acid.

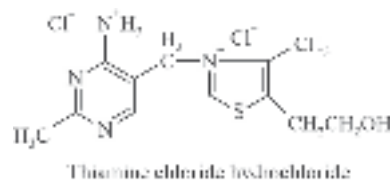


(viii) Finally, the structure of compound, **B** is confirmed by synthesis.



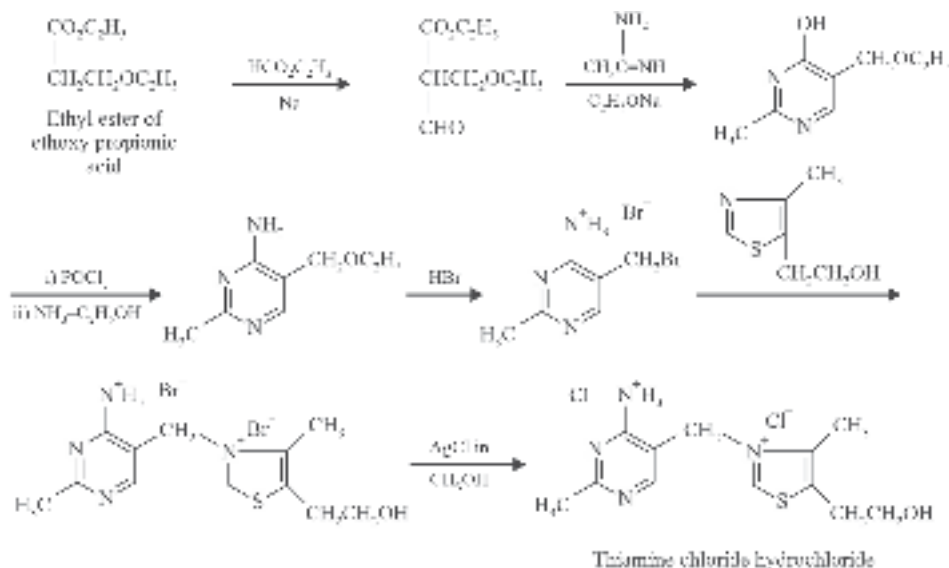
3. Point of Attachment of Compound A and B

To get the structure of thiamine, there is need to link both the compounds, **A** and **B**. As it is observed that sulphonic acid group is introduced in compound **B**, indicating the point of attachment at the CH_2 group at C-5 position in pyrimidine moiety. To account the formation of 6-amino-5-aminomethylpyrimidine, fragment, **B** must be linked to the nitrogen atom of fragment, **A**. In this position, nitrogen atom of thiazole ring is in a quaternary state and so account for the chloride hydrochloride of thiamine. The structure is given alongside.



4. The Proposed Structure has been Confirmed by Synthesis

Synthesis: The separate syntheses of the pyrimidine and thiazole moieties are carried out and then linked together to get the thiamine. The following method of synthesis has been used commercially for the production of thiamine.



Spectral Data

IR (cm^{-1}): 3450, 3325, 3100, 3000, 2600, 1650, 1627, 1425, 1100.

$^1\text{H-NMR}$ (δ): 2.35 (3H, CH_3) (attached to thiazole ring), 2.41 (3H, CH_3 , attached to pyrimidine ring), 3.03 (2H, CH_2), 3.73 (2H, CH_2), 5.3 (2H, linking CH_2 group), 8.0 (1H, Ar-H), 9.27 (1H, $\text{N}=\text{CH}$); $^{13}\text{C-NMR}$ (δ): 12, 22, 30, 50.97, 61.28, 107.06, 137.48, 143.81, 146.48, 154.70, 155.60, 156.02, 163.95, 164.66.

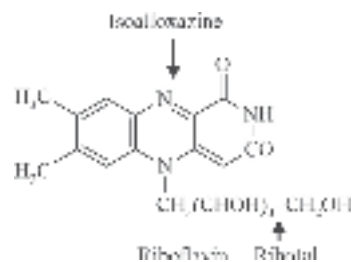
Vitamin B₂

Vitamin B₂, also commonly called riboflavin, gets its name from its color. The reason of naming riboflavin is the Latin word 'flavus' meaning 'yellow.' (One of the naturally occurring colouring agents in milk, called lactoflavin, is also a usable form of this vitamin). When a person's urine becomes bright yellow following high level supplementation with B-complex vitamins, excess riboflavin excreted in the urine is often responsible for this change in color.

Excellent sources of vitamin B₂ include mushrooms, and calf liver, spinach. Very good sources include romaine lettuce, asparagus, mustard greens, broccoli, turnip greens, chicken eggs, yogurt and cow's milk.

Functions of vitamin B₂

It is required by the body to use oxygen and the metabolism of amino acids, fatty acids, and carbohydrates. Riboflavin is further needed to activate vitamin B₆ (pyridoxine), helps



to create niacin and assists the adrenal gland. It may be used for RBCs formation, antibody production, cell respiration, and growth.

Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are the two coenzymes of riboflavin used in various biological process.

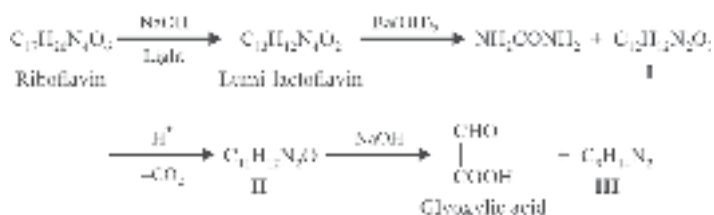
A shortage of this vitamin may manifest itself as cracks and sores at the corners of the mouth (cheilosis), eye disorders, inflammation of the mouth and tongue, and skin lesions.

Structural Elucidation of Vitamin B₂

(i) **Molecular formula:** It is found to be C₁₇H₂₀N₄O₆.

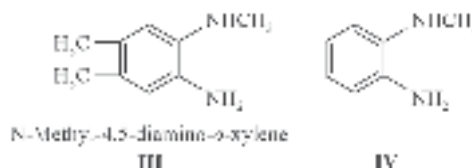
(ii) On exposure to light, riboflavin in sodium hydroxide solution forms mainly lumi-lactoflavin, C₁₃H₁₂N₄O₂ (this is soluble in chloroform). Lumi-lactoflavin, on boiling with barium hydroxide solution, is hydrolyzed to give one molecule of urea and one molecule of the barium salt of a β-keto carboxylic acid, **I**, C₁₂H₁₂N₂O₃. The nature of this acid is shown by sequence of reactions as shown below.

On acidification of this barium salt, the free acid immediately eliminates carbon dioxide to form the compound, **II**, with molecular formula, C₁₁H₁₂N₂O. This compound showed the properties of a lactam and on vigorous hydrolysis by boiling with sodium hydroxide solution, it produces one molecule of glyoxylic acid and one molecule of the compound C₉H₁₄N₂, **III**.

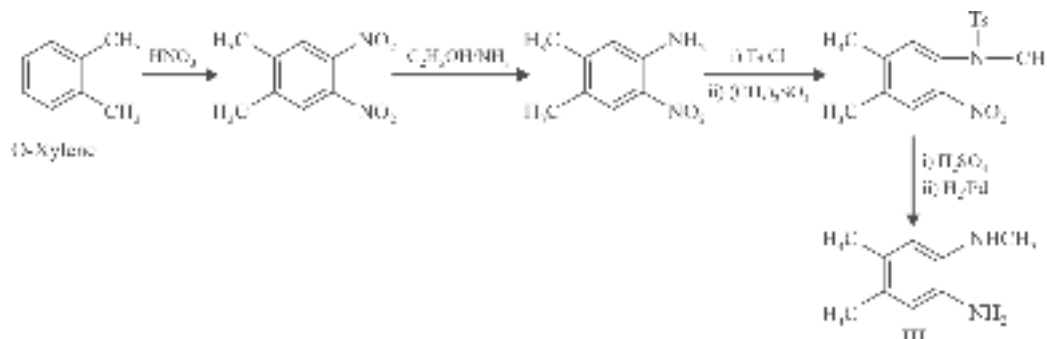


The structure of compound, **III** is elucidated as follows on the basis of following facts:

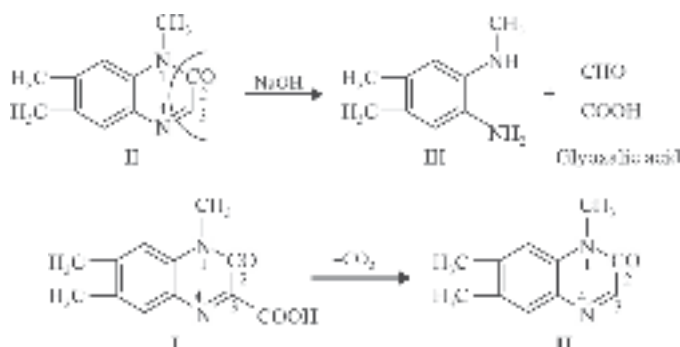
- Preliminary tests showed that compound, **III** is an aromatic diamino compound.
- It gives a blue precipitate with ferric chloride. This color reaction is characteristic of monomethyl-*o*-phenylenediamine, **IV**.



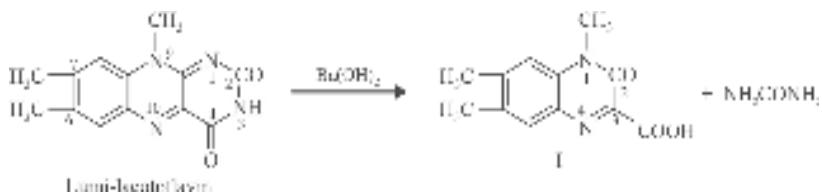
(c) The molecular formula of compound, **IV** and **III** are C₇H₁₀N and C₉H₁₄N₂ respectively, mean two compound differ by C₂H₄ moiety. On the basis of several synthetic experiments done by Kuhn et al., the structure is assigned to the compound, **III**. It is found to be N-methyl-4, 5-diamino-*o*-xylene. The proposed structure of compound, **III** is confirmed by its synthesis from *o*-xylene.



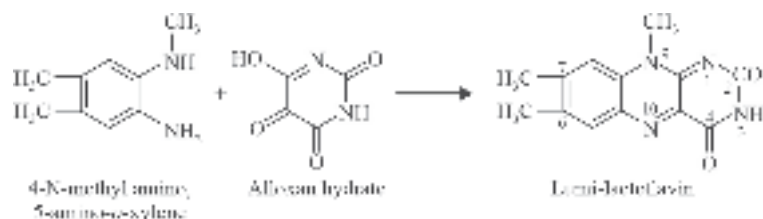
- (iii) On the basis of above points, the structure to the compound, **II** as precursor of compound, **III** which is formed from the compound, **II** along with glyoxalic acid is assigned. The compound, **II** is formed from ketocarboxylic acid, **I** as given below.



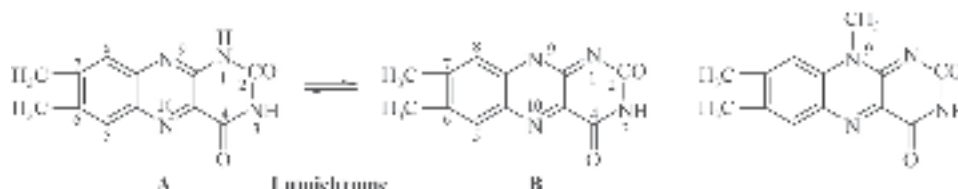
The compound, **I** and molecule of urea can be obtained if following structure is assigned to the lumi-lactoflavin (6,7,9-trimethylisoalloxazine).



- (iv) Finally, the structure of lumi-lactoflavin is confirmed by the condensation of N-methyl-4,5-diamino-*o*-xylene with alloxan hydrate in aqueous solution at 50°C temperature.

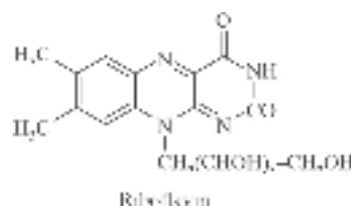


- (v) **Nature of side chain:** Exposure of a neutral solution of lactoflavin to light gives lumichrome, $C_{12}H_{10}O_2N_2$. On exposure to the same treatment as given to lumi-lactoflavin, it is found to be 6,7-dimethylalloxazine (**A**). The isoalloxazine (structure **B**) is a tautomer of alloxazine (structure). The structure, **B** does not exist as such, but this structure is fixed when there is a substituent at position-9.



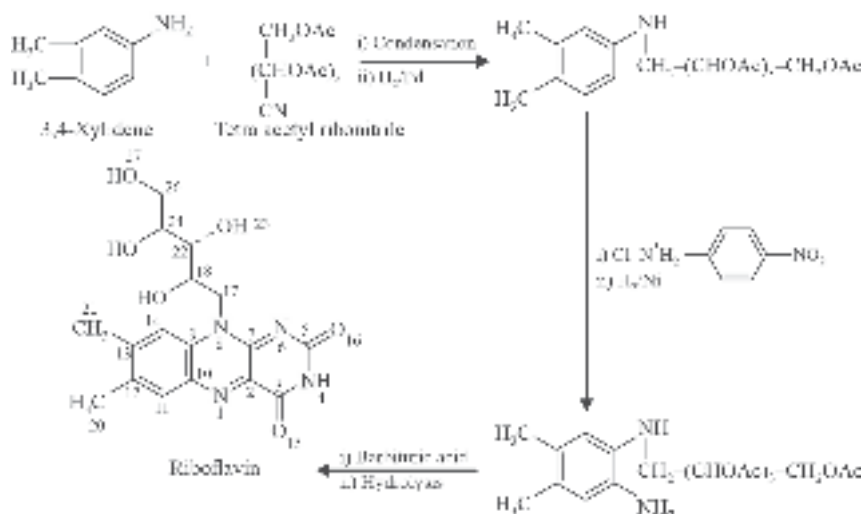
Thus, lumochrome is lumi-lactoflavin with a hydrogen atom instead of methyl group at position 9. This suggests that riboflavin contains a side chain (of five carbon atoms) attached to N-9. The presence of four hydroxyl groups is confirmed by the formation of tetraacetate derivatives from silver salt of riboflavin. Furthermore, tetra-acetate derivative on oxidation produces formaldehyde, indicating the presence of terminal primary alcoholic group. Formation of diisopropylidene derivative on reaction with acetone indicates the presence of 1,2-glycol system.

As per Zerewitinoff procedure, there are five active hydrogen atoms. Out of these, four hydrogen atoms are adjusted as hydroxyl groups and one active hydrogen atom is present as NH group at position N-3. On the basis of above facts, the following structure is assigned to riboflavin, which is further confirmed from synthesis.



Synthesis of Riboflavin (Vitamin B₂)

Tishler and Wellman developed the reaction scheme to produce good yields of pure riboflavin. 3,4-xylidene is condensed reductively with tetra acetyl ribonitrile. The condensation product is couple with *p*-nitrophenyl diazonium chloride followed by reduction to the diamine. Condensation with barbituric acid followed by hydrolysis of the acetyl groups gives riboflavin.



Spectral Data

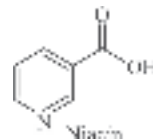
UV (CH₃OH) (nm): 446, 270, 222.

IR (cm⁻¹): 3100–3500 (N–H str., O–H str. mostly intramolecular H-bonded), 2850–3000 (Alkane C–H str.), 1680–1760 (C = O str.), 1620–1690 (C = N), 1400–1600 (aromatic C = C), 1000–1300 (alcoholic C–O str.), 817 (meta disubstituted aromatic ring).

$^1\text{H-NMR}$ (D_2O (δ): 2.23 ($\text{C}^{21}\text{-H}_3$), 2.34 ($\text{C}^{20}\text{-H}_3$), 3.73 ($\text{C}^{26}\text{-H}_2$), 3.84 ($\text{C}^{20}\text{-H}$), 3.89 ($\text{C}^{26}\text{-H}$), 3.96 ($\text{C}^{24}\text{-H}$), 4.25 (C-H), 4.48 ($\text{C}^{17}\text{-H}_2$), 7.36 ($\text{C}^{14}\text{-H}$), 7.39 ($\text{C}^{11}\text{-H}$).

Vitamin B₃

It is also known as niacin (nicotinic acid) and has 2 other forms, niacinamide (nicotinamide) and inositol hexanicotinate, which have different effects from niacin.

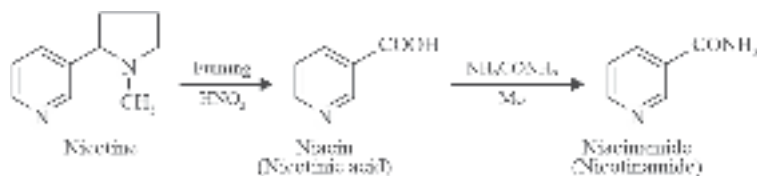


The best food sources of vitamin B₃ are found in beets, Brewer's yeast, beef liver, beef kidney, fish, salmon, swordfish, tuna, sunflower seeds, and peanuts.

Niacin (vitamin B₃ or nicotinic acid) derivatives include nicotinamide adenine dinucleotide (NAO) and nicotinamide adenine dinucleotide phosphate (NADP), which act as coenzyme in oxidation-reduction reactions vital in cell metabolism. Dietary niacin deficiency causes pellagra, disease characterized by dermatitis, gastrointestinal disorders, and mental disturbances. Primary deficiency results from extremely inadequate intake of both niacin and the amino acid tryptophan which usually occurs in areas, where maize (Indian corn) constitutes a substantial part of the diet.

Structural Elucidation of Niacin

- (i) **Molecular formula:** It is found to be $\text{C}_6\text{H}_5\text{O}_2\text{N}$.
- (ii) **Presence of carboxylic acid:** Formation of monosodium salt or monoester indicates the presence of carboxylic acid.
- (iii) **Nature of nitrogen atom:** Niacin forms addition salt either with HBr or HCl which indicate nitrogen is basic in nature.
- (iv) **Structure of niacin:** The structure of nicotinic acid is determined on the basis of its synthesis from nicotine by the oxidation reaction. Nicotine or nicotinic acid is converted to nicotinamide by heating with urea in presence of molybdenum as catalyst.



Spectral Data

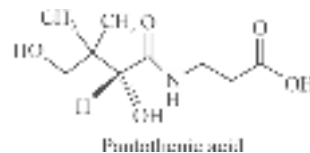
UV spectrum (nm) (H_2O): 350, 288

IR (cm^{-1}): 3095–3030 ($-\text{CH}$ str. for pyridine, 1710, 1596, 1417, 1323, 1041, 812.

$^1\text{H-NMR}$ ($\text{H}_2\text{O}/(\delta)$): 7.50 (t), 8.23 (d), 8.59 (d), 8.93 (s).

Vitamin B₅

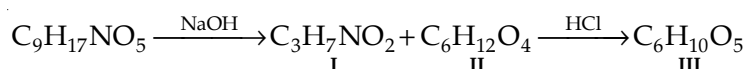
Vitamin B₅, also called pantothenic acid, is one of the 8 types of vitamin B. Pantothenic acid gets its name from the Greek root pantos, meaning 'everywhere', because it is available in a wide variety of foods major quantity of vitamin B₅ is lost when food is processed, however. Fresh meats, vegetables, and whole unprocessed grains have more vitamin B₅ than refined, canned, and frozen food. The best sources are brewer's yeast, corn, cauliflower, kale, broccoli, tomatoes, avocado, legumes, lentils, egg yolks, beef (especially organ meats such as liver and kidney), chicken, milk, split peas, peanuts, soybeans, sweet potatoes, and sunflower seeds.



Pantothenic acid is needed to form coenzyme-A (CoA), which is critical in the metabolism and synthesis of carbohydrates, proteins, and fats.

Structural Elucidation of Pantothenic Acid

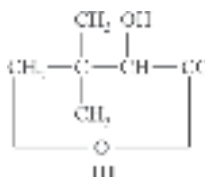
- (i) **Molecular formula:** It is found to be $C_9H_{17}NO_5$.
- (ii) **Presence of carboxylic group:** On esterification, pantothenic acid forms monoester, indicating the presence of one carboxylic group.
- (iii) **Presence of hydroxyl groups:** Usual tests show that pantothenic acid contains two hydroxyl groups. Since the acid condenses with benzaldehyde, there is possibility of two hydroxyl groups in either the 1, 2 or 1, 3 position. Since periodic acid has no action, the possibility of 1, 2-glycol is eliminated. Hence, there is 1, 3-glycol system.
- (iv) **Hydrolytic reaction:** On warming with dilute hydrochloric acid, pantothenic acid is hydrolyzed into two compounds, **I** and **II**.



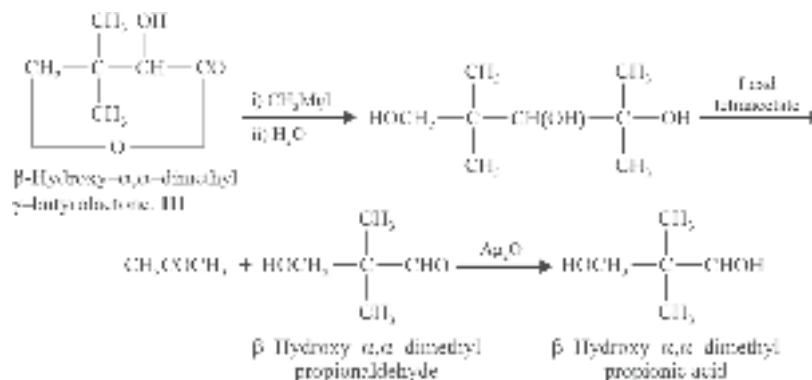
On structural elucidation, the compound, **I** is found to be β -alanine. On the other hand, on hydrolysis with alkali, pantothenic acid forms β -alanine (**I**) and the salt of an acid, **II** which, on acidification, spontaneously forms the lactone, **III**. Thus, acid may be either γ or δ -hydroxycarboxylic acid.

The fast rate of its hydrolysis indicates the possibility of γ -hydroxycarboxylic acid for the compound, **II**.

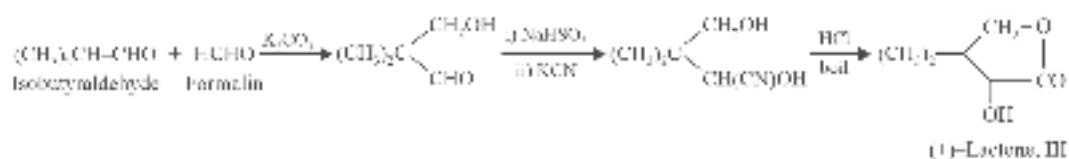
- (v) **Positions of hydroxyl groups:** As indicated in point (iii), pantothenic acid contains two hydroxyl groups. The position of one of the hydroxylic group is assigned γ to carboxylic group. The position of second hydroxylic group is assigned on the basis of following facts:
 - (a) The sodium salt of acid of the lactone, **III** gives a canary-yellow color with ferric chloride. It is a characteristic test for α -hydroxy acids.
 - (b) On warming pantothenic acid with Conc. H_2SO_4 , there is liberation of carbon monoxide. This also indicates the presence of α -hydroxy acid. Thus, the compound, **III** is probably γ -lactone of α -hydroxyl acid.
- (vi) The compound, **III** is found to contain one active hydrogen atom and *gem*-dimethyl group. The presence of *gem*-dimethyl group is confirmed by the formation of acetone when the lactone is oxidized with barium permanganate. On the basis of these facts, the following structure is assigned to compound, **III**.



The proposed structure is again confirmed as follows. The reaction of lactone with methyl magnesium iodide, followed by hydrolysis, produces the trihydric alcohol which on oxidation with lead tetra-acetate, gives an aldehyde and acetone. The aldehyde on oxidation gives β -hydroxy- α , α -dimethylpropionic acid. The complete sequence of reactions can be organized as shown here:

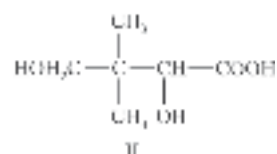


(vii) Finally, the proposed structure for compound, **III** is confirmed by synthesis.

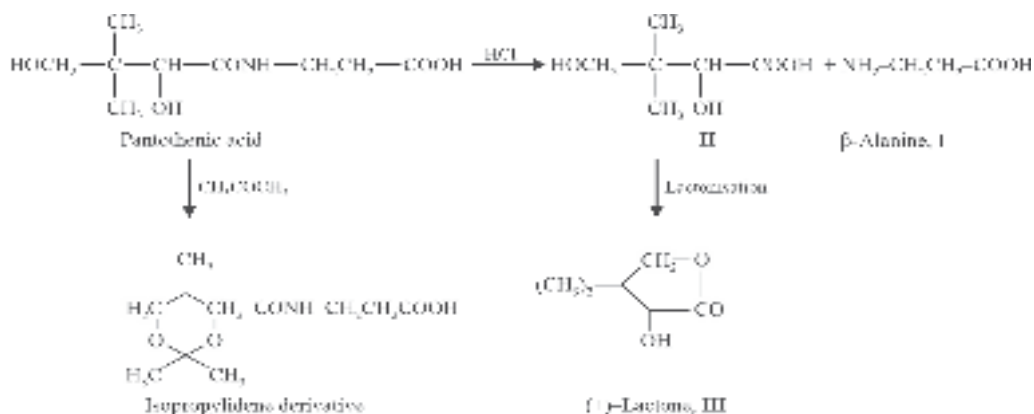


The lactone obtained from hydrolysis of pantothenic acid is laevorotatory while from synthetic method, racemic form is produced. It is resolved with the help of quinine hydrochloride.

After assigning structure to lactone compound, **III**, the structure to compound, **II** is assigned.



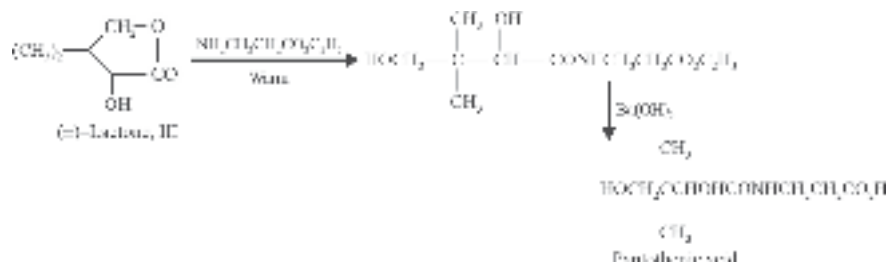
(viii) **Assignment of structure to pantothenic acid:** In pantothenic acid, nitrogen is weakly basic. On hydrolysis, free amino group (as β -alanine) is generated. This indicates the presence of amide group ($-\text{CO}-\text{NH}-$) as shown below by assigning the structure of pantothenic acid. The proposed structure also explains the formation of isopropylidene derivative on reaction with acetone.



(ix) Finally, the proposed structure of pantothenic acid is proved by synthesis.

Synthesis of Pantothenic acid

A better yield of pantothenic acid is obtained by warming the dry lactone (synthesized as above) with the ethyl ester of β -alanine (point vii).



Spectral Data

IR (cm^{-1}) (KBr): 3343 (OH, NH), 2961, 2955, 2876, 1667, 1647, 1565, 1466, 1077, 989.

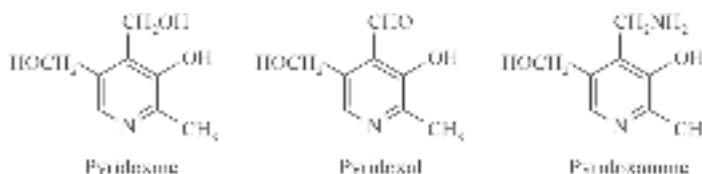
$^1\text{H-NMR}$ (δ) (D_2O): 0.904 (3H, CH_3), 0.935 (3H, CH_3), 2.42 (2H, $\text{CH}_2\text{-C}$), 3.42 (1H, $\text{CH}_2\text{-OH}$), 3.45 (2H, CH_2), 3.49 (1H, CH), 3.99 (1H, CH-OH); **$^{13}\text{C-NMR}$ (δ) (D_2O):** 20, 21, 36.96, 37.48, 39.44, 69.24, 76.68, 175.61, 181.60.

VITAMIN B₆

Vitamin B₆ consists of a group of closely related compounds: pyridoxine, pyridoxal, and pyridoxamine. These three compounds are metabolically and functionally interrelated and are readily interconverted.

They are metabolized in the body to pyridoxal phosphate (PLP), which acts as a coenzyme in many important reactions in blood. It is needed for:

- Decarboxylation of amino acids to yield amines, many of which are important neurotransmitters and hormones.
- Cellular metabolism of carbohydrate, protein and fat.
- Formation of neurotransmitters.
- Production of nicotinic acid (vitamin B₃) from tryptophan.
- Formation of alpha levulinic acid, a precursor to heme.



Deficiency of vitamin B₆ can cause seizures by inhibiting the synthesis of γ -aminobutyric acid (GABA). GABA is an inhibitory neurotransmitter and seizures can result if it is present in insufficient amounts.

Vitamin B₆ deficiency has been shown to increase urinary oxalate excretion. This represents the body's inability to convert glyoxalate to glycine, which is necessary to synthesize glycine and serine.

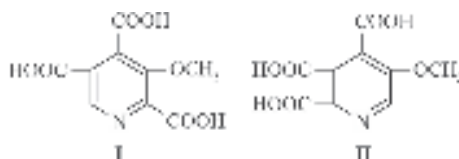
Good food sources of vitamin B₆ include chicken, beef liver, milk, cheese, lentils, beans, spinach, carrots, brown rice, bran, sunflower seeds, wheat germ, and whole-grain flour.

Pyridoxine

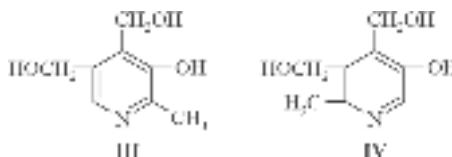
Pyridine is a weakly basic substance having m.p. 160°C. Chemically, it is 2-methyl-3-hydroxy-4,5-bis(hydroxy-methyl) pyridine.

Structural Elucidation of Pyridoxine

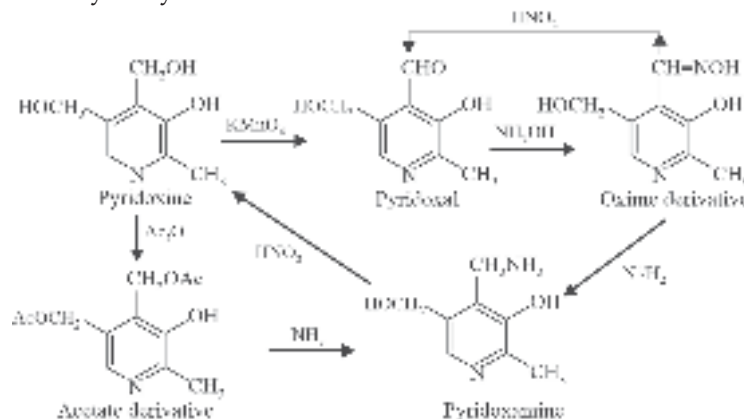
- (i) Its molecular formula is found to be $C_8H_{11}O_3N$.
- (ii) Usual Zerewitinoff test shows the presence of three active hydrogen atoms. The reaction with diazomethane, pyridoxine forms the monomethyl ether which on acetylation gives the diacetylated product. This shows that one active hydrogen atom is present as phenolic hydroxyl group, hence easily methylated to form monomethyl ether. Two hydrogen atoms are present as alcoholic hydroxyl group as shown by acetylation reaction.
- (iii) On comparison of UV spectra, it is observed that UV spectrum of pyridoxine is similar to 3-hydroxypyridine, indicating the presence of phenolic hydroxyl group at 3-position. Lead acetate has no action on the monomethyl ether of pyridoxine, indicating the absence of two hydroxyl groups on adjacent carbon atoms.
- (iv) On oxidation with alkaline $KMnO_4$ monomethyl ether of pyridoxine forms methoxy pyridine tricarboxylic acid, which gives blood red color with ferrous sulphate solution. It is a characteristic test for pyridine-2-carboxylic acid. Thus, out of three carboxylic groups, the position of one carboxylic group is assigned at C-2 position.
- (v) On oxidation with alkaline $KMnO_4$ under usual condition, monomethyl ether of pyridoxine forms anhydride of dicarboxylic acid, $C_8H_5O_4N$ along with evolution of CO_2 . Thus, two carboxylic groups are in *ortho* position. Further, this anhydride on hydrolysis, does not give the red color with ferrous sulphate solution, indicating the absence of carboxylic group at C-2 position in pyridine ring. It is, therefore, suggested that on decarboxylation, the tricarboxylic acid eliminates the carboxylic group present at C-2 position to form the anhydride. On the basis of following points, two structures are assigned to methoxy pyridine tricarboxylic acid, I or II.



- (vi) Pyridoxine methyl ether contains three oxygen atoms (one as methoxyl group and other two as alcoholic groups). It is therefore predicted that two carboxylic groups are generated from two $-CH_2OH$ groups and third from methyl group. Hence, two probable structures are assigned to pyridoxine III or IV.



- (vii) The one structure is selected on the basis of following evidence. When pyridoxine methyl ether is oxidized with barium permanganate, dicarboxylic acid, $C_9H_9O_5N$ is produced. It does not give the red color with ferrous sulphate solution, indicating the absence of carboxylic group at C-2 position. This dicarboxylic acid forms an anhydride so two carboxylic groups are present at *ortho* position. Thus, the structure of dicarboxylic acid is either V or VI.



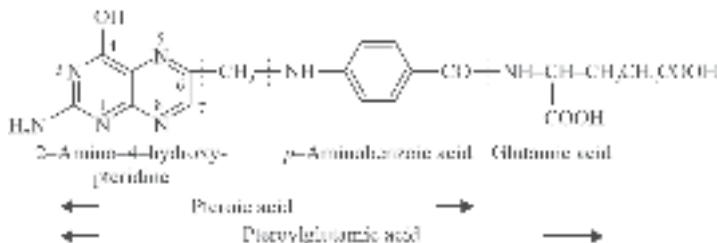
Vitamin B₉

The vitamin B₉, also called folic acid, is involved in maturation of red blood cells and the synthesis of purines and pyrimidines which are required for development of the fetal

nervous system. Adequate folic acid intake before conception and throughout the first trimester of pregnancy helps in preventing certain brain and spinal cord defects such as spina bifida. Folate is absorbed in the duodenum and upper jejunum. Deficiency produces megaloblastic anemia indistinguishable from that due to vitamin B₁₂ deficiency. A deficiency of folate in old age significantly increases the risk of developing dementia.

There are many food sources containing folic acid, the most important being:

• Green leafy	Vegetables beans
• Liver	Yeast extract
• Whole grains	Egg yolk
• Milk and milk products	Oranges and orange juice
• Beets	Wholemeal bread



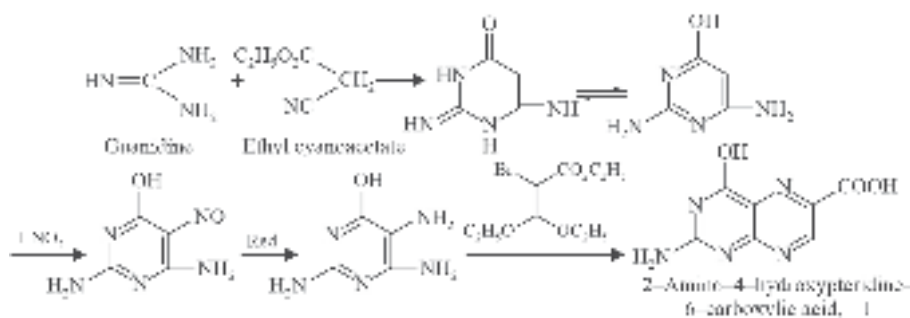
Structural Elucidation of Vitamin B₉ (Folic Acid)

The folic acid structure is determined on the basis of chemical reactions performed on yeast *L casei* factor. Structurally, it is similar to folic acid except folic acid contains one molecule of glutamic acid while *L casei* factor contains three molecules of glutamic acid.

On alkaline hydrolysis of fermentation *L casei* factor, in the presence of air, two compounds I and II are produced.

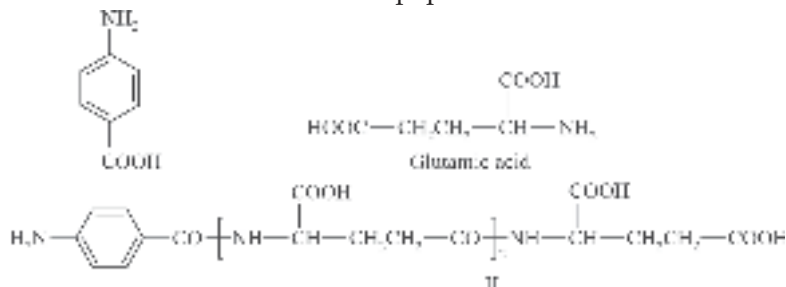
Structural Elucidation of Compound, I

- (i) **Molecular formula:** It is found to be $C_7H_5N_5O_3$.
- (ii) The substance, I is found to be monocarboxylic acid. On comparison of UV spectra, it is found to be pteridine derivative.
- (iii) The usual tests indicates the presence of hydroxyl and amino groups.
- (iv) Oxidation with chlorine water, followed by hydrolysis with HCl gives guanidine, $NH = C(NH_2)_2$. This reaction indicates the presehce of amino group at C-2 position.
- (v) On decarboxylation, the compound, I gives 2-amino-4-hydroxy pteridine, indicating the presence of hydroxyl group at 4 position.
- (vi) Finally, its structure is suggested as 2-amino-4-hydroxy pteridine-6-carboxylic acid which is further confirmed by synthesis.



Structural Elucidation of Compound, II

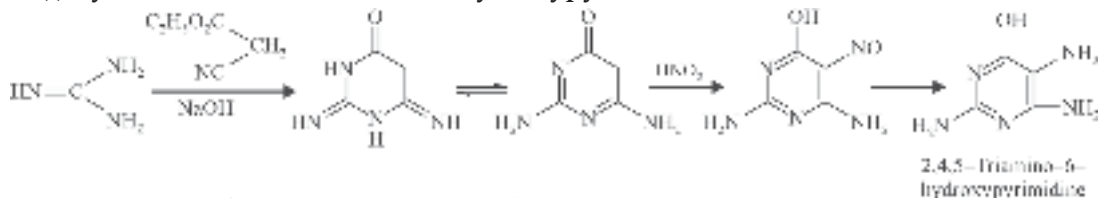
- (i) **Presence of amino group:** The compound, **II** is found to be primary aromatic amine.
- (ii) On hydrolysis, it gives one molecule of *p*-aminobenzoic acid and three molecules of glutamic acid. It indicates that it is a tripeptide of PABA.



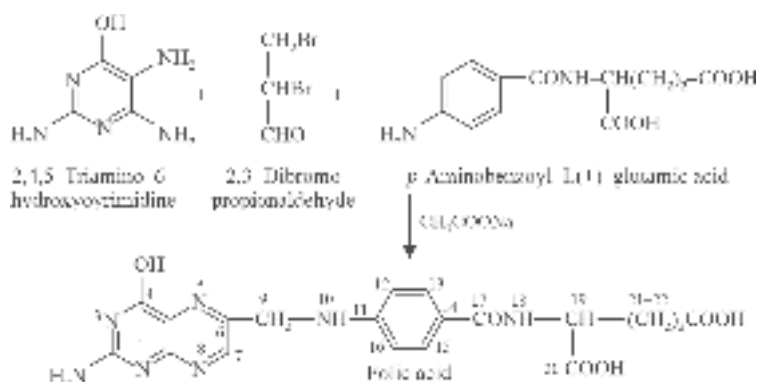
On the basis of above points, the structure **II** to L. Casei factor is assigned, confirmed by synthesis.

Synthesis: It is carried out in two steps:

(i) Synthesis of 2,4,5-Triamino-6-hydroxypyrimidine:



(ii) Synthesis of Vitamin B₉ (Folic acid)



Spectral Data

UV (nm): $\lambda_{\max}$ 250 (in water).

IR (cm^{-1}): 3545, 3415, 3323, 2925, 1693 ($-\text{C}=\text{O}$), 1619 (Aromatic $\text{C}=\text{C}$), 1604 (NH bending), 1485, 1194, 839.

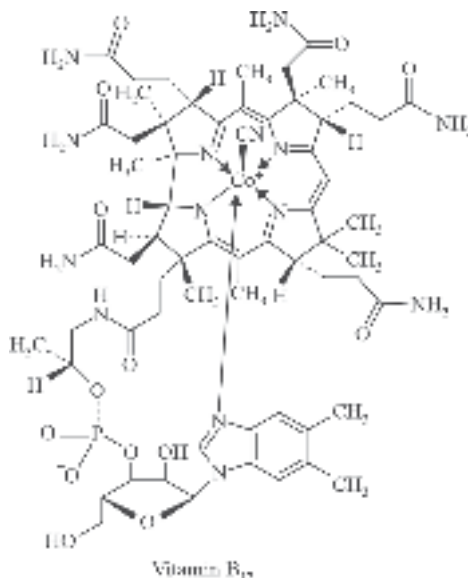
$^1\text{H-NMR}$ (DMSO- d_6): 2.01 (H-21B), 2.15 (H-21A), 2.42 (H-22), 4.44 (H-19), 4.59 (H-9), 6.74 (H-16), 6.74 (H-12), 8.22 (H-18), 8.75 (H-7).

Vitamin B₁₂

Vitamin B₁₂ has the most complicated structure among all vitamins. Since vitamin B₁₂ contains cobalt, it is also known as cobalamin. The vitamin can only be synthesized by bacteria, so it can be found mostly in dairy products, but also in eggs and some meat.

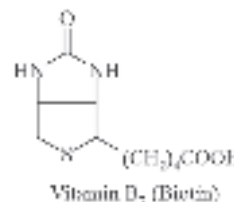
Cobalamin (cobal(t) + (vit)amin) is a compound containing the substituted corrin ring (polyaromatic ring consisting of 4 pyrrole subunits which are joined by methylene links on opposite sides and the two of the pyrroles are joined directly) and 5,6-dimethylbenzimidazole, as the aglycon attached by a glycosyl link. Vitamin B₁₂ is a cyanide ion substituted cobalamin derivative (cyanocobalamin) having cobalt mineral at 6 position. Hydroxycobalamin and methylcobalamin contain a hydroxyl group and a methyl group substituent, respectively.

These compounds are involved in nucleic acid metabolism, methyl transfer, and myelin synthesis and repair. They are necessary for the formation of normal red blood cells. Vitamin B₁₂ is released in the stomach's acid environment and is bound to R protein. Pancreatic enzymes cleave the B₁₂-R protein complex in the small intestine. After cleavage, intrinsic factor, secreted by parietal cells in the gastric mucosa, binds with vitamin B₁₂. Intrinsic factor is required for absorption of vitamin B₁₂, which takes place in the terminal ileum. The recommended dietary allowance (RDA) is 2.4 micrograms, which is the amount found in 3 ounces (85 grams) of meat. Vitamin B₁₂ is found in oysters, turkey, chicken, beef, and pork. Deficiency of vitamin B₁₂ causes megaloblastic anemia, damage to the white matter of the spinal cord and brain, and peripheral neuropathy which is characterized by tingling or numbness in the hands or feet.



Vitamin B₇

Vitamin B₇, also called biotin, acts as a coenzyme for carboxylation reactions involved with fat and carbohydrate metabolism. Adequate intake of vitamin B₇ for adults is 30 µg/day. Liver, egg yolks, green vegetables, and whole grains are rich sources of biotin.



Deficiency symptoms includes:

- (i) Hair loss.
- (ii) Dry scaly skin.
- (iii) Cracking in the corners of the mouth (called cheilitis).
- (iv) Swollen and painful tongue with magenta in coloration (glossitis).
- (v) Dry eyes.
- (vi) Loss of appetite.
- (vii) Fatigue, insomnia, and depression.

Structural Elucidation of Biotin

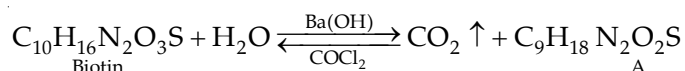
- (i) Molecular formula is found to be C₁₀H₁₆N₂O₃S.
- (ii) It is saturated compound as biotin does not decolorize bromine water.
- (iii) Biotin forms monomethyl ester on reaction with methanol. Hence, biotin is a monocarboxylic acid.

(iv) The sulphur is present as thioether as it is inert in nature (non-reactive). It is also proved as below:

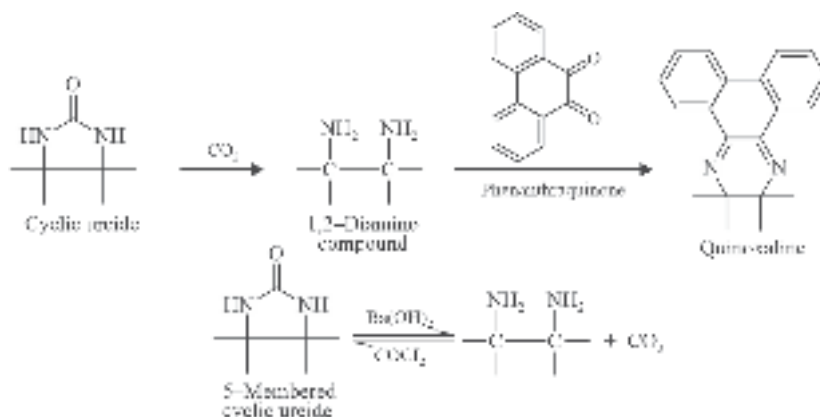
(a) Biotin on oxidation gives sulphone.

(b) Methyl ester of biotin with CH_3I gives a sulphonium iodide.

(v) Biotin on heating at 140°C is hydrolyzed by either with aq. HCl or aq. $\text{Ba}(\text{OH})_2$ into diaminocarboxylic acid (**A**), $\text{C}_9\text{H}_{18}\text{N}_2\text{O}_2\text{S}$ with the evolution of CO_2 . This acid is shown to contain two primary amino groups and also forms the dibenzoyl derivative which is soluble in an alkali solution. This reaction indicates that biotin contains a cyclic ureide structure. This is further confirmed by the formation of biotin from diaminocarboxylic acid on reaction with carbonyl chloride.



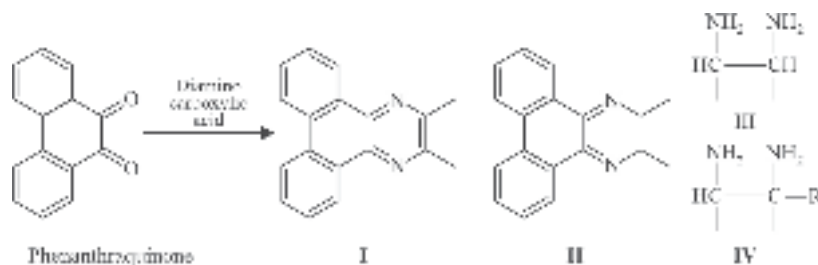
(vi) On condensation with phenanthraquinone, this acid, **A** forms a quinoxaline derivative, which shows that two amino groups are in the 1,2-positions and thus cyclic ureide is five membered.



(vii) On treatment of this diaminocarboxylic acid with alkaline KMnO_4 , adipic acid is formed. The formation of adipic acid takes place either may be due to an aliphatic side chain present in biotin or from the opening of six-membered ring. In the first option, the carboxyl group will appear in adipic acid, but in the latter option neither of the carboxyl group will be appear in biotin.

