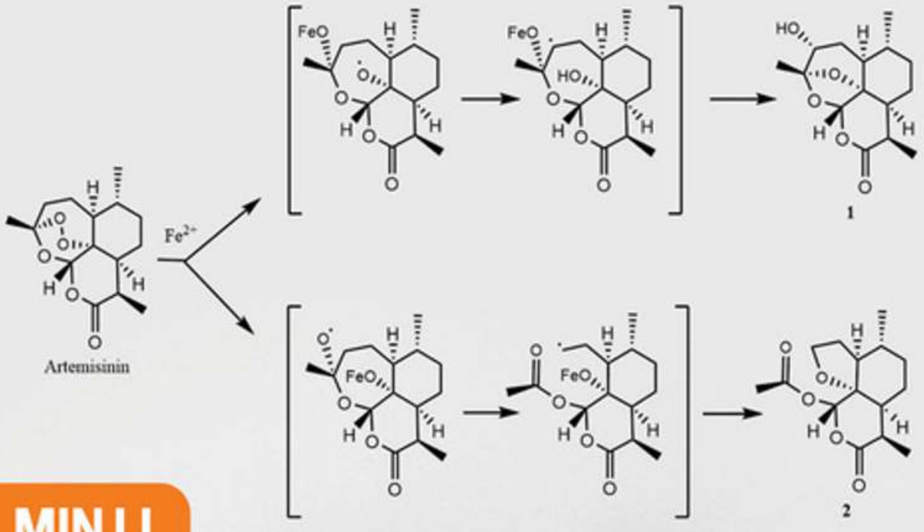
Compendium of Drug Degradation Pathways



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Compendium of Drug Degradation Pathways

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To Beihong and Emily

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Preface

My first book, *Organic Chemistry of Drug Degradation*, was published in September 2012, and it was intended to be a systematic summary of drug degradation chemistry based on the types of degradation pathways such as hydrolytic, oxidative, and photochemical degradation. Each type of drug degradation discussed in a particular chapter of that book is substantiated by examples of the specific degradation pathway(s) of a drug or drugs related to the subject of the chapter. Hence, for the drug molecules discussed in those examples, there are no summary or compilation of *all* of their known or reported degradation pathways. In other words, the degradation chemistry of a particular drug was not intended to be presented in its entirety in that book. Although *Organic Chemistry of Drug Degradation* can be used as a textbook for graduate students majoring in pharmaceutical sciences or anyone who is interested in a systematic study of drug degradation chemistry, it has occurred to me that in many occasions the practicing scientists, particularly those working in the pharmaceutical industry, would also like to have a convenient reference book in which the degradation chemistry of many commonly prescribed drugs can be readily found. Hence, for the past 10 years or so, it has been my goal to try to fulfill that need by summarizing the known or reported degradation pathways of representative small molecule drugs in another book, as such a book would be complementary to *Organic Chemistry of Drug Degradation* from a different perspective. Furthermore, such a book might also be supplementary to official pharmacopeias, where the degradation chemistry of drugs is usually not included, despite the fact that the supplementation would be quite limited.

During the course of pondering the contents of such a book, I decided to choose approximately 300+ representative small molecule drugs and then to summarize their degradation pathways in respective monographs that are organized in alphabetical order of the generic names of these drugs. The selection of these representative drugs in the book was based upon the following several factors: the numbers of their prescription, sales status, and inclusion in WHO List of Essential Medicines (the ones that "satisfy the priority health care needs of the population" per World Health Organization's definition). Many drugs in the same therapeutic categories share the same or similar core structures and functional groups and thus would have the same or similar degradation pathways. For this reason, these drugs are typically covered in the same respective monographs, with the best known or most widely used drugs as the main entries of the monographs. Such examples include, but not limited to, the monograph for benzodiazepines, the monograph for omeprazole and related azoles, the monograph for opioids, and the monograph for penicillins.