(viii) When the carboxymethyl group of methyl ester of biotin is replaced by an amino group by means of Curtius reaction (ester $\rightarrow$ hydrazide $\rightarrow$ azide $\rightarrow$ urethane $\rightarrow$ NH_2) and then the resulting amino product is hydrolyzed in a similar way with barium hydroxide solution, a triamine is obtained which do not give adipic acid on oxidation with KMnO_4 . On this basis, it is inferred that carboxylic group of adipic acid is original one, means same carboxylic group is present in biotin. Hence, biotin contains $-(\text{CH}_2)_4\text{COOH}$ side chain (*n*-valeric acid side chain).

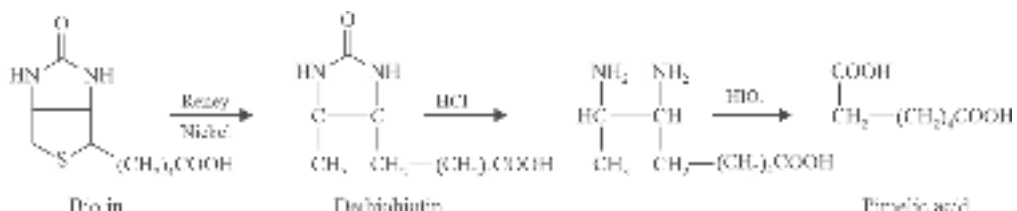
(ix) The UV spectrum of quinoxaline derivative (formed from phenanthraquinone and the diaminocarboxylic acid) showed that it is quinoxaline, **I** and not dihydro-quinoxaline, **II**. Hence, diaminocarboxylic acid contains the structure **III** and not **IV**. It also indicates that *n*-valeric side chain cannot be attached to a carbon atom joined to amino group.



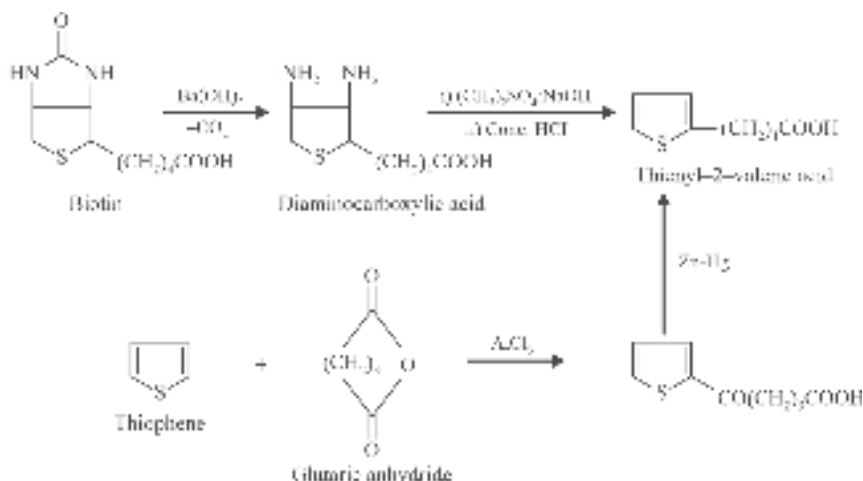
(x) The biotin is suggested to have bicyclic structure. It is deduced from the following facts:

- Biotin does not contain double bond.
- The carboxylic group is present as substituent so its molecular formula becomes $C_9H_{16}ON_2S$.
- Due to cyclic ureide, it contains two NH groups which may be replaced by two CH_2 groups so its molecular formula becomes $C_{11}H_{18}OS$.
- CO group may be replaced by CH_2 group and sulphide group also replaced by CH_2 . Hence, its molecular formula becomes $C_{12}H_{22}$, corresponds to general formula, C_nH_{2n-2} of bicyclic ring structure.

(xi) Biotin on heating with raney nickel forms dethiobiotin, which on hydrolysis with HCl produces diaminocarboxylic acid. The resulting product on oxidation with periodic acid gives pimelic acid. All these reactions can be explained by assuming the sulphur atom in the ring and n -valeric acid side chain at α -carbon atom with respect to sulphur atom.

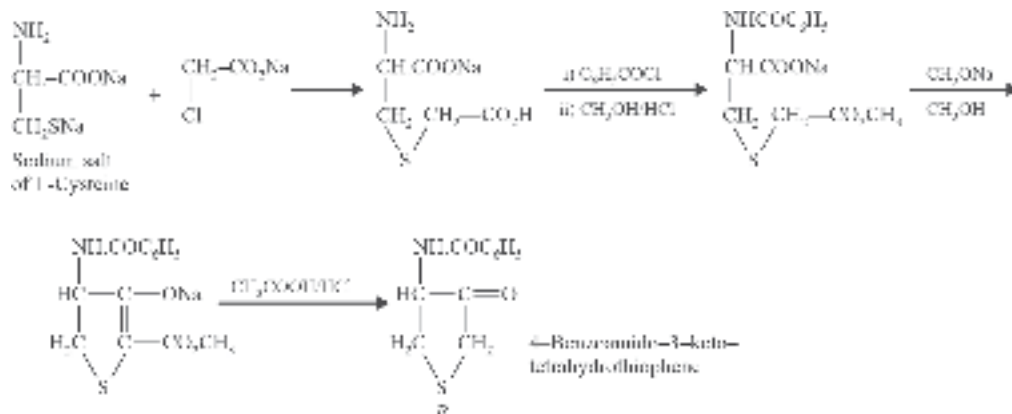


(xii) Further evidence for this structure is given by the exhaustive methylation of diaminocarboxylic acid (produced from biotin) to get δ -(2-thienyl) valeric acid, the structure of same is proved on the basis of its synthesis from glutaric anhydride.

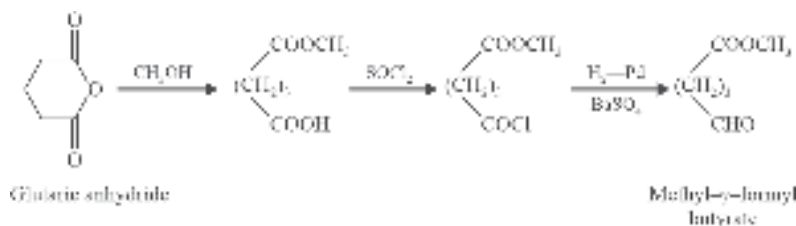


- (xiii) Finally, the above structure for biotin was confirmed by the synthesis which involves the three different steps: (a) Synthesis of 4-benzoamido-3-keto-tetrahydrothiophene (B); (b) Synthesis of methyl- γ -formyl-butyrate (C); (c) Condensation of (B) and (C) to give biotin.

(a) Synthesis of 4-benzoamido-3-keto-tetrahydrothiophene (B)

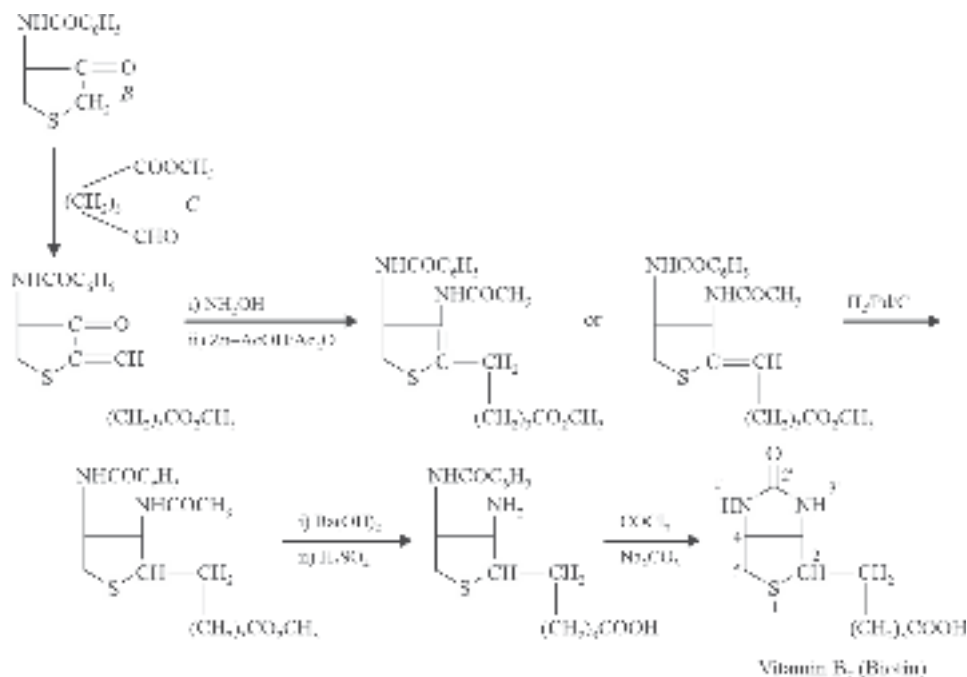


(b) Synthesis of methyl- γ -formyl-butyrate (C)



(c) Condensation of B and C in presence of piperidine acetate.

The compounds 'B' and 'C' are condensed to get biotin.



Spectral Data

IR (cm⁻¹) (KBr): 3306 (NH, OH), 2831 (–CH), 1710 (CO), 1639, 1481, 1316, 1269, 1020.

¹H-NMR (D₂O) (δ): 1.2 (CH₂), 1.5 (2H, CH₂), 1.55 (1H_a, –CH₂), 1.6 (1H_b, –CH₂), 2.1 (2H₁–CH₂–CO), 2.7 (1H_a, Ring CH₂), 2.9 (1H_b, Ring CH₂), 3.2 (1H, t ring CH), 4.4 (1H, –CH), 4.5 (1H, –CH); ¹³C-NMR (δ) (D₂O), 26.34, 28.34, 28.92, 38.00, 40.38, 56.00, 60.91, 62.66, 166.07, 184.45.

SHORT AND LONG QUESTIONS

1. What are vitamins? Enumerate the differences between vitamins and hormones? Discuss the biological importance of vitamins.
2. Discuss the structure of riboflavin
3. Classify the vitamins. Describe the structure elucidation of ascorbic acid.
4. Write the structures of vitamin B₁, B₂, B₆ and B₁₂. Give the importance of each in the biological system.
5. Classify the vitamins with examples. Write about the constitution of biotin in detail.
6. What is pantothenic acid. Discuss its chemistry.
7. Give the sufficient chemical evidences in favor of ascorbic acid.

MULTIPLE CHOICE QUESTIONS

1. Vitamin A or retinal is a:
 - a. Steroid
 - b. Polyisoprenoid compound containing a cyclohexenyl ring
 - c. Benzoquinone derivative
 - d. 6-Hydroxychromane
2. Niacin or nicotinic acid is a monocarboxylic acid derivative of:
 - a. Pyridine
 - b. Pyrimidine
 - c. Flavin
 - d. Adenine
3. The chemical structure of vitamin B₁ consists of:
 - a. aminopyridine and thiazolinum ring
 - b. aminopyridine and thiazolinum ring linked by methylene bridge
 - c. metylaminopyridine and thiazolinum ring
 - d. None of above

Answers

1. (b) 2. (a) 3. (b)

Polynuclear Aromatic Hydrocarbons

Polynuclear aromatic hydrocarbons, also called polycyclic aromatic hydrocarbons, are the hydrocarbons consisting of multiple benzene rings. These are, mostly associated with heavy petrolatum, including diesel fuel. Some examples of polynuclear hydrocarbons are given in Fig. 12.1.

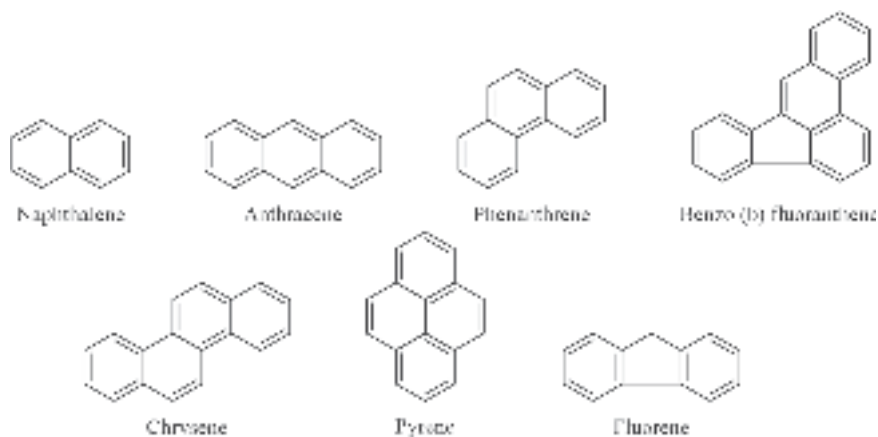


Fig. 12.1: Structures of some polynuclear aromatic hydrocarbons

Classification of Polynuclear Hydrocarbons

These are classified as given in Fig. 12.2.

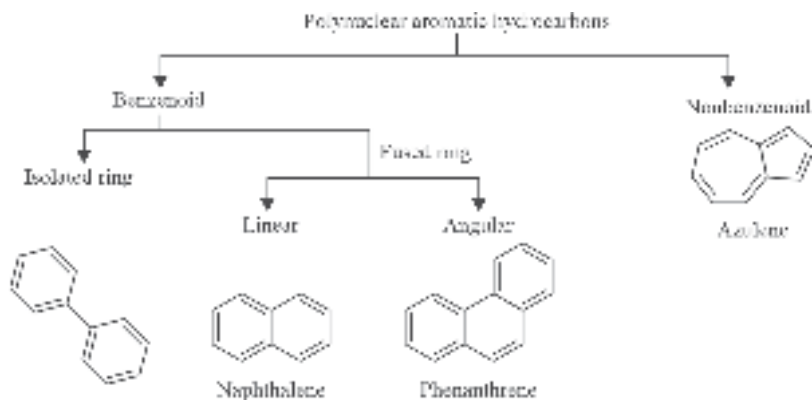
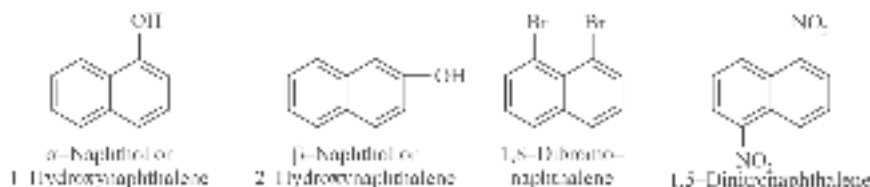
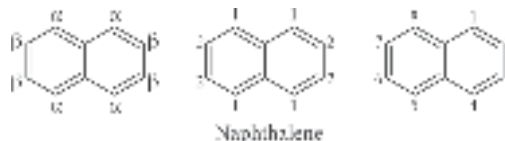


Fig. 12.2: Classification of polynuclear hydrocarbons.

NAPHTHALENE

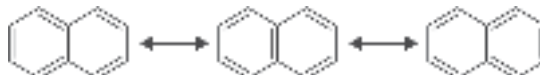
It is the first member of polynuclear hydrocarbon in which two benzene rings are fused together at ortho position.

The monosubstituted naphthalene derivatives are designated by either using prefixes alpha or beta or using numbering system. The numbering system begins with number '1' at the alpha position and number '2' in place of beta position.



Characteristics of Naphthalene

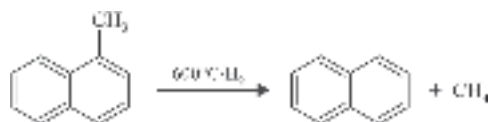
1. Molecular formula is $C_{10}H_8$.
2. Presence of Kekule type formula.
3. Like benzene, it is usually stable.
4. Its heat of combustion is 61 kcal.
5. It is resonance hybrid of 3 canonical form.



In naphthalene, π -electron system is delocalized which lower the electronic energy, i.e. 61 kcal giving stability and more reactivity to naphthalene than benzene.

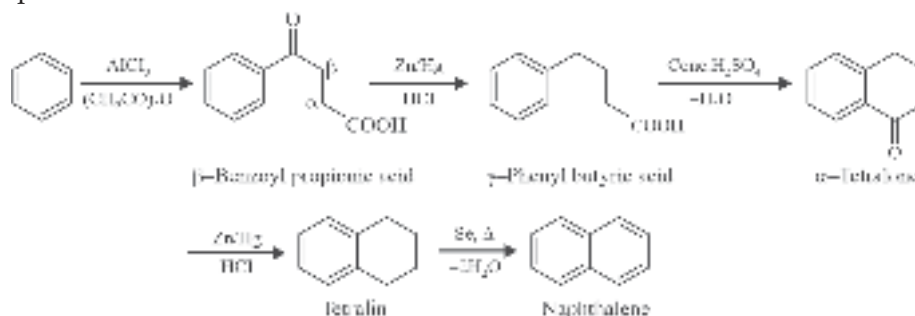
Preparations

1. **From coal tar:** The intermediate fraction of coal tar is chilled to form crystal of naphthalene which are separated by centrifugation. The crude crystals are melted and treated with H_2SO_4 to remove basic impurities. Then, aqueous NaOH solution is added to remove the phenols and excess H_2SO_4 . Finally, the contents are distilled to get the pure naphthalene.
2. **From petroleum:** The intermediate distillate of petroleum on catalytic cracking and reforming yield naphthalene and methyl naphthalene. The methyl naphthalene on heating to $600^\circ C$ temperature in presence of hydrogen is dealkylated to give naphthalene.



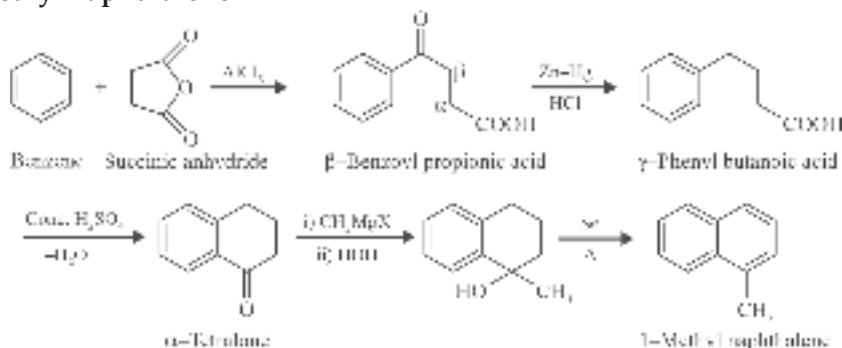
3. **Howarth synthesis:** Naphthalene can be prepared by **Freidel Craft acylation** of benzene with succinic anhydride in presence of anhydrous $AlCl_3$. The benzene is acylated to get β -benzoyl propionic acid, which is subjected to Clemenson reduction followed by cyclisation with conc. sulphuric acid gives the α -tetralone. The

α -tetralone on reduction, followed by dehydrogenation with selenium gives the naphthalene.

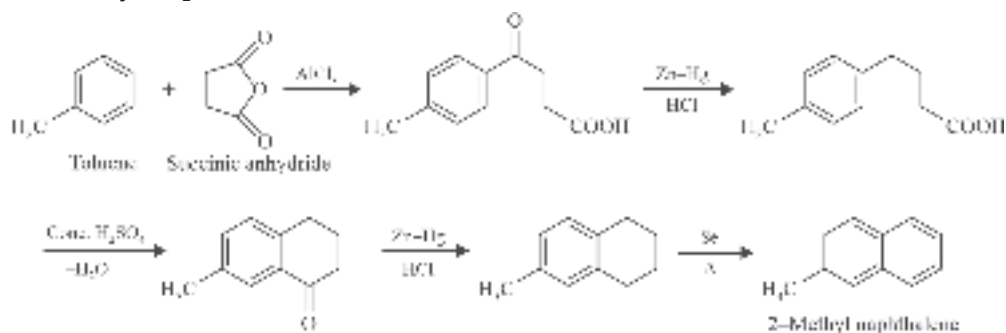


The alkylnaphthalene can also be prepared by acylation reaction as given here:

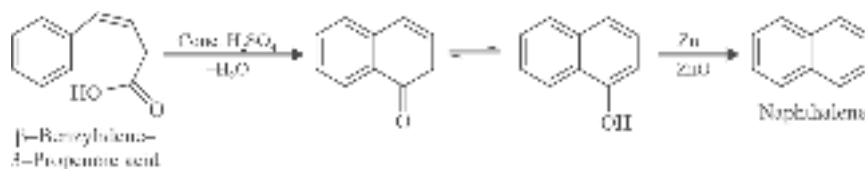
1-Methyl naphthalene:



2-Methyl naphthalene:



4. From β -benzylidene propenoic acid: Naphthalene can be prepared from beta benzylidene propenoic acid by reaction with sulphuric acid, followed by treatment with zinc metal.



Physical properties

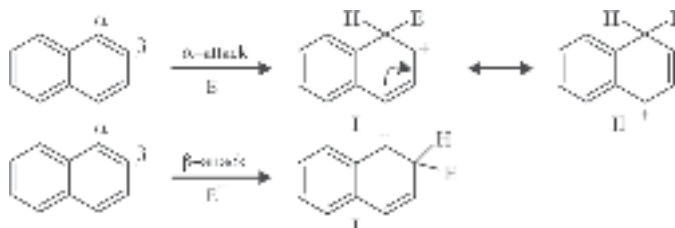
- Colorless solid which forms shining flaked crystals (m.p. 80.2° C, b.p. 217.9° C).
- Familiar odor of moth balls.
- Volatile and sublimes slowly at room temperature.

- Insoluble in water, moderately soluble in alcohol, very soluble in ether and benzene.
- Burns with smoky flame.
- Its λ_{max} is 314 nm in comparison to benzene, 255 nm.

Chemical Properties

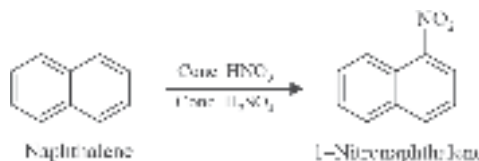
It closely resembles to benzene in chemical reaction.

1. **Electrophilic substitution reaction:** It gives usual aromatic electrophilic substitution reactions. The alpha products predominates in comparison to beta substituted product.

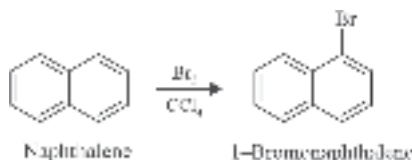


In case of alpha attack, the intermediate is more stable and so the product with substituent at alpha position predominates. Two resonating structures can be predicted for the intermediate carbonium ion obtained from alpha attack (without involving the other ring) whereas only one such structure is possible at beta position. Hence, substitution at beta-position occurs only when reaction is carried out at high temperature (e.g. sulphonation) or when bulkier solvent (e.g. Friedel-Craft reaction) is used.

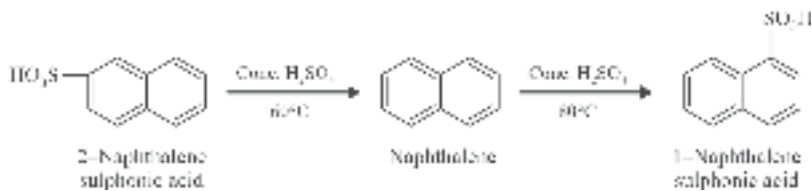
- (a) **Nitration:** Naphthalene forms α -nitronaphthalene or 1-nitronaphthalene on heating with conc. HNO_3 and H_2SO_4 mixture.



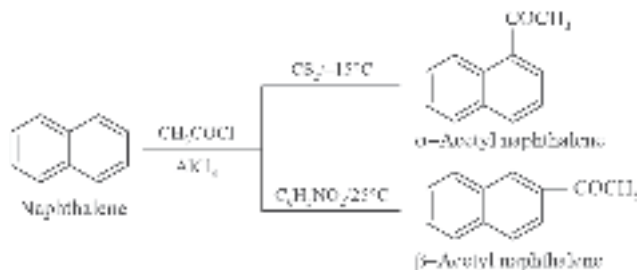
- (b) **Halogenation:** It reacts with chlorine/bromine in CCl_4 solution to give α -chloro or α -bromo-naphthalene.



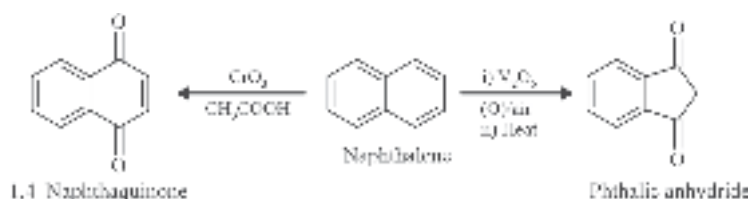
- (c) **Sulphonation:** On treatment with cone. sulphuric acid, it gives α or β -naphthalene sulphonic acid depending on the temperature.



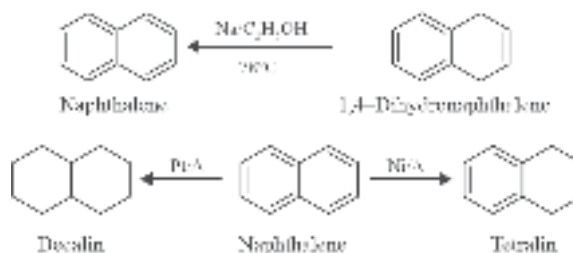
(d) *Freidel-Craft acylation*: Naphthalene undergoes acylation reaction to give α - or β -acylated product depending on the solvent used.



2. Naphthalene on mild oxidation by chromic acid in acetic acid forms 1,4-naphthaquinone and by V_2O_5 yield phthalic acid with the destruction of one ring. The phthalic acid on heating is converted into phthalic anhydride.



3. **Reduction:** The reduction of naphthalene with $\text{Na}/\text{C}_2\text{H}_5\text{OH}$ forms 1,4-dihydronaphthalene. Catalytic hydrogenation gives 1,2,3,4-tetrahydronaphthalene (tetralin) or decahydronaphthalene (decalin) depending on catalyst used.



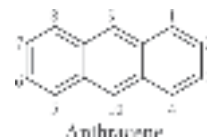
Uses

- It is used as moth-repellant.
- Used for production of phthalic anhydride.
- For making alpha naphthol (insecticide) and beta naphthol (tanning agent).
- For manufacture of dyes, explosives and synthetic resins.

ANTHRACENE

Anthracene is a tricyclic aromatic hydrocarbon. in which three benzene rings are arranged in a linear fashion. For naming anthracene derivatives, the numbering system is followed as shown here.

It is stabilized by five resonating structures (Fig. 12.3). The resonance energy is 84 kcal/mol, i.e. 28 kcal/mol per ring. This is substantially lower than that of benzene (36 kcal/mole). That is why anthracene is much less aromatic than benzene and gives both substitution and addition reactions with the same ease.



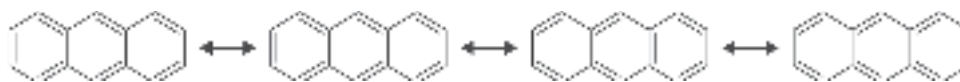
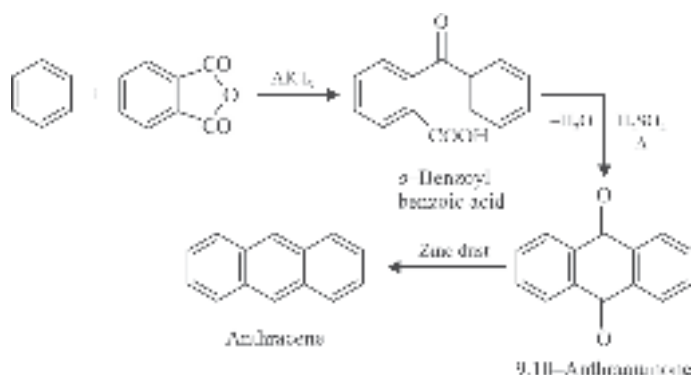


Fig. 12.3: Resonating structures of anthracene

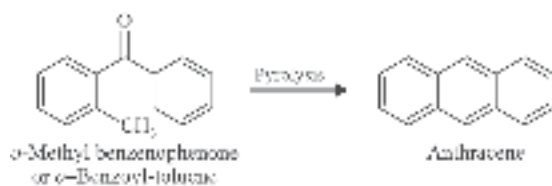
Preparations

1. **From anthracene oil:** The oil is chilled to crystallize out anthracene. The crude anthracene contains phenanthrene (an isomeric hydrocarbon) and carbazole (C_6H_4NH). It is washed with solvent naphtha (to remove phenanthrene) and pyridine (to remove carbazole). Finally, anthracene is purified by sublimation.
2. **Howarth synthesis:** Anthracene is synthesized by Freidel-Craft acylation of benzene with phthalic anhydride.

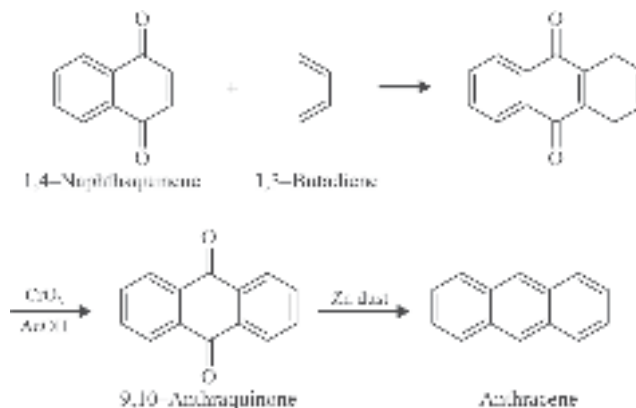


The benzene is acylated with phthalic anhydride to get *o*-benzoyl benzoic acid which on cyclisation gives the 9,10-anthraquinone. This on dehydrogenation with zinc dust gives the anthracene.

3. **Elbe reaction:** The pyrolysis of *o*-methyl benzophenone yields the anthracene.



4. Anthracene can also be prepared from the reaction of 1,4-naphthaquinone with 1,3-butadiene.

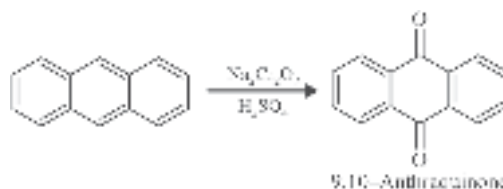


Physical Properties

- Colorless solid (m.p. 217°C, b.p. 340°C).
- When crystallized from benzene, it forms lustrous plates exhibiting blue fluorescence.
- Insoluble in water but soluble in alcohol and ether.
- λ_{max} 380 nm.

Chemical Properties

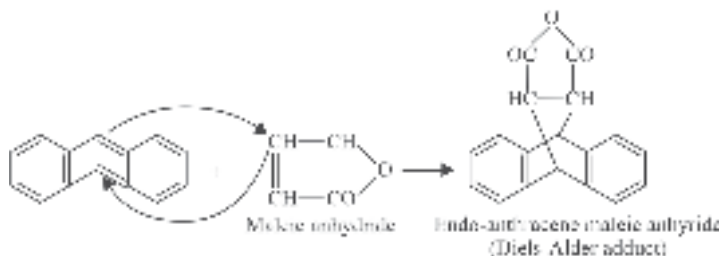
1. **Oxidation:** Anthracene on oxidation with acidified sodium dichromate gives 9,10-anthraquinone.



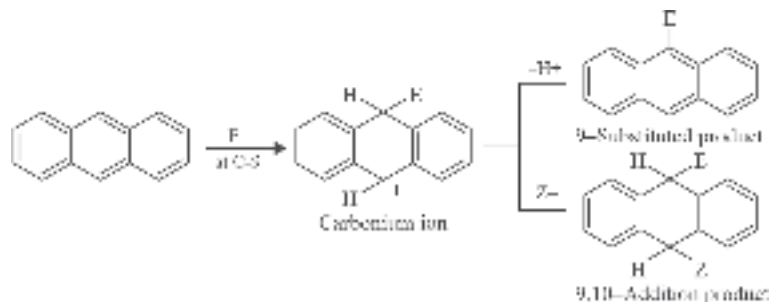
2. **Reduction:** On reduction with sodium and alcohol or on catalytic hydrogenation, it forms 9,10-dihydroanthracene. Further reduction is difficult.



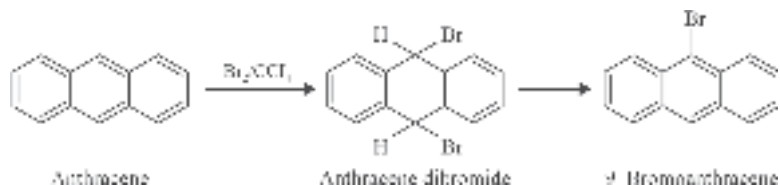
3. **Diels-Alder reaction:** Anthracene forms adduct on reaction with maleic anhydride.



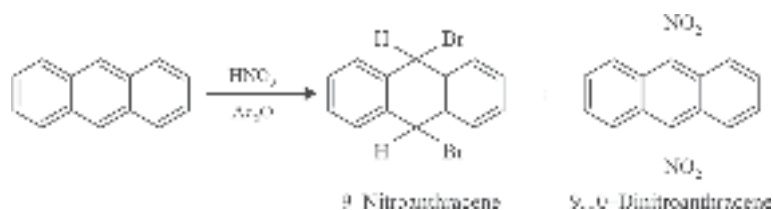
4. **Electrophilic substitution:** Anthracene is even more reactive than phenanthrene and has a greater tendency to add at 9,10-position than to substitute. However, the addition products of nitration and halogenations readily undergo elimination to form 9-substitution products.



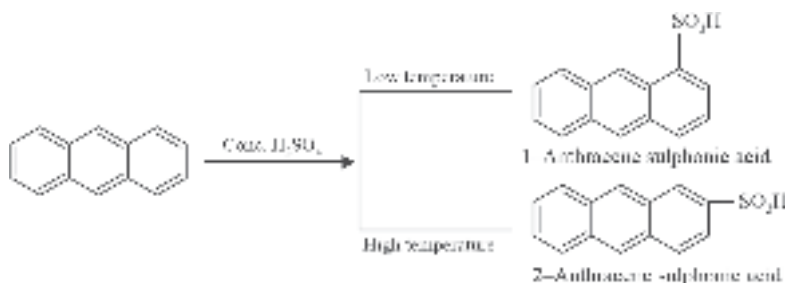
- (a) **Halogenation:** Anthracene reacts with chlorine or bromine in the absence of a catalyst to form anthracene dihalide. For example, anthracene reacts with bromine in CCl_4 gives anthracene dibromide. This on decomposition gives 9-bromo anthracene.



- (b) **Nitration:** On treatment with conc. nitric acid in acetic anhydride, anthracene forms 9-nitroanthracene and 9,10-dinitroanthracene.



- (c) **Sulphonation:** On sulphonation, it gives 1 or 2-anthracene sulphonic acid depending on the temperature.



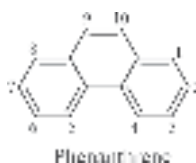
Uses

Anthracene is used for:

- Manufacture of anthraquinone.
- Making dyes (alizarin).
- In smoke screens.

PHENANTHRENE

Phenanthrene is also a tricyclic aromatic hydrocarbon, in which three benzene rings are fused angular fashion.



It is stabilized by five resonating structures as given in Fig. 12.4. The resonating energy is 92 kcal/mol.

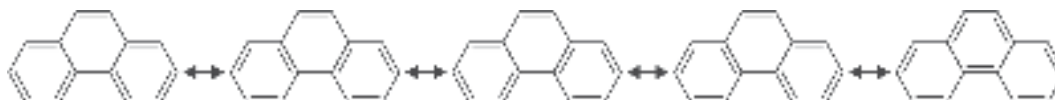
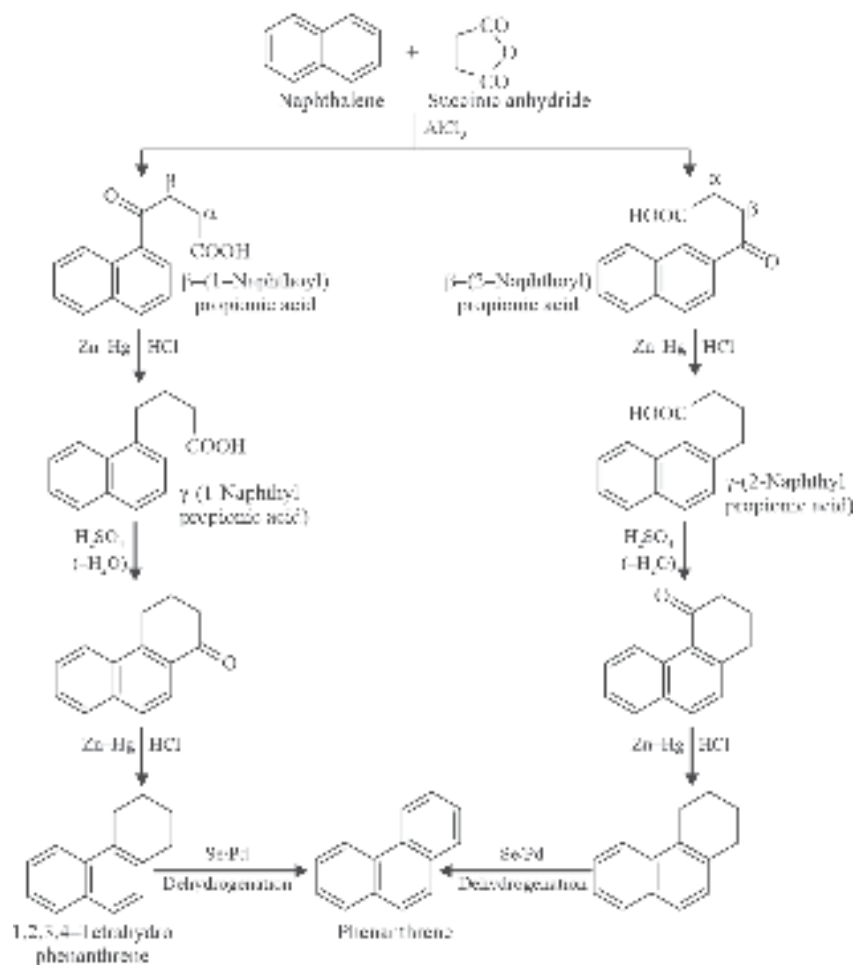


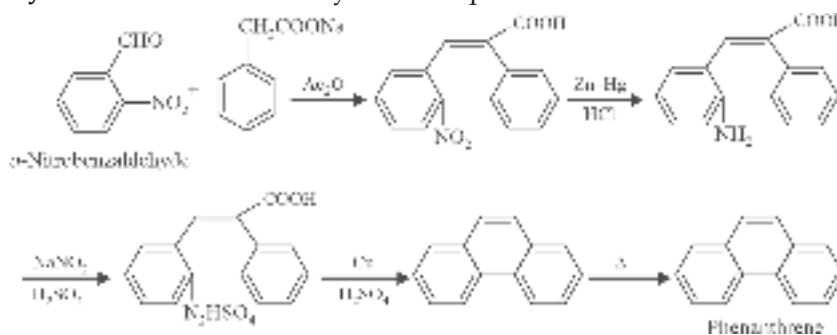
Fig. 12.4: Resonating structures of phenanthrene

Preparations

Howarth synthesis: It is synthesized by Howarth synthesis, which consists of Friedel-Craft acylation of naphthalene.



Pschorr synthesis: It includes the synthesis of phenanthrene from *o*-nitrobenzaldehyde.

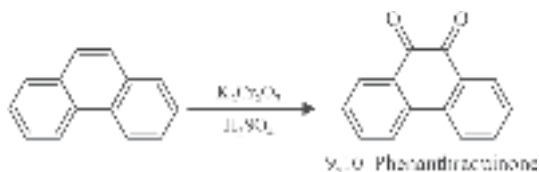




 2-Phenylcyclohexanol $\xrightarrow{P_2O_5}$ 2,10-Dihydro-phenanthrene $\xrightarrow{\Delta}$ Phenanthrene

- Solid (m.p. 100°C, b.p. 340°C).
- Soluble in organic solvent.
- Solution exhibits blue fluorescence.
- λ_{max} values nonnally are at shorter wavelength than those of their linear isomers.

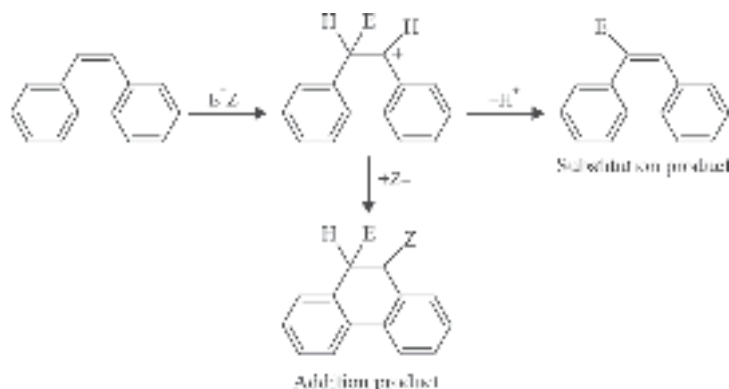
1. **Oxidation:** On oxidation with potassium dichromate, it gives 9,10-phenanthraquinone.



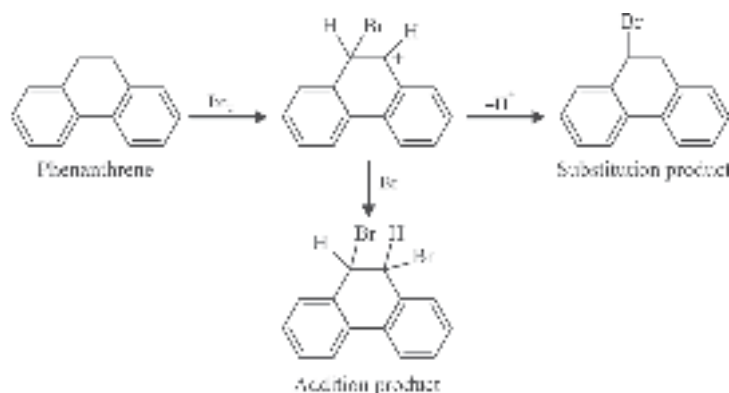




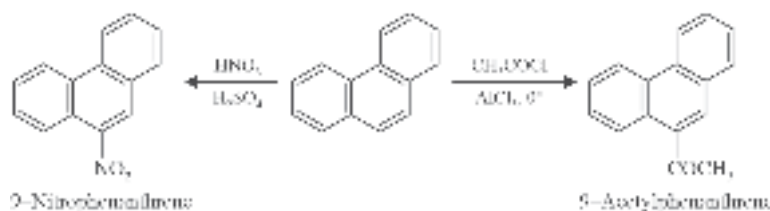
3. **Electrophilic substitution:** The reactions of phenanthrene with electrophilic reagents are more complex than of naphthalene. For example, phenanthrene can be nitrated and sulphonated and the products are mixtures of 1-, 2-, 3-, 4-, and 9-substituted phenanthrene.



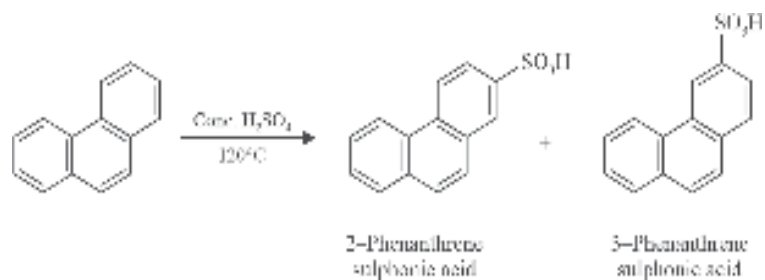
However, the bond 9,10 bond in phenanthrene is quite reactive; in fact it is almost as reactive as alkene double bond. Addition therefore occurs fairly readily; halogenations can give 9,10-addition and 9-substitution products by the following scheme.



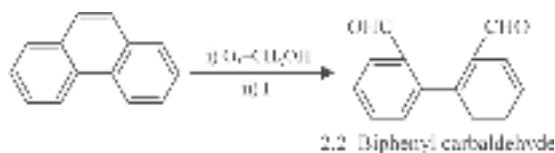
On Friedel Craft acylation with acetyl chloride in presence of anhydrous AlCl_3 at low temperature, 9-acetyl-phenanthrene is obtained.



Nitration of phenanthrene gives 9-nitrophenanthrene. Phenanthrene on sulphonation with concentrated sulphuric acid at 120°C temperature gives a mixture of 2-phenanthrene-sulphonic acid and 3-phenanthrene sulphonic acid.



4. **Ozonization:** The double bond character of 9,10-bond in phenanthrene is particularly evident in ozonization which leads to formation of a dialdehyde when ozonide is reduced with iodide ion.



Uses

Phenanthrene is used in the synthesis of dyes (e.g. alizarin), explosives and drugs (e.g. dithranol, used as antifungal drug). It is raw material of phenanthraquinone, widely used in the synthesis of dyes, agrochemical and preservatives.

SHORT AND LONG QUESTIONS

- What are polycyclic compounds. Classify them. Draw the resonating structures for the following.
 - Naphthalene
 - Anthracene
- Predict the product.
 - Naphthalene CrO_3/AcOH
 - Naphthalene $\text{O}_2/\text{V}_2\text{O}_5$, at $460\text{--}480^\circ\text{C}$
 - Naphthalene conc. H_2SO_4 , at 160°C
 - Naphthalene Cl_2
 - Naphthalene $\text{HNO}_3/\text{H}_2\text{SO}_4$, at $50\text{--}60^\circ\text{C}$
 - Naphthalene H_2SO_4
 - Naphthalene H_2/Pt
 - Naphthalene CH_3COCl , $\text{AlCl}_3/\text{CCl}_4$
- Naphthalene is aromatic in nature, explain.
- Draw the canonical structures for naphthalene and anthracene.
- Give the Haworth's synthesis of naphthalene.
- How will you convert naphthalene into:
 - 1,4-Naphthaquione
 - Decalin
 - β -Naphthalene sulphonic acid
 - α -bromonaphthalene
- Discuss the electrophilic substitution reactions of naphthalene.
- Give the methods of preparation of anthracene. Discuss its electrophilic substitution reactions.
- At which position electrophilic substitution takes place in phenanthrene and why?
 - Give the expected product which is formed on ozonization of phenanthrene.

MULTIPLE CHOICE QUESTIONS

- Which one of these is simplest polynuclear hydrocarbon?
 - Pyrene
 - Dibenzoanthracene

- c. Naphthalene
 - d. Benzacephenanthrylene
2. At what position in phenanthrene, substitution reaction takes place?
- a. 9,10 position
 - b. 2,3 position
 - c. 6,7 position
 - d. 4,5 position
3. Naphthalene on reaction with Ni/H₂ give:
- a. *cis*-decalin
 - b. *trans*-decalin
 - c. *cis*-tetralin
 - d. *trans*-tetralin
4. Naphthalene on oxidation with KMnO₄ in acidic medium gives:
- a. Phthaldehyde
 - b. Phthalic anhydride
 - c. Phthalic acid
 - d. Phthalonic acid
5. Ozonolysis of phenanthrene gives:
- a. Phenanthraquinone
 - b. Diphenic acid
 - c. Phenanthranic acid
 - d. Diphenylaldehyde
6. Anthracene on heating with oleum at 160°C temperature gives:
- a. 1-Sulphonic acid
 - b. 2-Sulphonic acid
 - c. 6-Sulphonic acid
 - d. 7-Sulphonic acid

Answers

1. (c) 2. (a) 3. (a) 4. (c) 5. (d) 6. (b)

Heterocyclic Compounds—Nomenclature, Synthesis, Properties and Medicinal Importance

Acyclic organic compound containing only carbon atoms in ring formation is referred to as a *carbocyclic compound*. If at least one atom, other than carbon atom, is a part of the ring system then it is designated as a *heterocyclic compound*. Nitrogen, oxygen and sulphur are the most common hetero atoms but heterocyclic rings containing other hetero atoms are also widely known. An enormous number of heterocyclic compounds are known and this number has been increasing rapidly day by day. Heterocyclic compounds may be classified into *aliphatic* and *aromatic*. The aliphatic heterocyclics are the cyclic analogues of amines, ethers, thioethers, amides, etc. Their properties are particularly influenced by the presence of strain in the ring. These compounds generally consist of small (3- and 4-member) and common (5–7 member) ring systems. The aromatic heterocyclic compounds, in contrast, are those which have a heteroatom in the ring and behave in a manner similar to benzene in some of their properties. Furthermore, these compounds also comply with the general rule proposed by Huckel. *This rule states that aromaticity is obtained in cyclic conjugated and planar systems containing $(4n + 2)$ π -electrons.*

The conjugated cyclic rings contain six π -electrons as in benzene, and this forms a conjugated molecular orbital system, which is thermodynamically more stable than the non-cyclic conjugated system. This extra stabilization results in a diminished tendency of the molecule to react by addition, but a larger tendency to react by substitution in which the aromatic ring remains intact.