Preceding the monographs, the main body of the current book *Compendium of Drug Degradation Pathways*, a general chapter entitled “Introduction” is included, in which a brief overview of the regulatory perspective on drug stability and impurities, as well as an overview of drug degradation chemistry is provided. In addition, the role and limitation of stress testing, often referred to as forced degradation study as well, is also discussed. In the ensuing monographs, each one is started with the structure(s) of the drug molecule(s), and compilation of their key information, including chemical names, brand names, formulas, protonated monoisotopic mass-over-charge ratios, average molecular weights, and CAS numbers, as applicable. The above information is mostly derived from the following publicly accessible resources: Pubchem (pubchem.ncbi.nlm.nih.gov), Wikipedia (wikipedia.org), Drugbank online (go.drugbank.com), BMRB (Biological Magnetic Resonance Data Bank, https://bmrb.io/metabolomics/mol_mass.php), and Chemspider (chemspider.com). In the main body of each monograph, the known or reported degradation chemistry of a drug or drugs is summarized in text, which is followed by schematic presentation of the drug degradation pathway(s). Quite often, the reported degradation chemistry of a drug was based upon stress or forced degradation studies of the drug. As it is discussed in Introduction of this book, the degradation chemistry predicted from the forced degradation studies may not necessarily represent the one under real-life scenario, i.e., the degradation chemistry observed under the long-term storage conditions as represented by the stability conditions recommended by the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the World Health Organization (WHO). The quality for the prediction of real-life degradation chemistry relies upon a clear understanding of the principles of drug degradation chemistry, so that the forced degradation study would be conducted under the conditions that are relevant to the drug degradation in real-life scenarios. The degradation pathways obtained by utilizing these conditions, sometimes referred to as purposefully designed conditions or mechanism-based conditions, would most likely to contain the real-life degradation profiles. In other words, the real-life degradation pathways of a drug would more likely be a subset of its degradation pathways obtained under the forced degradation conditions purposefully designed by taking into consideration of the perceived degradation mechanisms and kinetics. To borrow the terms from the quality-by-design paradigm, the degradation pathways of a drug obtained under the purposefully designed forced degradation conditions would be the design space, while the real-life degradation pathways would be the control space, a subset of the design space. Due to the limitation that many forced degradation studies reported in the literature were not necessarily performed under purposefully designed conditions, readers are cautioned in places where artificial degradation may occur in these forced degradation studies.

To facilitate organization of the book contents, the alphabetically arranged monographs are further sequentially grouped into eight sections, following Section 1, Dedication and Preface, and Section 2, Introduction.

It has been quite a long time since my initiation for the writing of this book, which far exceeds the originally proposed timeline for the completion; this is primarily due to the change of my career path and increasing responsibilities, particularly during the last 10 years at Huahai Pharmaceutical Co., Ltd. For this reason, I sincerely apologize for the delay, while being very grateful for the patience of the editors of Wiley. Meantime, I am also very appreciative for the opportunities provided by Huahai that allowed me to create Center of Excellence for Modern Analytical Technologies (CEMAT), the analytical core group within Huahai that is dedicated to investigating and solving the most technically challenging problems occurring in the stages of pharmaceutical research and development, scale up, regulatory filing, and commercial production. CEMAT was modeled after my former organizations at Merck & Co., Inc. (Department of Regulatory and Analytical Sciences–Analytical, or RAS-A), Schering-Plough Corp. (Department of Global Quality Services–Analytical Sciences, or GQS-AS), and Merck again (Analytical Chemistry in Development and Supply–Supply Analytical Sciences, or ACDS-SAS). CEMAT was formally established in early 2016, and it currently has a staff of approximately 150 scientists and is equipped with state-of-art analytical instrument and equipment. Over the years, members of CEMAT have performed thousands of investigations, many of which are related to elucidating drug degradation pathways and mechanisms. The results obtained from these investigations have further expanded my own knowledge in drug degradation chemistry. Among the many young and energetic scientists who have made significant contributions toward the establishment, operation, and continuous improvement of CEMAT, I would like to extend my special gratitude to Mr. Wenquan Zhu, who helped me from the very beginning for the establishment, organization, daily operation, as well as future expansion of CEMAT. My appreciation also goes to the following CEMAT members, some of whom have moved on to other career opportunities: Mr. Wenbin Chen, Dr. Jinsheng Lin, Mr. Jian Ye, Mr. Jianyang Jin, Dr. Lewei Zheng, Ms. Dan Li, Dr. Jichao Wang, Dr. Jian Ma, Mr. Wenping Hu, Ms. Qianqian Lv, Mr. Zijian Kuang, Mr. Shaolan Wang, Mr. Zijian Wang, Mr. Qiang Zhou, Ms. Hong Cai, and Ms. Qiaohong Guo.