A heterocyclic ring may comprise of three or more atoms which may be saturated or unsaturated. Also the ring may contain more than one hetero atom, which may be similar or dissimilar. The chemistry of heterocyclic compounds is as logical as that of aliphatic or aromatic compounds. Their study is of great interest both from the theoretical as well as practical standpoint. Heterocyclic compounds occur widely in nature and in a variety of non-naturally occurring compounds. A large number of heterocyclic compounds are essential to life. Various compounds such as alkaloids, antibiotics, essential amino acids, the vitamins, hemoglobin, the hormones and a large number of synthetic drugs and dyes contain heterocyclic ring systems. The knowledge of heterocyclic chemistry is useful in biosynthesis and in drug metabolism as well nucleic acids are important in biological processes of heredity and evolution. There are a large number of synthetic heterocyclic compounds with additional important applications and many are valuable intermediates in synthesis.

Nomenclature System in Heterocyclic Compounds

In heterocyclic chemistry, there is a special name for each individual ring system and a trivial name for each compound. Trivial names convey little or no structural information but they are still commonly used. The systematic name, in contrast, is designed so that one may deduce from it the structure of the compound. They tend to be long. However, a systematic nomenclature is still indispensable. In recent years, the International Union of Pure and Applied Chemistry (IUPAC) has made efforts to systematize the nomenclature of heterocyclic compounds.

According to this system, single three-to-ten-member rings are named by combining the appropriate *prefix* or *prefixes* (listed in Table 13.1) with a stem from Table 13.2.

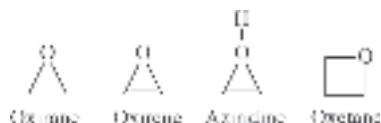
Table 13.1: Prefix for hetero atoms

Hetero atom	Valence	Prefix
O	2	Oxa
N	3	Aza
S	2	Thia
Se	2	Selena
Te	2	Tellura
P	3	Phospha
As	3	Arsa
Si	4	Sila
Ge	4	Germa

Table 13.2: Common name endings for heterocyclic compounds

Ring size	Suffixes for fully unsaturated compounds		Suffixes for fully saturated compounds	
	With 'N'	Without 'N'	With 'N'	Without 'N'
3	-irine	-irene	-iridine	-irane
4	-ete	-ete	-etidine	-etane
5	-ole	-ole	-olidine	-olane
6	-ine	-in	—	-ane
7	-epine	-epin	—	-epane
8	-ocine	-ocin	-ocane	

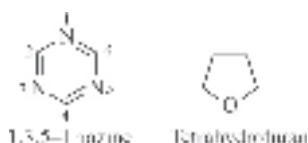
The endings in Table 13.2 also indicate the size of the ring and the state of hydrogenation with or without the presence of a nitrogen atom. Accordingly some examples of compounds named on the basis of above two tables are also given.



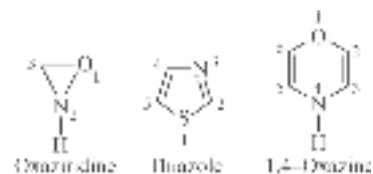
In all these examples, the letter 'a' has been omitted. However, in the following cases, letter 'a' has been retained.



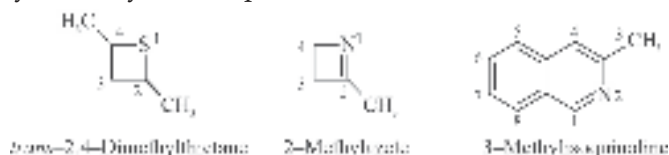
Saturated or hydrogenated ring systems are named by varying the ending or by placing prefixes such as '*dihydro*-' , '*tetrahydro*-' , etc. The ending of the name will depend on the presence or absence of nitrogen (Table 13.2).



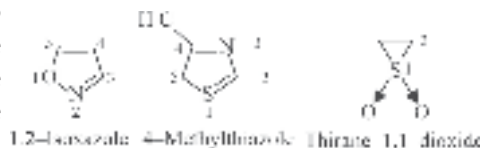
Two or more similar atoms contained in a ring are indicated by the prefixes 'di-', 'tri', etc. placed before the appropriate 'a' term (Table 13.2). If two or more different hetero atoms occur in the ring, then it is named by combining the prefixes as in Table 13.1 with the ending in Table 13.2 in order of their preference, i.e. O, S and N. This is illustrated by the following examples:



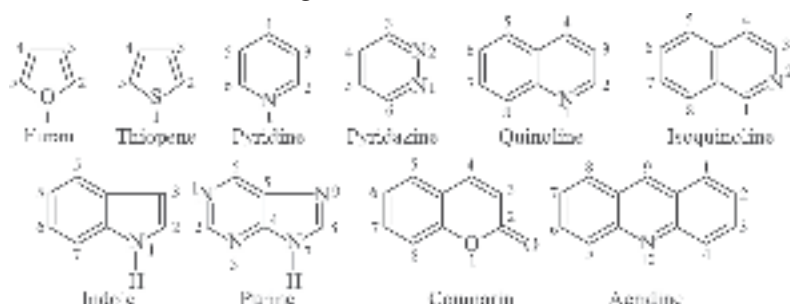
The position of a single hetero atom controls the numbering in a monocyclic compound but not necessarily in a bicyclic compound.



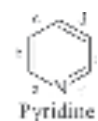
Numbering of the heterocyclic rings becomes essential, when substituents are placed on the ring. Conventionally, the hetero atom is assigned position 1 and the various substituents are then counted around the ring in a manner so as to give them the lowest possible numbers. While writing the name of the compound, the substituents are placed in an alphabetical order. In case the heterocyclic ring containing more than one hetero atom, the order of preference for numbering is O, S and N. The ring is numbered from the atom of preference in such a way so as to give the smallest possible number to the other hetero atoms in the ring. As a result the position of the substituent plays no part in determining how the ring is numbered in such compounds. The following examples illustrate this rule:



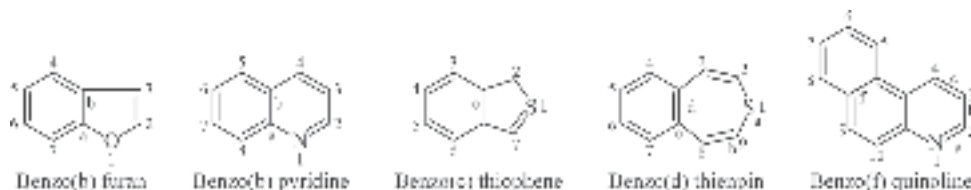
There are a large number of important ring systems, which do not possess any systematic names rather non-systematic or common names are used for them. Some of such examples include the following:



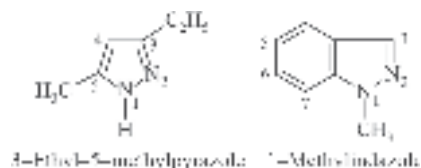
The numbering and nomenclature of heterocyclic rings become more complicated for condensed or fused ring systems, i.e. when a part of one ring is also a part of another ring. Such ring systems, however, are known by nonsystematic or common names, such as indole, isatin, isoquinoline, etc. as indicated in the preceding paragraph. There is yet another system of nomenclature for fused rings that is commonly employed. According to this system, the side of the heterocyclic ring is labelled by the letters a, b, c, etc. starting from the atom numbered 1. Therefore, side 'a' being between atoms 1 and 2, side 'b' between atoms 2 and 3, and so on as shown here for pyridine.



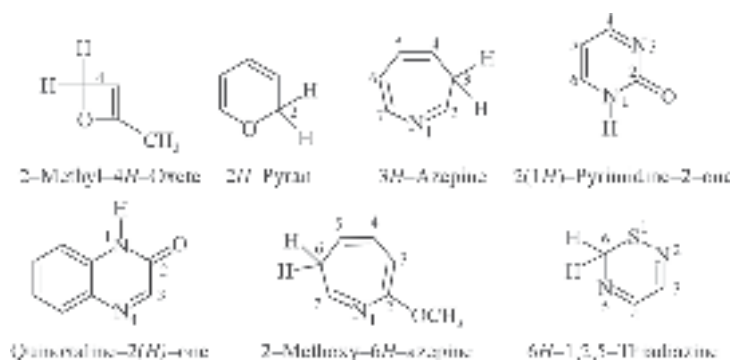
The name of the heterocyclic ring is chosen as the parent compound and the name of the fused ring is attached as a prefix. The prefix in such names has the ending 'o', i.e. benzo, naphtho and so on. The following examples explain this rule.



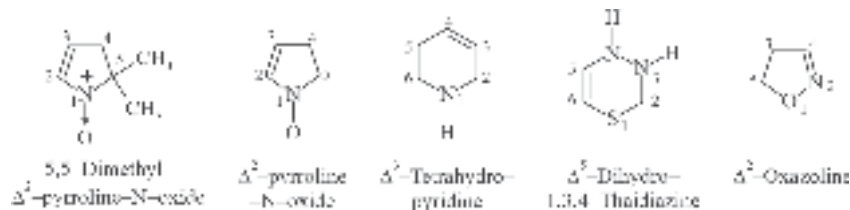
In a heterocyclic ring, other things being equal, numbering preferably commences at a saturated rather than at an unsaturated hetero atom, as depicted in the following examples:



In a heterocyclic ring with maximum unsaturation, if the double bonds can be arranged in more than one way, then their positions are specified by numbering those nitrogen or carbon atoms which are not multiply-bonded, i.e. bear an 'extra' hydrogen atom, by italic capital '1H', '2H', '3H', etc. The numerals indicate the position of these atoms having the extra hydrogen atom. The following examples illustrate this rule:



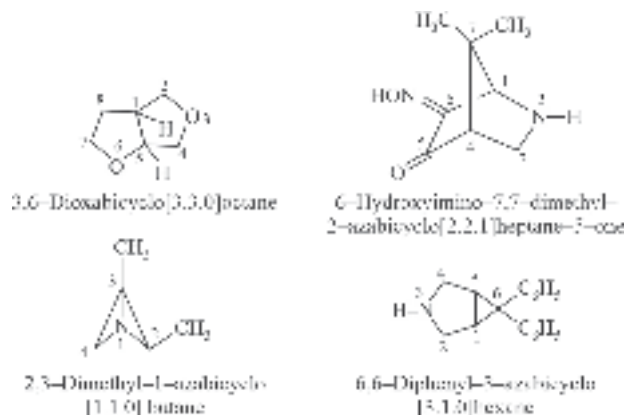
The position of the hydrogen atom in a partially saturated heterocyclic ring can be indicated by writing 1,2-dihydro, etc. with the name of the compound. Alternatively, the position of the double bond can also be specified as Δ^1 , Δ^2 , Δ^3 , etc. which indicates that 1 and 2; 2 and 3; 3 and 4 atoms respectively have a double bond.



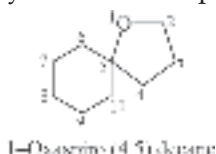
A positively charged ring is denoted by the suffix "-ium". Groups such as C = S and C = NH present in the ring are denoted by the suffixes "-thione" and "-imine".

Bicyclic bridged structures are quite common in heterocyclic chemistry. The nomenclature of such a structure consists of the prefix bicyclo, followed in square brackets the number of carbon atoms, separating the bridge heads by the three possible routes in

descending numerical order. This is followed by the alkane containing the same number of carbon as the whole bicyclic heterocyclic skeleton. The following examples illustrate the use of this rule.



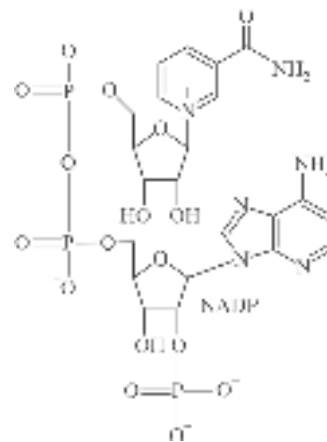
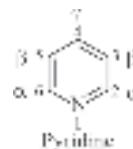
In 'spiro' fused compounds, bridgehead positions are on the same carbon. Hence the word 'spiro' is placed for word 'bicyclo'. For example:



SIX-MEMBERED HETEROCYCLIC RINGS WITH ONE HETERO ATOM

Pyridine

Pyridine compounds are characterized by the presence of a six-membered heterocyclic ring consisting of five carbon atoms and one nitrogen atom. The carbon valencies not taken up in forming the ring are satisfied by hydrogen atoms. The arrangement of atoms is similar to benzene except that one of the carbon "hydrogen ring sets has been replaced by a nitrogen atom. The parent compound is pyridine itself. Substituents in the pyridine ring are indicated either by the numbering shown here. 1 through 6, or by the Greek letters, α , β or γ . The Greek symbols refer to the position of the substituent relative to the ring nitrogen atom, and are usually used for naming monosubstituted pyridines. The 'o' (ortho), 'm' (meta), and 'p' (para) nomenclature commonly used for disubstituted benzenes is not used in naming pyridine compounds. Pyridine and its simple derivatives are stable and relatively unreactive liquids, with strong unpleasant odors. They are much used as solvents and bases, especially pyridine itself, in reactions such as N- and O-tosylation and acylation. Pyridine is used as solvent for paint, rubber, pharmaceuticals, etc. Pyridine and the monomethyl pyridines (picolines) are miscible with water. Pyridine is first isolated, like pyrrole, from bone pyrolysates: the name is constructed from the Greek for fire, pyr and the suffix, idine which was at the time being used for all aromatic bases—phenetidine, toluidine, etc.



The pyridine ring plays a key role in several biological processes, most notably in the oxidation/reduction coenzyme nicotine adenine dinucleotide (NADP) and also form basic skeleton of many drugs (Fig. 13.1). The vitamin niacin (or the corresponding acid) is required for its biosynthesis. Pyridoxine

(vitamin B₆) plays a key role as the coenzyme in transaminases. Nicotine, a highly *toxic* alkaloid, is the major active component in tobacco, and the most addictive drug known. Many synthetic pyridine derivatives are important as therapeutic agents, for example isoniazid is a major antituberculosic agent. Sulphapyridine is one of the sulphonamide antibacterials. Pralidoxime is an antidote for poisoning caused by organophosphates, and amlodipine is one of several antihypertensive 1,4-dihydropyridines. Some herbicides (Paraquat) and fungicides (Davicil) are also pyridine derivatives.

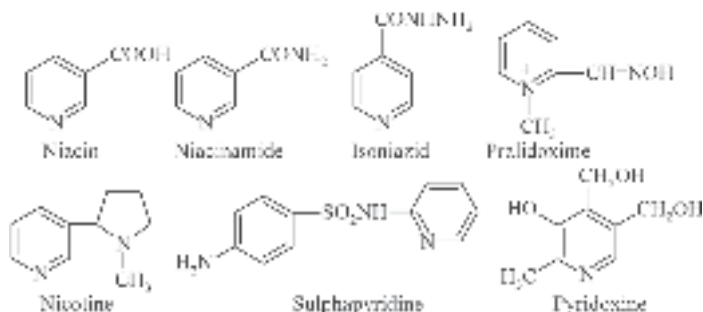


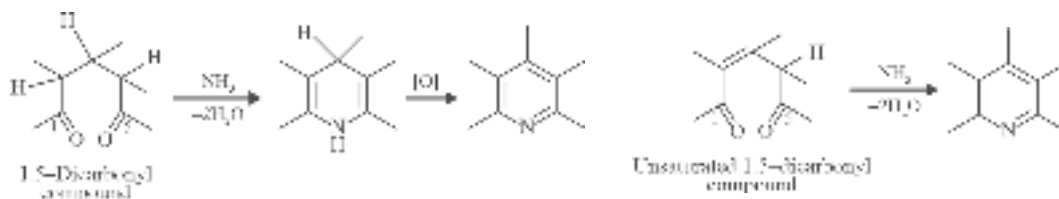
Fig. 13.1: Examples of pyridine ring containing drugs

Synthesis of Pyridines

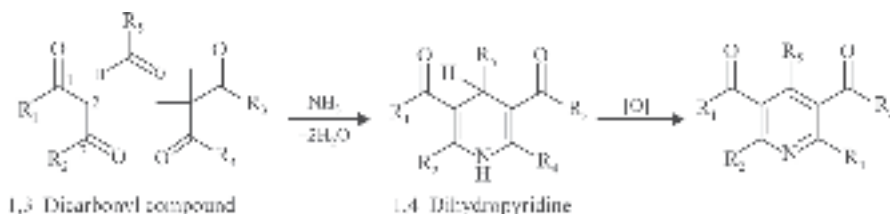
Pyridine and its simple alkyl derivatives were for a long time produced by isolation from coal tar. In recent years, this source has been displaced by synthetic processes: pyridine itself, for example, can be produced on a commercial scale in 60–70% yields by the gas-phase high-temperature interaction of crotonaldehyde, formaldehyde, steam, air and ammonia over a silica-alumina catalyst.

Various other methods of synthesis of pyridines are as follows:

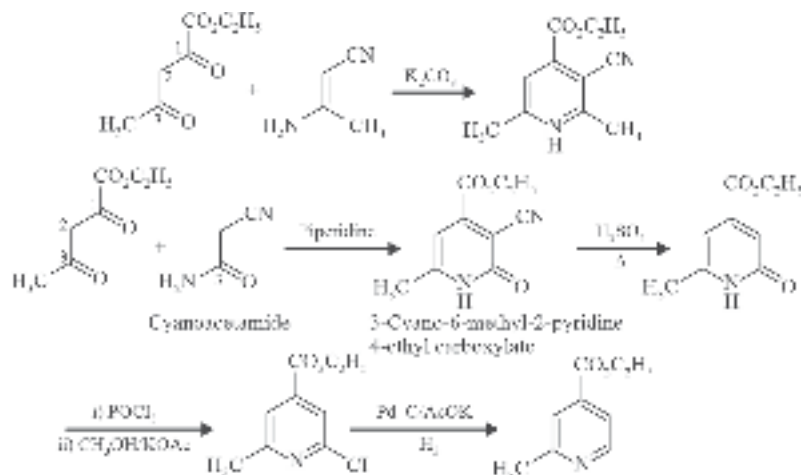
- (i) **From 1,5-dicarbonyl compounds and ammonia:** Ammonia reacts with 1,5-dicarbonyl compounds to give unstable 1,4-dihydropyridines, which on dehydrogenation produces pyridines. In a similar manner, the unsaturated 1,5-dicarbonyl compounds, react with ammonia to give pyridines directly.



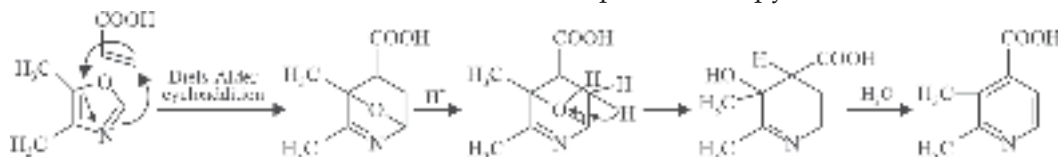
- (ii) **Hantzsch synthesis:** Symmetrical 1,4-dihydropyridines are produced from the interaction of ammonia, an aldehyde, and two equivalents of a 1,3-dicarbonyl compound (which must have a central methylene moiety). Dihydropyridines on oxidation give pyridines.



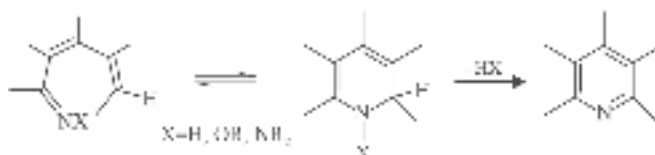
- (iii) **From 1,3-dicarbonyl compounds and nitriles:** Pyridines are formed from the interaction of 1,3-dicarbonyl compound with nitriles under mild condition. It is known as **Guareschi synthesis**.



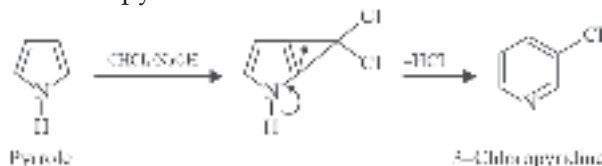
- (iv) **By cycloadditions:** Various electrocyclic additions, with subsequent extrusion of a small molecule have been used to get pyridines. The below given example shows the addition of small molecule to oxazole to produce the pyridine.



- (v) **By thermal electrocyclization:** Electrocyclization of 1-aza-1,3,5-trienes give dihydropyridines which can be oxidised to pyridines. If an oxime or hydrazine derivative is used, elimination of water or an amine *in situ* gives the pyridine directly.



- (vi) **Ring expansion:** Pyrrole undergoes ring expansion after reaction with dichlorocarbene to give 3-chloropyridine.

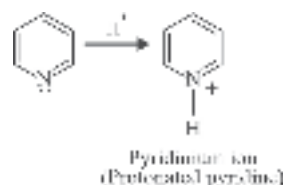


Physical properties

Pyridine is colorless liquid or slightly yellow, b.p. 115.5°C with characteristic odor. It is miscible with water.

Basicity of Pyridine

Low basicity of pyridine is due to the fact that the lone pair of electrons on the nitrogen are in a sp^2 hybridized orbital, while



those in an alkyl are in a sp^3 -hybridized orbital. The electrons in an orbital with more 's' character are held more closely to the nucleus and are less available for bonding (sp^2 -hybridized orbital, 33% s character, sp^3 -hybridized orbital, 25% s character). The weakly electron donating methyl group always enhances the basicity and the effect is larger from the 2- or the 4-position than from the 3-position.

Chemical Properties

Electrophilic substitution reactions: Pyridine is a six-membered aromatic heterocycle and undergoes electrophilic substitution, e.g. nitration, sulphonation, halogenation, formylation, etc. but the ring is highly deactivated (about million times) compared to benzene because the more electronegative nitrogen pulls the electrons towards itself, thus reducing the electron density on the ring carbons.

The electrophilic substitution takes place at C-3 position in pyridine. This orientation can be explained by comparing the relative stabilities of the intermediates arising from attack at C-3, C-4 or C-2 position (Fig. 13.2). The electrophilic attack at C-3 position gives a carbocation which is hybrid of three resonance structures in which the positive charge is on the carbon atoms only.

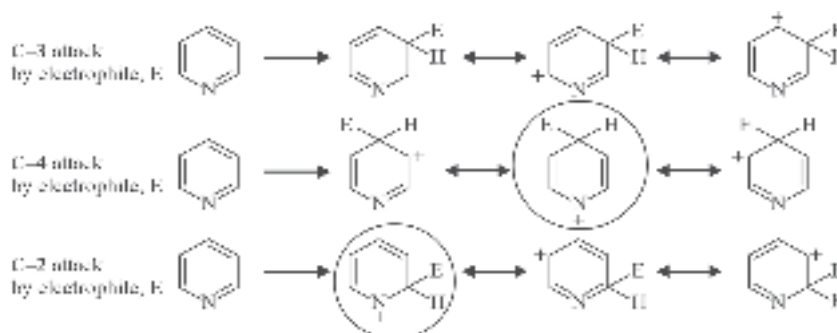


Fig. 13.2: Electrophilic substitution reactions in pyridine

The electrophilic attacks at C-4 or C-2 position also give an ion, which is hybrid of three resonance structures but one of the resonance structures in each contains positively charged nitrogen which is a *sextet* and so unstable and hence does not contribute significantly to the resonance hybrid. Thus, the resonance hybrid resulting from electrophilic attack at C-3 position, is more stable and the preferred site of electrophilic attack.

Addition to Nitrogen

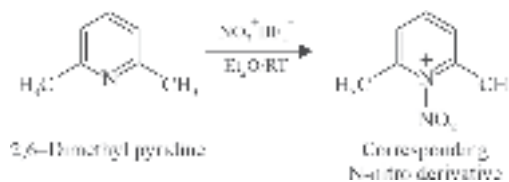
In the reactions, such as protonation and quaternarization which involve bond formation using the lone pair of electrons on the ring nitrogen, pyridines behave just like tertiary aliphatic or aromatic amines. When a pyridine reacts as a base or a nucleophile, it forms a pyridinium cation in which the aromatic *sextet* is retained and the nitrogen acquires a formal positive charge.

(i) Protonation of Nitrogen

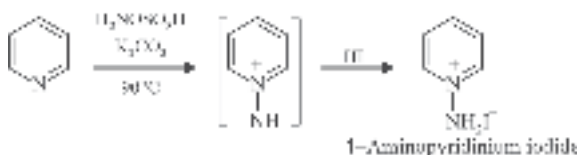
Pyridines form crystalline salt, frequently hygroscopic with most of the protic acids. The positive charge is retained by nitrogen atom. Pyridine itself, with pK_a 5.2 in water, is a weaker base than saturated aliphatic amines which have pK_a values between 9 and 11.

(ii) Nitration at Nitrogen

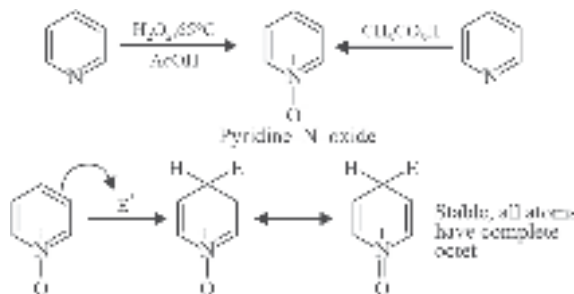
This occurs readily by reaction of pyridines with nitronium salts, such as nitronium tetrafluoroborate. Protic nitrating agents such as nitric acid cause exclusively N-protonation. 1-Nitro-2,6-dimethylpyridinium tetra-fluoroborate is one of several N-nitropyridinium salts, which can be used as non-acidic nitrating agents with good substrate and positional selectivity.

**(iii) Amination of Nitrogen**

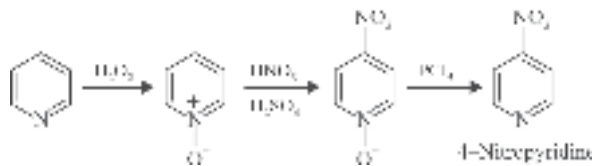
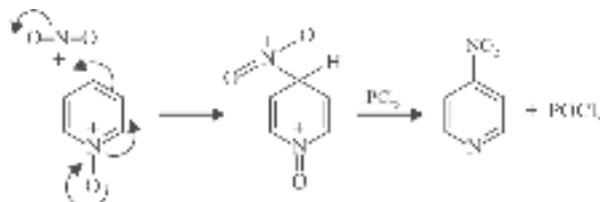
The introduction of amino group at nitrogen can be achieved with hydroxylamine *o*-sulphate.

**(iv) Oxidation of Nitrogen**

Pyridine reacts with percarboxylic acids or hydrogen peroxide to give N-oxides. Pyridine-N-oxide is more reactive towards electrophilic and nucleophilic substitution reactions than pyridine. The electrophilic or nucleophilic attack takes place preferably at the C-4 position.

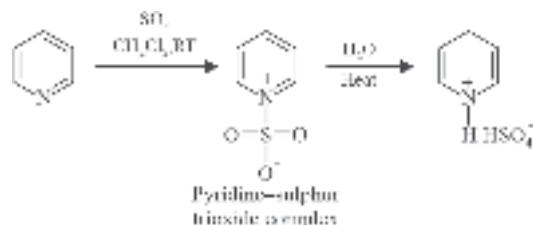


Direct nitration of pyridine to produce 4-nitro pyridine is not quite possible, but this can be done via nitration of pyridine-N-oxide followed by the treatment with PCl_5 as follows:

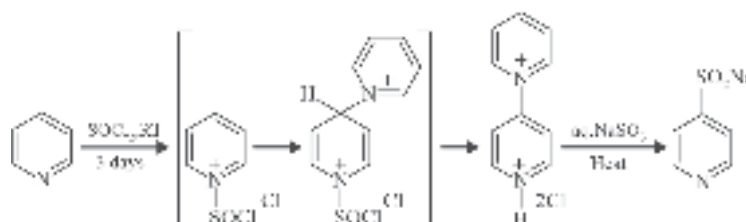
**Mechanism****(v) Sulphonation at Nitrogen**

Pyridine reacts with sulphur trioxide to give crystalline, zwitter ionic pyridinium-1-sulphonate, known as pyridine sulphur trioxide complex. This compound is quite active

and hydrolysed in hot water to give sulphuric acid and pyridine, but usefully it serves as a mild sulphonating agent. It also serves as source of sulphur trioxide.

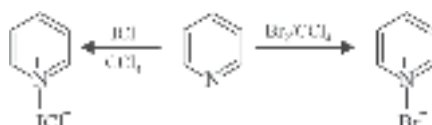


When pyridine reacts with thionyl chloride, a synthetically useful dichloride salt is formed, which can be transformed into pyridine-4-sulphonic acid. The reaction is believed to involve initial attack by sulphur at nitrogen, followed by nucleophilic addition of a second pyridine at C-4 position.



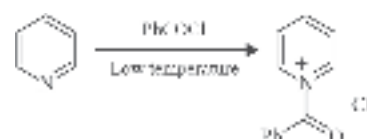
(vi) Halogenation at Nitrogen

Pyridines react with halogens to give crystalline compounds, which are undissociated when dissolved in non-polar solvents such as carbon tetrachloride.



(vii) Acylation at Nitrogen

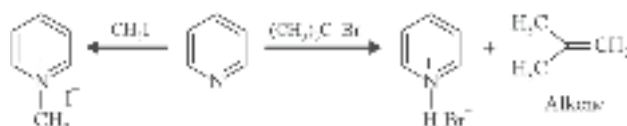
Carboxylic, arylsulphonic acid and alkyl halides react easily with pyridines to produce 1-acyl- and 1-aryl-sulphonylpyridinium salts in solution. These are very reactive and widely used as acylating agent.



The usual acylation reaction procedure involves the addition of alcohol or amine solution to a solution prepared by addition of the acyl chloride to an excess of pyridine.

(viii) Alkylation at Nitrogen

Alkyl halides and sulphates react with pyridines to give quaternary pyridinium salts. As with aliphatic tertiary amines, increased substitution around the nitrogen, or around the halogen-bearing carbon, causes an increase in the alternative competing, elimination process which gives alkene and N-proto-pyridinium salt.



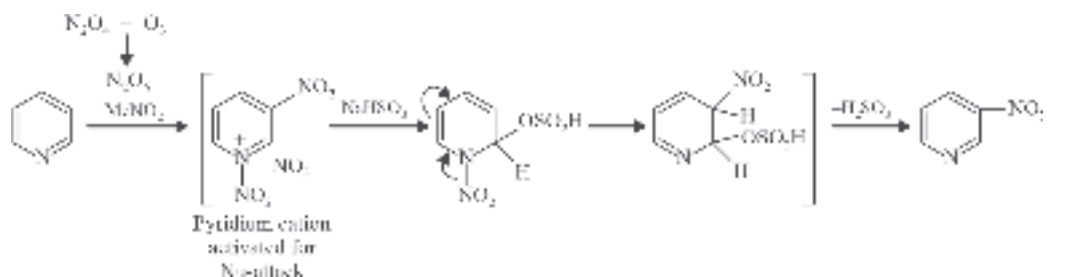
Electrophilic Substitution at Carbon

Electrophilic substitution of pyridines occurs less frequently than corresponding substituted benzene, because of electrophilic reagent, or a proton in the reaction medium, adds preferentially to the pyridine nitrogen, generating a pyridinium cation, which is very resistant to a further attack by an electrophile. Electrophilic substitution at carbon must involve attack on a pyridinium cation or free pyridine base. Some of the typical electrophilic substitution like Friedel-Crafts alkylation and acylation reactions do not occur.

(i) Nitration

Pyridine on nitration gives only a small yield of 3-nitropyridine under vigorous conditions. Steric or inductive inhibition of N-Nitration allows C-substitution using nitronium tetrafluoroborate.

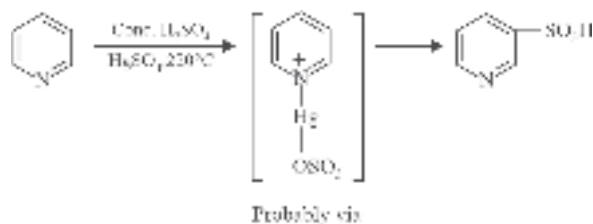
3-Nitropyridine and some of its substituted derivatives are prepared by reaction with dinitrogen pentoxide.



The initially formed N-nitropyridinium nitrate suffers addition of a nucleophile-sulphur dioxide when this is used as solvent or co-solvent forming a 1,2-dihydropyridine. Transfer of the nitro group to a 3- or 5-position, via a (1,5]-sigmatropic migration produce 3 or 5-nitro pyridine.

(ii) Sulphonation

Pyridine is very resistant to sulphonation by using concentrated sulphuric acid or oleum. Very low yields of 3-sulphonic acid is produced at 320°C, while addition of mercuric sulphate in catalytic quantity gives rapid sulphonation.



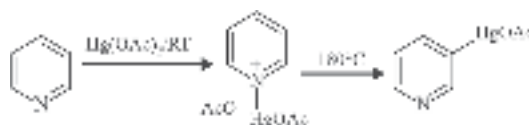
(iii) Halogenation

3-Bromopyridine is produced by the action of bromine in oleum. The process involves pyridinium-1-sulphonate as the reactive species as no bromination occurs in 95% sulphuric acid. 3-Chloropyridine can be produced by chlorination at 100°C in the presence of aluminium chloride.

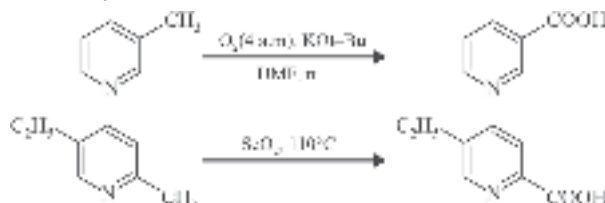


(iv) Acetoxymercuration

The salt formed by the interaction of pyridine with mercuric acetate at room temperature can be rearranged to 3-acetoxymercurypyridine by heating at 180°C. This process involves C-position attack by a relatively weakly electrophilic reagent.

**Reactions with Oxidizing Agents**

The pyridine ring is resistant to oxidising agents. In vigorous conditions, pyridine itself is oxidised by neutral aqueous potassium permanganate to give carbon dioxide at the same rate as benzene. In acidic solution, pyridine is more resistant than benzene, but in alkaline media more rapidly oxidised, than benzene.

**Nucleophilic Substitution Reactions**

Pyridine undergoes nucleophilic substitution reactions much more readily than benzene because the ring has lower electron density than benzene due to electron withdrawal by nitrogen, which is also responsible for its lower reactivity towards electrophiles. The high reactivity of pyridine towards nucleophilic substitution can cause displacement of even powerful hydride (H^-) ion, e.g. the reaction of pyridine with sodamide gives 2-aminopyridine.

The nucleophilic substitution in pyridine takes place at C-2 and C-4 positions compared to electrophilic substitution at C-3 (Fig. 13.3). Attack of nucleophile at C-2, C-3 and C-4 gives different resonating structures.

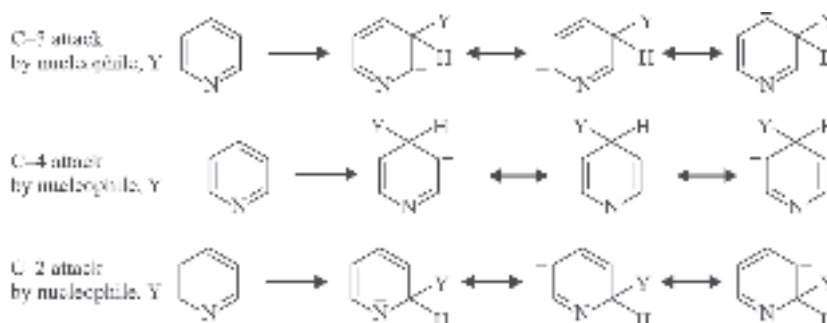
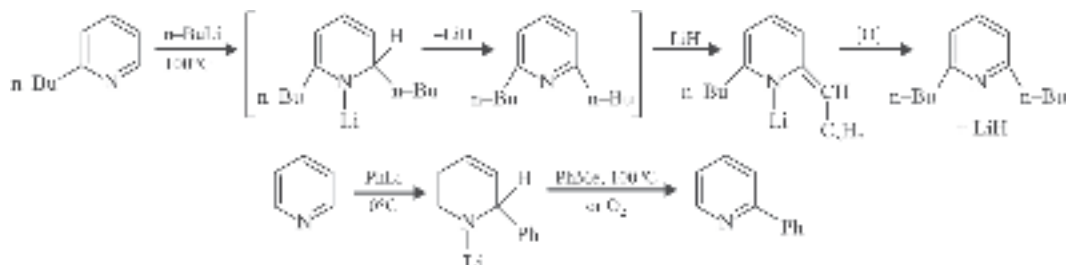


Fig. 13.3: Nucleophilic substitution reactions in pyridine

It is obvious from the above structures that nucleophilic attack at C-2 and C-4 gives hybrid of three resonance structures, in which one of the contributory structures contains negative charge on nitrogen which is more electronegative atom and therefore contributes significantly towards its stabilization unlike nucleophilic attack at C-3 in which the negative charge is present on only carbon atoms. Since the intermediate resulting from nucleophilic attack at C-2 and C-4 is more stable, nucleophilic substitution at these positions is preferred.

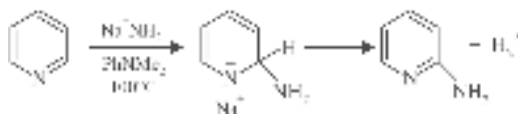
(i) Alkylation and Arylation

Reaction with alkyl- or aryl-lithiums proceeds in two steps addition to give a dihydropyridine N-lithio-salt, which converted into the substituted aromatic pyridine by oxidation, or elimination of lithium hydride.



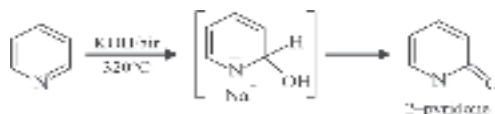
(ii) Amination

Amination occurs at a position α to the nitrogen. It is called the **Chichibabin reaction**. The pyridine reacts with sodamide with the evolution of hydrogen and the 'hydride' transfer. In simple cases α -substitution occurs but where both the α -positions are occupied, it is possible to get γ -aminopyridine.



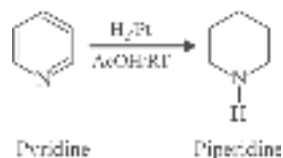
(iii) Hydroxylation

Hydroxide ion is weaker nucleophile than amide so attacks pyridine only at very high temperatures to produce a low yield of 2-pyridone. The corresponding reaction with isoquinoline proceeds much more smoothly.

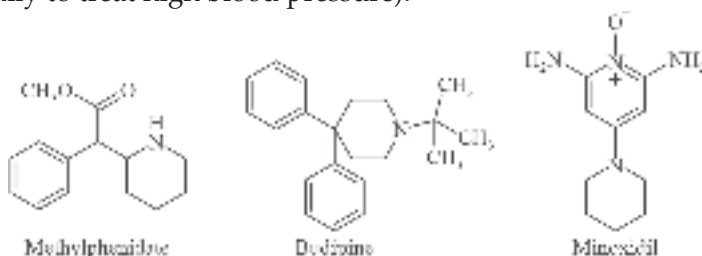


Reactions with Reducing Agents

Pyridines are much more easily reduced than benzene. Catalytic reduction usually proceeds to completion at atmospheric pressure at ambient temperature to give piperidine. Industrially, piperidine is produced by the hydrogenation of pyridine, usually over a molybdenum disulphide catalyst. Pyridine can also be reduced to piperidine by sodium in ethanol.



Piperidine, hexahydropyridine, is an organic compound with the molecular formula, $(CH_2)_5NH$. This heterocyclic amine consists of a six-membered ring containing five methylene units and one nitrogen atom. It is a colorless fuming liquid b.p. 106°C with an ammoniacal or pepper like odor; the name comes from the genus name *Piper*, which is the Latin word for pepper. It is soluble in water, alcohol, and ether. It is a basic moiety of some drugs such as methylphenidate (central nervous system stimulant), bupropion (antiparkinsonian drug) raltegravir (selective estrogen receptor modulator), minoxidil (a drug, given orally to treat high blood pressure).



SIX MEMBERED HETEROCYCLIC RINGS WITH TWO HETERO ATOMS

Pyridazine

Pyridazine, also called 1,2-diazine contains a six-member ring with two adjacent nitrogen atoms.

Pyridazine has no household use. It is mainly used in research and industry as building block for more complex compounds. The pyridazine structure is found within a number of herbicides such as credazine, pyridafol and pyridate. It is also found within the structure of several drugs such as cefozopran (antibacterial), cadralazine (vasodilator), minaprine (psychotropic), hydralazine (antihypertensive), and cilazapril (antihypertensive) (Fig. 13.4).

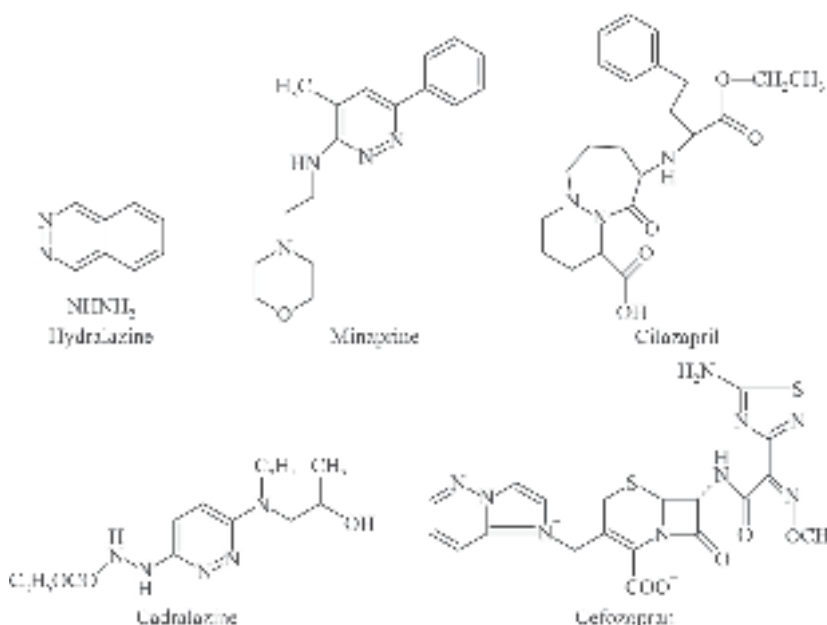
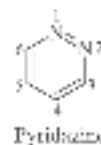
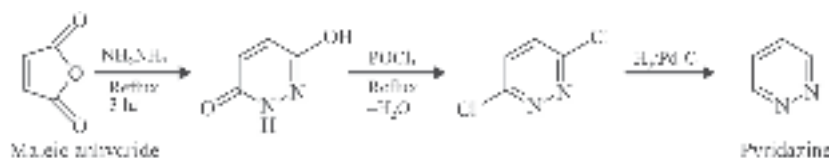


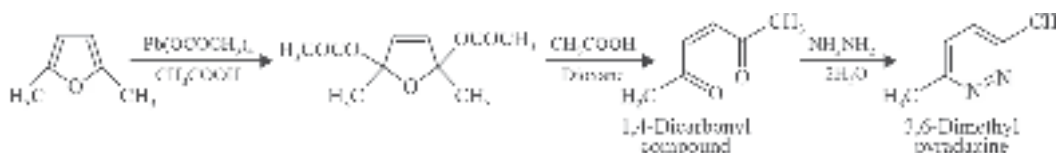
Fig. 13.4: Pyridazine ring containing drugs

Synthesis of Pyridazines

- (i) **From maleic anhydride:** The reaction of maleic anhydride with hydrazine yields pyridazine.



- (ii) **From 1,4-dicarbonyl compounds:** The reaction of 1,4-dicarbonyl with hydrazine also yields pyridazine.

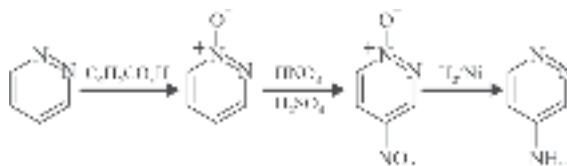


Physical Properties

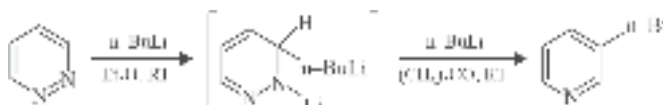
It is a colorless liquid b.p. 208°C with pyridine like odor. The pyridazine is completely miscible with water and alcohol.

Chemical Reactions

- (i) **Reaction with acids:** It is a weak base; thus form readily salts with mineral acids. The protonation of second nitrogen atom is difficult because of the high energy required to generate two positive charges on adjacent atoms.
- (ii) **Electrophilic substitution reactions:** The C-3, 4, 5 and 6 positions in pyridazine nucleus are electron deficient due to inductive effect. Hence, electrophilic substitution can undergo reaction only under drastic condition. No sulphonation or nitration of pyridazine has been reported. The conversion into 1-oxide offers a mean of preparing various pyridazine substitution products.



- (iii) **Reaction with nucleophilic reagents:** The diazines in general, are very susceptible to the action of nucleophilic reagents. The presence of a second nitrogen atom makes the carbon atoms of the ring even more electron deficient in comparison to pyridine.

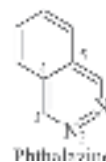
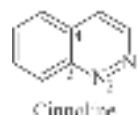


Aminopyridazines are prepared by the direct displacement of the halo group with CN^- , ammonia or amines.

Fused Pyridazines

The pyridazine ring can be fused into a benzene ring in two ways:

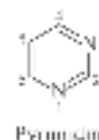
- (i) Fusion at 3,4-position: gives cinnoline (a weak base and has antibacterial action against *E. coli*).
- (ii) Fusion at 4,5 position: gives phthalazine (more reactive than pyridazine and can undergo direct electrophilic substitution at position-5 and 8).