Between June 2020 and May 2023, I was also responsible for managing Shanghai Synergy Pharmaceutical Sciences, the Huahai subsidiary dedicated to small molecule innovative drug research. During that three-year tenure, my team advanced two novel drug candidates into clinical stage and developed several preclinical drug candidates. Toward these achievements, I am very much indebted to the following team members at Shanghai Synergy: Dr. Yu Huang, Dr. Jia Chen, Dr. Chengjun Deng, Dr. Xin Xu, Dr. Zhan Cai, and Dr. Zhezhou Yang. Over the past 10 years or so, I also have had the opportunities to work with many capable and hard-working colleagues at different functional areas of Huahai and its subsidiaries. Their expertise, contributions, and friendship have made my journey of the last 10 years more fruitful and enjoyable. Among them, the most notable ones are Mr. Jun Du, Ms. Linda (Lihong) Lin, Dr. Xiaodi Guo, and Mr. Jay (Jie) Wang. Jun was the founder and CEO of Huahai US and also a longtime friend of mine for 20 years, who not only introduced me to Huahai but also gave me firm support and trust throughout the time we had worked together. Linda and her Regulatory Affairs team have worked closely with my CEMAT team on a great number of projects, particularly during their regulatory filing stages. Together, we have overcome many challenges in dealing with pharmaceutical impurities in terms of their assessment, control strategies, analytical methods, and specifications. Xiaodi and his formulation team performed formulation development for HHT-120, a novel orally available anticoagulant developed by my teams initially at Huahai US and later at Shanghai Synergy. HHT-120 demonstrated comparable antithrombotic efficacy but significantly less bleeding risk as compared to an approved drug in animal models. Jay, a congenial colleague often with a sense of subtle humor, was a vice president for Huahai's API Sales Department. In 2016, I worked with him to help resolve analytical issues associated with a key API product for a major client, during which process I introduced the first liquid chromatography – triple quadruple mass spectrometer into Huahai to ensure the method for a trace impurity with sufficient sensitivity, specificity, and robustness.

My special thanks go to the editors of Wiley for their excellent job in the production of this book, in addition to their patience. Last but not the least, my profound gratitude goes to my family, especially my wife Beihong, for her love, support, and unwavering confidence in me for more than 30 years.

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Introduction

The stability of a drug product, which includes chemical and physical stability, is a key attribute for its efficacy and safety. This book focuses on the chemical aspect of drug stability. To have a drug product with desirable stability during production, shipment, storage, and throughout its expiration requires a clear understanding of drug degradation chemistry, so that a reasonably stable molecule can be selected as the pharmaceutical development candidate in the first place. This candidate will then be formulated with excipients to impart adequate pharmacokinetic properties onto the drug product. The excipients may stabilize, do nothing with, or destabilize the drug molecule. It would be desirable that the excipients would stabilize the drug molecule, or at least would not destabilize it. To achieve that goal, it is necessary to understand the chemical and physical interactions between the drug molecule and excipients, which also fall into the scope of drug degradation chemistry, or at least the part of the chemical interactions. In my previous book, *Organic Chemistry of Drug Degradation*, drug degradation chemistry was systematically summarized based on the types of degradation pathways such as hydrolytic, oxidative, and photochemical degradation. The current book, *Compendium of Drug Degradation Pathways*, is intended to be complementary to *Organic Chemistry of Drug Degradation* from the perspective of summarizing the known or reported degradation pathways of representative drugs in their entirety. There are more than 300 drugs that are discussed in the ensuing monographs. Before proceeding to the monographs, it would be helpful to have a brief overview of the regulatory perspective on drug stability, impurities, as well as drug degradation chemistry. Readers may need to refer to *Organic Chemistry of Drug Degradation* from time to time for a more comprehensive discussion on a particular degradation mechanism or pathway.

Drug Stability and Regulatory Requirements

The chemical stability of a drug is characterized by the persistence of its active ingredient(s), i.e., not degrading into degradation products, over its intended or registered shelf-life. The study of drug stability probably dates back to when

Compendium of Drug Degradation Pathways, First Edition. Min Li.

modern drug discovery and development started. The first publications on drug stability were released in the 1950s, according to Jamrógiewicz and Merchel [1]. In 1978, the pioneering book, *Chemical Stability of Pharmaceuticals*, was first published, and the second edition was released in 1986; the book provided "a collection of selected drug-stability data from the pharmaceutical literature" [2]. Nevertheless, it was not until 1987 when the US FDA issued the first guidance that required stability data in the submission of investigational new drug applications (INDs) as well as new drug applications (NDAs) [3]. Subsequently in April 1990, representatives of the regulatory agencies and industry associations from Europe, United States, and Japan convened in Brussels to inaugurate the international organization for pharmaceutical regulatory guidance – ICH, which stands for International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (formerly known as International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) [4]. In the early 1990s, ICH started to issue a series of guidance documents for performing the stability study and submission of stability data during the registration of new drug substances and products, starting from Q1, *Stability Testing of New Drug Substances and Products*. And the code Q1 was later revised to Q1A (and the most updated version is Q1A(R2)), as additional guidance documents on stability, Q1B through Q1E, were issued. The other ICH guidance documents that directly relate to the drug stability and drug impurities (including both process impurities and degradation products) are Q3A(R2), *Impurities in New Drug Substances*, and Q3B(R2), *Impurities in New Drug Products*. The above ICH guidance documents are summarized in Table 1.1 [5].

The main environmental factors influencing the drug stability over time are temperature, humidity, and light. Accordingly, Q1A(R2) stipulates the temperature and humidity requirements for typical long-term and accelerated stability studies to be carried out at 25°C, 60% relative humidity (RH) and 40°C, 75% RH, respectively, in order to assess the thermal stability of drug substances and products. For the drug

Table 1.1 ICH Guidance documents on drug stability and impurities.

Code	Document title
Q1A(R2)	Stability Testing of New Drug Substances and Products
Q1B	Stability Testing: Photostability Testing of New Drug Substances and Products
Q1C	Stability Testing for New Dosage Form
Q1D	Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products
Q1E	Evaluation of Stability Data
Q3A(R2)	Impurities in New Drug Substances
Q3B(R2)	Impurities in New Drug Products

Table 1.2 Storage conditions and time periods in Q1A(R2) and Q1B(R2) for stability studies of drug substances and products at the time of NDA submission.

Study[a]	Storage condition[b]	Minimum time period required at NDA submission
Long term	25°C, 60%RH	12 months
Intermediate	30°C, 65%RH	6 months
Accelerated	40°C, 75%RH	6 months

[a] Applicants of NDA may choose the intermediate condition as the long-term condition as appropriate, due to climate zone differences.
[b] The temperature variation allowed is ±2°C and the humidity variation allowed is ±5%.

substances or products that undergo significant degradation under the accelerated conditions of 40°C, 75% RH, an intermediate condition of 30°C, 65% RH is recommended for the stability study. Likewise, Q1B outlines the photolysis requirements, i.e., sources of light and duration of exposure, for photostability studies of drug substances and products, in order to assess the photostability of drug substances and products with and without packaging materials. The requirements of Q1A(2R) and Q1B are outlined in Table 1.2 [5].

The World Health Organization (WHO) also issued guidance on drug stability, particularly for Climate Zone III (hot and dry) and Climate Zone IV (hot and humid) regions, which provides the options of selecting 25°C/60%RH, 30°C/65%RH, or 30°C/75%RH as the long-term stability condition dependent upon the climate zones, where the drug products are intended to be marketed [6].

In order to help elucidate the intrinsic stability of drug molecules, stress testing, also referred to as forced degradation, is required in Q1A(R2), among which photostability testing is an integral part of the stress testing. The subject of stress testing will be discussed in detail later in this introduction.