Pyrimidine

Pyrimidine is an organic compound which is heterocyclic and aromatic in nature. The structure of pyrimidine is much more similar to benzene and pyridine. It includes two such nitrogen atoms in C-1 and C-3 position of six-membered ring. The pyrimidine structure is isomeric with other forms of diazine structure: Pyridazine, with the nitrogen atoms in positions 1 and 2 and pyrazine, with the nitrogen atoms in positions 1 and 4. Nucleotides of pyrimidine derivatives are found in nucleic acids. Three such pyrimidine derivatives are as follows:

- Cytosine (C)
- Thymine (T)
- Uracil (U)



It is the most important member of all the diazines as this ring system occurs widely in living organisms. Some important members, found in biological system, are folic acid (Chapter 11), vitamin B₁ and nucleoside bases-thymine, cytosine and uracil. The barbiturates (CNS drugs), pyrimethamine (antimalarial), trimethoprim (antibacterial) are also an examples of drug possessing pyrimidine moiety (Fig. 13.5).

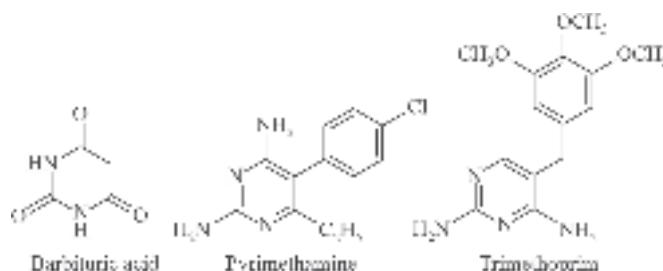
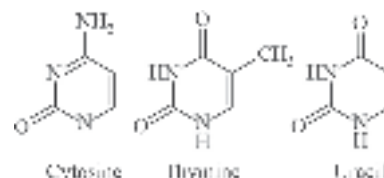
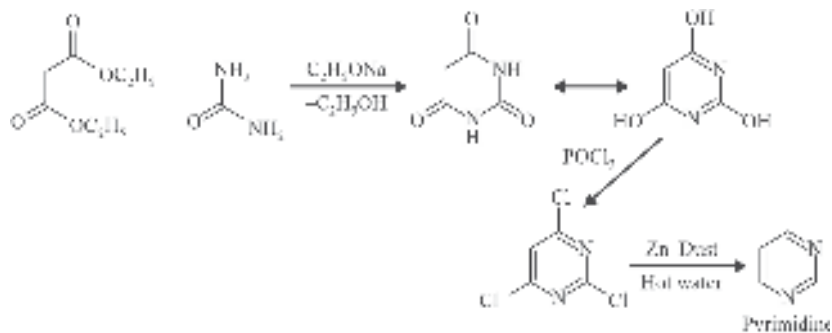


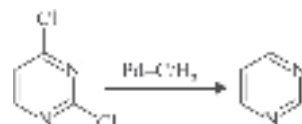
Fig. 13.5: Examples of pyrimidine ring containing drugs

Synthesis of Pyrimidines

- (i) **From malonic esters:** The reaction of diethyl malonic acid with urea followed by series of reactions yield the pyrimidine.



- (ii) The catalytic reductive dechlorination of 2,4-dichloropyrimidine, with hydrogen under pressure in the presence of Pd-C and magnesium oxide also yields pyrimidine.



- (iii) It can be also prepared by reaction of 1,1,3,3-tetraethoxypropane with formamide.



Physical Properties

Its melting point and boiling point are $20\text{--}22^\circ\text{C}$ and $124\text{--}125^\circ\text{C}$, respectively. It is a weak base. The decrease in basicity is due to the electron withdrawing effect of the second nitrogen atom present in the ring. The presence of alkyl group, however enhance the basicity.

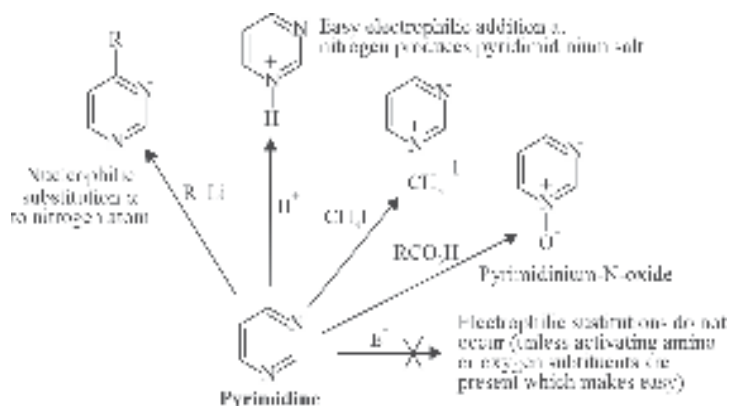
Chemical Properties

- (i) It is neutral in solution, however, form salts with acids.
- (ii) It is probably resonance hybrid of the following resonating structure.



The C-5 position has the greatest electron density. It can be therefore expected that the attachment by electrophilic reagents will be difficult but nucleophilic reagents (at position 2, 4 and 6) may be facilitated.

Chlorine atom at 2, 4 and 6 are readily replaced by a hydroxyl group or amino group and an amino group in position 2 or 4 is readily replaced by a hydroxyl group merely on boiling with water. Some of the reactions of pyrimidine are shown here:



Pyrazine

Pyrazine or 1,4 diazine is as symmetrical molecule as the nitrogen atoms occupy the 1,4-position. Derivatives like phenazine are well known for their antitumor, antibiotic diuretic activity. Pyrazinamide is a well known antitubercular drug having pyrazine moiety. Aspergillic acid is an antibiotic, isolated from *Aspergillus flavus*. Tetramethylpyrazine (also known as ligustrazine) is reported to scavenge superoxide anion and decrease nitric oxide production in human polymorphonuclear leukocytes (Fig. 13.6). It is also a part of pteridine moiety, which constitute folic acid.

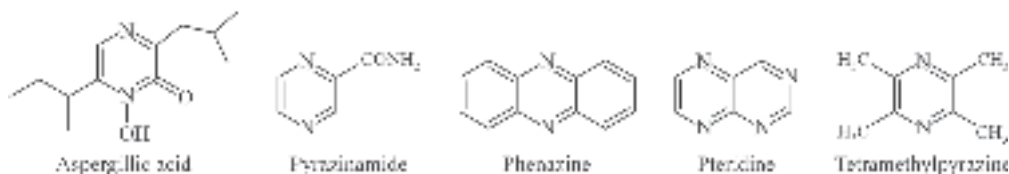
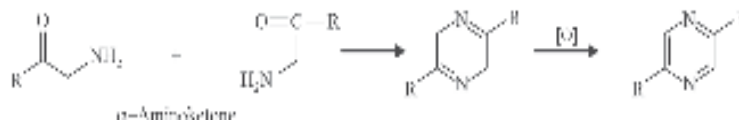


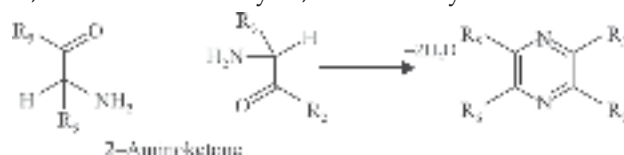
Fig. 13.6: Examples of pyrazine ring containing drugs/moiety

Synthesis of Pyrazines

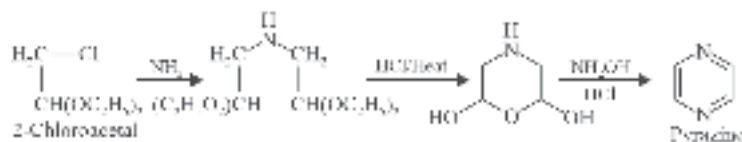
- (i) Pyrazine may be prepared by the self-condensation of α -aminoketone in the presence of an oxidizing agent such as mercuric chloride; the intermediate dihydro compound is readily oxidized to the pyrazine.



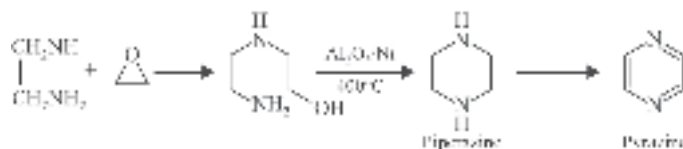
- (ii) **From the self-condensation of a 2-amino-ketone:** Symmetrical pyrazines result from the spontaneous self condensation of two mole equivalents of a substituted 2-amino ketone, or 2-amino aldehyde, followed by an oxidation.



- (iii) Pyrazine itself may be prepared from chloroacetal as follows:



- (iv) From the reaction of ethylenediamine and ethylene oxide. The reaction of ethylenediamine and ethylene oxide yields the pyrazine.



Physical Properties

Pyrazine is a stable and colorless solid, m.p. 54°C which is soluble in water. Pyrazine is less basic in nature than pyridine, pyridazine and pyrimidine. Its canonical structures are shown in Fig. 13.7.

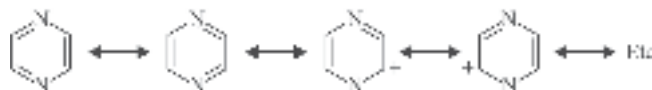
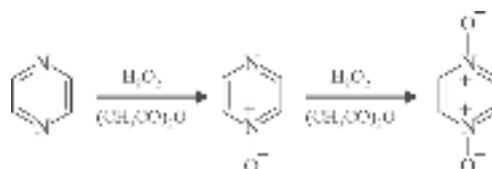
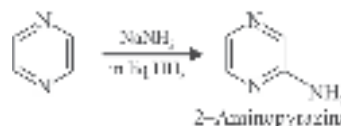
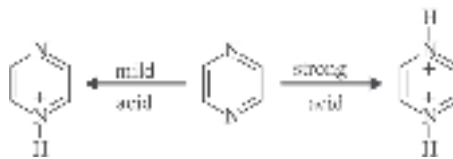


Fig. 13.7: Canonical structure of pyrazine

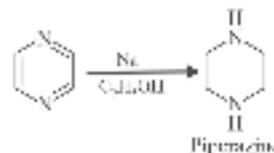
Chemical Properties

- (i) **Reaction with acid:** Pyrazine basicity is slightly lower than pyridine one. Like other amines, it can form salts with mineral and carboxylic acids. Pyrazine is protonated at N-1 position. Diprotonation is possible in presence of strong acids.
- (ii) **Electrophilic substitution:** The presence of two nitrogen atoms deactivates the ring towards electrophilic attachments. Presence of electron donating groups, however facilitate electrophilic attachments.
- (iii) **Reaction with nucleophilic reagents:** Pyrazine reacts in manner similar to pyridine in the **Chichibabin** reaction. Thus, it reacts with sodium amide, NaNH_2 in liquid ammonia, resulting in 2-aminopyrazine.
- (iv) **Oxidation:** Pyrazine can be oxidized with hydrogen peroxide to form N-oxides on one or both nitrogen atoms:



N-oxides are used for preparing pyrazine derivatives. N-oxide readily interacts with phosphorus oxytrichloride POCl_3 to form 2-chloropyrazine-1-oxide, which is converted into 2-hydroxy pyrazine-1-oxide by action of dilute sodium hydroxide solution. Then, N-oxide function is easily removed by reduction.

- (v) Pyrazine is readily reduced by sodium and ethanol to hexahydro-pyrazines or **piperazine**.

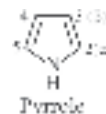


Piperazine occurs as a white, crystalline powder that may have a slight odor. It is soluble in water and alcohol. Piperazine is available commercially in a variety of salts, including citrate, adipate, phosphate, hexahydrate and dihydrochloride. Each salt contains a variable amount of piperazine (base): adipate (37%), chloride (48%), citrate (35%) dihydrochloride (50–53%), hexahydrate (44%), phosphate (42%) and sulphate (46%).

Piperazine acts as anthelmintic drug. It is thought to exert “curare-like” effects on susceptible nematodes, thereby paralyzing or narcotizing the worm and allowing it to be passed out with the feces. The neuromuscular blocking effect is believed to be caused by blocking acetylcholine at the myoneural junction. In ascaris, succinic acid production is also inhibited.

FIVE-MEMBERED HETEROCYCLIC RINGS WITH ONE HETERO ATOM

Pyrrole



Pyrrole is the most important among the various five member heterocyclic compounds. Pyrrole itself is readily available commercially, and is manufactured by alumina-catalysed gas-phase interaction of furan and ammonia. Pyrrole is first isolated from coal tar in 1834 and then in 1857 from the pyrolysate of bone by a process which is similar to an early laboratory method for the preparation of pyrrole—the pyrolysis of the ammonium salt of the sugar acid, mucic acid. The word pyrrole is derived from the Greek for red, which refers to the bright red color.

They are abundant in nature and are of great interest as subunits of natural products ranging from photosynthesis to oxygen transport in the blood (see porphine, heme and chlorophyll in Fig. 13.8). The pyrrole polymers have been used as conducting polymers and materials for nonlinear optics. The pyrrole is also a part of drug like Atorvastatin and Tolmetin.

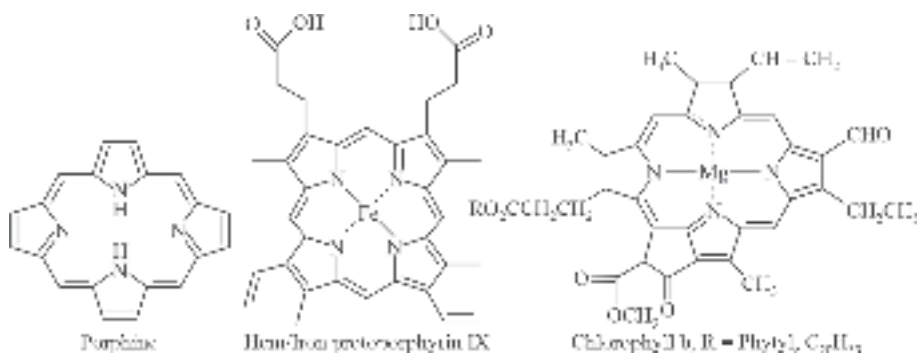
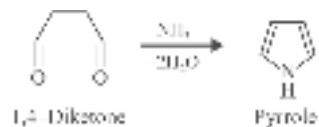


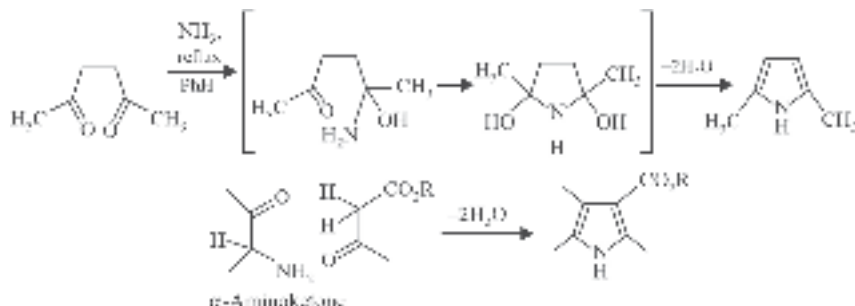
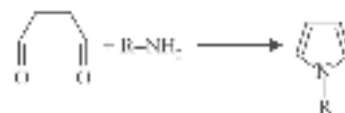
Fig. 13.8: Pyrrole ring containing biologically important substances

Synthesis of Pyrroles

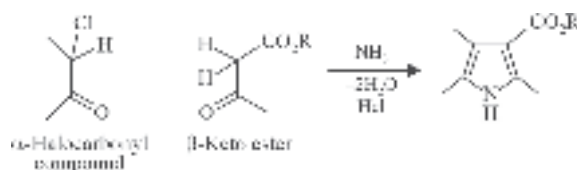
- (i) **Paal-Knorr synthesis:** Pyrroles can be obtained by heating 1,4-diketones with ammonia (or primary amines).



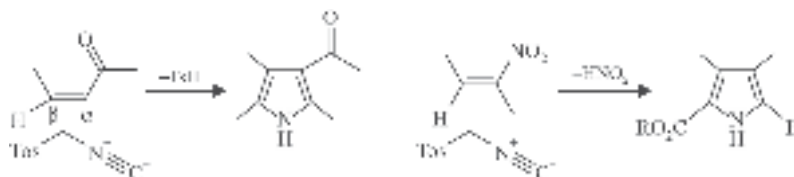
- (ii) **From α -aminocarbonyl compounds and activated ketones:** The activated ketones give pyrrole on heating with ammonia. α -Aminoketones react with carbonyl compounds (having an α -methylene grouping) to give appropriate pyrrole.



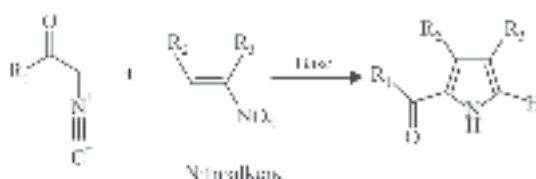
- (iii) **From α -halocarbonyl compounds:** In this process, a pair of two-carbon units (for example, an α -halocarbonyl compound, and β -keto ester) and ammonia give pyrrole ring. It is referred as **Hantzsch synthesis**.



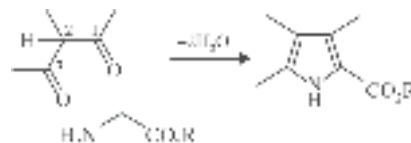
- (iv) **From tosylmethylisocyanide and β -unsaturated esters or ketones or from isocyno acetates and β -unsaturated nitro compounds:** Tosylmethyl isocyanide anion reacts with α,β -unsaturated esters, ketones, or sulphones with the loss of toluenesulphinate. Isocynoacetates react with α,β -unsaturated nitro compounds with the loss of nitrous acid. It is referred as **Van Leusen synthesis**.



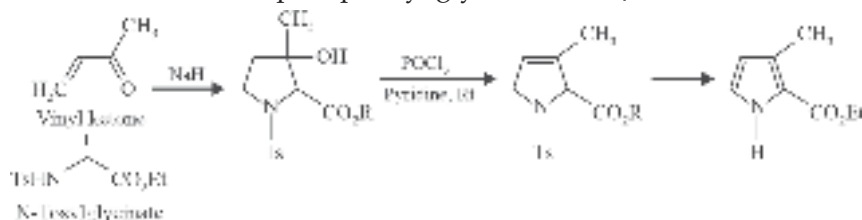
- (v) **Barton-Zard synthesis:** The **Barton-Zard synthesis** method of pyrrole synthesis is a cyclisation reaction between a nitroalkene and ethyl α -isocyanoacetate under basic conditions.



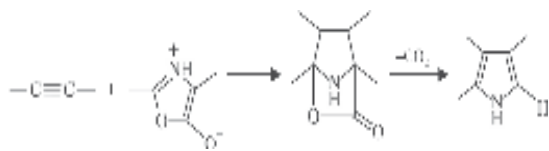
- (vi) **From 1,3-dicarbonyl compounds and glycine esters:** 1,3-Dicarbonyl compounds, react with glycine esters to give pyrrole-2-esters.



- (vii) **Kenner synthesis:** Kenner et al. has reported a method for the synthesis of pyrrole from the ester of 'toluene-p-sulphonyl glycine with α,β -unsaturated ketone.

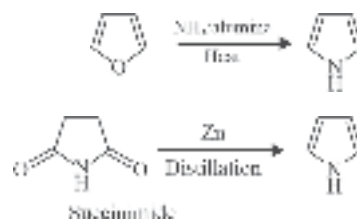


- (viii) **From alkynes and oxido-oxazoliums:** Dipolar cycloaddition of alkynes to mesoionic oxido-oxazoliums, followed by removal of carbon dioxide, yields pyrroles.



- (ix) Pyrrole can be produced by heating a mixture of furan, ammonia and steam on alumina.

- (x) Pyrrole can be obtained by distillation of succinimide over zinc dust.



Physical Properties

Pyrrole and the simple alkyl pyrroles are colorless liquids with aniline like odor, darken by autoxidation. Pyrrole has relatively high boiling point as compared to furan and thiophene due to presence of intermolecular hydrogen bonding.

Basicity of Pyrrole

Pyrrole has very low basicity compared to conventional amines and some other aromatic compounds like pyridine. The lone pair of electron on the N-atom in pyrrole is contributed towards the π -electron cloud forming the aromatic sextet. As a result, pyrrole cannot accept a proton and hence, has very little basic character: $pK_b = 13.6$.

Chemical Reactions

Electrophilic substitution reactions: Pyrrole, furan and thiophene undergo electrophilic substitution reactions like nitration, sulphonation, halogenation, etc. characteristic of aromatic rings. The carbons in 5-membered heterocyclic rings have higher electron density compared to benzene and hence undergo electrophilic substitution more readily than benzene. The electrophilic substitution takes place preferentially at C-2 position (α). The substitution at α -position is preferred because intermediate formed by electrophilic attack at C-2 is stabilized by charge delocalization to a greater degree than the intermediate from C-3 attack (Fig. 13.9). From the Hammond postulate, it may be inferred that the activation energy for substitution at the former position is less than the latter substitution.

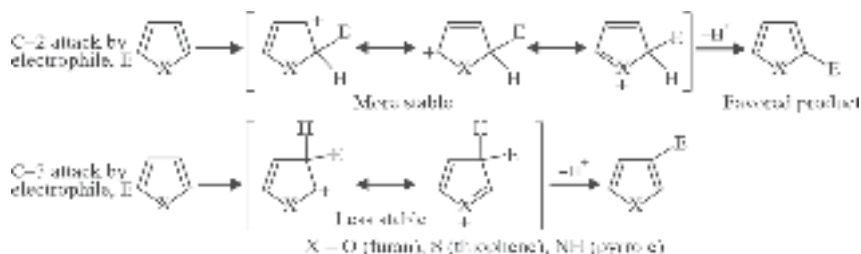
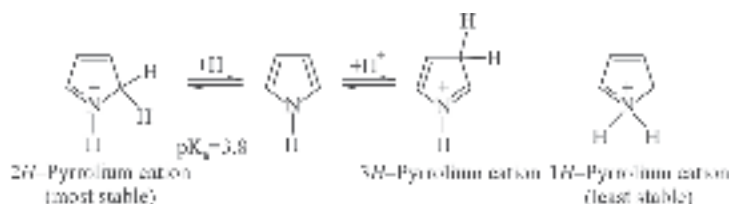


Fig. 13.9: Electrophilic substitution reactions in pyrrole, furan and thiophene

Note: Resonance stabilization of C-2 substitution intermediate is greater than that of the C-3 substitution intermediate.

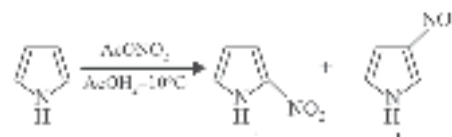
Protonation

Reversible proton addition occurs at all positions in solution, reaction is fastest at the nitrogen, and about twice as fast as at C-2 and C-3. In the gaseous phase, protonation takes place only on carbon and having higher proton affinity at C-2 than at C-3 position.



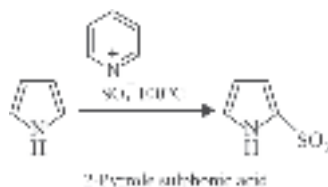
Nitration

Nitrating mixture causes complete decomposition of pyrrole; reaction occurs smoothly with acetyl nitrate at low temperature, giving mainly 2-nitropyrrole. This nitrating agent is fuming nitric acid with acetic anhydride, which forms acetyl nitrate and acetic acid.



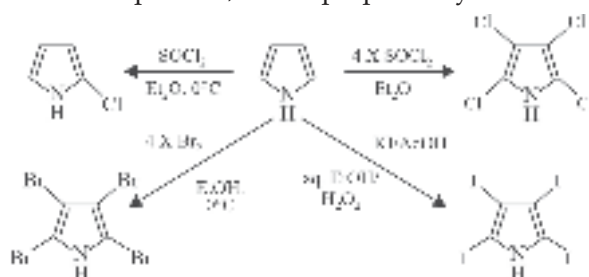
Sulphonation

For sulphonation, reagent of low acidity is used. For example, pyridine-sulphur trioxide compound easily converts pyrrole into the 2-sulphonate derivative. The beta sulphonation occurs when both alpha positions are blocked.



Halogenation

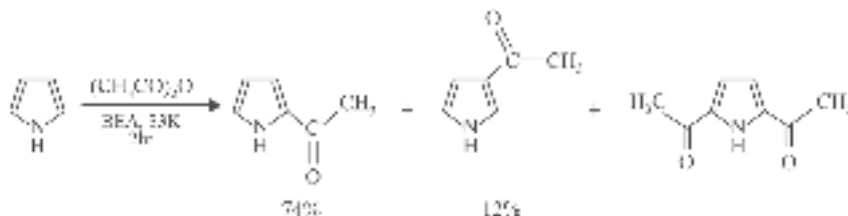
Pyrrole halogenates easily in controlled conditions and tetrahalopyrroles are the only isolable products. Monohalogenation of simple alkylpyrroles fail, because of side-chain halogenation and the formation of extremely reactive pynylalkyl halides, 2-Bromo- and 2-chloropyrroles, unstable compounds, can be prepared by direct halogenation of pyrrole.



Acylation

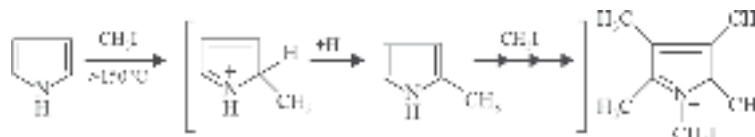
Direct acylation of pyrrole is done with acetic anhydride at 200°C to yield 2-acetyl pyrrole as main product with some 3-acetylpyrrole and 2,5-diacetylpyrrole and no N-acetyl

pyrrole. A small proportion of N-acetylpyrrole may be formed by heating above 100°C in presence of sodium acetate. Alkyl substitution facilitates acylation, for example, 2,3,4-trimethyl pyrrole yields the 5-acetyl derivative even on refluxing in acetic acid.



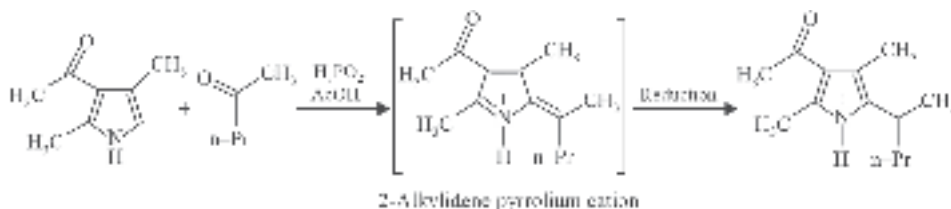
Alkylation

Simple pyrrole do not react with methyl iodide below 100°C temperature. Above about 150°C temperature, a series of reactions occur leading to a complex mixture made up of mostly of polymeric material together with some polyalkylated pyrrole.

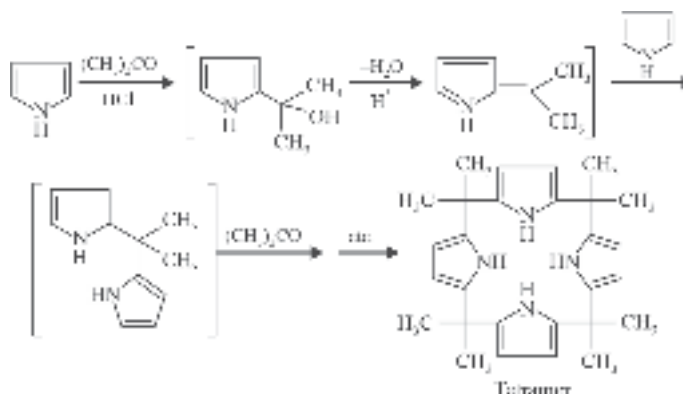


Condensation with Aldehydes and Ketones

Condensation of pyrroles with aldehydes and ketones occurs in presence of acid catalyst but the resulting product pyrrolyl carbinols cannot be isolated. Under the reaction conditions, proton-catalysed loss of water produces 2-alkyldenepyrrolium cations, which are highly reactive electrophiles. Thus, in case of pyrrole itself, reaction with aliphatic aldehyde inevitably gives resin, probably linear polymer.

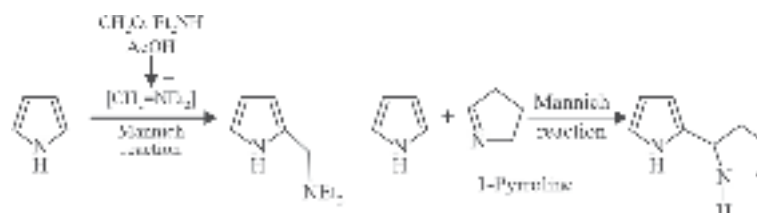


Acetone, however, in reacting in comparable way, gives a high yield of a cyclic tetramer.



Condensation with Imines and Iminium Ions

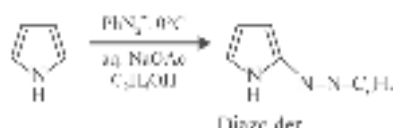
The imine and iminium functional groups undergo the Mannich reaction with pyrrole to produce dialkylaminomethyl derivatives. The iminium electrophile is generated *in situ* from formaldehyde, dialkylamine, and acetic acid. The reaction of pyrrole with 1-pyrroline take place via electrophilic attack by neutral C=N.



Diazo Coupling

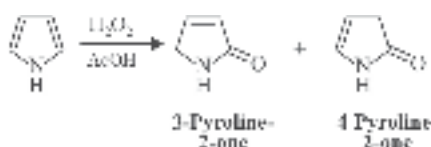
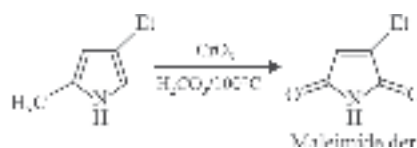
The pyrroles react with benzenediazonium salts due to its high reactivity. Pyrrole gives a mono-azo derivative by reacting as a neutral species below pH 8.

The azo coupling fail with furan, showing its lower reactivity compared with pyrrole.



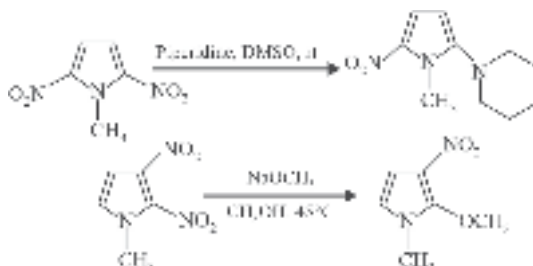
Reaction with Oxidizing Agents

Simple pyrroles undergo reaction with strong chemical oxidizing agents to form maleimide derivatives with the elimination of 2- and 5-substituent if present. This kind of oxidative degradation is used in early porphyrin structure determination, in which chromium trioxide in aqueous sulphuric acid or fuming nitric acid is used as oxidizing agents. Hydrogen peroxide is a more selective reagent and convert pyrrole itself into a tautomeric mixture of pyrrolin-2-ones.



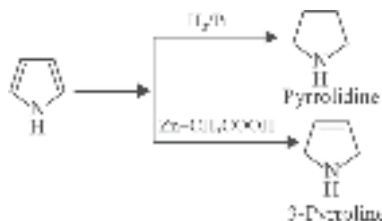
Reactions with Nucleophilic Reagents

Pyrrole and its derivatives do not react with nucleophilic reagents by addition or by substitution but in extreme condition give substituted products.



Reduction

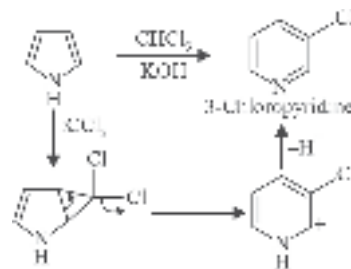
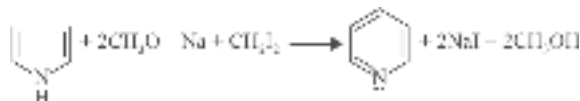
Pyrrole can be reduced to pyrrolidine by catalytic hydrogenation.



Ring Expansion of Pyrrole

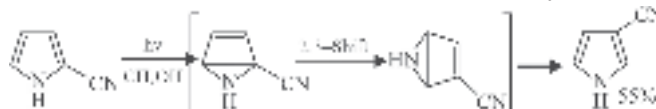
Pyrrole adds on dichlorocarbene generated *in situ* from chloroform and base to give a bicyclic compound which undergoes ring expansion to give 3-chloropyridine.

The treatment of pyrrole with sodium methoxide and CH_2I_2 , pyrrole is converted into pyridine by ring expansion.



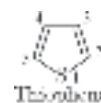
Photochemical Reactions

The ring skeletal rearrangement takes place. The photo-catalysed rearrangement of 2- to 3-cyanopyrroles is considered to involve a 2,3-shift in an initially-formed bicyclic aziridine.

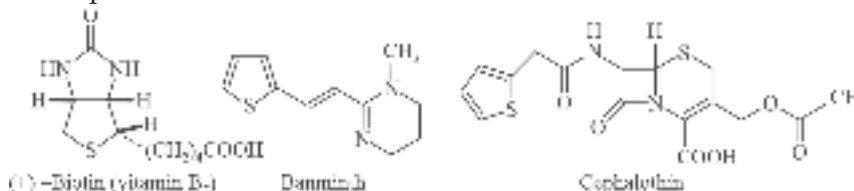


THIOPHENE

The simple thiophenes are stable liquids which closely resemble to the corresponding benzene compounds in boiling points and smell. They occur in coal tar distillates. The name thiophene is derived from **theion**, the Greek word for sulphur, and another Greek word **phaino** which means shining, a root first used in phenic acid (phenol) because of its occurrence in coal tar, a byproduct of the manufacture of illuminating gas.

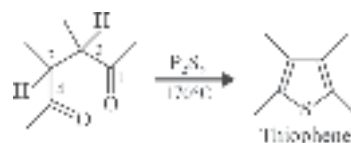


Biotin, one of vitamins, is a tetrahydrothiophene and Banminth (Pyrantel), a valuable anthelmintic used in animal husbandry, are the few thiophene compounds. Thiophene is also found in cephalothin antibiotic.



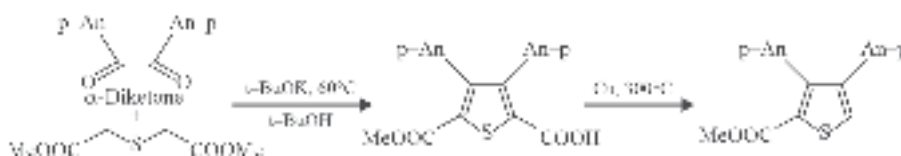
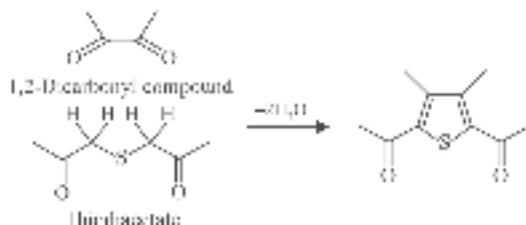
Synthesis of Thiophenes

- (i) **From 1,4-dicarbonyl compounds:** The reaction of a 1,4-dicarbonyl compound with a source of sulphur (like phosphorus sulphides, bis (trimethylsilyl) sulphide etc), gives thiophenes.

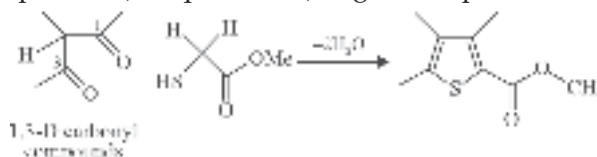


- (ii) **From thiodiacetates and 1,2-dicarbonyl compounds:** 1,2-Dicarbonyl compounds condense with thiodiacetates (or thiobismethyleneketones) to give thiophene-2,5-diacids (diketones).

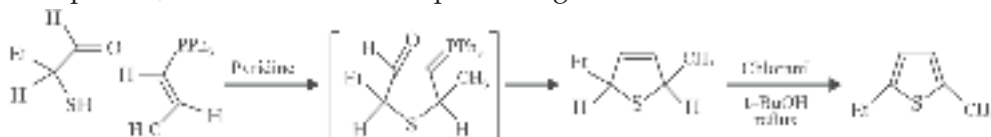
- (iii) **Hinsberg synthesis:** Formation of thiophene carboxylic acids takes place from reaction of α -diketones and dialkyl thiodiacetates. This on decarboxylation gives the corresponding thiophene derivatives.



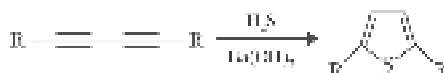
- (iv) **From thioglycolates and 1,3-dicarbonyl compounds:** Thioglycolates react with 1,3-dicarbonyl compounds (or equivalents) to give thiophene-2-carboxylic esters.



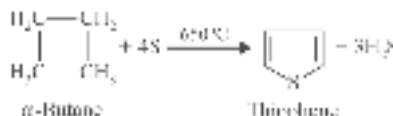
- (v) **From thiocarbonyl compounds:** 2-Keto-thiols add to alkanylphosphonium ions to form ylides. The ring closure takes place via Wittig reaction to give 2,5-dihydrothiophenes, which then form thiophene ring.



- (vi) 1,3-Dienes and hydrogen sulphide can also be converted to substituted thiophenes.



- (vii) **Commercial method:** Heating of sulphur with butane at high temperature ($650^\circ C$) gives the thiophene.



Physical Properties

Thiophene is a toxic, flammable and colorless liquid, b.p. $84^\circ C$. It is insoluble in water.

Chemical Properties

Electrophilic substitution reactions:

Thiophene undergoes electrophilic substitution reactions like nitration, sulphonation, halogenation, etc. characteristic of aromatic rings. It has already been

observed in pyrrole that carbon atoms in 5-membered heterocyclic rings have higher



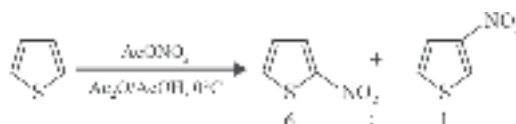
electron density compared to benzene and hence undergo electrophilic substitution more readily than benzene. The electrophilic substitution takes place preferentially at C-2-position.

The electrophilic substitution at C-2 position in thiophene can also be accounted in the same manner (Fig. 13.9). Furan is not as reactive as pyrrole in electrophilic substitution reactions because the oxygen, in furan is more electronegative than nitrogen in pyrrole and therefore, does not enhance the electron density of carbons as much as pyrrole. Thiophene is less reactive than furan towards electrophilic substitution because the p-electrons of sulphur are in 3p orbital which overlaps less effectively than the 2p orbital of nitrogen or oxygen with 2p orbitals of carbon. The relative reactivity towards electrophilic substitution follow the order.



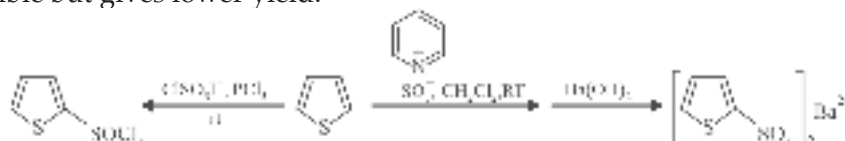
Nitration

Nitration of thiophene occurs in presence of acetyl nitrate or nitronium tetrafluoroborate because normal nitrating mixtures give explosive reaction.



Sulphonation

Sulphonation gives thiophene-2-sulphonic acid on reacting with sulphuric acid; use of the pyridinesulphur trioxide complex is probably the best method. Chlorosulphonation also possible but gives lower yield.



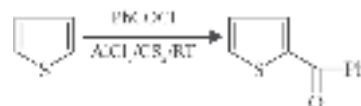
Halogenation

Halogenation of thiophene occurs very readily at room temperature and also occurs even at 30°C in the dark. Tetra substitution occurs easily. The rate of halogenation of thiophene, at 25°C, is about 10 times as compared to benzene. Both 2,5-dibromo- and 2,5-dichlorothiophenes, 2-bromo, and 2-iodothiophene can be produced under various controlled conditions.



Acylation

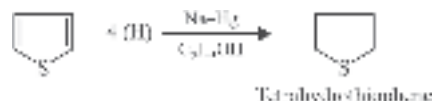
The Friedel-Crafts acylation of thiophenes gives good yields under controlled conditions. Anhydrous aluminium chloride and to a lesser extent, stannic chloride reacts with thiophene to produce intractable tars. However, this undesirable resinification is largely avoided by adding the catalyst gradually to



mixture of thiophene and the acylating agent when the AlCl_3 preferentially reacts with the acylating agent to give the active electrophile.

Reduction

Thiophene may be hydrogenated to form tetrahydrothiophene.

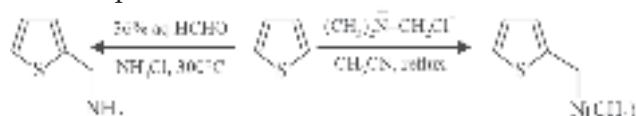


Condensation with Aldehydes and Ketones

Acid-catalysed reaction of thiophene with aldehydes and ketones occurs very easily while reaction product is unstable under the reaction conditions. Chloroalkylation of thiophenes carrying electron withdrawing groups can be achieved with the use of zinc chloride.

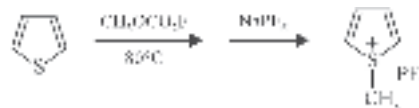
Condensation with Imines and Iminium Ions

Amino methylation of thiophene involves Mannich reaction.



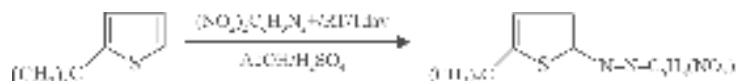
Addition at Sulphur

Thiophene sulphur can add electrophilic species but thiophenium salts not produced from thiophene itself. The sulphur in such salts in tetrahedral sp^3 hybridized.



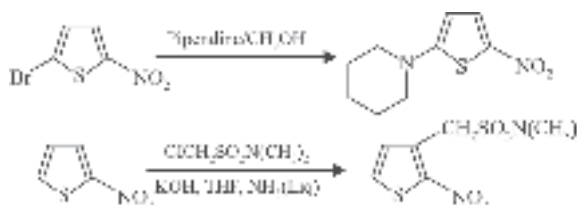
Diazo Coupling

Thiophene itself insufficiently shows coupling reaction, even with reactive diazonium salt such as the 2,4-dinitrodiazonium ion, only 2-mono- and 2,5-diarylation is observed.

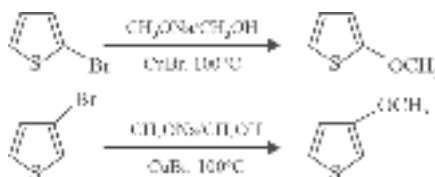


Reactions with Nucleophilic Reagents

Nitro-substituents activate the removal of leaving groups like halide in thiophene nucleus. Nucleophilic displacements in thiophene proceed at least 10 times faster than for benzenoid system due to participation of the sulphur in the delocalisation of charge in the Meisenheimer intermediate.



Copper and copper (I) salts have been used extensively in thiophene chemistry to catalyse displacement of bromine and iodine, but not chlorine, in simple halothiophenes.



Furan



The term “furan” refers to a class of aromatic organic compounds featuring a five-member ring. A furan ring consists of four carbon atoms plus one oxygen atom. Structurally the simplest compound to possess a furan ring is, itself, called furan, C_4H_4O . For purposes of identification, the ring is numbered starting with oxygen, counter clockwise. If a methyl group replaces the hydrogen atom on ring atom two, the compound is called 2-methylfuran. When the methyl group is located on ring at C-3 position, the compound is called 3-methylfuran.

A number of 5-nitrofurfural derivatives are important chemotherapeutic agent; Nitrofurazone, a bactericide is a simple example. It is also found in diuretic drug, frusemide and antiulcer drug, ranitidine (Fig. 13.10).

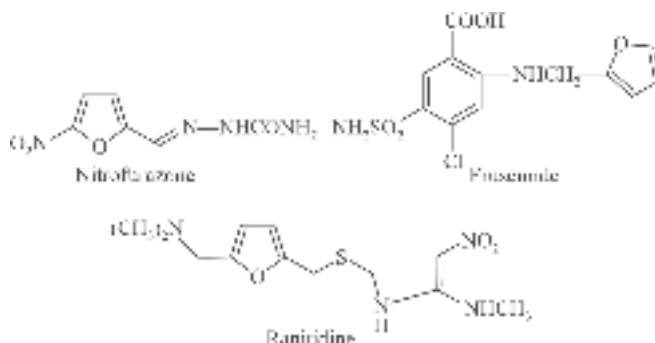
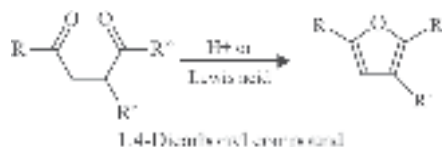


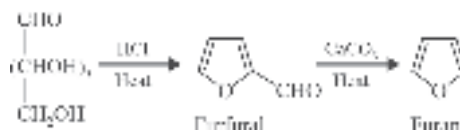
Fig. 13.10: Examples of drugs possessing furan ring skeleton

Synthesis of Furan

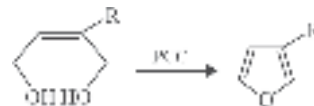
- (i) **Paal-Knorr Furan synthesis:** The acid-catalyzed cyclization of 1,4-dicarbonyl compounds known as the Paal-Knorr synthesis is one of the most important methods for the preparation of furans.



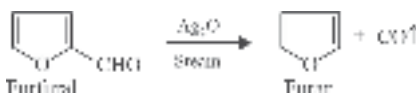
- (ii) Furfural can be prepared by dehydration and cyclization of pentoses. Furfural undergoes decarboxylation to give furan.



- (iii) 3-Substituted furans can be prepared by cyclization of Z-butene-1,4-diols with pyridinium chlorochromate (PCC).



- (iv) **Decarboxylation of furfural:** Decarboxylation of furfural in steam in presence of Ag_2O catalyst gives the furan. It is a commercial method.



Physical Properties

It is a clear colorless liquid with b.p. $31.3^\circ C$, turning brown upon standing, with characteristic odor. The vapour is heavier than air. It is slightly soluble in water.

Chemical Properties

Electrophilic substitution reactions: Furan undergoes electrophilic substitution reactions like nitration, sulphonation, halogenation, etc. characteristic of aromatic rings. The 5-membered ring heterocycles furan like pyrrole and thiophene is π -electron rich aromatics (6p electrons over 5 atoms) compared to benzene and hence undergo electrophilic substitution more readily than benzene. The electrophilic substitution takes place preferentially at C-2 position. There are 3 important resonance structures that justify the reactivity of the C-2 position (Fig. 13.11).

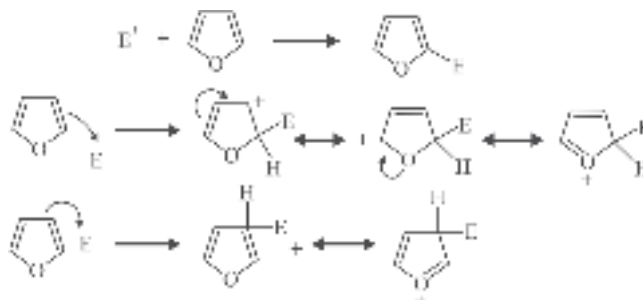
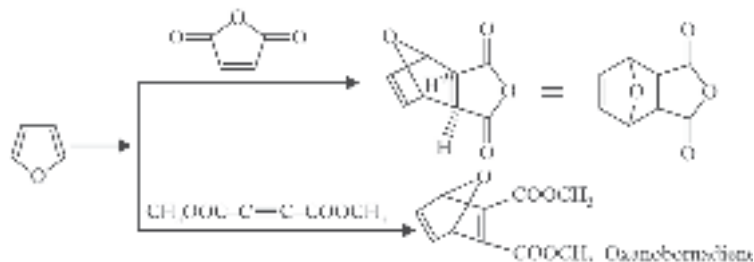


Fig. 13.11: Electrophilic substitution reactions in furan



- (ii) **Reduction:** Furan can be reduced to tetrahydrofuran by catalytic hydrogenation.
- (iii) **Diels-Alder reaction:** Furan is least aromatic of heterocycles in comparison to thiophene as pyrrole and therefore behaves as a diene in a number of Diels-Alder reactions. Furan reacts with maleic anhydride to form bicyclic Diels-Alder adduct. Similarly, it reacts with acetylenic dienophile to form the oxanobornadiene.



- (iv) **Oxidation:** Air oxidation of furan results in 2,5-addition product, i.e. a bridged peroxy adduct, which on further oxidation yield succinaldehyde.



- (v) **Reactions with diazonium salts:** 2-Phenyl furan is formed in place of azo compound.

FIVE-MEMBERED HETEROCYCLIC RINGS WITH TWO HETERO ATOMS

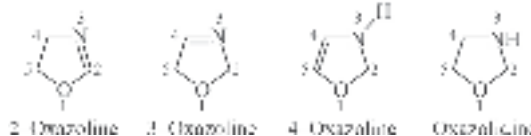
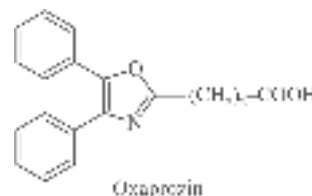
1,3-AZOLES

OXAZOLE

Oxazole is a 1,3-azole having an oxygen atom and a pyridine type nitrogen atom at the 3-position in a five-membered ring.

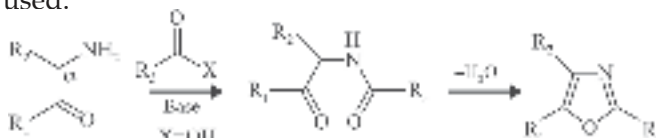
Oxazole does not found in nature and thus, does not play any part in fundamental metabolism as do imidazole and thiazole. The antiinflammatory drug, oxaprozin (cox-2-inhibitor) is a good example of oxazole ring containing drug. The aleglitaz (antibiotic), ditazole (platelet aggregation inhibitor) and mubritinib (tyrokinase inhibitor) are the other examples of oxazole derivatives.

Partially reduced oxazoles are called oxazolines and three types are possible depending on the position of the double bond. These are 2-oxazoline, 3-oxazoline and 4-oxazoline. The fully saturated system is called oxazolidine. Oxazolidines are solids and behave as bases and are readily hydrolysed by water.

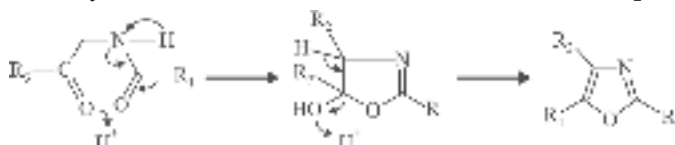


Synthesis of Oxazoles

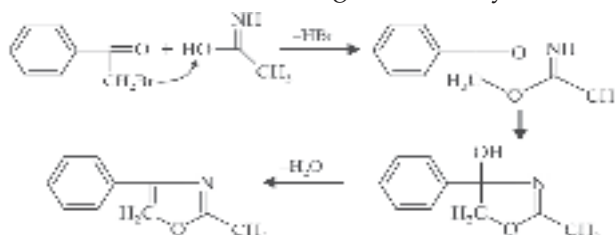
- (i) **By the action of acid on α -acylaminoketone:** For oxazoles, the **Robinson-Gabriel** synthesis is used:



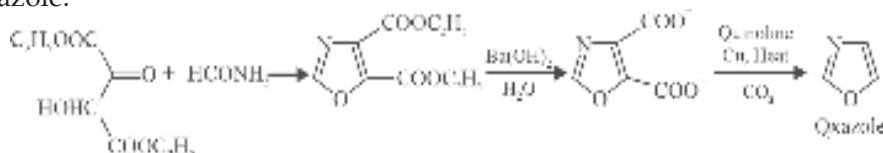
Dehydration can be carried out by a wide range of acids, such as phosphoric acid, phosgene or thionyl chloride. The mechanism of reaction is depicted below:



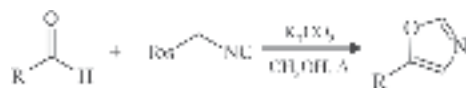
- (ii) The reaction of an acid imide and α -halogenoketone, yields oxazole.



- (iii) The reaction of diethyl α -hydroxy keto succinate with formamide also yields oxazole.



(iv) **van Leusen oxazole synthesis:** The Van Leusen oxazole synthesis allows the preparation of oxazoles from aldehydes by reaction with tosylmethyl isocyanide (TosMIC).



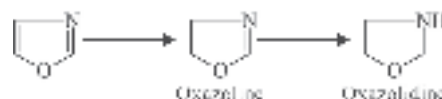
Physical Properties

Oxazole is a clear, colorless liquid with boiling point, melting point, density, flash point and PK_a are $69\text{--}70^\circ\text{C}$, -84 to -87°C , 1.05, 18°C and 0.8, respectively. It is polar and weakly basic, and is weakly aromatic. It is a skin and eye irritant but there is no evidence of mutagenicity.

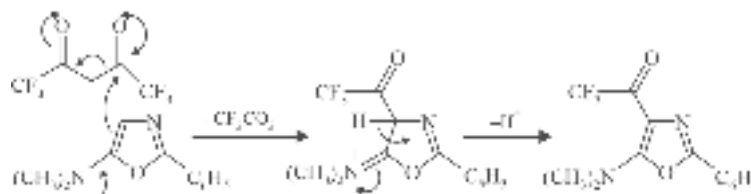
Although oxazole possess a sextet of electrons but the delocalization is incomplete as a result it has little aromatic character.

Chemical Reactions

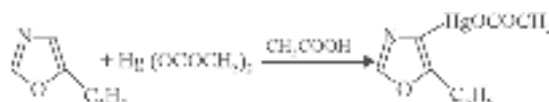
Reduction: Oxazoles are stable but can be reduced to oxazolidine treated with sodium in ethanol.



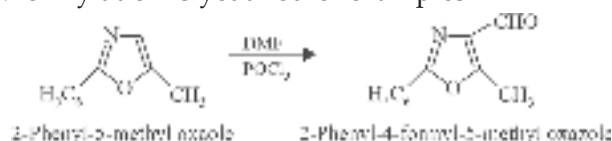
Electrophilic substitution reactions: Oxazole (1,3-azoles) is not very reactive to electrophilic attack due to the deactivating effect of the pyridine-like nitrogen atom. Electron-donating groups can facilitate this reaction.



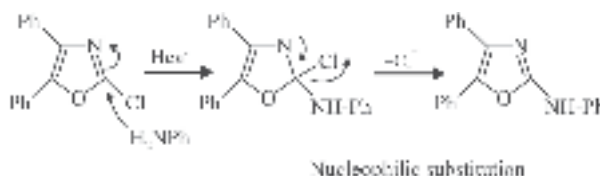
However, Oxazole is more reactive towards electrophilic substitution than thiazole but less than imidazole. Nitration and sulphonation of oxazole itself are difficult because of presence of pyridine type nitrogen. Oxazoles are mercurated in acetic acid and mercuric acetate, the ring position reacts in order of $5 > 4 > 2$.



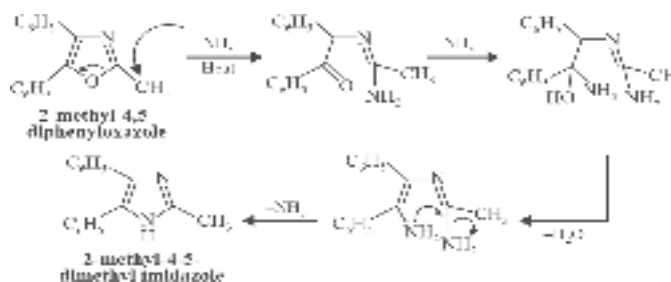
Vilsmeier-Haack formylation is yet another examples.



Nucleophilic substitution reactions: As a consequence of lower electrophilic reactivity, these are more reactive to nucleophiles. They require no activation with EWG like furans etc. The ease of displacement of halogen on the oxazole ring is $\text{C2} > \text{C4} > \text{C5}$. The cleavage of the oxazole ring by



nucleophiles is rather a more frequent reaction than nucleophilic substitution. In some cases a cyclic intermediate may be isolated while in others it may cyclise to afford another ring system.

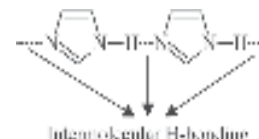


IMIDAZOLE

Imidazole (1,3-diaza-2,4-cyclopentadiene) is a planar five-member ring system with 3C and 2N atoms at 1 and 3 positions. The imidazole (molecular formula $C_3H_4N_2$) itself, is the simplest member of imidazoles family.



The systemic name for the compound is 1,3-diazole, one of the annular N bear a H atom and can be regarded as a pyrrole type N. It is colorless liquid having a high b.p. of 256°C in comparison to all other 5-membered heterocyclic compounds due to the intermolecular H-bonding, where there is linear association of molecules. It is soluble in water and other polar solvents. It exists in two equivalent tautomeric forms because the hydrogen atom can be located on either of the two nitrogen atoms. Imidazole is a highly polar compound, as evidenced by a calculated dipole of 3.61 D. The compound is classified as aromatic due to the presence of a sextet of π -electrons, consisting of a pair of electrons from the protonated nitrogen atom and one from each of the remaining four atoms of the ring. Imidazole is amphoteric, i.e. it can function as both an acid and as a base. As an acid, the pK_a of imidazole is 14.5, making it less acidic than carboxylic acids, phenols, and imides, but slightly more acidic than alcohols. The acidic proton is located on N-1. As a base, the pK_a of the conjugate acid (cited above as pK_{BH^+} to avoid confusion between the two) is approximately 7, making imidazole approximately sixty times more basic than pyridine. The basic site is N-3.



Imidazole is incorporated into many important biological molecules. The most pervasive example is the amino acid "histidine", which has an imidazole side chain. Histidine is present in many proteins and enzymes and plays a vital part in the structure and binding functions of hemoglobin. Histidine can be decarboxylated to histamine, which is also a common biological compound. It is also found in carnosine (dipeptide, found in brain and muscle), metronidazole (antiamoebic), azathioprine (Fig. 13.12), etc.

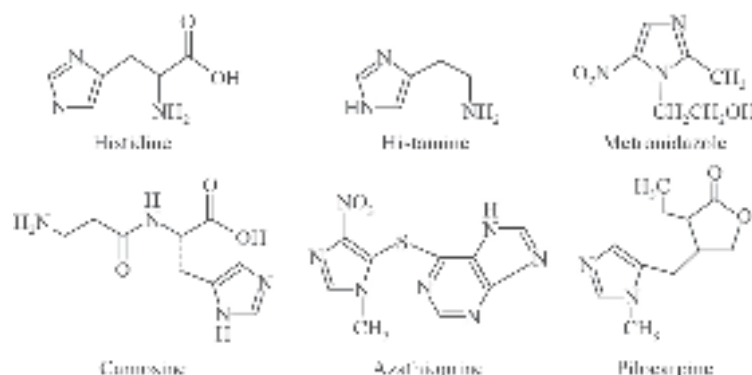
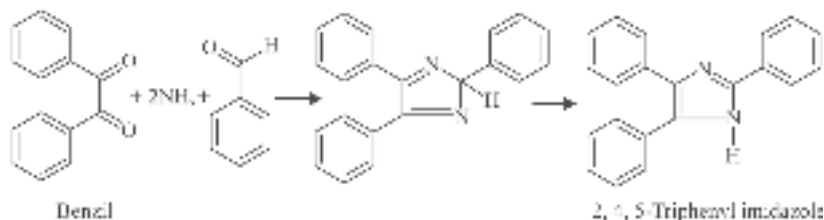


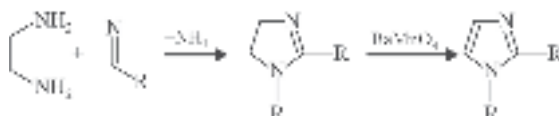
Fig. 13.12: Examples of drug possessing imidazole ring skeleton

Synthesis of Imidazoles

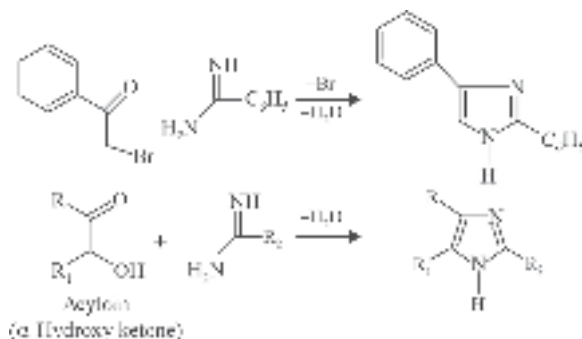
- (i) **Radiszewski synthesis:** It consists of condensing a dicarbonyl compound such as glyoxal, α -keto aldehyde or α -diketones with an aldehyde in the presence of ammonia. For example, benzil for instance with benzaldehyde and two molecule of ammonia reacts to yield 2,4,5-triphenylimidazole. Formamide often proves a convenient substitute for ammonia.



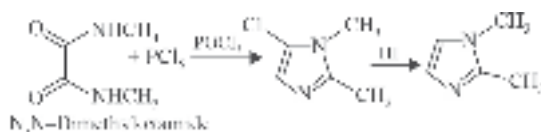
- (ii) **Dehydrogenation of imidazoline:** Knapp et al. have reported a milder reagent barium manganate for the conversion of imidazolines to imidazoles in presence of sulphur. Imidazolines obtained from alkyl nitriles and 1,2 ethanediamine on reaction with BaMnO_4 yield 2-substituted imidazoles.



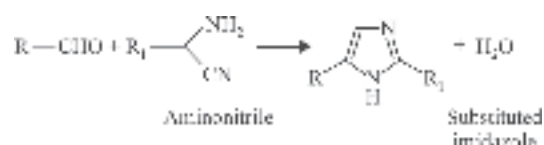
- (iii) **From α -halo ketone:** This reaction involves an interaction between an imidine and α -halo ketones. This method has been applied successfully for the synthesis of 2,4- or 2,5-biphenyl imidazole; for example, phenacyl bromide and benzimidine according to this method afford 2,4-diphenyl imidazole. Similarly, amidine reacts with acyloin or α -hydroxyketones to yield imidazoles.



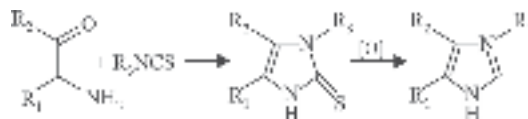
- (iv) **Wallach synthesis:** When N,N' -dimethyloxamide is treated with phosphorus pentachloride, a chlorine containing compound is obtained which on reduction with hydroiodic acid give N -methyl imidazole.



- (v) **From aminonitrile and aldehyde:** The aminonitrile reacts with aldehyde to yield imidazole.

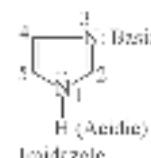


(vi) **Markwald synthesis:** The preparation of 2-mercaptoimidazoles from α -amino ketones or aldehyde and potassium thiocyanate or alkyl isothiocyanates is a common method for the synthesis of imidazoles. The sulphur can readily be removed by a variety of oxidative method to give the desired imidazoles. The starting compounds, α -amino aldehyde or ketone, is not readily available, and this is probably the chief limitation of the **Markwald** synthesis.



Chemical Reactions

Imidazole can be considered as having properties similar to both pyrrole and pyridine. The electrophilic reagent would attack the unshared electron pair on N-3, but not that on the 'pyrrole' nitrogen since it is the part of the aromatic sextet. While the imidazole ring is rather susceptible to electrophilic attack on an annular carbon, it is much less likely to become involved in nucleophilic substitution reaction unless there is a strongly electron withdrawing substituents elsewhere in the ring. In the absence of such activation, the position most prone to nucleophilic attack is C-2. The fused benzene ring in benzimidazoles provides sufficient electron withdrawing property to allow a variety of nucleophilic substitution reaction at C-2.



Electrophilic substitution reactions: Imidazoles shows increased reactivity towards electrophilic attack. It is more susceptible to electrophilic attack than pyrazole or thiazole and more so than from furan and thiophene also. From the following resonance structure of the intermediate ion; it is evident that the attack takes place at the 4th and 5th position in imidazole ring (Fig. 13.13). It may be noticed that the attack at C-2 involves a canonical form, which is highly unfavoured at positive N at position-3.

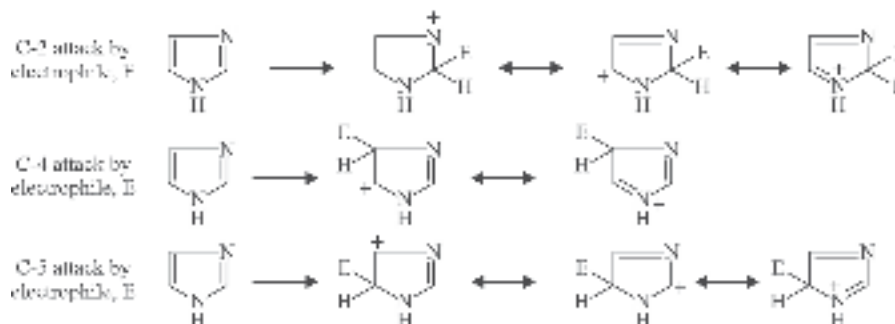
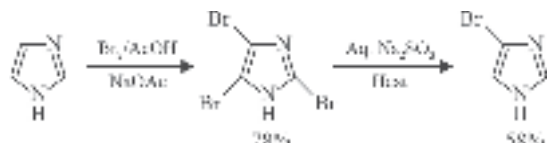


Fig. 13.13: Electrophilic substitution reactions in imidazole

Imidazoles undergo nitration which takes place at C-4 position; undergo sulphonation with disulphuric acid at 100°C temperature at C-5 and C-4 position.



Imidazoles are brominated easily and bromination at multiple positions can occur.



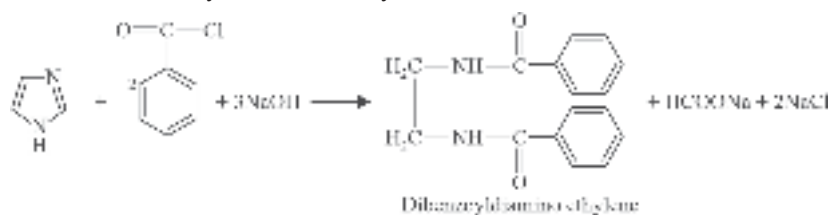
Bromination of imidazole in organic solvents, i.e. CHCl_3 gives 4 and 5 substituted compounds if positions 4 and 5 are blocked so that the substitution takes place at C-2 position.

Nucleophilic substitution reactions: Nucleophilic attack with the replacement of halogen by nucleophile is possible only, if substituted with electron withdrawing group or quaternized.

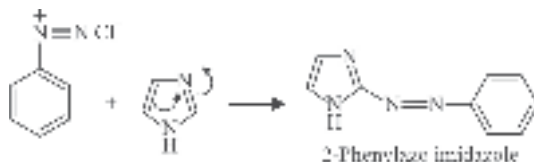
The halogen atoms at C-4 or C-5 position are normally unreactive but may be activated if electron withdrawing substituent is present either at α or β position.

Oxidation: It is extremely stable towards oxidizing agent and reducing agent. However, H_2O_2 readily opens the ring to form oxamide.

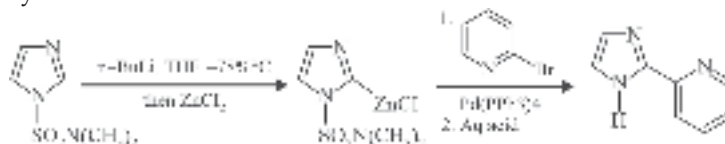
Acetyl chloride and acetic anhydride have no action on imidazole but benzoyl chloride in the presence of NaOH opens the ring of imidazole to form dibenzoyldiamino ethylene.



Diazo coupling: It takes place at C-2 position.



Imidazole-metallation: It occurs preferentially at either the 2- or 5-position. Through this reaction, arylation can be done.



THIAZOLE

Thiazole, $\text{C}_3\text{H}_3\text{NS}$, first described by Hantzsch and Weber in 1887, is a five-membered aromatic heterocyclic (contains both sulphur and nitrogen atoms in



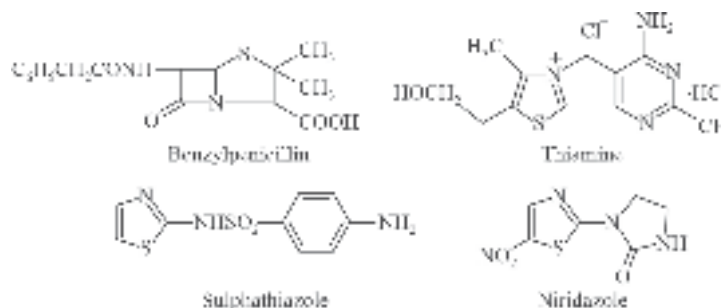


Fig. 13.14: Examples of drugs possessing thiazole ring skeleton

the ring) organic compound that is useful in the manufacture of pesticides, drugs, dyes and other compounds.

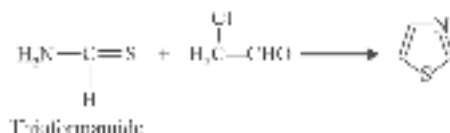
Some of them exhibit anti-tumor, schistozomicidal and anti-viral properties. The thiazole ring is part of the vitamin B₁ (thiamine) structure, as well as that of penicillins Niridazole and sulphathiazole (Fig. 13.14). Many substituted thiazoles contribute to the flavors and aromas of food. A number of thiazoles: 2-methylthiazole, 4-methylthiazole, 5-methylthiazole, and 5-butylthiazole, are all found in roasted peanuts. They are formed in foods by the action of sulphur-containing amino acids interacting with carbohydrates. Some thiazole derivatives also contribute to the flavor of brewed coffee.



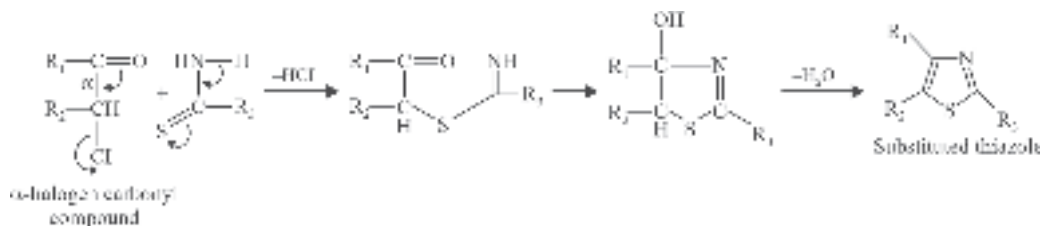
The partially reduced thiazoles are called thiazolines such as 2,3 and 4-thiazoline.

Synthesis of Thiazoles

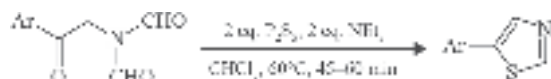
- (i) Thiazole is straight forwardly made by the reaction of thioformamide with chloroacetaldehyde.



The substituted thiazole is prepared by the condensation reaction between α -halogen carbonyl compound and thioamides.



- (ii) Treatment of N,N-diethylaminomethyl aryl ketones with phosphorus pentasulphide and triethylamine in chloroform gives 5-arylthiazoles in good yield. The 5-aryl-1,3-thiazole compound has been successfully functionalised at the 2-position to yield, over two steps, a large array of 5-aryl-2-arylsulphonyl-1,3-thiazoles.



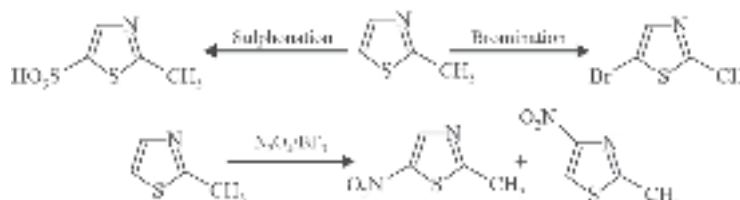
Physical Properties

Thiazole is a yellowish liquid, b.p. 116 to 118°C. It possesses an odor distinctly similar to pyridine. It is soluble in alcohol and ether. Although thiazole possesses only a five-member ring, the lone pair of electrons on the sulphur atom delocalizes and completes the required Huckel number of six π -electrons for aromaticity. This aromaticity is evidenced by the chemical shift of the ring protons in proton NMR spectroscopy (between 7.27 and 8.77 ppm), clearly indicating a strong diamagnetic ring current. The calculated pi-electron density marks C-5 position as the primary site for electrophilic substitution, and C-2 as the site for nucleophilic substitution.

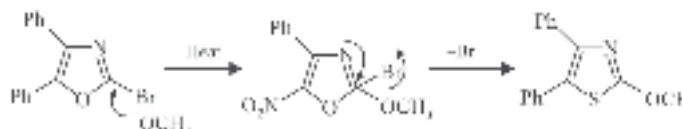
Chemical Properties

Thiazole is very resistant to substitution reaction however, if C-2 position is substituted, then the molecule is readily attacked by the usual electrophilic reagents to form 5-substitution products.

For example, thiazole does not brominated easily but 2-alkylthiazoles brominated at the C-5 position. Similarly, these may be nitrated and sulphonated with oleum to the corresponding 5-derivative.

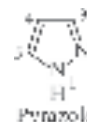


Nucleophilic substitution reactions: Displacement of halogen occurs easily.



1,2-AZOLES

PYRAZOLE



Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic diazole series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions.

The name 'Pyrazole' is given by "Ludwig Knorr" to this class of compounds in 1883. The reduction products, named as are other rings of five atoms, are pyrazoline and pyrazolidine.

Pyrazole derivatives constitute an interesting class of organic compounds, which are associated with diverse chemical and pharmacological properties. Pyrazolines have received considerable attention in recent years. Pyrazoline derivatives occupy a unique place in field of medicinal chemistry due to a wide range of biological activities exhibited by them. Several derivatives like phenylbutazone (anti-inflammatory), oxyphenbutazone (anti-inflammatory), analgin (analgesic), sulphinpyrazone (uricosuric), propylphenazone (analgesic), celecoxib (COX-II inhibitor), etc. of these systems find useful in medicine (Fig. 13.15).

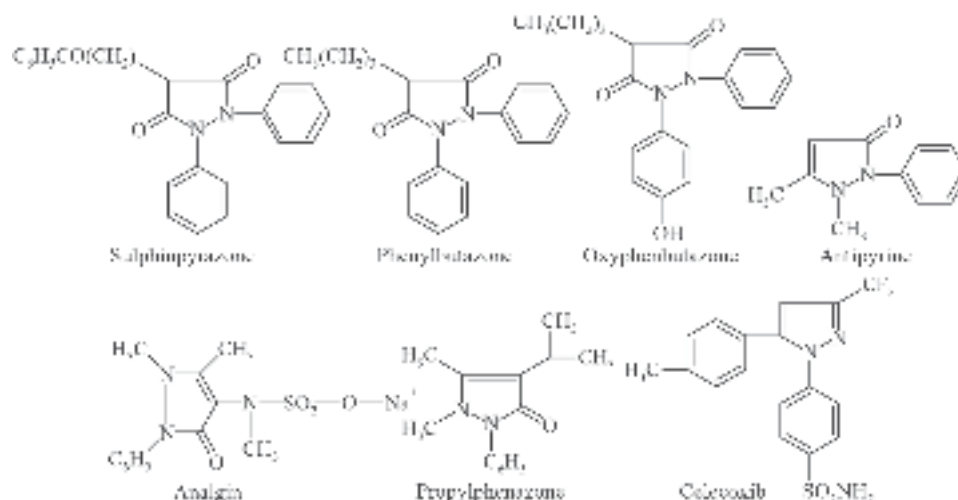
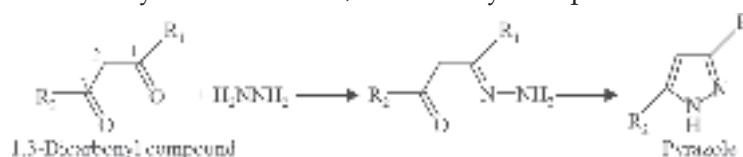


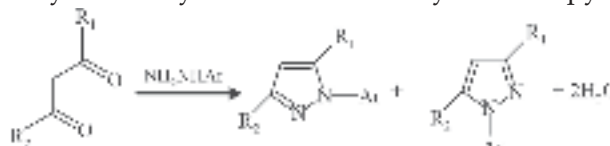
Fig. 13.15: Examples of drugs possessing pyrazole ring skeleton

Synthesis of Pyrazoles

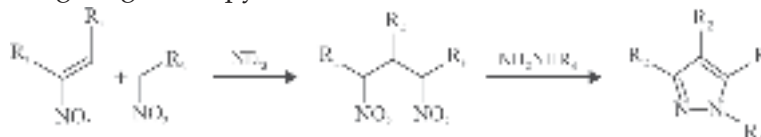
- (i) **Knorr Pyrazole synthesis:** The pyrazole derivatives are synthesized from condensation reaction of hydrazines with 1,3-dicarbonyl compounds.



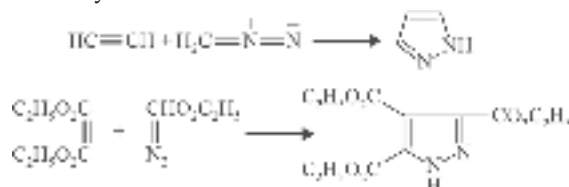
The substituted hydrazines yield two structurally isomeric pyrazoles.



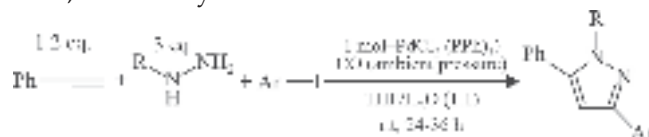
- (ii) Aliphatic nitro compounds have proved to be useful starting materials in organic synthesis. The appropriate substituted nitro compounds can cyclize to yield five membered heterocyclic compounds. 1,3-Dinitroalkanes can be viewed as synthetic equivalents for 1,3-dicarbonyl compounds, and therefore could be ultimately converted into azole heterocycles. For example 1,3-dinitroalkanes react with hydrazines giving rise to pyrazoles.



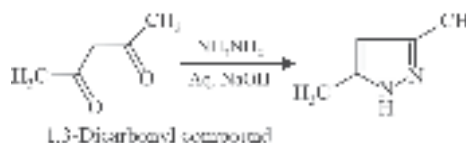
- (iii) **Pechmann Pyrazole synthesis:** The pyrazoles are formed by reaction of acetylenes with diazomethane. The analogous addition of diazoacetic esters to the triple bond yields pyrazole-carboxylic acid derivatives.



- (iv) Pyrazole or isoxazole derivatives are prepared by a palladium-catalyzed four-component coupling of a terminal alkyne, hydrazine, carbon monoxide under ambient pressure, and an aryl iodide.

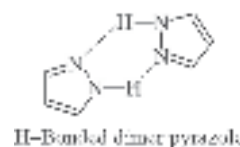


- (v) **From 1,3-dicarbonyl compounds:** Pyrazole can be prepared from condensation reaction of 1,3-dicarbonyl compounds with hydrazine.



Physical Properties

It is available as white to yellow crystals or crystalline powder, m.p. 67–70°C; soluble in water. Only one nitrogen atom is pyrrole-like nitrogen while the second nitrogen which has no hydrogen is described as pyridine-like. Only one nitrogen atom can contribute two electrons to the aromatic sextet. The lone pair on pyrrole-like nitrogen is delocalized round the ring while that on the pyridine-like nitrogen H-Bonded dimer pyrazole is localized in sp^2 orbital on nitrogen. Thus, these compounds have properties intermediate between those of pyrrole and pyridine. Pyrazole has higher boiling point hydrogen bonding. Pyrazole can form dimers only.



Pyrazoles are much weaker bases than imidazoles. The difference is due to the fact that the positive charge in the pyrazolium ion is less delocalized than in the imidazolium ion. The basicity order is imidazole > pyrazole > pyridine > pyrrole.

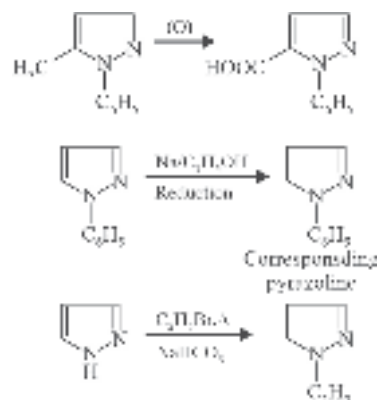
Chemical Properties

Oxidation: Pyrazole is resistant to oxidizing agents but the side chain may be oxidized to carboxylic acid group in presence of potassium permanganate.

Reduction: Pyrazole ring system can be reduced with molecular hydrogen and metal catalyst. Pyrazoline and pyrazolidine are stronger bases than pyrazole.

Alkylation: There is a free N-H group in pyrazole. The free -NH group can be alkylated with alkylating agents (alkylhalides, diazomethane or dimethyl sulphate).

Electrophilic aromatic substitutions: Pyrazole readily undergoes electrophilic substitution readily at C-4 position (which is the least deactivated towards electrophiles) through the intermediate formation of arenium ion. The canonical structures for intermediates of reaction at C-3 and C-5 position include the very unfavored positively charged sextet azomethine: canonical structures (pyrazole, isoxazole, isothiazole) for the intermediate at C-4 do not involve the azomethine system (Fig. 13.16).



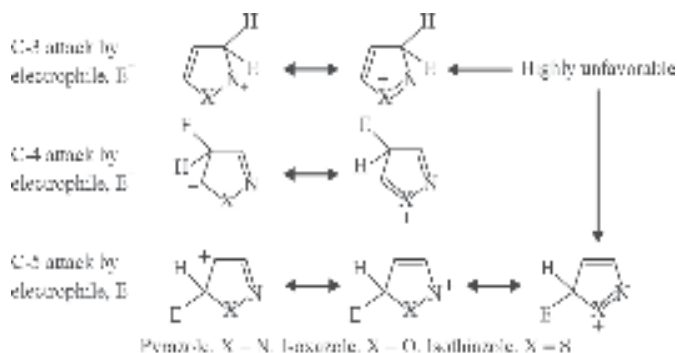
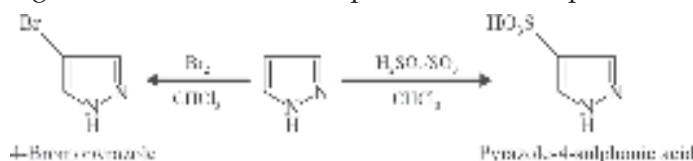
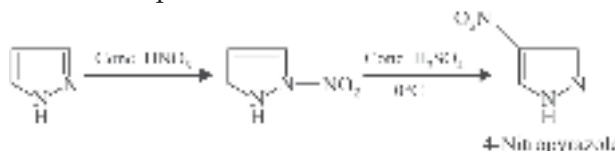


Fig. 13.16: Electrophilic substitution reaction in pyrazole, isoxazole and isothiazole

Some examples of electrophilic substitution reaction in pyrazole are shown here. Pyrazole undergoes bromination and sulphonation at C-4 position.

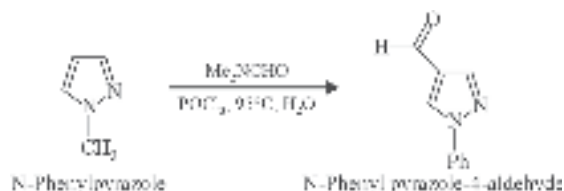


1-Nitropyrazole is formed in good yield by treatment of pyrazole with the mild nitrating reagent, acetyl nitrate. 1-Nitropyrazole can be rearranged to give 4-nitropyrazole by treatment with acid at low temperature.

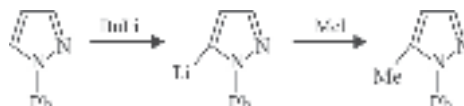


Formylation

Only N-substituted pyrazoles can be C-acylated directly.



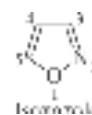
Pyrazole-metallation: 1-Substituted pyrazoles and isothiazoles can be lithiated and alkylated at the C-5 position.



Reaction of pyrazoles with nucleophiles: Halogens of pyrazole derivatives do not undergo substitution reaction. But the presence of electron-withdrawing groups assists nucleophilic substitution at 3 or 5 position.

ISOXAZOLE

Isoxazole is a 1,2-azole with an oxygen atom next to the nitrogen. Isoxazole rings are found in some natural products, such as ibotenic acid, found in mushroom. Isoxazoles also form the basis for a number of drugs, including the sulphamethoxazole. COX-2 inhibitor, valdecoxib (Bextra) (Fig. 13.17). A derivative, furoxane, is a nitric oxide donor.



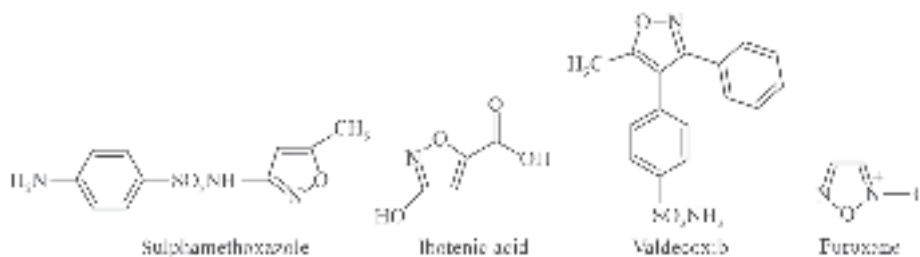
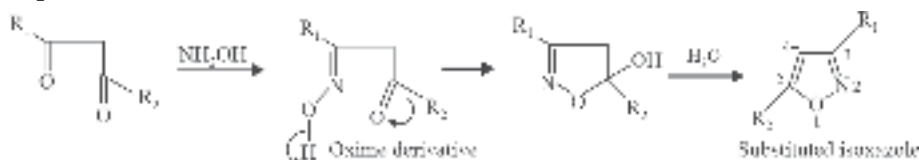


Fig. 13.17: Examples of drugs possessing Isoxazole ring skeleton

Synthesis of Isoxazole

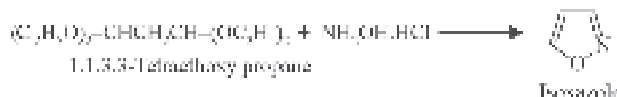
- (i) Isoxazole is prepared by the reaction between hydroxylamine and 1,3-dicarbonyl compound.



- (ii) Isoxazole itself may be prepared by the action of hydroxylamine on propargyl aldehyde.



- (iii) Reaction between 1,1,3,3-tetraethoxypropane and hydroxylamine hydrochloride also yield isoxazole.

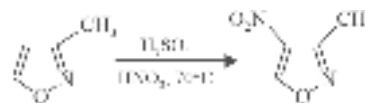


Physical Properties

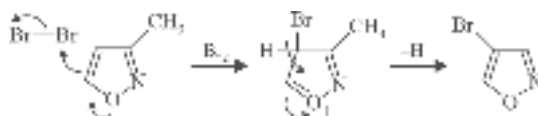
It is a colorless liquid, b.p. 95°C with pyridine like odor.

Chemical Reactions

Electrophilic substitution reactions: The 1,2-azoles are generally much less reactive than 1,3-azoles. Isoxazoles are more reactive than isothiazoles but less than pyrazoles. The electrophilic attack occurs readily at C-4 position and frequently fails if this position is occupied. Presence of both the heteroatom influences the rate of electrophilic substitution in the isoxazole ring. Due to electron withdrawing nature of the nitrogen atom, the electrophilic attack is retarded as in pyridine. The electron donating property of the oxygen atom on the other hand facilitates such reactions. For example, isoxazole nitrates in very low yield, but 3-methyl-isoxazole is sufficiently reactive to undergo nitration at the C-4 position.

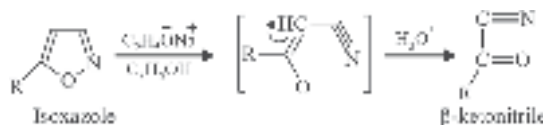


Similarly, isoxazole gives low yield of 4-bromoderivative on bromination.



Reaction with Nucleophilic Reagents

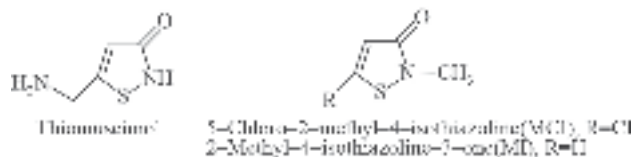
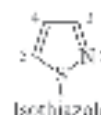
Isoxazole is very liable towards the action of nucleophilic reagent or strong bases. Chichibabin reaction fails in the case of isoxazole as it opens the isoxazole ring. Strong bases result in the cleavage of the isoxazole ring, yielding β -keto nitriles as the end products.



ISOTHIAZOLE

Isothiazole, sometimes known as 1,2-thiazole, is first discovered in 1956. It is a stable heteroaromatic compound of which chemistry is well known.

Several applications of isothiazoles are known: 5-(aminomethyl) isothiazol-3-one (thiomuscimol) is active in the central nervous system as an agonist of receptors for γ -aminobutyrate (GABA). Certain isothiazol-3(2H)-ones (marketed as Kathon) act as biocides and preservative in industrial applications and for stabilizing skin preparations. For example, Kathon is combination of isothiazoline biocides containing 5-chloro-2-methyl-4-isothiazoline (MCI) and 2-methyl-4-isothiazolin-3-one (MI).

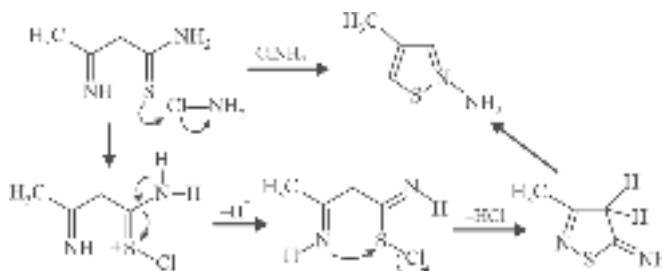


Isothiazole is an aromatic compound that can be represented by the resonance forms shown here.

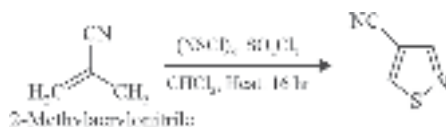


Synthesis of Isothiazoles

- (i) Isothiazoles are usually prepared by routes involving formation of the N-S bond in the cyclisation step. This is often set up by oxidation of the sulphur atom, as in the following:

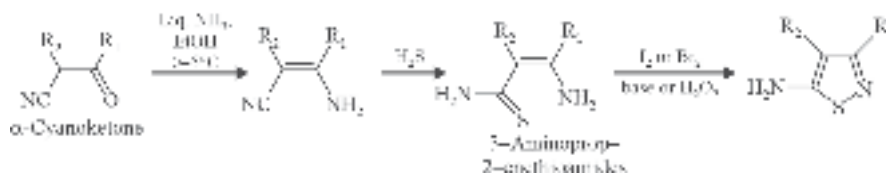


- (ii) Isothiazole-4-carbonitrile can be prepared in 78% yield by the action of trithiazyl trichloride and sulphuryl chloride on 2-methylacrylonitrile in boiling chloroform.

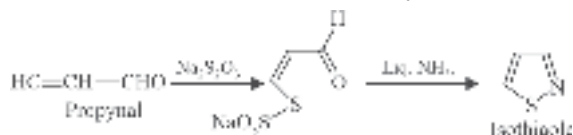


- (iii) **From α -Cyano ketones and ammonia:** A very familiar reaction sequence starts by reacting an α -cyano ketone with ammonia and treating the product, 3-amino-2-

enenitrile with hydrogen sulphide. This affords the corresponding 3-aminoprop-2-enethioamide, which can then be cyclized oxidatively to the appropriate isothiazol-5-arnines by the action of various oxidizing agents, including air, chloroamine, hydrogen peroxide, ammonium persulphate, peracids, bromine, and iodine; other reagents such as thionyl chloride or sulphuryl chloride can also be employed.



- (iv) The best method for the preparation of isothiazole itself involves another reaction sequence that forms the S–N bond in the last step. Here, propynal is reacted with sodium thiosulphate to give the sodium salt of Z-3-thiosulphatoprop-2-enal. This compound, on treatment with liquid ammonia, affords isothiazole, probably via the intermediate imine or its equivalent; the overall yield is 61%.



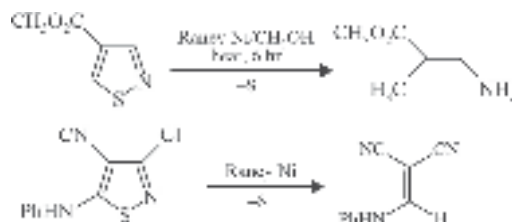
Properties

Isothiazole b.p. 114°C is a planar, heteroaromatic compound with a pyridine-like odor. It has a solubility of about 3.5% in water and is miscible with most organic solvents.

Isothiazoles are generally electron-rich heteroarenes and undergo electrophilic substitution reactions with electrophiles. Isothiazoles are most susceptible to electrophilic substitution at C-4 position. Thus, nitration in a mixture of nitric and sulphuric acids gives mainly 4-nitroisothiazole. Similar results are noted for sulphonation with fuming sulphuric acid (oleum) or with sulphur trioxide.

Halogenation is less controllable and may occur at nitrogen. However, although polyhalogenated products can form, it is possible to obtain 4-chloroisothiazoles in modest yield. For example, isothiazole can be chlorinated directly to afford 4-chloroisothiazole in 30 ± 40% yield.

Direct S-oxidation of isothiazoles is possible and either 1-oxides or 1, 1-dioxides can be formed depending on the oxidant used and the nature of the starting isothiazole.



Treatment of isothiazoles with Raney nickel normally induces the elimination of sulphur and ring opening. For example, methyl isothiazole-4-carboxylate, on heating with Raney nickel in methanol, gives methyl 3-amino-2-methylpropanoate whereas 5-anilino-3-chloroisothiazole-4-carbonitrile, on similar treatment, affords 2-anilinoethene-1,1-dicarbonitrile in low yield.

5-MEMBERED HETEROCYCLIC RINGS WITH MORE THAN TWO HETERO ATOM

TRIAZOLES

Triazole refers to either one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$, having a five-membered ring of two carbon atoms and three nitrogen atoms. The two isomers are:

- 1,2,3-Triazole
- 1,2,4-Triazole

Triazoles have revolutionized the prevention and treatment of invasive fungal infections. The triazoles available for routine clinical use include fluconazole, itraconazole, voriconazole and posaconazole, with ravuconazole and isavuconazole (Fig. 13.18) currently in development.

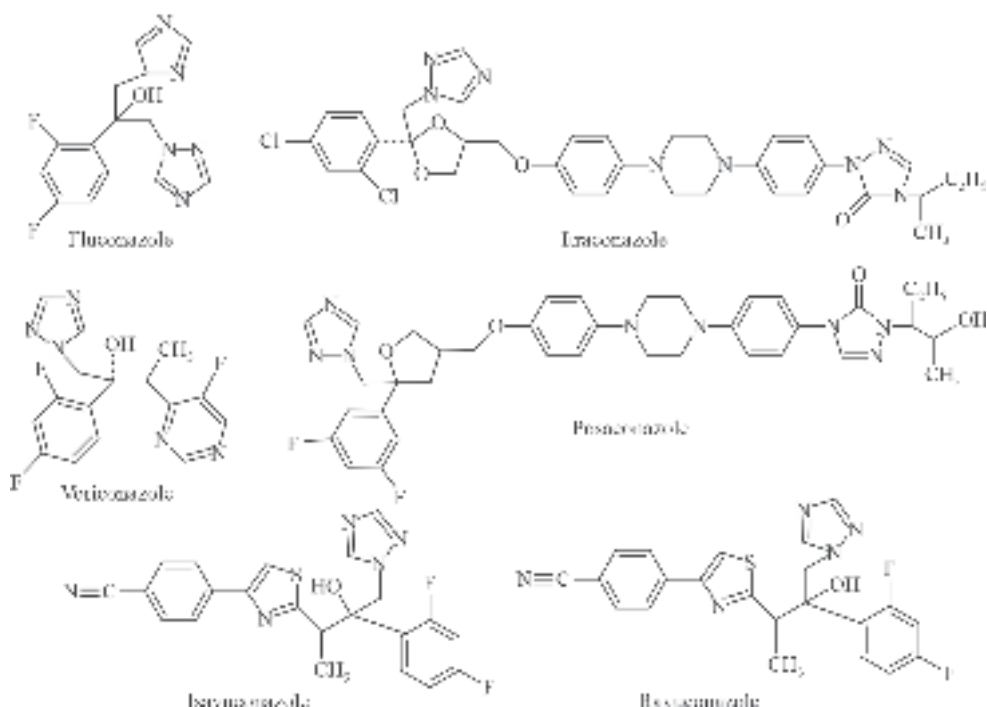
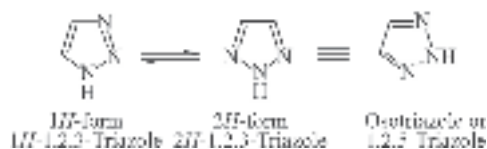


Fig. 13.18: Examples of drugs possessing triazole ring

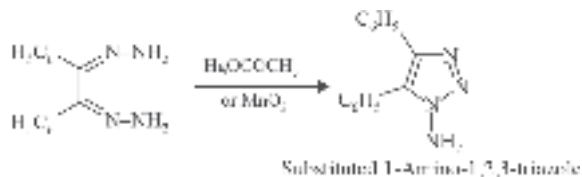
1,2,3-Triazoles

It is a planar five member heterocyclic system with three nitrogen atoms (one pyrrole type and two pyridine type) in the 1,2- and 3-positions. 1,2,3-triazole exists in two isomeric forms: 1H- and 2H-forms. 1H-form was considered more stable than 2H-form but spectral studies have confirmed the presence of 2H-form.

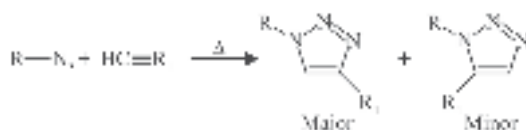


Synthesis of 1,2,3-Triazoles

- (i) **From diketones:** Bis-hydrazones of diketones on treatment with mercuric acetate or manganese dioxide cyclise to give 1-amino-1,2,3-triazoles.

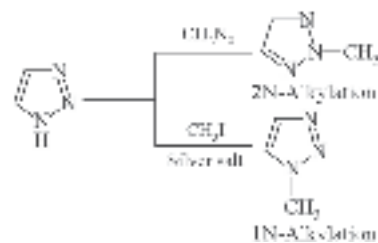


- (ii) **From 1,3-Dipolar cycloaddition:** Many of the starting monosubstituted alkynes and organic azides are available commercially, many others can easily be synthesized with a wide range of functional groups, and their cycloaddition reaction selectively gives 1,2,3-triazoles.

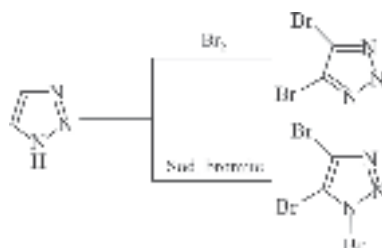


Properties

1,2,3-triazole is a weak base, but it can also behave as a weak acid of similar strength to phenol. 1,2,3-triazole undergoes electrophilic substitution at a two ring carbon atoms or at a ring nitrogen. It undergoes N-alkylation with variety of alkylating agent. The ratio of isomers depends upon the nature of alkylating agents and reaction conditions. The 1N-alkylated and 2N alkylated products are obtained depending upon the nature of alkylating agents.

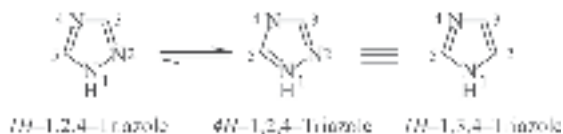


1,2,3-Triazole on bromination with bromine gives 4,5-disubstituted product but with an excess of sodium bromite, bromination-reaction gives 1,4,5-1,2,3-triazole.



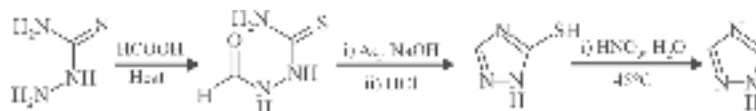
1,2,4-Triazoles

These are cyclic hydrazines with hydrogen atom on either hydrazide nitrogen or an amide nitrogen. Parent 1,2,4-triazole (1H-form) is in tautomeric equilibrium with 1,3,4-triazole (4H-form).

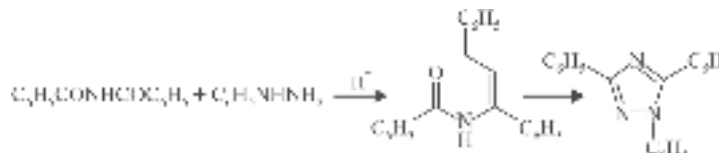


Synthesis of 1,2,4-Triazoles

- (i) **From Thiosemicarbazide:** The thiosemicarbazide gives 1,2,4-triazole via 1-Formyl-3-thiosemicarbazide, and 1,2,4-triazole-5-thiol.



(ii) **Einhorn-Brunner reaction:** The **Einhorn-Brunner reaction** is the chemical reaction of imides with alkyl hydrazines to form a mixture of isomeric 1,2,4-triazoles.

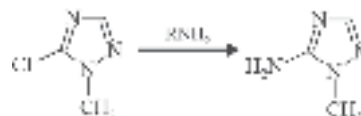


Properties

It is available as white solid, m.p. 120–121°C; very soluble in water. The 1,2,4-triazole is slightly less acidic but more basic than 1,2,3-triazole.

Electrophilic substitution reactions: It undergoes halogenations to give 3-halo-1,2,4-triazole, probably via N-halo-1,2,4-triazole formation.

Nucleophilic substitution reactions: The 3 or 5-halo substituted triazole undergoes nucleophilic displacement. The ease of nucleophilic substitution is facilitated with the quaternization of nitrogen or with the presence of additional electron withdrawing substituent on the ring carbon atom.



TETRAZOLES

Tetrazoles are a class of synthetic organic heterocyclic compound, consisting of a 5-membered ring of four nitrogen and one carbon atoms. The simplest is tetrazole itself, CN_4H_2 . They are unknown in nature. There are several pharmaceutical agents which are tetrazoles. Angiotensin II receptor blockers, in particular, often contain tetrazoles, such as losartan, valsartan and candesartan. A well-known tetrazole is MTT, which is a dimethyl thiazolyl diphenyl tetrazolium salt (Fig. 13.19). This tetrazole is used in the MTT assay to quantify the cellular metabolic activity as an indicator of cell viability.

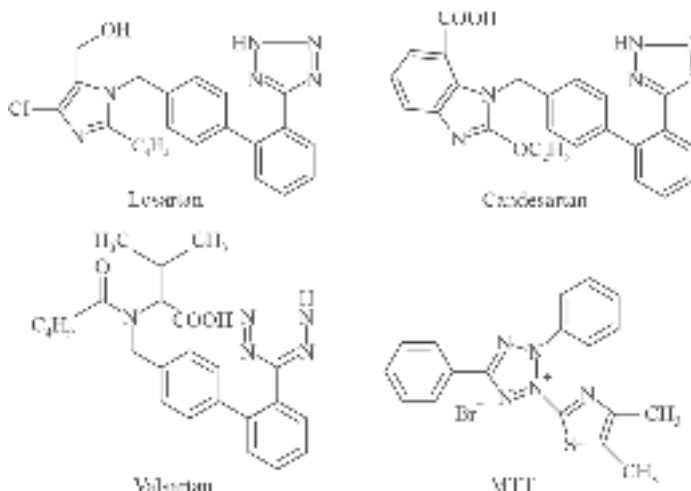
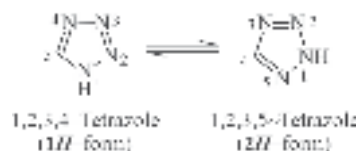


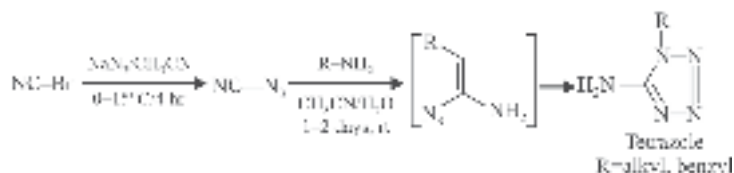
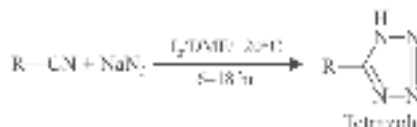
Fig. 13.19: Examples of drugs possessing tetrazole ring

Tetrazole exists in two tautomeric forms: 1,2,3,4-tetrazole (1*H*-form) and 1,2,3,5-tetrazole (2*H*-form). The 1*H*-form shows its predominance.



Synthesis of Tetrazoles

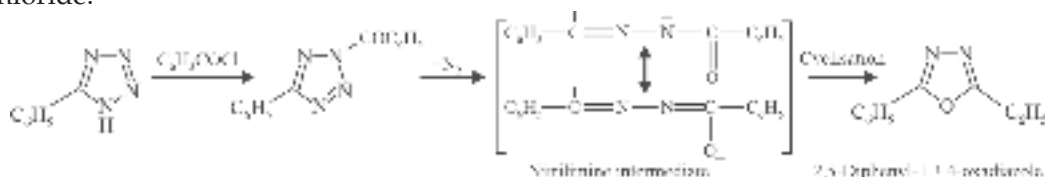
- (i) **From Nitriles:** Treatment of organic nitriles with NaN_3 in the presence of iodine or silica-supported sodium hydrogen sulphate as a heterogeneous catalyst gives 5-substituted 1*H*-tetrazoles.
- (ii) **From Cyanogen azide:** The reaction of cyanogen azide and primary amines generates imido azides as intermediates in acetonitrile/water. After cyclization, these intermediates give 1-substituted aminotetrazoles in good yield.



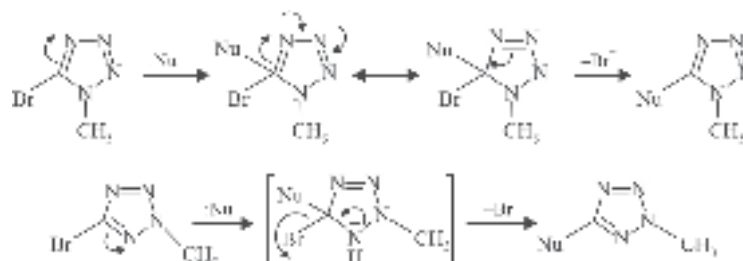
Properties

The majority of tetrazoles are crystalline solids. There is considerable variation in thermal stability, viz. derivatives which melt above 150°C with decomposition, while 5-guanylamino tetrazole does not melt at 300°C . In general, most of the tetrazoles are acids and often yield explosive salts. Tetrazoles are generally soluble in polar solvents, and insoluble in non-polar solvents. 5-Substituted tetrazoles are generally soluble in polar organic solvents such as ethylacetate and DMSO, but under basic conditions, they can be easily extracted into the water phase as a salt, like the carboxylic acid. 5-Substituted tetrazoles display acidity comparable with the corresponding carboxylic acids. One difference between the tetrazole ring and the carboxylic acid group is the annular tautomerism of the tetrazoles. Substituents at C-5 have effects similar to those for carboxylic acids, while in general, 5-aryltetrazoles are stronger acids. The increased acidity is ascribed to enhanced resonance stabilization in the 5-phenyltetrazole anion relative to benzoate.

Tetrazole contains one pyrrole type and three pyridine type nitrogen atoms. The pyrrole type exert electron releasing effect and pyridine type nitrogen exert electron withdrawing effect and thus deactivating the affect which makes the tetrazole ring- π -electron deficient. The π -electron deficiency in tetrazole facilitate the nucleophilic attack. Electrophilic attack in tetrazole ring generally occurs on the nitrogens which results in the ring fragmentation with the loss of nitrogen. It is evidenced by the reaction of 5-phenyl tetrazole with benzoyl chloride.



Nucleophilic substitution reactions: The halo substituted tetrazoles undergo nucleophilic substitution. The kinetic studies have shown that 1,5-disubstituted tetrazoles undergo nucleophilic substitution more easily than 2,5-disubstituted tetrazoles.



BENZFUSED HETEROCYCLES

Heterocycles with fused five membered ring: Five membered aromatic heterocycles namely pyrrole, furan and thiophene, fused with benzene ring give rise to various possible structures, given in Fig. 13.20.

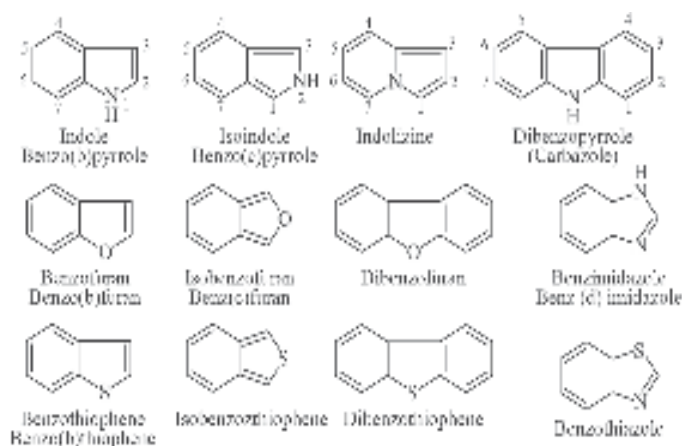
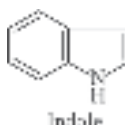


Fig. 13.20: Heterocycles with fused five-membered ring

Indole, benzofuran and benzothiophene are planar aromatics which contain 10π -electrons including the non-bonding electron pair of heteroatom as in monocyclic heterocycles. These 10π -electrons are delocalized over the ring. Because of the involvement of non-bonding lone pair of heteroatom in aromatization, it makes the 5-membered rings more prone to attack by electrophilic reagents.

HETEROCYCLES FUSED WITH FIVE MEMBERED RING

INDOLE



Indole is the most abundant bicyclic heterocycle found in nature—found in tryptophan (essential amino acid), indole-3-acetic acid (plant growth hormone), 5-Hydroxytryptamine (5-HT) (neurotransmitter), natural products-strychnine (stimulant), LSD (psychoactive drug), indomethacin (antiinflammatory drug), sumatriptan (used in migraine attack) (Fig. 13.21).

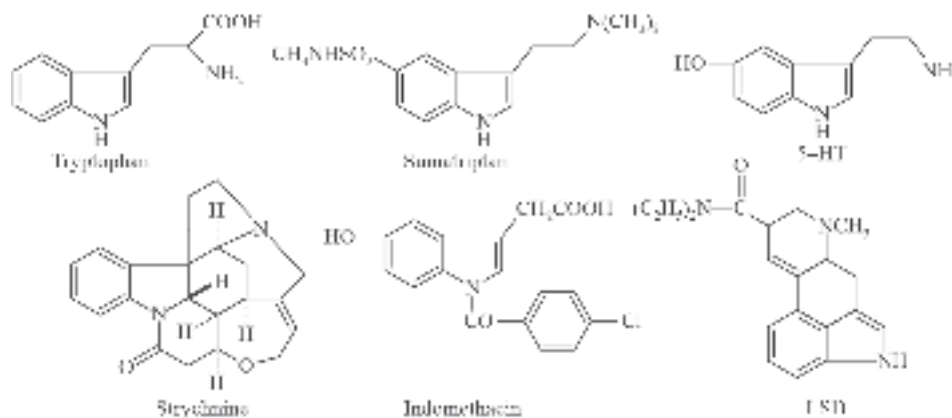


Fig. 13.21: Examples of drugs possessing indole ring skeleton

Synthesis of Indole

- (i) **Fischer-Indole synthesis:** It is the most important synthesis of indole which has been very widely investigated. The Fischer-indole synthesis is carried out by heating phenyl hydrazone or substituted phenyl hydrazone of an aldehyde or ketone. The reaction is catalyzed by zinc chloride, polyphosphoric acid, sulphuric acid or boron trifluoride and proceeds with elimination of a molecule of ammonia. Different mechanisms have been proposed depending on reaction conditions and nature of hydrazone. The most acceptable mechanism is depicted in Fig. 13.22.

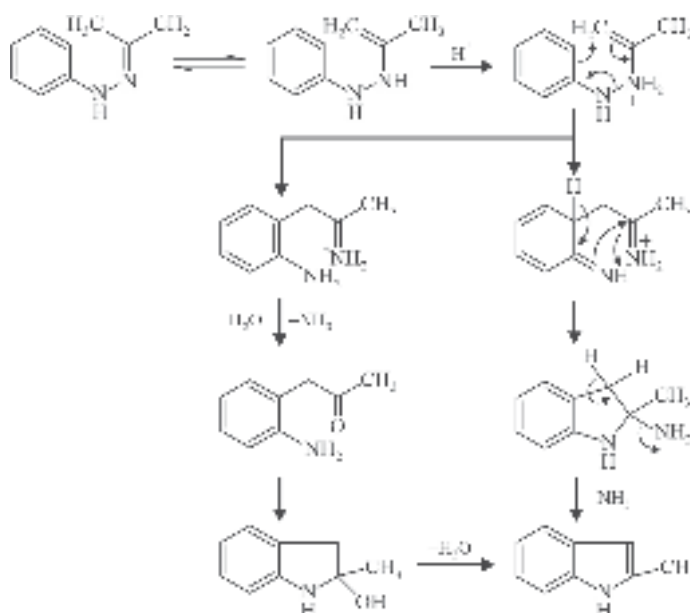
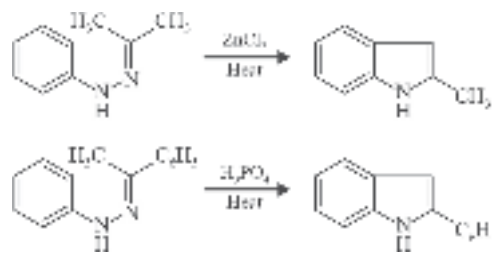
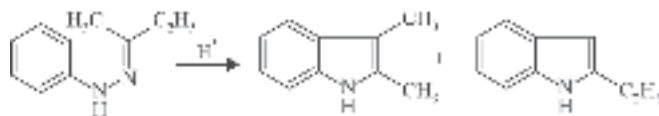
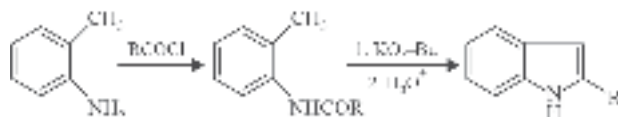


Fig. 13.22: Mechanism of Fischer-indole synthesis

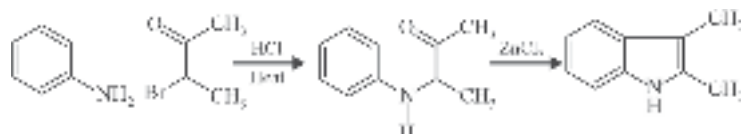
Unsymmetrical phenyl hydrazones give a mixture of two differently substituted indoles, the ratio of which depends on steric factors and reaction conditions.



- (ii) **Madelung synthesis:** Reaction of *o*-toluidine with acyl chloride gives *o*-acylamino toluene which can be cyclized with strong bases followed by dehydration to give indole or 2-substituted indoles.



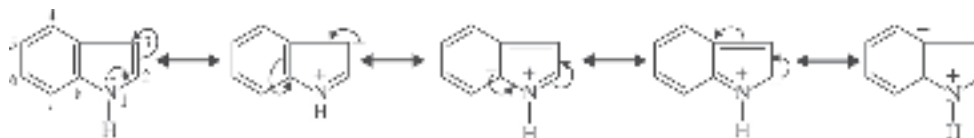
- (iii) **Bischler synthesis:** The Bischler synthesis involves reaction of aniline or substituted aniline with α -halo ketone or aldehyde to give α -aryl aminoketone or aldehyde, which can be cyclized by heating with an acid or zinc chloride to give substituted indoles.



It is a good method to prepare the 2 or 3 or 2,3 substituted indole derivatives. However this method does not work to prepare benzene compounds having electron withdrawing group on the ring.

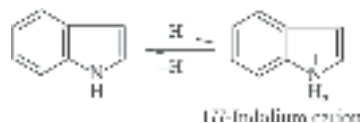
Physical Properties

Indole is a pleasant smelling solid which melts at 52°C and is used as a perfume base. It is a planar aromatic molecule with conjugated 10 π -electrons contributed by eight carbon atoms and a lone pair contributed by nitrogen. Its resonance energy is 47–49 kcal/mol. Because of the involvement of lone pair of nitrogen, charge separated canonical forms can be written for indole as shown here.



Chemical Reactions

Indole is a very weak base like pyrrole because of the involvement of lone pair of nitrogen in aromatization. However, in dilute solutions it undergoes protonation of nitrogen to give indolium cation in which the aromaticity of benzene ring is retained. Indole does not undergo easy alkylation of nitrogen unlike amines for the same reason.



- (i) **Electrophilic substitution reactions in indole:** The electron density of carbons in heterocyclic ring of indole is higher due to contribution from nitrogen as in case of pyrrole. Therefore, the heterocyclic ring of indole is more reactive towards electrophiles compared to its benzene ring. The electrophilic substitution in indole

takes place at C-3 and not at C-2 as in pyrrole. This can be explained from the following observations. Electrophilic attack at C-2 and C-3 gives different intermediates as shown in Fig. 13.23.

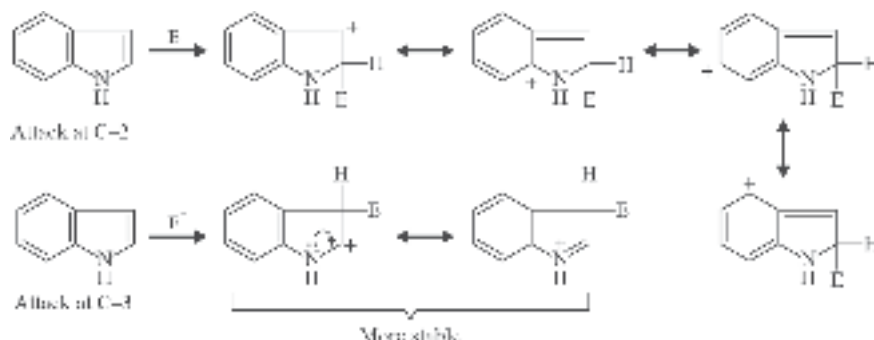


Fig. 13.23: Electrophilic substitution reactions in indole

The carbocation resulting from electrophilic attack at C-2 is less favourable than the carbocation resulting from electrophilic attack at C-3 because though the former has more resonance structures, the aromaticity is completely lost whereas in the latter intermediate, the positive charge resides on heterocyclic ring carbon or the nitrogen atom without affecting the benzene ring. Some typical examples of electrophilic substitution of indole are given in Fig. 13.24.

- (ii) **Acylation:** Acylation of indole at C-3 does not take place as the reaction with acyl halide leads to acylation of nitrogen. If C-3 of indole is blocked, then electrophilic substitution takes place at C-2 and if both C-2 and C-3 are blocked, then electrophilic substitution takes place preferably at C-6 in aromatic ring.
- (iii) **Oxidation and reduction of Indole:** Indoles are easily oxidized by air and give a mixture of products. The double bond in between C-2 and C-3 in indole is labile and can be easily cleaved by ozonolysis, peracids and sodium hypiodate, etc. Indole

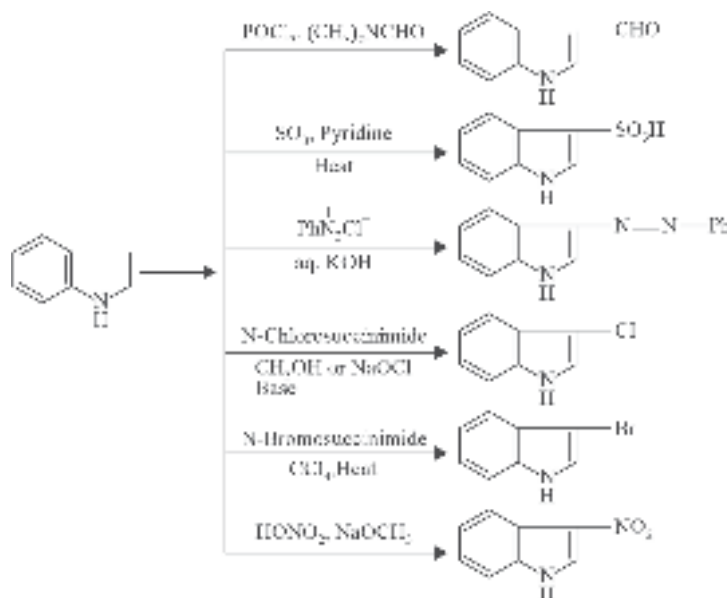
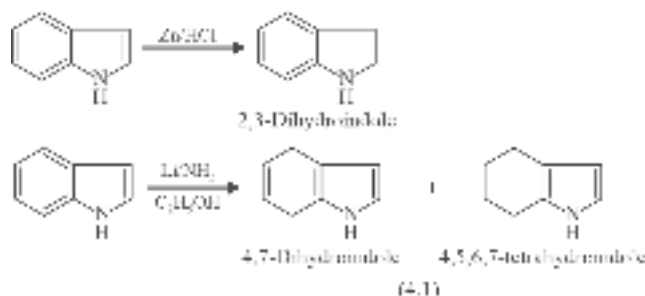


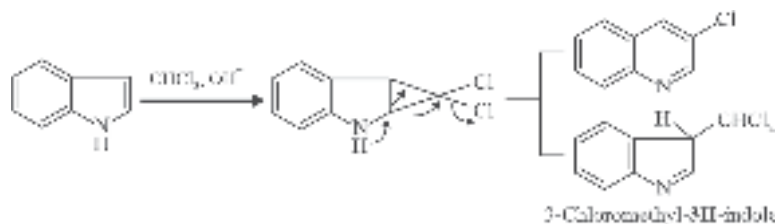
Fig. 13.24: Electrophilic substitution reactions of indole

gives a resinous material whereas 3-substituted or 2,3-disubstituted indoles undergo 2N-C3 cleavage as in case of ozonolysis.

Indoles can be selectively reduced in five-membered ring or six-membered ring. The five-membered ring is reduced by a number of reagents in acidic media, e.g. Zn, Sn, $\text{BH}_3\text{-NMe}_3$ complex while six-membered ring can be reduced by Birch reduction using Li-ammonia in ethanol to give 4,7-dihydroindole as major product.



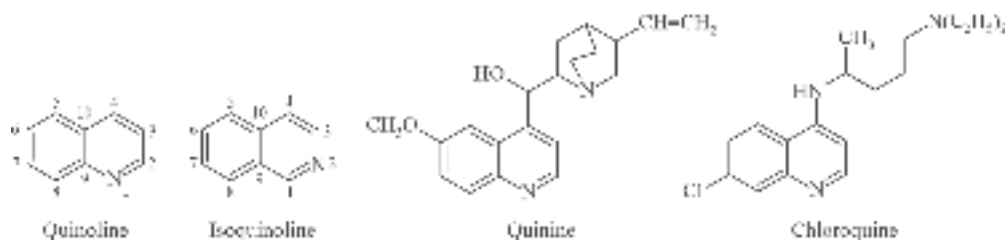
- (iv) **Reaction with Carbenes:** Indole and substituted indoles undergo addition of dihalocarbene on electron rich $\text{C}_2\text{-C}_3$ double bond to give an intermediate which undergoes ring expansion to give 3-chloroquinoline or 3-membered ring cleavage to give 3-substituted 3H-indole derivative.



HETEROCYCLES WITH FUSED SIX MEMBERED RING

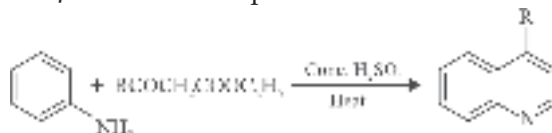
Quinoline and Isoquinoline

Quinoline and isoquinoline are two fused heterocycles derived by fusion of pyridine ring with a benzene ring. Both ring systems occur naturally and is also found in medicinally important drugs. Quinine and chloroquine are the examples of antimalarial drugs having quinoline ring structure.

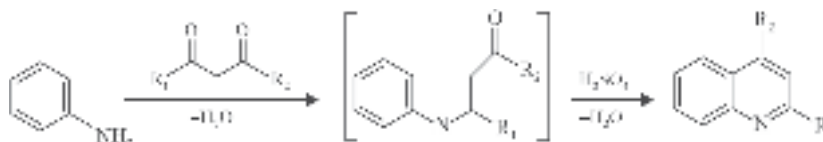


Papaverine (vasodilator), quinapril (antihypertensive) are the examples of important drugs possessing isoquinoline moiety.

- (iii) **Knorr Quinoline synthesis:** 4-Substituted quinolines can also be prepared by heating aniline with β -keto esters in presence of an acid.

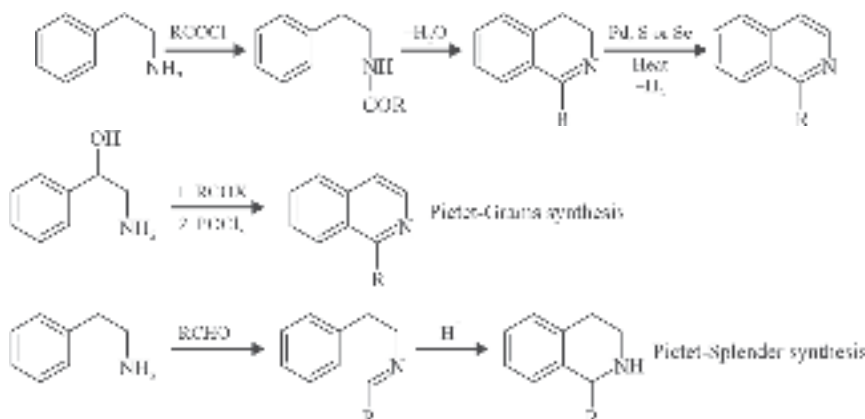


- (iv) **Combes synthesis:** Aniline on heating with 1,3-diketones in presence of an acid gives 2,4-disubstituted quinoline after an acid catalyzed ring closure of an intermediate Schiff base.

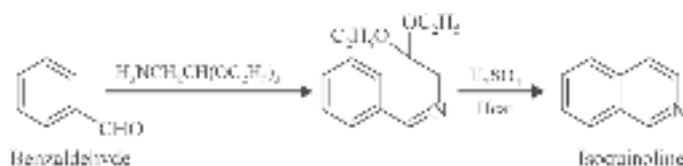


Synthesis of Isoquinoline

- (i) **Bischler-Napieralski synthesis:** The Bischler-Napieralski synthesis involves reaction of β -phenyl ethylamine with acyl halide to give *p*-phenyl ethylamide which undergoes cyclodehydration in presence of POCl_3 , P_2O_5 , H_3PO_4 or ZnCl_2 to give 3,4-dihydroisoquinoline. Dehydrogenation of 3,4-dihydroquinoline over Pd, S or Se yields 1-substituted isoquinoline. There are various modifications in this method. The two modifications are **Pictat-Gams synthesis** (in which a hydroxy group is introduced in the starting β -phenyl ethylamine) and **Pictet-Spengler synthesis** (in which the β -phenyl ethyl amine is reacted with an aldehyde) to give an imine which is cyclized in presence of an acid to give tetrahydroisoquinoline.



- (ii) **Pomeranz-Fritsch reaction:** This is carried out by condensation of an aromatic aldehyde with an amino acetal, which is then cyclized in presence of sulphuric acid to give isoquinoline.



Alternatively, benzylamine can be condensed with acetal followed by cyclization to give isoquinoline.

Physical Properties

Quinoline is high boiling liquid (b.p. 237°C) and smells like pyridine while isoquinoline is a low melting solid (m.p. 26.5°C b.p. 243°C). Both quinoline and isoquinoline are planar 10 π -electron aromatic systems in which all atoms are sp^2 hybridized and contribute one electron each in orthogonal p-orbitals for delocalization over the rings.

Both quinoline and isoquinoline are aromatic with resonance energies of 198 and 143 kJ/mol respectively as depicted by various contributing structures as shown in Fig. 13.25. The first three structures (i–iii) of both quinoline and isoquinoline are of lower energy and contribute significantly to aromatic character compared to charge separated structures (iv and v). Quinoline and isoquinoline are weak bases but slightly more basic than pyridine but less basic than anilines since the nitrogen in quinoline and isoquinoline is more electronegative being sp^2 hybridized compared to sp^3 hybridized nitrogen of anilines. Isoquinoline has higher dipole moment (2.60 D) compared to quinoline (2.10 D).

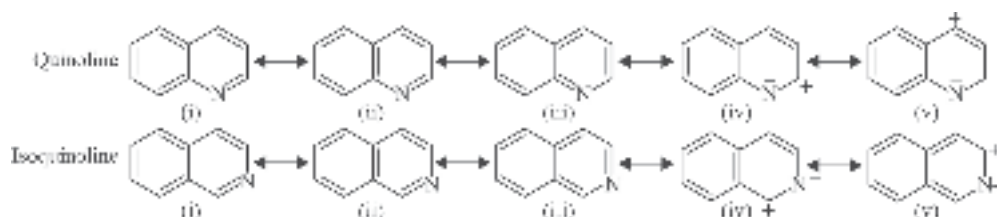
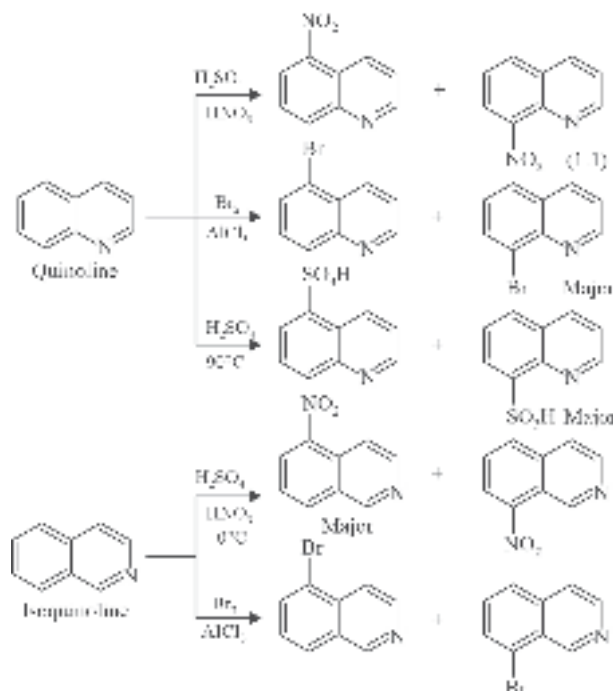


Fig. 13.25: Canonical structures of quinoline and isoquinoline

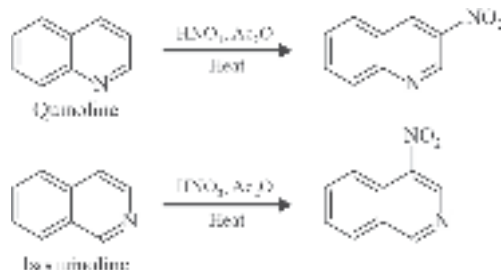
Chemical Reactions of Quinoline and Isoquinoline

Quinolines and isoquinolines behave similar to pyridine. Both are weakly basic and undergo protonation of nitrogen without affecting the aromaticity of the ring. Quaternary ammonium salts are formed on nitrogen by alkylation.



- (i) **Electrophilic aromatic substitution:** The nitrogen of the quinoline and isoquinoline has deactivating effect on the ring towards electrophilic substitution as in case of pyridine as discussed earlier. However, electrophilic substitution of quinoline and isoquinoline requires less vigorous conditions than pyridine. Electrophilic substitution of protonated quinoline and isoquinoline takes place on the carbocyclic ring at C-5 or C-8 positions. Some typical examples of electrophile substitution in quinoline and isoquinoline are given here.

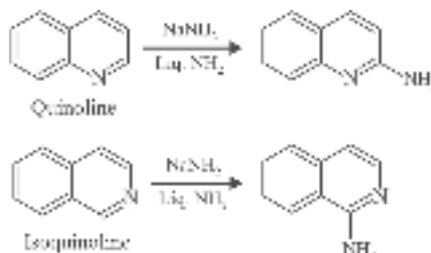
Quinoline and isoquinoline undergo reaction with nitric acid in presence of acetic anhydride to give 3-nitroquinoline and 4-nitroisoquinoline, respectively.



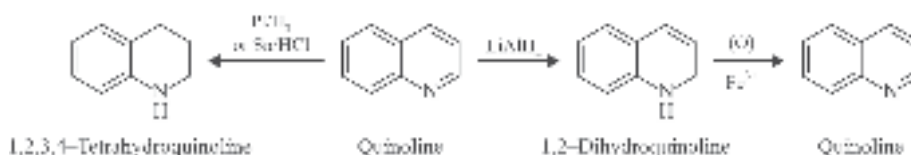
- (iii) **Nucleophilic aromatic substitution reaction:** Quinoline and isoquinoline undergo facile nucleophilic substitution reaction as in pyridine ring, in which C-2 position is the preferred site of attack in case of quinoline and C-1 position in case of isoquinoline. Quinoline undergoes Chichibabin reaction to give 2-aminoquinoline while isoquinoline undergoes Chichibabin reaction to give 1-amino isoquinoline. The preferred site of attack on C-1 of isoquinoline is explained by the canonical form, **II** which has a benzenoid nucleus while structure **I** does not have any benzenoid nucleus.

The preferred site of attack on C-2 position in quinoline is indicated by its canonical form, **I** (has styrene type of conjugation), which is absent in form, **II**.

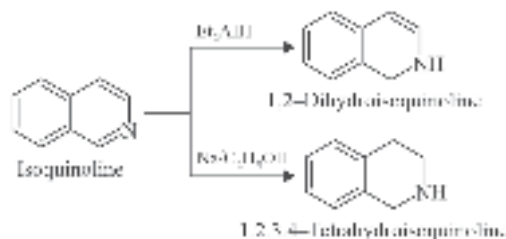
Isoquinoline undergoes substitution faster than quinoline. The reaction proceeds in a manner analogous to pyridine.



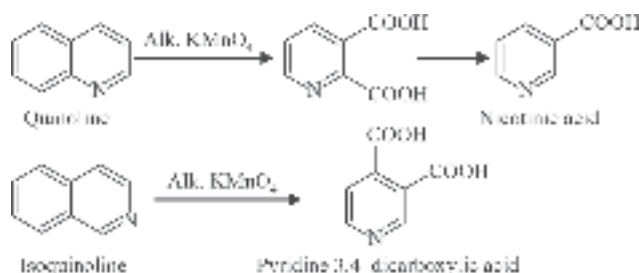
- (iii) **Reduction:** Quinoline can be selectively reduced at 1,2-bond by reaction with lithium aluminium hydride but the 1,2-dihydroquinolines is easily oxidized back to give quinoline with ferric ion. Quinoline can be converted to 1,2,3,4-tetrahydroquinoline by catalytic hydrogenation or with tin and hydrochloric acid.



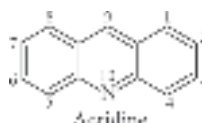
Isoquinoline can also be converted to 1,2-dihydro or 1,2,3,4-tetrahydroisoquinoline with diethyl aluminium hydride and sodium-ethanol, respectively.



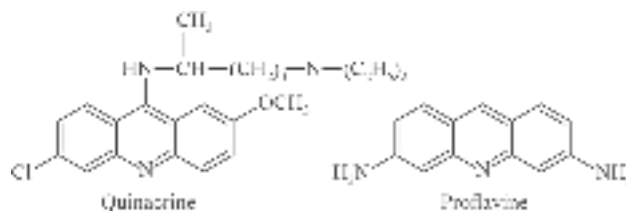
- (iv) **Oxidation:** Quinoline and isoquinoline undergo oxidative cleavage with alkaline potassium permanganate to give pyridine-2,3-dicarboxylic acid and pyridine-3,4-dicarboxylic acid, respectively. However, pyridine-2,3-dicarboxylic acid is not stable and undergoes decarboxylation to give nicotinic acid. Quinoline and isoquinoline both form N-oxides when treated with hydrogen peroxide in acetic acid or with organic peracids in a manner exactly analogous to pyridines.



ACRIDINE

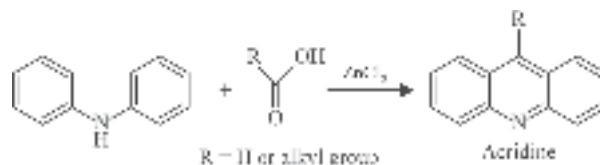


Acridine ($\text{C}_{13}\text{H}_9\text{N}$): It is tricyclic nitrogen atom containing heterocycle. Acridine is also used to describe compounds containing the C_{13}N tricyclic structure. Acridine is structurally related to anthracene with one of the central CH groups is replaced by nitrogen or pyridine fused with two benzene rings. Acridine is first isolated from coal tar. It is a raw material used for the production of dyes and some valuable drugs. Many acridines, such as proflavine, also have antiseptic properties. Quinacrine having acridine nucleus, has antimalarial action.

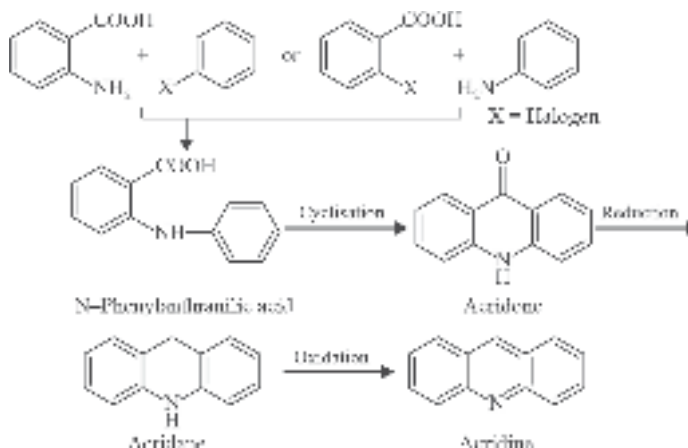


Many synthetic processes including **Bernthsen acridine synthesis** are known for the production of acridine and its derivatives. Bernthsen condensed diphenylamine with carboxylic acids in the presence of zinc chloride to get acridine. With formic acid as the

carboxylic acid, the reaction yields acridine itself, and with the higher homologues the derivatives substituted at the meso carbon atom are generated.



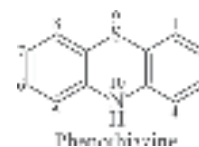
The cyclization of N-phenylanthranilic acid also gives the acridone. The acridone on reduction followed by oxidation yields the acridine. This method is referred as **Ullmann synthesis**.



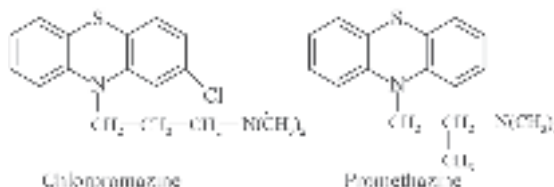
Acridine is available as colorless needle like crystalline solid, m.p. 106–109°C and slightly soluble in hot water.

PHENOTHIAZINE

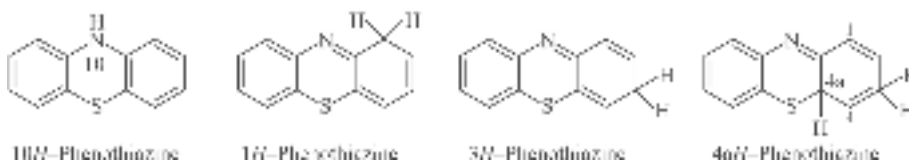
Phenothiazine is a tricyclic heterocyclic compound having sulphur and nitrogen atoms. The compound is related to the thiazine-class of heterocyclic compounds.



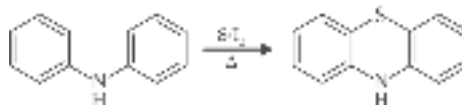
The phenothiazine structure occurs in various neuroleptic drugs, e.g. chlorpromazine (Largactil), and antihistamine drugs, e.g. promethazine (phenergan). The term “phenothiazines” describes the largest of the five main classes of neuroleptic antipsychotic drugs. These drugs have antipsychotic and, often, antiemetic properties.



Phenothiazine occurs in four isomeric forms as given here.



The compound was originally prepared by Bernthsen in 1883 via the reaction of diphenylamine with sulphur in presence of I_2 (catalyst), but more recent synthesis rely on the cyclization of 2-substituted diphenyl sulphide. It is important to note that pharmaceutically significant derivatives of phenothiazine are not prepared from phenothiazine.



It occurs as yellow colored crystalline solid, m.p. 185.1°C , soluble in acetic acid, benzene, and ether. All phenothiazines are readily oxidized particularly in presence of sunlight and moisture. Therefore, phenothiazines are good antioxidant.

COUMARIN

Coumarin is a oxygen atom containing heterocyclic compound, called benzopyrones. The coumarin chemical structure contain a benzene ring fused to an alpha-pyrone ring. Umbelliferone, esculetin and scopoletin are the most widespread coumarins found in nature. Coumarin and its derivatives are principal oral anticoagulants. Warfarin and dicoumarol are the examples of anticoagulant drug (Fig. 13.26).

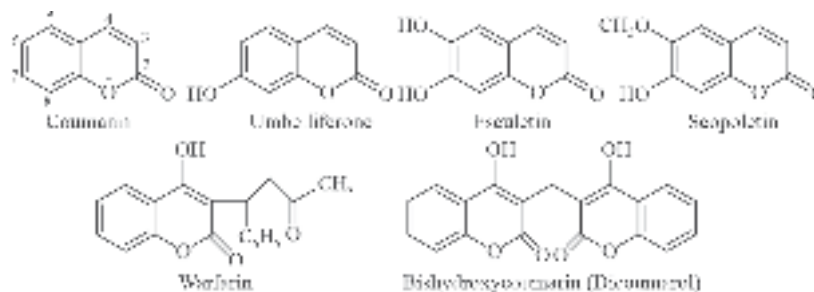
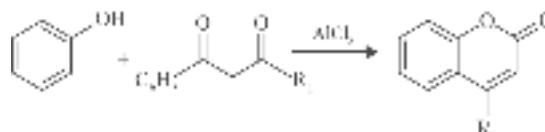


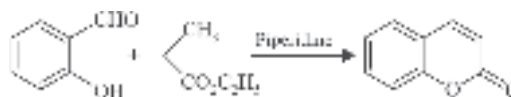
Fig. 13.26: Examples of drugs possessing coumarin ring skeleton

Synthesis of Coumarins

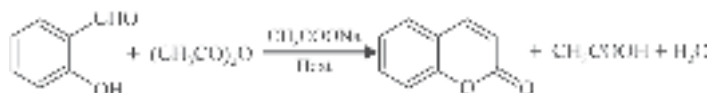
Pechmann coumarin synthesis: The Pechmann condensation allows the synthesis of coumarins by reaction of phenols with β -keto esters.



Knoevenagel condensation reaction: Condensation of salicylaldehyde or its derivative with various derivatives of ethyl acetate in the presence of piperidine leads to the synthesis of coumarins.

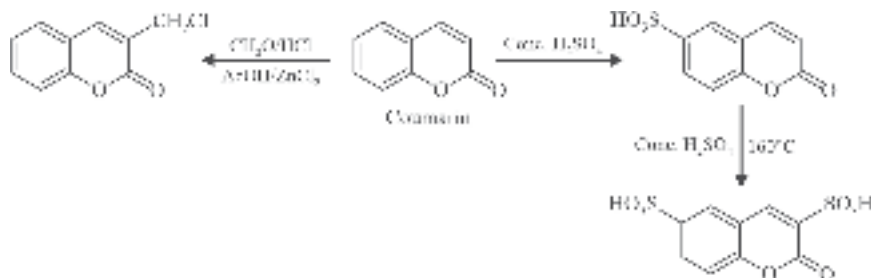


Perkin reaction: Perkin reaction involves the interaction of salicylaldehyde and acetic anhydride in the presence of sodium acetate as given here.

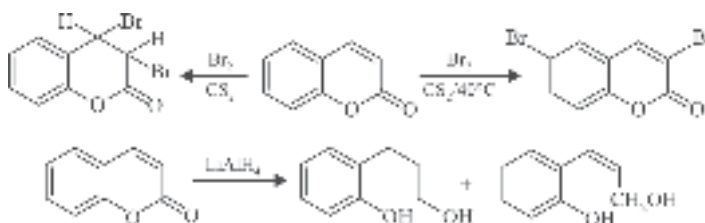


Properties

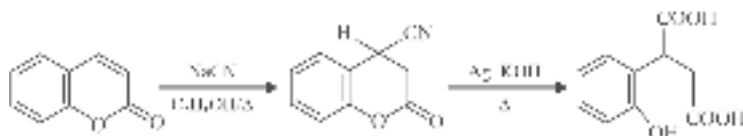
It is white crystalline solid, m.p. 69–73°C with vanilla like odor. It is slightly soluble in water. On nitration and sulphonation, coumarins give mainly C-6 monosubstituted product and under more vigorous condition, further substitution at C-3 also occurs. Similarly, Friedel-Crafts acylation occurs at C-6 position. Chloromethylation occurs at C-3 position.



On bromination under mild condition, it gives addition product. The reduction with lithium aluminium hydride is possible with opening of ring.



Mild carbon nucleophiles, such as cyanide anion, usually add to the β -carbon of α,β -unsaturated carbonyl compounds, react with coumarin at C-4 position.



SHORT AND LONG QUESTIONS

- Define the following terms:
 - Heterocyclic compound
 - Fused hydrocarbons
- Discuss the principles of the nomenclature of heterocyclic compounds with examples.
- Predict the product:**
 - Furan H_2/Ni ?
 - Thiophene $\text{CH}_3\text{COCl}/\text{AlCl}_3$?
 - Thiophene CHCl_3/KOH ?
 - Thiophene H_2/Ni ?
 - Pyrrole CHCl_3/KOH ?
 - Pyrrole H_2/Ni ?
 - Pyridine $\text{H}_2\text{SO}_4/350^\circ\text{C}$?
 - Pyridine H_2/Pt ?

- q. Pyridine $\text{HNO}_3/\text{H}_2\text{SO}_4$?
 - r. Furan SO_3 /pyridine?
 - s. Pyridine Br_2 ?
 - t. Pyrrole H_2SO_4 ?
 - u. Pyrrole $\text{CH}_3\text{COCl}/\text{AlCl}_3$?
 - v. Thiophene H_2SO_4 ?
 - w. furan CHCl_3/KOH ?
3. Explain Hantzsch pyridine synthesis with mechanism. Electrophilic substitution reaction in pyridine takes place at position no 3. Explain.
 4. What is the action of following reagent on Pyridine? Give balance eq".
 - a. H_2/Ni
 - b. $\text{Br}_2/300^\circ\text{C}$
 - c. $\text{H}_2\text{SO}_4/350^\circ\text{C}$.
 5. What is the action of following reagent on Naphthalene? Give balance eq".
 - a. Conc. $\text{H}_2\text{SO}_4/80^\circ\text{C}$
 - b. H_2/Pt
 - c. $\text{HNO}_3/\text{H}_2\text{SO}_4$, $50-60^\circ\text{C}$
 - d. Br_2
 - e. Conc. $\text{H}_2\text{SO}_4/160^\circ\text{C}$
 - f. $\text{Na}/\text{C}_5\text{H}_{11}\text{OH}$, Reflux.
 - g. $\text{O}_2/\text{V}_2\text{O}_5$, $460-480^\circ\text{C}$
 - h. CrO_3/AcOH , R.T.
 6. Give the reaction of KOH/CHCl_3 on
 - a. Thiophene
 - b. Pyrrole
 - c. Furan
 7. How will you synthesize furan from mucic acid and furfural?
 8. Give two methods of preparation of pyrrole.
 9. Give two methods of preparation of thiophene.
 10. Give two methods of preparation of pyridine
 11. How will you convert following:
 - a. Pyridine into piperidine
 - b. Pyrrole into pyrrolidine.
 - c. Furan into furfural
 - d. Thiophene into 2-benzoyl thiophene.
 - e. Pyrrole into 2-pyrrole sulphonic acid
 - f. Acetylene into pyridine
 - g. Pentamethylene diamine into pyridine
 - h. Furfural into furan
 - i. Acetylene into pyrrole
 - j. Acetylene into thiophene
 12. What are heterocyclic compounds? Give one method of synthesis of:
 - a. Furan
 - b. Thiophene
 - c. Pyrrole
 - d. Pyridine

13. Explain sulphonation of naphthalene at different temperature. Why β -isomers predominates at high temp?
14. Give synthesis of thiophene, pyrrole and pyridine from acetylene.
15. Attempt the following:
 - a. Why imidazole has higher boiling point?
 - b. Why piperidine is more basic than pyridine?
 - c. Why pyrrole does not give Diels-Alder reaction?
 - d. Why pyridine N-oxide is more reactive than pyridine towards the electrophilic and nucleophilic substitutions.
16. Write a note on following:
 - a. Coumarins and their medicinal importance
 - b. Quinolines and their medicinal importance
17. a. Give the examples of 1,2-azole and 1,3-azoles.
b. Discuss the various reactions of imidazole.
18. a. Describe the methods of preparation of quinoline.
b. At what position electrophilic substitutions take place in quinoline and why. Illustrate with examples
19. Discuss the aromatic character in pyrrole, furan and thiophene.
20. How do pyridine and pyrrole differ in their reactions with sulphuric acid.

MULTIPLE CHOICE QUESTIONS

1. Which of the following five membered rings is most resonance stabilized?
 - a. Furan
 - b. Thiophene
 - c. Pyrrole
 - d. Pyridine
2. What type of ring system is found in the compound pyridine?
 - a. Aromatic
 - b. Aliphatic
 - c. Heterocyclic
 - d. Cyclic
3. What type of heterocyclic compound is found in the compound purine?
 - a. Pyridine
 - b. Aniline
 - c. Pyrimidine
 - d. Pteridine
4. Furan, pyrrole and thiophene undergo electrophilic substitution at position.
 - a. 1
 - b. 2
 - c. 3
 - d. 4
5. Thiophene can be prepared from
 - a. Ethylene
 - b. Butane

- c. Sodium succinate
 - d. Ethane
6. Skraup synthesis is used to synthesize which of the following heterocycles?
- a. Isoquinoline
 - b. Quinoline
 - c. Indole
 - d. Imidazole
7. What is the name of starting material used for Paal-Knoor synthesis of furan?
- a. 1,4-diketo compound
 - b. 1,5-diketo compound
 - c. All of the above
 - d. None of the above
8. Following is the correct order of aromaticity.
- a. Thiophene > pyrrole > furan
 - b. Thiophene < pyrrole < furan
 - c. Furan > pyrrole > thiophene
 - d. Pyrrole > furan > thiophene
9. Quinoline on oxidation with alkaline potassium permanganate gives
- a. Pyridine-3-carboxylic acid
 - b. Pyridine-2,3-dicarboxylic acid
 - c. Pyridine-N-oxide
 - d. All of the above
10. Reaction of quinoline to 2-aminoquinoline, which of the following reagents is used
- a. NaNH_2 /liquid ammonia
 - b. Sodium azide
 - c. Ammonia
 - d. None of the above

Answers

1. (a) 2. (c) 3. (c) 4. (b) 5. (b) 6. (b) 7. (a) 8. (a) 9. (b) 10. (a)

Chapter 14

Antibiotics—Chemistry and Medicinal Importance

The word 'antibiotic' comes from the Greek word *anti* meaning 'against' and *bios* meaning 'life' (a bacterium is a form of life). Antibiotics are specific substances, derived from or produced by living organism. These substances in small concentration are capable of inhibiting the life processes of other organisms.

To date, in the literature more than three thousands, about two thousands originating from microorganism have already been reported. Only a few dozens, however, are used in medicine, because, besides of other reasons, most of antibiotics are devoid of selective toxicity. Most of the antibiotics are produced by microorganisms, but some have resulted from chemical modification of known antibiotics or microbial metabolites; for example the semi-synthetic penicillins and cephalosporins, the modified tetracyclines. etc. Furthermore, some antibiotics are now completely obtained from synthetic procedure, for example, chloramphenicol, quinolones, etc.

Antibiotics are the most widely prescribed class of drugs in the present scenario. Most of the recent medical prescriptions essentially contain an antibiotic. They are used for several purposes: (i) circulatory, respiratory, genitourinary, gastrointestinal, ophthalmic, soft tissue, skeletal, and topical infections; (ii) prophylaxis of infections. either in healthy or in ill persons; (iii) diagnosis of malignancies-tetracycline has been used for this purpose.

History of Antibiotics

The clinical application of molds, now known to contain antibiotics, is very old. Centuries before our era, folk medicine used them rather frequently. But the phenomenon of antibiotics was studied scientifically, only after **Pasteur** laid down the foundation of bacteriology. As early as 1877, **Pasteur** and **Joubert** reported that a liquid culture of aerobic bacteria inhibited the growth of *B. anthracis*.

Flemming in 1929, observed by chance and then reported the inhibitory action of the *Penicillium notatum* on the growth of staphylococci. His discovery did not have immediate practical application, because he could neither able to isolate penicillin, the substance responsible for this action, nor could produce it in great yields. This task was accomplished 10 years later by **Florey** and **Chain**. Penicillins was first used clinically in 1941. In 1944, another landmark was set in the history of natural penicillin's at Northern Regional Research Laboratories. **Meyer** and **Coghill** discovered that deep culture in corn steep liquor increased yields of penicillin's in a substantial percentage. In 1959, Beecham Lab obtained 6-aminopenicillanic acid (6-APA) from *P. chrysogenum* molds. The availability of 6-APA made possible the introduction of semi-synthetic penicillin's, with new and better properties than the natural ones.

In 1939 **Dubos**, at the Rockefeller institute, isolated from a strain of *Bacillus brevis* antimicrobial substances, which he named tyrothricin. Actually, this substance contains two antibiotics: gramicidin and tyrocidin. **Waksman**, who was Dubois's professor at Rutgers University. In his systematic search for antimicrobial substances from actinomycetes, he was successful in isolating several new antibiotics: actinomycins (1940), streptothricin (1942), streptomycin (1943), and neomycin (1949).

The development of basic knowledge and experimental techniques, related to the newly introduced field of antibiotics coupled with the important chemotherapeutic uses of penicillin's and streptomycin, prompted several investigations in many parts of the world to search for better antibiotics. This effort resulted in the introduction of the following ones in a rapid succession: bacitracin in 1943 by **Johnson and Meleney**; chloramphenicol in 1947 by Burkholder; chlortetracycline in 1947 by **Duggar**, then oxytetracycline in 1950 by **Finley**; erythromycin in 1952 by **McGuire**; oleandomycin in 1954 by **Sobin**; vancomycin by **Eli Lilly and Company** in 1956; kanamycin in 1957 by **Umezawa**; rifampin in 1959 by **Sensi**. For this reason the period 1940–1959 has been wisely called the Golden Era of Antibiotic Discovery.

The next decade (1960–1970) saw a decline in the isolation of therapeutically useful antibiotics except fusidic acid in 1961 by **Godfredson** and associates; lincomycin in 1962 by **Mason** and coworkers; gentamicin and capreomycin in 1963. However, during this period, existing antibiotics were chemically or biochemically modified. Some molecular modifications are very successful, resulting in new therapeutic agents, such as semi-synthetic penicillins, cephalosporins, rifampin, tetracyclines, and lincomycin, for instance.

In this decade, efforts are being made to discover new antibiotics by the following approaches: (i) molecular modification of useful antibiotics; (ii) isolation of new antibiotics; (iii) structural variation of toxic or poorly efficacious antibiotics. These approaches have been very fruitful, as will be seen in the next sections of this chapter.

Classification of Antibiotics

Among the several criteria used to classify antibiotics, the main ones are (i) biosynthesis; (ii) spectrum of activity; (iii) chemical structure.

A. Biosynthesis

According to biosynthesis, antibiotics can be divided into following classes:

1. *Antibiotics derived from amino acids*: Cycloserine, chloramphenicol, penicillins, cephalosporins, gramicidin, polymyxin, viomycin, and capreomycin.
2. *Antibiotics derived from carbohydrates*: Streptomycin, kanamycin, neomycin, gentamicin, lincomycin, and spectinomycin.
3. *Antibiotics derived from acetate and propionate*: Fusidic acid, griseofulvin, macrolide antibiotics, polyene antibiotics, tetracyclines.
4. *Miscellaneous antibiotics*: Novobiocin, puromycin, vancomycin.

B. Spectrum of Activity

1. *Broad spectrum antibiotic*: These antibiotics are effective against a variety of micro-organism, both gram-positive and gram-negative and may also be rickettsias and even protozoa, etc. Some penicillins (e.g. ampicillin, carbenicillin), some cephalosporins (cephaloridine). Chloramphenicol, macrolides, tetracyclines,

sparsomycins, some aminoglycosides (neomycin, kanamycin, gentamicin, paromomycin).

2. *Antibiotics active predominantly against gram positive bacteria:* Bacitracin, most penicillin's (e.g. benzylpenicillin, cloxacillin, dicloxacillin, oxacillin), fusidic acid, erythromycin, lincomycin.
3. *Antibiotics active against gram-negative bacteria:* Polymixin B, Sulphomycin.
4. *Anti-fungal antibiotics:* Amphotericin B, candidin, fumagillin, haymycin, nystatin.
5. *Anti-amoebic antibiotics:* Paromomycin, puromycin.
6. *Anti-neoplastic antibiotics:* Adriamycin, bleomycin, daunorubicin, mitomycin C, actinomycins.

C. Chemical Structure

Here, antibiotics are classified based on chemical structure. None of classification is satisfactory. However, in the present chapter, this classification is followed to classify the antibiotics into penicillins, cephalosporins, chloramphenicol and derivatives, tetracyclines, polyene antibiotics, macrolide antibiotics, aminoglycoside antibiotics, antracyclines, lincomycin group, nucleoside antibiotics.

PENICILLINS

Structural Features

Penicillins as well as cephalosporins are usually referred as β -lactam antibiotics. These are characterized by three fundamental requirements: (a) the fused β -lactam structure, (b) a free carboxylic group, and (c) one or more properly substituted amino side chain groups.

All the β -lactam antibiotics with high antibacterial activity possess a continuous $-C-CO-NH-CCO-N-C-COO^-$ linkage, because of the side chain ($-C-CO-NH-$), and containing β -lactam and the thiazolidine or dihydrothiazine nucleus.

All penicillins have the same β -lactam-thiazolidine general structure that contains three asymmetric centers. Therefore, theoretically this structure could present eight optically active forms. However, natural isomer, presumably the only one with biological activity has the following stereochemistry:

The fused rings are not coplanar but folded along the C-5, N-4 axis. This non-planarity suppresses normal amide resonance. The amide carrying carbon atom (C-6) has the L configuration, whereas the carbon atom bearing the carboxyl group (C-3) has the D configuration. The absolute stereochemistry of the penicillins is designated as 3S 5R:6R. The natural penicillins are derived from a cysteinylvaline precursor.

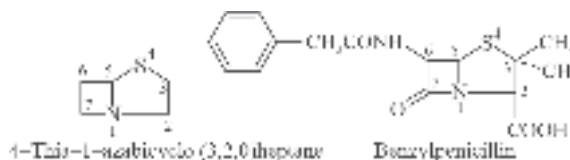


Nomenclature

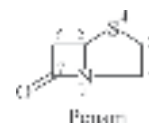
Penicillins are named in the following ways:

1. The penicillins are named as 4-Thia-1-azabicyclo [3,2,0] heptanes. According to this, benzylpenicillin is 6-(2-phenyl acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3, 2, 0] heptane-2-carboxylic acid. The number 1 and 5 represent bridgehead atoms. There are three carbons atoms on one side of the bridgehead atom, two on the other

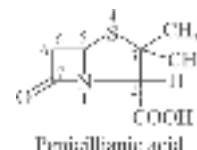
and none in-between. The bicyclic system possesses seven atoms, thus the bicycle [3, 2, 0] heptane notation.



2. **Penam derivatives:** The name 'penam' is given to unsubstituted bicyclic lactam ring as mentioned here. According to this system, benzylpenicillin is named as 6-Phenylacetamido-2,2-dimethyl penam-3-carboxylic acid.



3. **Penicillanic acid derivatives:** Penicillanic acid is the duly substituted penam. Thus, benzylpenicillin is 6-phenyl acetamido penicillanic acid.



4. **Penicillin derivatives:** It is the most commonly used nomenclature; penicillin is the name given to the general structure without R. Based on the presence of benzyl group, the name Benzylpenicillin is given to penicillin G. Usually, the side chain R is an alkyl, aryl or heterocyclic grouping (Table 14.1).

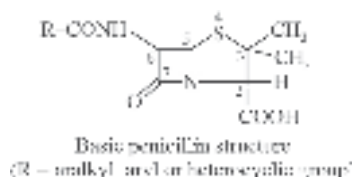
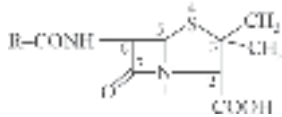
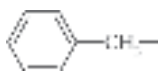
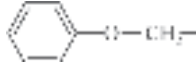
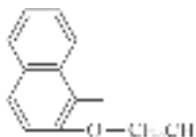


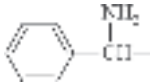
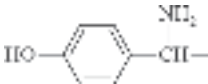
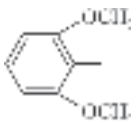
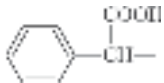
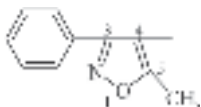
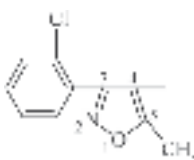
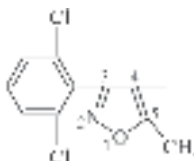
Table 14.1: Examples of penicillins



R group	Chemical name	Generic name
	Benzylpenicillin	Penicillin G
	Pheoxymethylpenicillin	Penicillin V
	Pheoxyethylpenicillin	Phenethicillin
	2-Ethoxy-1-naphthyl penicillin	Naficillin

(Contd.)

(Contd.)

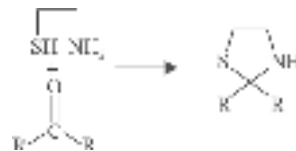
<i>R</i> group	<i>Chemical name</i>	<i>Generic name</i>
	α -Amino benzylpenicillin	Ampicillin
	<i>p</i> -Hydroxy- α -Amino benzylpenicillin	Amoxicillin
	2,6-Dimethoxy phenylpenicillin	Methicillin
	α -Carboxy benzylpenicillin	Carbenicillin
	5-Methyl-3-phenyl-4-isoxazolyl penicillin	Oxacillin
	5-Methyl-3-(<i>o</i> -chlorophenyl)-4-isoxazolyl penicillin	Cloxacillin
	5-Methyl-3-(2',6'-dichloro phenyl)-4-isoxazolyl penicillin	Dicloxacillin
	α -Sulphonyl benzylpenicillin	Sulbenicillin

Properties

Penicillins are white or slightly whitish crystalline powders. These are strongly dextro-rotatory. Owing to the carboxyl group attached to the fused ring, all the penicillins are relatively strong acids with pK_a value, 2.65. However, those that contain a basic group in the side chain exist as Zwitterions; such as ampicillin whose pK_a is 7.4. Therefore, penicillins are classified as monobasic and zwitterionic. Most of the penicillins are used as sodium, potassium, or other salt, which are water soluble. Penicillins and their salts have a strong tendency to form crystalline hydrates.

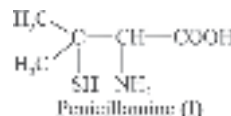
Owing to the strained amide bond in the fused β -lactam ring, penicillins are very reactive. They are extremely susceptible to nucleophilic attack, as well as to electrophilic attack. They are inactivated by hydrolysis particularly in the presence of heavy metal salts, acids and especially bases and also by the catalytic action of enzymes (like amidases and β -lactamases). The water or hydroxide ion forms pencilloic acid (Fig. 14.1). In strongly

(d) Penicillamine on reaction with acetone gives isopropylidene derivative. This isopropylidene derivative does not contain a free amino or thiol group and it can be reconverted into the penicillamine on hydrolysis. This reaction also suggests that the amino and thiol group in penicillamine are just opposite to each other, indicated by easy formation of thiazolidine derivative.

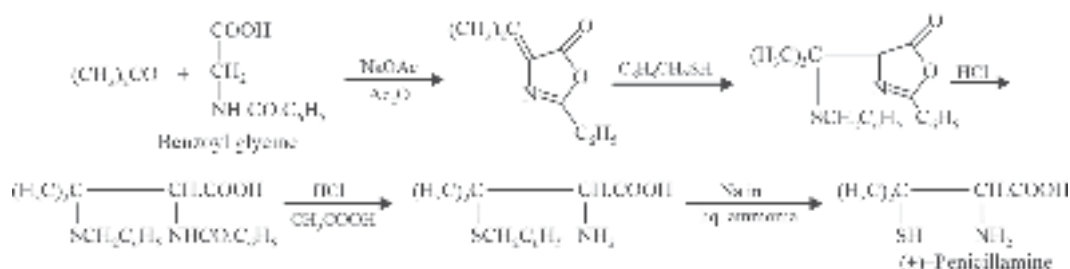


(e) On the basis of above evidences and synthesis, Penicillamine is found to be β,β -dimethylcysteine.

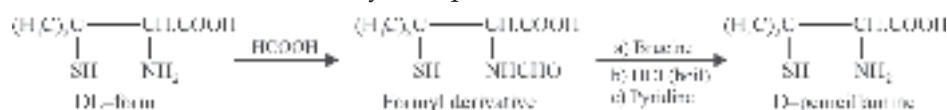
The proposed structure is further confirmed on the basis of its synthesis.



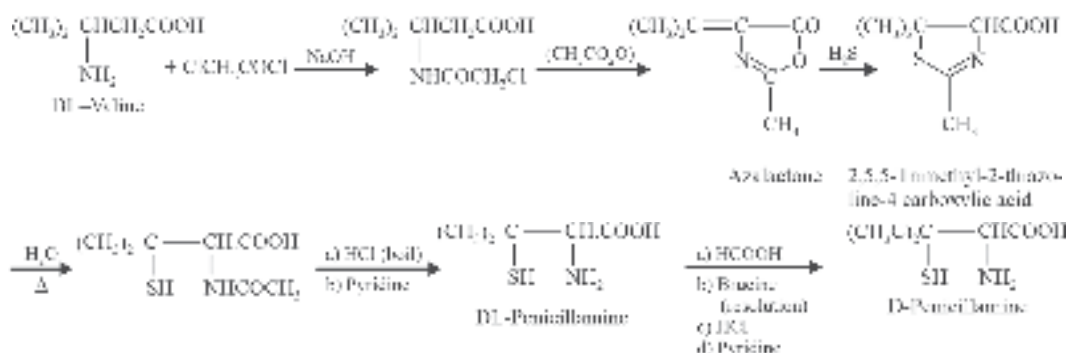
Synthesis of D-penicillamine from Benzoyl glycine:



The resolution of this amino acid is best achieved through fractionation of the brucine salt of the N-formyl compound.



Other method:



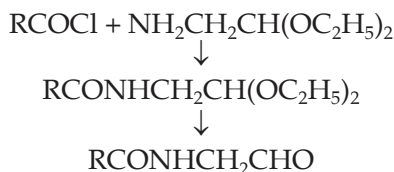
(f) Penicillin on treatment with diazomethane forms methylester which on treatment with aqueous solution of mercuric chloride gives methylester of penicillamine. Thus, the carboxyl group in the Penicillamine is the carboxyl group in penicillin itself.

Structural elucidation of Penilloaldehyde:

(a) On vigorous hydrolysis, all penilloaldehydes give a substituted acetic acid and aminoacetaldehyde.



This reaction has been confirmed by the synthesis from acid chlorides and aminoacetal.



(b) As pointed out above, acid hydrolysis of the penicillin yields penicillamine and penilloaldehyde with the evolution of CO_2 . The formation of CO_2 molecule is indicative of the fact that some unstable acid is formed as intermediate which undergoes decarboxylation to give CO_2 . On this basis, presence of β -keto acid can be predicted. Hence, penilloaldehyde-carboxylic acid (Penaldic acid) must be formed as intermediate in the hydrolysis of Penicillin.

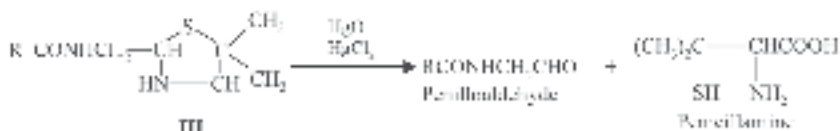


VII. Linkage of Penicillamine and Penilloaldehyde: This can be established on the basis of the following facts:

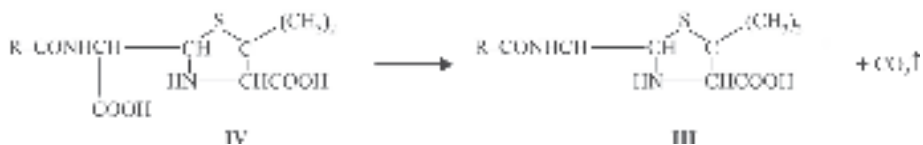
The hydrolysis of penicillin with dilute alkali or with the enzyme, penicillinase produces penicilloic acid (a dicarboxylic acid), which readily eliminates a molecule of CO_2 to form penilloic acid. This suggests that carboxyl group is in the β -position with respect to an electron attracting group.

Penilloic acid, on hydrolysis with aqueous mercuric chloride gives penicillamine and penilloaldehyde. This hydrolysis is the characteristic of thiazolidine ring.

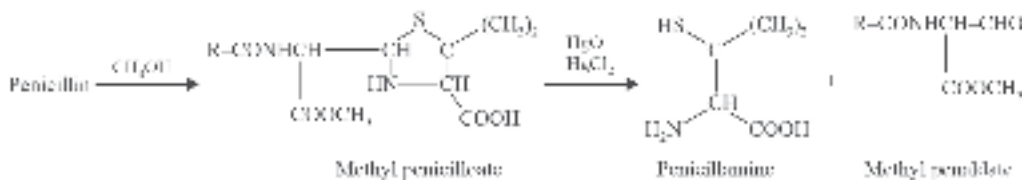
On the basis of the above evidences, the structure, **III** is assigned to Penilloic acid which can produce the required products—penicillamine and penilloaldehyde.



Hence if the structure **III** is penilloic acid, then penicilloic acid would be structure **IV**.

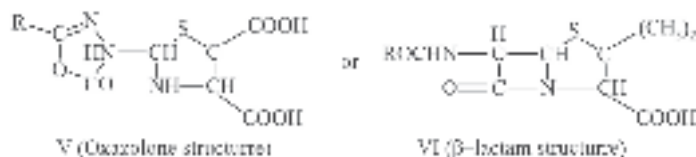


The structure, **IV** of penicilloic acid has been further confirmed by the fact that penicillin on treatment with methanol gives methyl penicilloate, which on hydrolysis with aqueous mercuric chloride yields methyl penaldate.

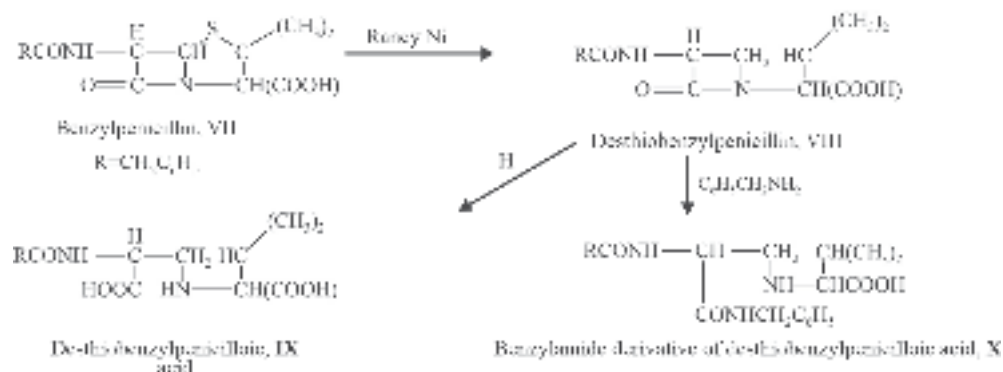


VIII. As it is already stated in point **II** that penicillin is monocarboxylic acid, the carboxyl group is present in the penicillamine molecule, which is coming from the

thiazolidine nucleus, the second carboxyl group of the Penicilloic acid may be present either as oxazolone or a β -lactum and thus, Penicillin might be **V** or **VI**.

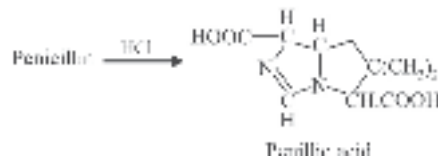


- IX. The evidence for the existence of the β -lactam ring in penicillin is obtained by desulphurization of the Benzyl penicillin with Raney nickel to give desthiobenzyl penicilloic acid, IX or on boiling with Benzylamine in dioxane solution to get benzylamide derivative of desthiobenzylpenicilloic acid, X.



The compound **IX** and **X** can be obtained by desulphurization (with Raney nickel) of benzyl-penicilloic acid and its benzylamide respectively.

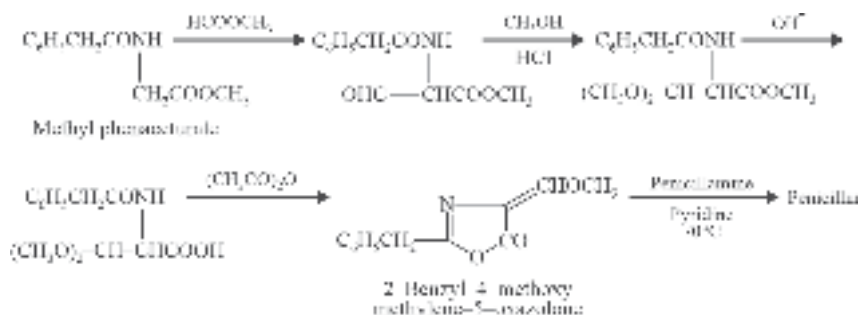
- X. However, the correct structure of penicillin cannot be assigned on the basis of chemical degradation method, since penicillin readily undergoes molecular rearrangement. For example, on treatment with dilute acid penicillin rearranges to penillic acid.



The structure, **VII** to penicillin is assigned on the basis of IR and X-ray method. This can explain the formation of hydrolytic product.

Using the structure, the above mentioned chemical reaction can be designed as given in Fig. 14.2.

- XI. Finally, the structure of Penicillin is confirmed on the basis of synthesis from methyl phenacetate.




$$\text{C}_6\text{H}_5\text{CH}_2\text{CO}-\text{NH}-\text{CH}(\text{S})-\text{CH}^+ \quad m/e \ 192$$

Semi-synthetic Penicillins

There were four main areas, considered by medicinal chemists to target for developing semi-synthetic penicillins: **increased acid stability**, **increased spectrum** (improved activity against gram-negative bacteria), **increased activity against penicillin-resistant bacteria**, and **decreased allergic reactions**. They were successful in accomplishing the first three but not the fourth goal. The allergic reaction arises from a reaction with the β -lactam ring itself. The penicillin is broken down in the body, and some of the byproducts are bound to carrier proteins in the body. It is the complex of the byproduct and the carrier proteins that triggers the body's allergic reaction. Because it is not possible to alter the β -lactam ring without inactivating the penicillin, allergic reactions are not likely to be overcome in new penicillins.

Initially, penicillins were isolated from cultures of *Penicillium notatum* and *B. chrysogenum* species of fungi. Later on new penicillins were prepared by adding precursors, such as carboxylic acids or related compounds, to the fermentation mixture. Under certain condition, those precursors were incorporated into the side chains of the new penicillins. Unfortunately, this method got limited success.

A second approach, which also proved to yield good results, was to modify the available penicillins by chemical synthesis. Sheelan and Henery-Logan obtained the phenoxymethyl penicillin by synthetic process but yield was very low. For this reason, only few of the penicillins have been synthesized by total synthesis.

The isolation 6-aminopenicillanic acid (6-APA) in the Beecham Research laboratory in 1957 permitted the synthesis of vast number of semi-synthetic penicillins. These penicillins are synthesized by making the reaction of 6-APA with one of the following group of compound (i) carboxylic acid using N,N' -dicyclohexyl carboximide as condensing agent, at room temperature; (ii) acyl chloride in presence of triethylamine; (iii) acid anhydrides.

These semisynthetic are categorized as follows depending on their beneficial properties:

1. **Acid stable penicillins:** These have powerful electron attracting group attached to amino side chain, such as penicillin in which acidic rearrangement is prevented. This arrangement is illustrated in Fig. 14.3. Penicillin V, phenoxymethylpenicillin, etc. are examples of acid stable penicillins.

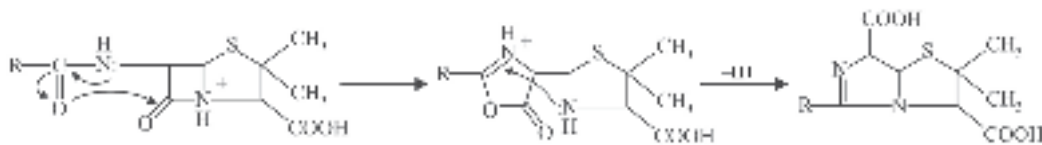


Fig. 14.3: Mechanism of acidic inactivation of penicillins

2. **β -Lactamase resistant penicillins:** These have bulky group attached to the amino acid side chain. By steric hindrance, this group interferes with the enzyme attachment to the penicillins and causes conformational changes in the enzyme which results in loss of enzymatic activity. Methicillin, azidocillin, flucloxacillin, etc. are common examples of this type.
3. **Acid and β -lactamase resistant penicillins:** These have electron withdrawing and bulky group attached to amino side chain. Examples are oxacillin, cloxacillin, dicloxacillin, nafcillin, cyclacillin, etc.
4. **Broad spectrum penicillins:** Gram-negative bacteria have channels called, porins in their cell wall, which allow certain substances to enter the bacterial cells.

Penicillin G is not able to enter through these porins because its R group does not have the right chemical makeup, so medicinal chemists changed the R group to allow the new penicillins to enter through the porins; in chemical language, the new R groups contain polar groups, such as NH_2 and COOH . Examples of these broad spectrum penicillins with gram-positive and gram-negative activity are: Ampicillin, Amoxicillin; Ticarcillin, Mezlocillin, Piperacillin, Carbenicillin, etc. The latter four drugs are known specifically for activity against *Pseudomonas aeruginosa*, and the R groups of some of them are shown in Table 14.1.

Therapeutic Uses

Penicillins are bactericidal agents, being more effective against gram-positive bacteria, because their cell wall structure is more susceptible to inhibitory action than gram-negative bacteria. They kill dividing rather than resting microorganism. Penicillins are excreted by both the liver and the kidneys, either unaltered or metabolized. Penicillins can be metabolized to penicilloic acids in man, the extent being dependent on the penicillin structure.

Most of the penicillins are active only against gram-positive microorganism. They are indicated for treatment and prophylaxis of infections caused by cocci (gonococci, β -hemolytic, streptococci, and group A (β -hemolytic streptococci), *Treponema pallidum*, *Bacillus anthracis*, *Clostridium*, *Corynebacterium diphtheriae*, and several species of *Actinomyces*. Other penicillins are effective against gram-negative bacteria: *Hemophilus influenzae*, most of strain of *Escherichia coli*, and some species of *Salmonella*, *Shigella* and *Pseudomonas*.

Penicillins should not be used topically and are often prone to cause hypersensitization.

Mechanism of Action of Penicillins

There is resemblances between a segment of penicillins and certain segment of N-acetylmuramic acid, D-alanyl-D-alanine, and L-alanyl-D-glutamic acid present in cell wall. This may be required for mechanism of action of penicillins. The last step in the bacterial cell wall synthesis is a cross-linking reaction between two nascent peptidoglycan units, catalyzed by enzyme, **transpeptidase**. Owing to their structural resemblance to the D-alanyl-D-alanine terminal group of the pentapeptide part of the nascent peptidoglycan units, both penicillins and cephalosporins according to the hypothesis advanced by Tipper and Strominger in 1965, inhibit this enzyme by binding covalently with it, thus preventing the formation of the bacterial cell wall (Fig. 14.4). As a result,

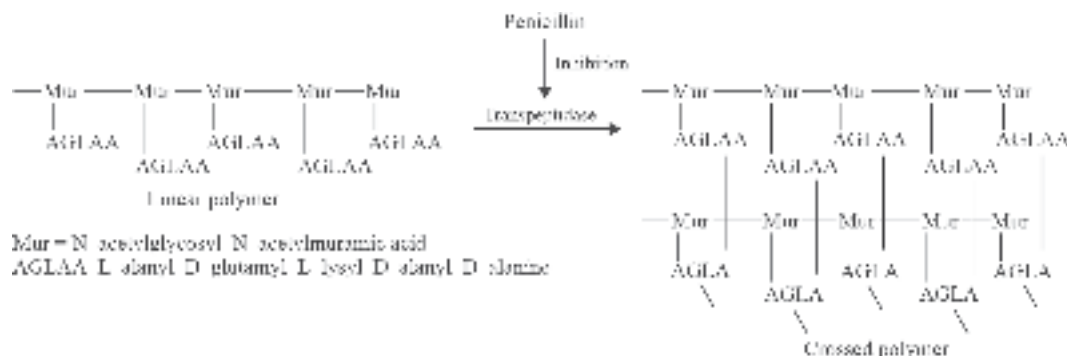


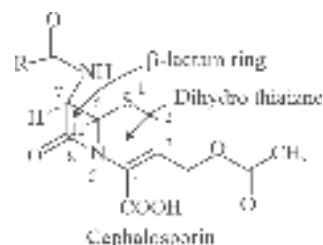
Fig. 14.4: Mechanism of action of β -lactam antibiotics

high internal turgor pressure of bacteria (about 20 atm for gram-positive and 5 for gram-negative types) causes a rupture of the bacterial cell wall and a bursting of the bacterial cytoplasm, with subsequent death of the microorganism. Therefore, these antibiotics kill only growing bacteria; they do not affect dormant or persisting forms. Since mammal cells have no cell walls, β -lactam antibiotics are highly specific to bacteria.

CEPHALOSPORINS

Structure and Properties

Cephalosporins are β -lactam antibiotics, with the same fundamental structural requirements as in penicillins. Cephalosporins have a six-membered dihydrothiazine ring instead of the five-member thiazolidine ring in penicillin, which causes the β -lactam ring to experience less stress than in penicillin. Because the β -lactam is under less stress, it becomes less of a substrate for protein binding. The fact that the cephalosporins are less susceptible for protein binding is compensated by the driving force from the reaction by the 3,4 double bond coupled with a good leaving group.



Cephalosporins contain two asymmetrical centers. Thus, four optically active forms are possible.

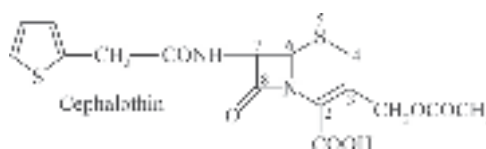
The fused rings are not coplanar but folded along the C-6, N-5 bond but less markedly than in penicillins. The amide bearing carbon atom (C-7) has the L configuration.

The physicochemical properties of cephalosporins are similar to those of penicillins. For instance, most cephalosporins are available as soluble salt or zwitterions. Those with an asymmetric carbon atom are stereospecific.

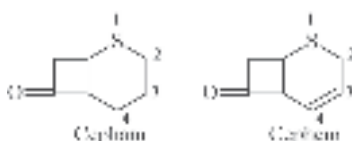
Nomenclature

Cephalosporins are named in the following ways:

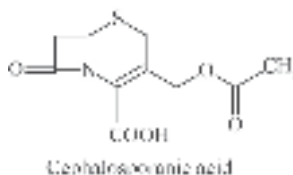
- (i) 5-Thia-1-azabicyclo [4,2,0] octanes in chemical abstract. Accordingly, cephalothin is 3-(acetoxymethyl)-8-oxo-7-[2-(2-thienyl) acetamido]-5-thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid.



- (ii) **Cephams derivatives:** Cephams is the name given to the unsubstituted bicyclic lactam. Thus, cephaloridine is 3-pyridinomethyl-7-(2-thiophene-2-acetamido)-3-cephem-4-carboxylate and cephalothin is 3-acetoxymethyl-7-(2-thiophene-2-acetamido)-3-cephem-4-carboxylate.



- (iii) **Cephalosporanic acid derivatives:** Cephalosporanic acid is the duly substituted cepham. According to this nomenclature, cephaloglycin is 7-(D- β -aminophenyl-acetamido) cephalosporanic acid:



However, this practice applies only to cephalosporins that have a 3-acetoxymethyl group.

Mechanism of Action of Cephalosporins

Cephalosporins are bactericidal and have the same mode of action as other β -lactam antibiotics (such as penicillins). Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity, especially in gram-positive organisms. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases, known as penicillin binding proteins (PBPs).

Therapeutic Uses and Adverse Reactions

Cephalosporins are used for the treatment of infections caused by most gram-positive cocci and many gram-negative bacteria, especially *Escherichia coli*, *Proteus mirabilis* and *Klebszella*. They are not effective in infections caused by pseudomonas, most of the species of proteus and enterobacter. Most cephalosporins are resistant to cephalosporinase, an enzyme that inactivates them by cleaving the β -lactam ring. The resistance is ascribed to the side chain at C-3.

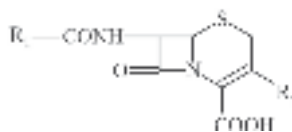
Adverse reactions include tubular necrosis, diarrhea, thrombophlebitis, and rarely anaphylaxis.

Semisynthetic Cephalosporins

Only cephalosporin C is found in nature, isolated from culture of fungi especially *Cephalosporium* species. In 1966 Woodward and associates reported the first total chemical synthesis of cephalosporins C, but their process is not found industrially feasible. The discovery of 7-aminocephalosporanic acid (7-ACA) (a product of hydrolysis of cephalosporin C) resulted in the introduction of several better cephalosporins (Table 14.2) which were obtained by molecular modification of the two side chains: 7-acylamide and 3-acetoxymethyl. Variation of the first side chain is performed by the same process used for the preparation of semi-synthetic penicillins but the method of choice is acetylation by acyl chlorides. The semi-synthetic cephalosporins are prepared to increase the acid stability, increase the absorption on oral administration, broaden the antimicrobial spectrum, etc.

Classification of Cephalosporins

Cephalosporins have classified as first, second or third generation, mainly on the basis of bacterial susceptibility pattern and resistance to β -lactamases. Commonly used cephalosporins are listed in Table 14.2.

Table 14.2: Examples of some Cephalosporins

Name	R_1	R_2

First generation cephalosporins: These are highly active against gram-positive bacteria and generally act as a substitute for penicillins that are resistant to the staphylococcal, penicillinase. They also have activity against *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae*.

Cephlothin		$-\text{CH}_2\text{OCOCH}_3$
Cephradine		$-\text{CH}_3$
Cephalexin		$-\text{CH}_3$
Cephradroxil		$-\text{CH}_3$
Cefazolin		$-\text{CH}_2-\text{S}-\text{C}_4\text{H}_4-\text{CH}_3$

Second generation cephalosporins: These display greater activity against three additional gram-negative organisms like *Hemophilus influenzae*, some *Enterobacter aerogenes* and *Neisseria* sp.

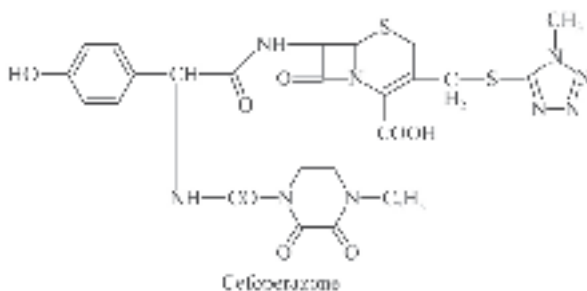
Cefaclor		$-\text{Cl}$
Cefoxitin		$-\text{CH}_2\text{OCONH}_2$ (OCH_3 at C-7 also)
Cefuroxime		$-\text{CH}_2\text{OCONH}_2$
Loracarbef		$-\text{Cl}$ (1-Sulphur is replaced also by CH_2)

Third generation cephalosporins: These cephalosporins are inferior to first generation cephalosporins with respect to their activity against gram-positive cocci but possess enhanced activity against gram-negative bacilli, including above and *Serratia marcescens*

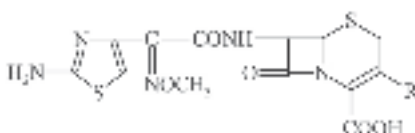
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Name	R_1	R_2

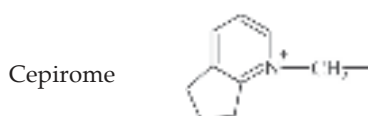
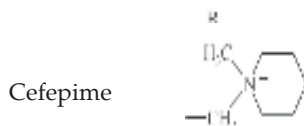
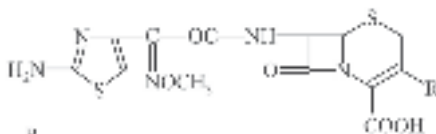
(i) containing different acyl residues.



(ii) containing 2-Aminothiazolyloximino moiety.

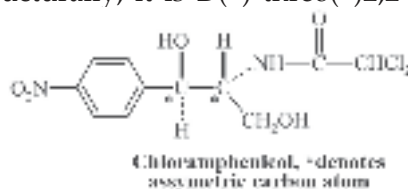


Fourth generation cephalosporins: Cefipime is the most clinically useful fourth generation cephalosporins and must be administered parenterally. It has wide spectrum being active against streptococci and staphylococci. It is also effective against gram-negative organism such as *Enterobacter*, *Escherichia coli*, *Klebsiella* and *Pseudomonas aeruginosa*.



CHLORAMPHENICOL

Chloramphenicol is a broad spectrum antibiotic. Structurally, it is D(-) threo(-)2,2-dichloro-N-[β -hydroxy- α -(hydroxymethyl)p-nitrophenyl]acetamide. It is first isolated from *Streptomyces venezuelae*, a microorganism that was found by Paul Burkholder in a sample of soil. This source is not in use nowadays. Since 1949, chloramphenicol has been obtained by total synthesis, which is less expensive than extraction from fermentation molds.



Properties

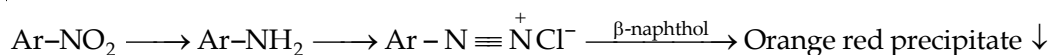
It is available as yellowish white colored needle like crystals, insoluble in water but highly soluble in lipids. It has very bitter taste. In order to mask its bitter taste and improve its physicochemical properties, several esters of chloramphenicol have been prepared and marketed. These esters are inactive *in vitro* but *in vivo*, they release the parent compound. Therefore, they are latent forms of Chloramphenicol. Some esters are insoluble in water but are devoid of the bitter taste of parent compound. Hence, they are indicated for pediatric use, e.g. palmitate, stearate, cinnamate. Other esters are soluble, suitable for parenteral administration, e.g. glycinate hydrochloride, sodium succinate, arginine succinate.

Mechanism of Action of Chloramphenicol

Chloramphenicol acts by inhibiting the protein synthesis at its initial phase.

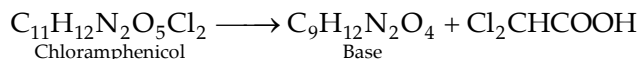
Structural Elucidation

- (i) Its molecular formula is found to be $C_{11}H_{12}Cl_2N_2O_5$.
- (ii) **Presence of nitro group:** Chloramphenicol on reduction with tin and HCl, followed by diazotisation and then coupling with β -naphthol gives orange-red colored precipitate. This indicates the presence of nitro group.

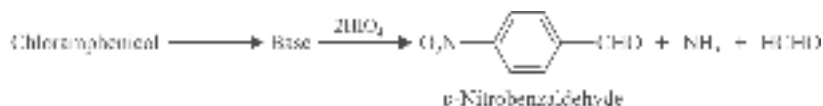


It is again confirmed by the UV spectrum, found similar to nitrobenzene.

- (iii) The UV spectrum of reduced chloramphenicol is found similar to p-toluidine. The solution responds to chlorine test. This indicates the presence of ionic chlorine. The overall reaction indicates:
 - (a) Chloramphenicol is p-nitrobenzene derivative.
 - (b) Chlorine atom is present in the side chain.
- (iv) Chloramphenicol contains neither free amino nor carbonyl group.
- (v) Chloramphenicol shows the presence of two hydroxyl groups as it forms diacetyl derivation on acetylation with acetic anhydride and pyridine.
- (vi) On hydrolysis with acid or alkalis, it produces dichloroacetic acid and an optically active base, $C_9H_{12}N_2O_4$.

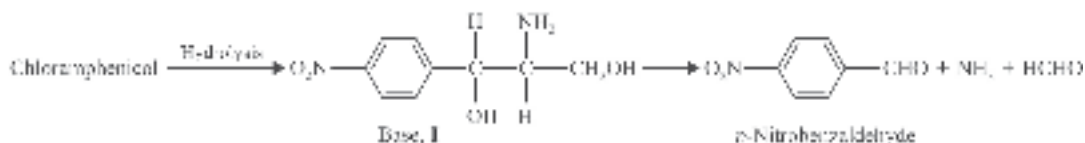


- (vii) (a) The base, $C_9H_{12}N_2O_4$ is found to possess one primary amino group.
- (b) On acetylation, it produces triacetyl derivative. It confirms the presence of two hydroxyl groups and one amino group in the base.
- (c) On oxidation with periodic acid, there is formation of ammonia, formaldehyde and p-nitrobenzaldehyde. In this reduction, two moles of periodic acid are consumed.

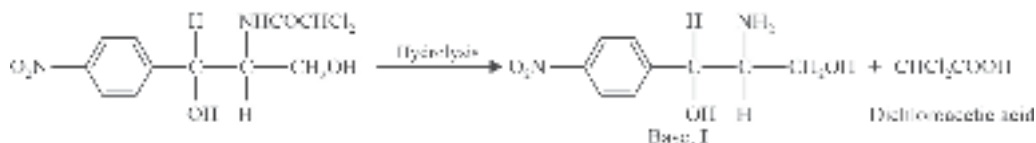


This reaction suggests that propyl group is in *para* position with respect to nitro group and amino group at C-2 position in propyl chain.

(viii) On the basis of above reactions, the structure, **I** is assigned to the base as 2-amino-1-*p*-nitrophenylpropane-1,3-diol.

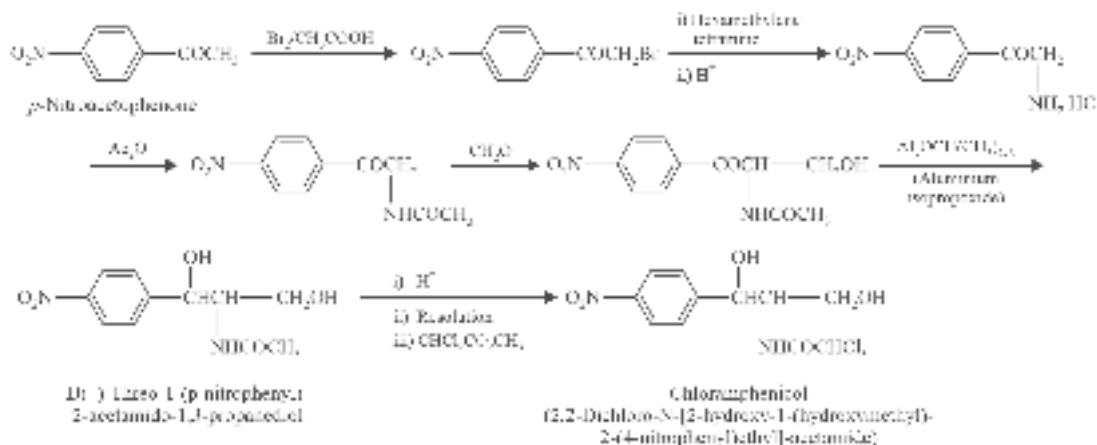


(ix) Chloramphenicol as such do not react with periodic acid, indicating the absence of free amino group, probably blocked by dichloroacetyl group. Hence, the structure is assigned to chloramphenicol as D(-) threo-2-dichloroacetamido-1-*p*-nitrophenylpropan-1,3-diol which explains the formation of base.



(x) Finally, the structure is confirmed by the synthesis.

Synthesis of Chloramphenicol



Spectral Data

IR (cm⁻¹): 3401, 3352(s), 3356(s) (OH stretching), 2927, 2910 (Aromatic C–H stretching), 1732 (C = stretch amide II), 1686 (Amide I), 1561 (N–O stretch, ArNO₂), 1071 (C–O stretch, Primary alcohol), 876 (C–N stretch).

¹H-NMR (δ) (CO(CD₃)₂): δ 3.76 (m, 2H), 4.17 (m, 1H), 4.30 (m, 1H), 5.25 (s, 1H), 5.33 (s, 1H), 6.35 (s, 1H), 7.55 (m, 1H), 7.70 (d, 2H, J = 7.6 Hz), 8.18 (d, 2H, J = 7.6 Hz).

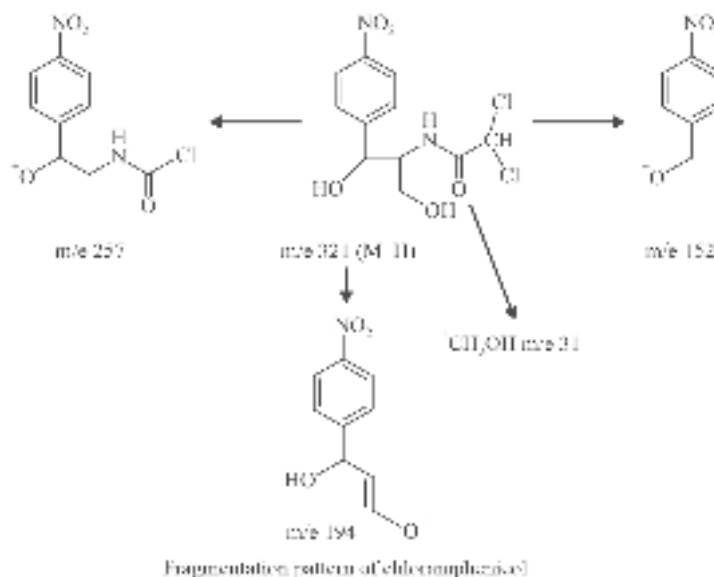
¹³C-NMR (75 MHz, CO(CD₃)₂): 52.8, 57.1, 62.3, 66.0, 118.5, 122.8, 142.7, 159.2.

MS (m/e): 322(M⁺), 257, 194, 170, 152, 31.

Therapeutic Uses and Adverse Reactions

Chloramphenicol is the most effective drug for treating acute typhoid fever and several other serious infections produced by *Salmonellae*. It is also effective against many strains of gram-positive and gram-negative bacteria, *Rickettsia* and psittacosis-lymphogranuloma group of viruses. However, owing to its serious adverse effects blood dyscrasia, including

aplastic anemia with pancytopenia-chloramphenicol should be reserved for those infections not susceptible to less dangerous drugs. It is not recommended for both premature and full term infants during the first two weeks of life, because it can cause gray-baby syndrome which may be fatal.



Tetracyclines

The tetracycline antibiotics (Table 14.3) are characterized by common octahydronaphthacene nucleus, a system formed by a four fused rings. There is highly functionalized A-ring that is cis-fused to the B-ring, a C-ring containing a tetrasubstituted stereogenic center and a D ring that is aromatic. Tetracyclines are known for their broader spectrum of action (Fig. 14.5). The first two members were isolated from *Streptomyces aureofaciens* (chlortetracycline) and *S. rimosus* (oxytetracycline). Tetracycline, the prototype of this class of antibiotics was first obtained by hydrogenolysis of 7-chlorotetracycline. In 1957, the 6-demethyltetracycline family was described by semi-synthetic procedures. In 1970, quelocardine, a new antibiotic of the tetracycline family, was explored.

There are five chiral centers (4, 4a, 5a, 6, 12a) are present in tetracycline. Thus, $2^5 = 32$ stereoisomers are possible for the compound.

Properties

Tetracyclines are amphoteric compounds, and many form water soluble salts with strong acids and strong bases. Acids salts, generally stable are formed through protonation of the dimethylamino group on C-4 position. Basic salts are formed with sodium or potassium hydroxides; these salts are generally unstable. The common chromophoric system imparts to them a yellow color. In each tetracycline, three ionizable groups can be distinguished with the approximate following pKa's 3.5, 7.7 and 9.5.

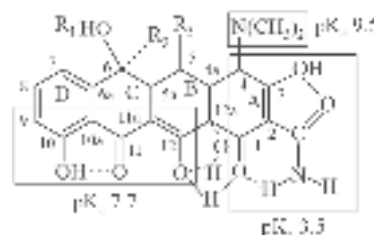
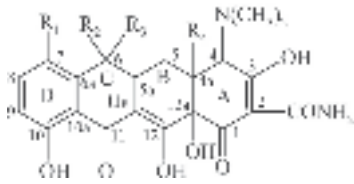


Fig. 14.5. Absolute configuration of tetracyclines showing pKa values and groups involved in hydrogen bonding properties

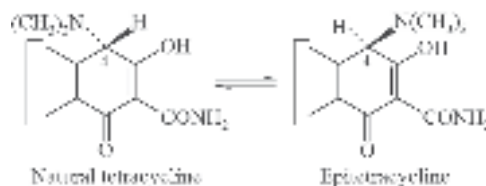
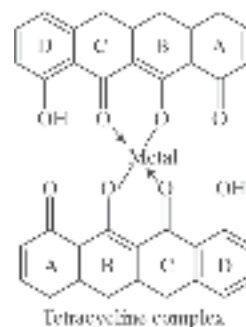
Table 14.3: Tetracyclines


Name	R ₁	R ₂	R ₃	R ₄
Chlortetracycline	Cl	CH ₃	OH	H
Oxytetracycline	H	CH ₃	OH	OH
Tetracycline	H	CH ₃	OH	H
Demeclocycline	Cl	H	OH	H
Doxycycline	H	CH ₃	H	OH
Minocycline	(CH ₃) ₂ N	H	H	H
Rolitetracycline*	H	CH ₃	OH	H

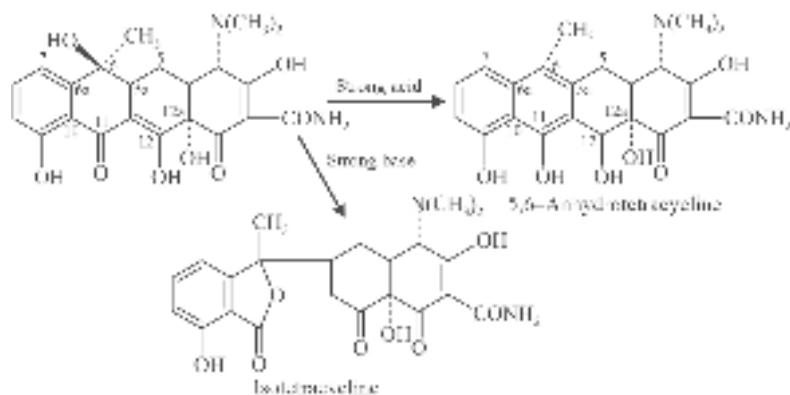
* Also pyrodinylmethyl group substitute one hydrogen of 2-amide group (see structure)

Tetracycline possesses a great tendency to form complexes with a number of chemical species, due to its B- and C-ring oxygen atoms.

It forms complexes most readily with Fe³⁺, Fe²⁺, Cu²⁺, Ni²⁺, Co²⁺, Zn²⁺, Mn²⁺, Mg²⁺, Ca²⁺, Be²⁺, Al³⁺ among metal ions. Therefore, for better absorption, they should not be administered with milk or milk products or antacids or other agents containing these salts. In solution of intermediate pH (2–6) tetracyclines undergo epimerization at carbon atom 4 and reach equilibrium when approximately equal amounts of isomers are formed. Epitetracyclines are much less active than the natural isomers. This fact explains the decrease of potency of aged solution.



Strong acids and strong bases inactivate tetracycline's having 6-hydroxyl group by forming **anhydrotetracyclines** and **isotetracyclines**, respectively. Strong acid causes dehydration through 6-hydroxy group and 5a-hydrogen atom. The double bond thus formed between C-5a and C-6 position induces a shift in the position to a C-11 and C-11a forming the energetically stable resonant system in inactive anhydrotetracyclines. Bases form the lactone ring through reaction of C-6 hydroxyl group and ketone group, present at C-11 position found in inactive tetracycline. Efforts to overcome this inactivation process resulted in the development of 6-deoxytetracyclines, which are more stable and more prolonged action. Examples are methacycline, doxycycline, sancycline.



Classification of Tetracyclines

Tetracyclines can be grouped into three classes:

- (i) Natural tetracycline's: chlorotetracycline, oxytetracycline, tetracycline, demeclocycline.
- (ii) Semisynthetic tetracycline with unchanged carboxamide group: doxycycline: methacycline, minocycline, sancycline.
- (iii) Latent form of tetracycline. This latentiation was accomplished in several ways: (a) by a Manich reaction at amide function: apicycline, clomycine, rolitetracycline; these salts are highly water soluble and suitable for both parenteral and oral administration, (b) by formation of salt: tetracycline lactate, tetracycline lauryl sulphate, tetracycline phosphate complex, which are insoluble in water, (c) by molecular association: etamocycline (addition of two molecules of tetracycline through an ethylenediamine bridge) and penimocycline (hybridization of tetracycline with methampicillin).

Mechanism of Action of Tetracyclines

Tetracycline inhibits cell growth by inhibiting translation. It binds to the 16S part of the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome. The binding is reversible in nature.

Therapeutic Uses

Tetracyclines are broad spectrum bacteriostatic agents. They are effective in treatment of infections by many species of gram-positive and gram-negative bacteria, spirochetes and some larger viruses.

Tetracyclines are also used for nontherapeutic purposes (a) promotion of growth rate of farmstock, (b) conservation of food; (c) microbiological control of fermentation

Adverse Reactions

At usual dosages tetracyclines are relatively safe drugs. Nausea, vomiting, anorexia, flatulence, pyrosis, and diarrhea are the most common side effects. Other minor reactions (glossitis enterocolitis, stomatitis) result from suppression of normal flora with overgrowth of other microorganism. Hypersensitivity and photosensitivity reactions are common. Superinfection may occur occasionally, especially moniliasis, caused by *Candida albicans*; this complication is inhibited by simultaneous administration of antifungal agents, such as nystatin or amphotericin B. A serious effect is the permanent staining of

teeth of children during calcification caused by deposition of the tetracyclines taken by young children or pregnant mothers, since these drugs readily cross the placenta and localize metallic calcium containing structure by forming a chelate with calcium phosphate.

Prolonged treatment with tetracyclines can give rise to resistant strains of microorganisms. Cross resistance among these antibiotics is also possible.

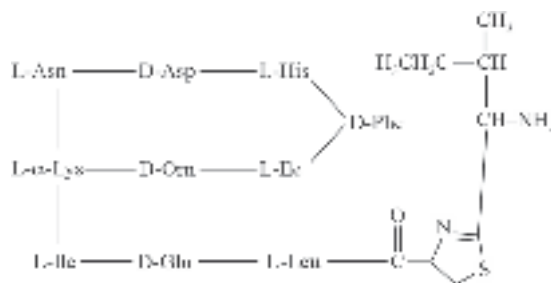
Polypeptide Antibiotics

These antibiotics have complex polypeptide structure, in which the peptide chain is often cyclic or branched. They are mainly isolated from the genera *Bacillus* and *Streptomyces* spp. These are resistant to animal and plant proteases. These contain lipid moieties besides amino acids that are not found in peptides of animal and plant origins. Although, there are so many polypeptide antibiotics but only few of them are in clinical use due to their nephrotoxicity. Polypeptide antibiotics can be divided according to several criteria. Based on their components, these may be either homomeric (if comprised only of amino acids) or heteromeric peptides (if constituted of amino acids plus other moieties). Based on ring components these may be divided into homodietic (only peptide linkage in the ring) and heterodietic (ring also contains other linkages). Thus, bacitracin is an example of homomeric homodietic polypeptide antibiotic, colistin is an example of heteromeric homodietic antibiotic and actinomycin is an example of heteromeric heterodietic antibiotic.

Polypeptide antibiotics act by disrupting the bacterial walls.

Some of the examples of polypeptide antibiotics like bacitracin, polymyxin, colistin are discussed here.

A. Bacitracin

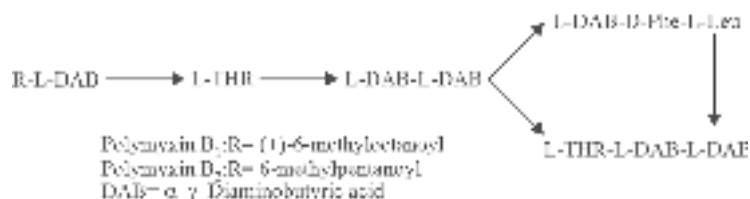


Chemical structure of bacitracin A

Bacitracin is a polypeptide antibiotic that inhibits cell wall synthesis and is active against gram-positive bacteria.

Properties and uses: Bacitracin is a white hygroscopic powder, soluble in water and alcohol. Bacitracin antibiotic is isolated from the fermentation broth of a culture of tracyl-1 strain of *Bacillus subtilis*. It is found to be a complex mixture of at least 10 polypeptides (A, A₁, B, C, D, E, F₁, F₂, F₃ and G), of which bacitracin A fraction is believed to be the most abundant and the most potent. A divalent ion Zn⁺⁺ enhances its activity. Although, bacitracin is occasionally employed for topical application (often in combination with neomycin, polymyxin, and tyrothricin) for the treatment of burns, ulcer, and wounds, it can cause serious necrosis of the kidney tubules; if it is given systematically (i.e. IV route) an oral administration is not feasible due to its lack of absorption from the GI tract. A variety of gram-positive *cocci* and *bacilli* are sensitive to bacitracin. It should be stored in airtight containers due to its hygroscopic nature.

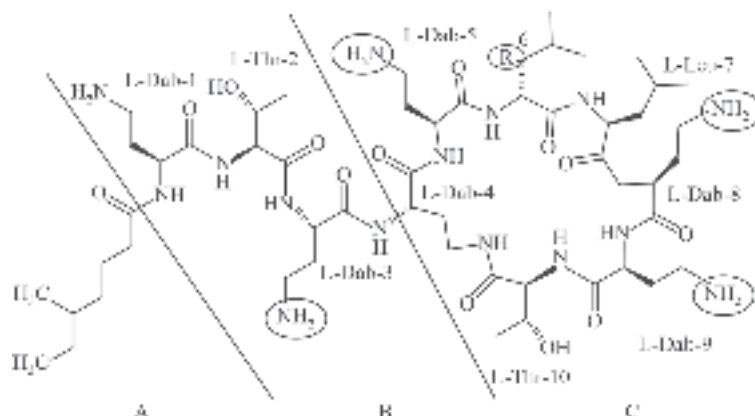
B. Polymyxin



Properties and uses: Polmyxin sulphate is a white hygroscopic powder, soluble in water, and slightly soluble in ethanol. The polymyxins are cyclic peptides holding a fatty acid side chain. This is a group of relatively simple basic, cationic, detergent peptides that are produced by *Bacillus polymyxa*. At least, five polymyxins (A, B, C, D, and E) are known, but only polymyxin B and polymyxin E are of clinical utility. Both polymyxin B and polymyxin E (colistin) are mixtures of two components and is used in the treatment of bacterial meningitis, urinary tract infection, burns, wounds, and gastroenteritis. Polymyxin may affect renal tubules and central nervous system, and because of their nephrotoxicity associated with their systemic use, they are primarily employed to treat topical infections.

C. Colistin

Colistin (polymyxin E) is a polymyxin antibiotic produced by *Paenibacillus polymyxa* var *colistinus* consisting of a cyclic heptapeptide ring with three positively charged amine groups, a tail tripeptide moiety with two positively charged amine groups, and a hydrophobic acyl chain tail. Colistin is an amphipathic molecule, with hydrophobicity mainly attributable to the fatty acyl moiety and hydrophilicity due to the five L-diaminobutyric acid (L-Dab) amino groups. The L-Dab molecules are positively charged in positions 1, 3, 5, 8, and 9.



Chemical structure of colistin is composed of three parts: (A) hydrophobic acyl tail (6-methylheptanoic acid), (B) linear tripeptide segment (C) hydrophilic, heptapeptide ring. Arabic numeral indicates the position of amino acids on the structure and the reactive amino groups are encircled. R^6 : D-phenylalanine in polymyxin B or D-leucine in polymyxin E (colistin)

Colistin (polymyxin E and polymyxin B) are cationic polypeptide antibiotics that induces changes in the permeability of cell wall by binding to the anionic lipopolysaccharide

molecules and displacing calcium and magnesium and thus, causing leakage of essential constituents and bacterial cell death.

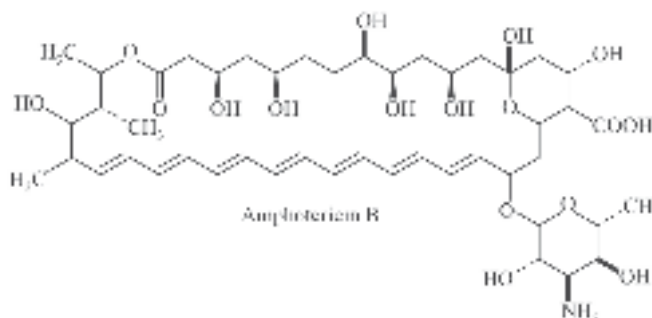
POLYENE ANTIBIOTICS

Polyene antibiotics are produced by various strains of *Streptomyces*. They are characterized by a large ring containing a lactone function and a sequence of conjugated double bonds, which is their chromophoric part. Owing to their macrolide structure, polyenes could be studied as a subclass of macrolide antibiotics and not as separate class. All are poorly water soluble, and in the presence of light, many undergoes autooxidation. They occur as pale yellow, crystalline compounds or powders. These may be acidic, basic and nonionic.

According to number of double bonds present in the conjugated system, polyenes are classified as tri, tetra, penta, hexa and heptenes.

Polyene antibiotics have no antibacterial or antirickettsial activity, but they are active against molds and yeasts.

Amphotericin B (Fungizone): It is a naturally occurring polyene macrolide antibiotic, produced by *Streptomyces nodosus*. In spite of its toxic potential, amphotericin B is the drug of choice used in the treatment of systemic mycoses.



For antifungal action several polyene (amphotericin) molecules bind to ergosterol present in cell membranes of sensitive fungal cell; disrupt the membrane function: allowing electrolytes (particularly potassium and small molecules) to leak from cell which result in death of fungal cell.

MACROLIDE ANTIBIOTICS

Macrolide antibiotics are produced by *Streptomyces* species. They are characterized by five common features.

- (i) A macrocyclic lactone, usually having 12–17 atoms, hence the name macrolide.
- (ii) A ketone group.
- (iii) One or two amino sugars glycosidically linked to the nucleus.
- (iv) A neutral sugar linked to amino sugar or nucleus.
- (v) Presence of the dimethylamino moiety on the sugar residue, which explains the basicity of these antibiotics and affords the possibility of preparing their salts.

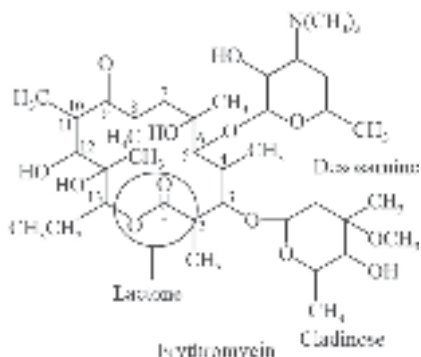
The pure macrolide antibiotics are colorless and usually crystalline. In neutral solution, they are stable, but in acidic media, they are hydrolyzed at the glycosidic bonds and in basic media, the lactone bonds are saponified. Erythromycin, oleandomycin, azithromycin, clarithromycin, etc. are common examples of this group.

Mechanism of Action of Macrolide Antibiotics

The macrolide antibiotics inhibit the protein synthesis by binding reversibly to the subunit 50s of the bacterial ribosome. This action is bacteriostatic.

Examples of Macrolide antibiotics

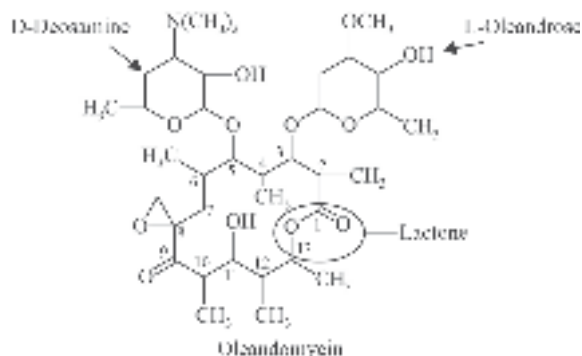
Erythromycin (Erythrocin): In structure, this macrocyclic compound contains a 14-membered lactone ring with ten symmetric centers and two sugars (L-cladinose and O-desosamine), making it a compound very difficult to produce via synthetic methods.



Erythromycin is produced from a strain of the actinomycete, *Saccharopolyspora erythraea*.

It is a very bitter or yellowish-white, sparingly water soluble crystalline powder.

It is used both as free base and in the form of salts actobionate, stearate or esters (esolate, ethyl carbonate, and ethyl succinate). The lactobionate salt is water soluble and administered parenterally. The stearate salt is insoluble in water and tasteless. It is used in tablets and suspension dosage form. Erythromycin is active against most of gram-positive bacteria and some of gram-negative organism. It is an alternative to penicillin, when high drug concentration is required. Its main use is in the treatment of group β -hemolytic streptococcal, staphylococcal, and pneumococcal infections. Adverse serious reactions occur rarely, but the esolate salt can produce jaundice. The usual dosage is calculated on the basis of the free erythromycin, 250 mg daily.



Oleandomycin: It is a natural 14-membered-ring macrolide produced by *Streptomyces antibioticus*. It is stable in acid conditions. It is less active than erythromycin. It contains two sugars attached to the lactonic aglycon, 1-oleandrose and d-desasamine. It is used as the phosphate, which is a white, water soluble, crystalline powder. Oleandomycin targets and reversibly binds to 50s subunit of bacterial ribosomes to prevent the protein

synthesis. Routes of administration are intravenous, intramuscular, and oral. Its main use is in treatment of infections like infections of respiratory tract, skin, soft tissue, etc. refractory to other antibiotics.

AMINOGLYCOSIDE ANTIBIOTICS

They are named aminoglycoside due to presence of amino sugar. All these consist of amino sugar in their structure linked glycosidically. All have at least one amino hexose and some have pentose sugar lacking an amino group (e.g. streptomycin, neomycin, paromomycin). Additionally, each of this possess either highly substituted 1,3-diaminocyclohexane ring (kanamycin, neomycin, gentamicin) or streptidine (streptomycin) or deoxystreptamine (e.g. tobramycin). The aminoglycosides are strongly basic, water-soluble and form crystalline salts. They are used as salts, especially the sulphate.

These antibiotics are active against *Escherichia coli* and most species of *Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella* and *Proteus*. They have no effect on fungi or viruses. Cross resistance is developed among all members of this class of antibiotics. A serious adverse reaction is permanent damage to cochlear and vestibular parts of the eighth cranial nerve. Most of these antibiotics are also used as local or topical antibacterial agents.

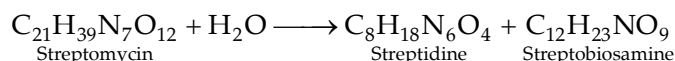
The most widely used antibiotics of this class include streptomycin, neomycin, paromomycin, gentamicin, kanamycin and tobramycin. Those which are derived from *Streptomyces* species are named with the suffix *mycin*, while those which are derived from *Micromonospora* are named with the suffix *micin*.

Streptomycin: It is isolated from *Streptomyces griseus*. The drug is effective against a broad spectrum of gram-negative and gram-positive organisms and tubercle bacillus. Chemically, it is composed of three units—N-methylglucosamine, streptose and streptidine. Later contains two guaindo moieties. It is triacidic base due to presence of two guainido and methyl amino groups. Acid hydrolysis yields streptidine and streptobiosamine (the compound that is combination of L-streptose and N-methyl-L-glucosamine). It is poorly absorbed when administered orally; 98% is eliminated unchanged in the feces. Due to its poor absorption in the gut, it is only given by injection. Because of incidence of nausea, dizziness, ototoxicity, rashes, fever, nephrotoxicity, pain at the site of injection and rapid development of bacterial resistance, streptomycin is not in common use. Its principal use is in the treatment of tuberculosis. Streptomycin is also occasionally used to treat brucellosis in combination with another antibiotic.

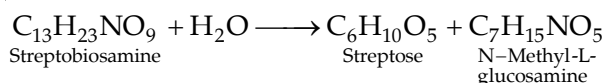
Chemistry

Streptomycin belongs to the glucosides in which a diguanido-group is linked to a nitrogen-containing disaccharide-like compound. It is a strong base, with three basic functional groups.

Upon hydrolysis, it splits into two compounds:

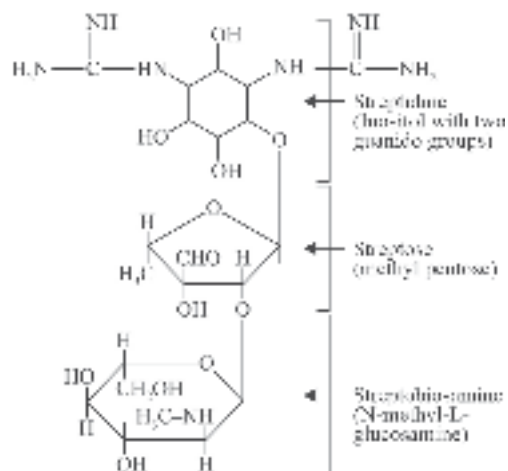


On further hydrolysis with strong mineral acids, the streptobiosamine gives a 6-carbon atoms sugar and glucosamine:

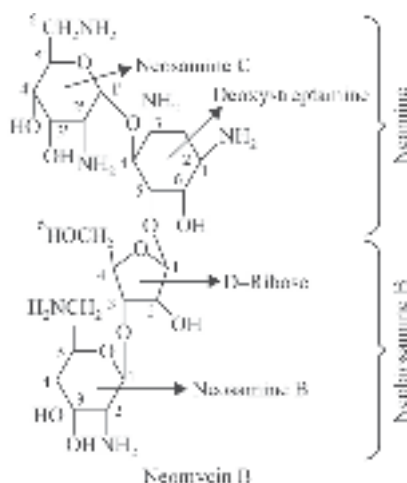


The basic nitrogen atom in the streptobiosamine is not present as a primary amino group.

The structure of streptomycin molecule is usually presented as follows:



Neomycin (Neobiotic): Neomycin is first isolated in 1949 by the American microbiologist **Selman Waksman** from a strain of the bacterial species *Streptomyces fradiae*. Neomycin is mainly used topically in the treatment of skin and mucous membrane infections, wounds, and burns. Although it is also used systemically, but highly toxic. Neomycin consists of a mixture of neomycin A, B and C. Neomycin A is a degradation product of neomycin B and C, and has no antimicrobial activity. Neomycin B differs from neomycin C by the nature of the sugar attached terminally to ribose. The sugar present in neomycin B and neomycin C are called neosamine B and neosamine C respectively. Both types of sugar differ in stereochemical features. The neosamine B contains α -L-idopyranosyl, while in neosamine C, the configuration is inverted and it is β -D-glucopyranosyl. Neomycin is effective in vitro against gram-positive and gram-negative organisms, in concentrations of 5–10 $\mu\text{g/mL}$ or less. However, it should be noted that certain strains of Streptococci, Pneumococci, *Pseudomonas*, clostridia, *Staphylococcus aureus*, and *Aerobacter aerogenes* have moderate to marked resistance to neomycin. Again, it is not active against viruses and fungi.



Neomycin is used to treat or prevent the bacterial infections of the intestine (which often cause diarrhea). Because this drug is not absorbed into the circulation, its effects are confined to the bacteria in the intestine.

Used topically in creams and ointments, usually in combination with other antibiotics, to treat or prevent bacterial infections of wounds, as well as infections of the skin, eyes and ears. Neomycin is sometimes used as oral treatment for certain diseases such as liver disease.

Patients receiving oral neomycin are reported to experience both oto- and nephrotoxic effects. The most serious adverse effect from neomycin is kidney damage.

Replacement of CH_2NH_2 structure with CH_2OH in neomycin B and C yields **paromomycin I** and **II** respectively. Paromomycin is similar to neomycin and streptomycin in all respects. In addition, however, it also displays activity against *Entamoeba histolytica*.

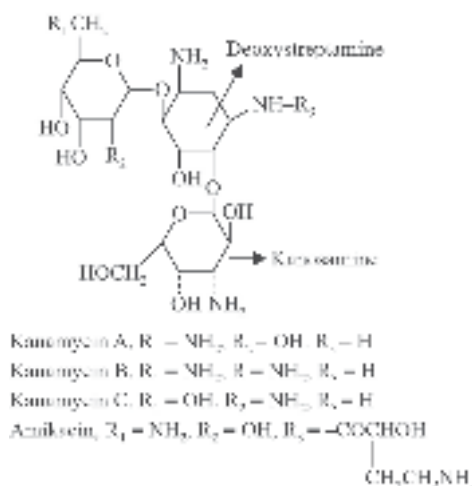
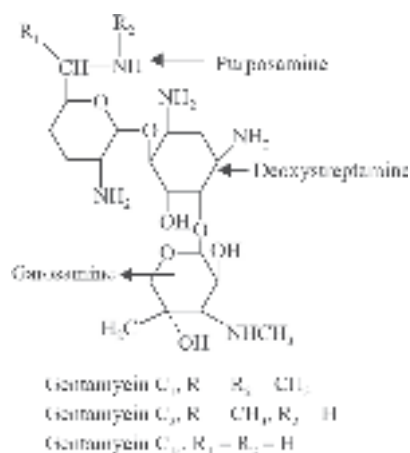
Gentamycin sulphate (Garamycin): It is obtained commercially from *Micromonospora purpurea*. It is a mixture of three different: gentamicins, C_1 , C_2 and C_{1a} , which occur as a water-soluble, white to buff-colored substance. All of these contain sugar-garosamine. Gentamicin C_1 contains 2N- CH_3 groups:

1-secondary C- CH_3 and 1-tertiary C- CH_3 groups; Gentamicin C_2 has 1 less N- CH_3 group while Gentamicin C_{1a} differs from C_2 by the absence of C- CH_3 groups. Its main systemic use is for the treatment of serious infections by gram-negative organisms. By injection, the usual dosage is 5 mg/kg of body weight daily. Topical use is not advisable. Gentamicin kills bacteria by preventing them from making proteins.

Kanamycin (Kantrex): It is obtained from *Streptomyces kanamyceticus*. Natural kanamycin is a mixture of three closely related structures: kanamycin A (major component), B and C (minor components). Commercially available kanamycin contains almost kanamycin A, which is least toxic.

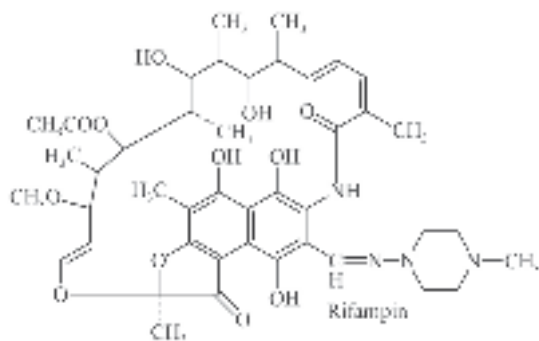
These kanamycins only differ from each other in the sugar moieties attached to the glycosidic oxygen atom on the C-4 position of the central deoxystreptamine. It is basic but used as sulphate, which is very water soluble and stable to heat and chemicals. It is used as an anti-tubercle's, antibacterial and anti-infective agent.

Amikacin (Amikin): It is a semi synthetic aminoglycoside, prepared from acylation of amino group in Kanamycin A by 4-amino-2-hydroxybutyryl chloride. It retains 50% activity of kanamycin A against sensitive strains of gram-negative bacilli. The L-isomer is more active. Only advantageous feature is its resistance toward most inactivating enzymes and, therefore, it is effective against the organism resistant to other aminoglycosides.



ANSAMYCINS

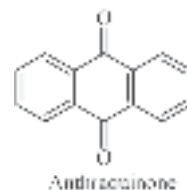
Ansamycins are marolide antibiotics containing an aliphatic ansa bridge, a bridge connecting two nonadjacent positions of an aromatic nucleus. This class includes geldanamycins, rifamycins, streptovaricins and maitansine. These antibiotics, which are notably active against gram-positive bacteria are produced by different strains of *Streptomyces* species.



Rifamycins have broad spectrum chemotherapeutic activity. Rifampin, which is a rifamycin, is already marketed for the treatment of tuberculosis. It contains a macrocyclic ring bridged across two non-adjacent (ansa) position of aromatic nucleus. Streptovaricins also have anti-tubercle activity, but they are too toxic. Streptovaricins are active against gram-negative bacteria. Geldanamycins are active against protozoa. Miatansine has shown anti-neoplastic activity.

ANTHRACYCLINES

Anthracyclines are antibiotics, characterized by presence of anthraquinone nucleus. Most of antibiotics of this group have anti-neoplastic activity, e.g. adriamycin, daunorubicin, etc.

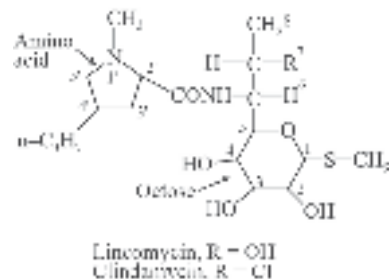


LINCOMYCIN

The lincomycin group of antibiotics includes clindamycin, lincomycin, etc. These are clinically used against the common gram-positive pathogens. Some halogenated lincomycins have shown activity in experimental malaria. This class of antibiotics is characterized by a 4-alkyl substituted, hygric acid, bound to an alkyl 6-ammo- α -thio-octapyranoside by an amide linkage.

Examples of Lincomycins

Lincomycin: It is an antibiotic that comes from the bacteria *Streptomyces lincolnensis*. It is a derivative of an amino acid and a sulphur-containing octose. It is used as hydrochloride monohydrate: water soluble, crystalline and stable solid in the dry state. It has been structurally modified to its more commonly known 7-chloro-7-deoxy derivative, clindamycin. Although similar in structure, antibacterial spectrum. and in mechanism of action to macrolides, they are also effective against other species as well, i.e. actinomycetes, **Mycoplasma**, and some species of *Plasmodium*. However, because of its adverse effects and toxicity, it is rarely used today and reserved for patients, who are either allergic to penicillin or where bacteria has developed resistance.



Clindamycin: It is available as phosphate hydrochloride hydrate and palmitate. Clindamycin is a derivative of lincomycin, being obtained by substituting the 7-hydroxy group by chlorine atom. Chlorination results in an increases in lipophilicity yielding

better absorption and penetration into the bacterial cell, thus broadening the structure of activity to include some protozoa. It has better intestinal absorbability, greater potency, and lower adverse effects than the parent antibiotic but the same indications. It is used to treat serious infections, caused by bacteria.

NUCLEOSIDE ANTIBIOTICS

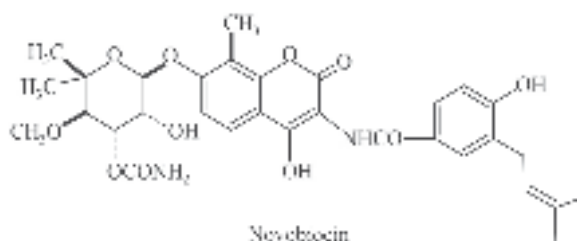
Cordycepin, nebularine, nucleocidin, puromycin, etc. are example of nucleoside antibiotics. Most of them are produced by different strain of *Streptomyces* species. Their chemotherapeutic activities vary. Some of them are broad spectrum whereas others have shown activity against tumor cells. However, none of this class of antibiotics is clinically used.

GLUTARIMIDE ANTIBIOTICS

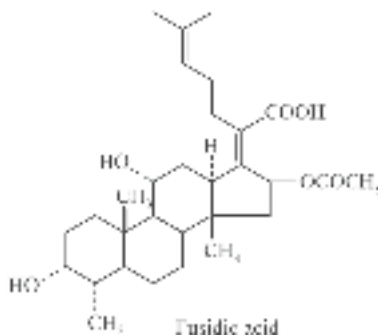
Glutarimide antibiotics are characterized by a common β -(2-hydroxyethyl)-glutarimide moiety, attached to a cyclic or acyclic ketone. They are produced by *Streptomyces* species. These have antifungal activity. The prototype of this class is cycloheximide (Actidione), used as an antifungal agent. Other important members of this class include acetoxycycloheximide, actiphenol, mycophenolic acid, niromycin, protomycin, etc.

MISCELLANEOUS ANTIBIOTICS

In this class, we can include other antibiotics that have useful action. Some are already being used as chemotherapeutic agents, especially against fungi and tumors. For example, novobiocin is a coumarin antibiotic act by inhibiting the DNA gyrase. Its calcium or sodium salt is used as antibacterial agent but due to the high incidence of adverse reactions it is not an antibiotic of choice.



Fusaric acid is a steroidal antibiotic isolated from *Fusidium coccineum*. It is highly potent against staphylococci, **Corynebacterium**, and **Clostridium** species.



It is often used topically in creams, or ointments and eye drops but may also be given systemically as tablets or injections.

SHORT AND LONG QUESTIONS

1. Define and classify antibiotics.
2. What are beta-lactam antibiotics? Elucidate the structure of benzylpenicillin.
3. Write a note of peptide antibiotics.
4. What are aminoglycoside antibiotics? Discuss the chemistry of two of them

MULTIPLE CHOICE QUESTIONS

1. The no of chiral carbon present in beta lactam antibiotics is:
 - a. 1
 - b. 2
 - c. 3
 - d. 4
2. Which one of the following is a peptide antibiotic?
 - a. Chloramphenicol
 - b. Bacitracin
 - c. Griesofulvin
 - d. Nystatin
3. Tetracyclines are derivatives of:
 - a. Hexahydro naphthalene
 - b. Octa hydro naphthacene
 - c. Octa tetrahydro naphthalene
 - d. Phenanthrene
4. Chemical name of Penicillin V is:
 - a. Phenyl penicillin
 - b. Benzyl penicillin
 - c. Phenoxoy penicillin
 - d. Phenoxymethyl penicillin
5. Which of the following is a second generation cephalosporin?
 - a. Cefaclor
 - b. Ceftazidime
 - c. Cephalexin
 - d. Cefotaxime
 - e. Cephalothin
6. Which of the following is not an appropriate target for antifungal drugs?
 - a. ergosterol
 - b. chitin
 - c. cholesterol
 - d. $\beta(1\rightarrow3)$ -glucan
7. Streptomycin is obtained from:
 - a. Streptomyces griseus
 - b. Streptomyces aureofaciens
 - c. Streptomyces venezulae
 - d. Streptomyces ramosus
8. Which of the following has the steroidal nucleus?
 - a. Fusidic acid
 - b. Amphotericin
 - c. Novobiocin
 - d. Oleandomycin

Answers

1. (c) 2. (b) 3. (b) 4. (d) 5. (a) 6. (c) 7. (a) 8. (a)

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Anees Ahmad Siddiqui MPharm, PhD is currently Professor, BM College of Pharmacy, Gurugram, Haryana. He is former Professor and Head, Department of Pharmaceutical Chemistry, School of Pharmaceutical Education and Research (SPER), Jamia Hamdard, New Delhi. He has more than 44 years of experience in teaching and research at undergraduate and postgraduate levels. He has attended several national and international conferences in India and abroad. He has to his credit several research publications and life membership of many national pharmaceutical associations. He has guided many MPharm and PhD students and is the author of several books in the field of pharmaceutical sciences: *Experimental Pharmaceutical Chemistry*, *Pharmaceutical Analysis* (Vols I and II), *Practical Pharmaceutical Chemistry*, *Selected Experiments of Pharmaceutical Chemistry: Laboratory Manual*, *A Precise Chemistry of Natural Products and Heterocyclic Compounds*, *Introduction to Organic Mass Spectrometry*, *A Textbook of Hospital and Clinical Pharmacy*, *GPAT-2012*, *Natural Products Chemistry Practical Manual*, *Organic Medicinal Chemistry: Practical Manual*, *Pharmaceutical Organic Chemistry* (Vols 1 and 2) and *Computer Aided Drug Design*.



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