On the other hand, Q3A(R2) and Q3B(R2) set the thresholds for impurities that need to be reported, identified, or qualified, once they exceed the respective thresholds, and these thresholds are summarized in Tables 1.3 and 1.4 [6].

Table 1.3 Thresholds for impurities in drug substances.

Maximum daily dose	Threshold (% of drug substance)		
	Reporting	Identification	Qualification
≤2 g	0.05%	0.10% or 1.0 mg/day intake (whichever is lower)	0.15% or 1.0 mg/day intake (whichever is lower)
>2 g	0.03%	0.05%	0.05%

Table 1.4 Thresholds for degradation products in new drug products[a].

	Threshold (% of drug substance or total daily intake)		
Maximum daily dose	**Reporting**	**Identification**	**Qualification**
≤1g	0.1%	–	–
>1 g	0.05%	–	–
<1 mg	–	*1.0% or 5 μg TDI*[b]	–
1–10 mg	–	*0.5% or 20 μg TDI*[b]	–
>10 mg–2 g	–	*0.2% or 2 mg TDI*[b]	–
>2 g	–	*0.10%*	–
<10 mg	–	–	**1.0% or 50 μg TDI**[b]
10–100 mg	–	–	**0.5% or 200 μg TDI**[b]
>100 mg–2 g	–	–	**0.2% or 3 mg TDI**[b]
>2 g	–	–	**0.15%**

[a] The reporting thresholds are set based on two ranges of maximum daily doses (i.e., ≤ 1 g/day and >1 g/day), while both the identification threshold and qualification threshold are set based on four ranges of maximum daily doses.
[b] Whichever amount is lower; TDI, total daily intake.

Brief Overview of Drug Degradation Chemistry

The most frequently occurring drug degradation types are hydrolytic and oxidative in nature, followed by various other types, such as isomerization/rearrangement, elimination, decarboxylation, cyclization, dimerization, and photochemical degradation. The categorization of the degradation types is not absolute, as they may be interrelated, e.g., oxidation may trigger decarboxylation, cyclization, and dimerization, while oxidation, isomerization/rearrangement, elimination, decarboxylation, cyclization, and dimerization can often occur under photochemical degradation conditions.

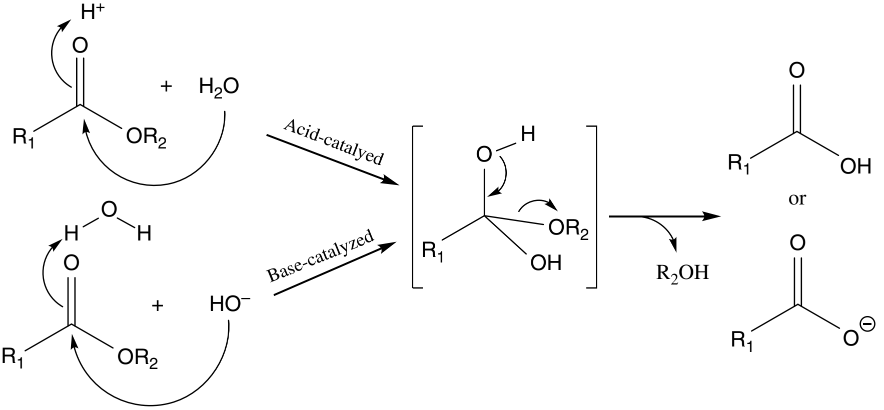
Types of Functional Groups or Structural Moieties Susceptible to Hydrolytic Degradation

The susceptibility to hydrolytic degradation may be estimated based on the hydrolysis activation energy (Ea) or enthalpy of activation ($\Delta H^{\neq}$) of a compound bearing a hydrolyzable functional group [7]. Although Ea and $\Delta H^{\neq}$ are obtained from the different models describing the same reaction process, their numerical values are usually quite close to each other [8]. For this reason, these two types of values may be used interchangeably to evaluate the hydrolytic susceptibility of different hydrolyzable drug molecules. The lower the activation energy or enthalpy of activation, the easier it would be for the drug molecule to undergo hydrolytic degradation, and vice versa. The common functional groups or structural moieties in this category include, but are not limited to, derivatives of carboxylic acid (esters, lactones, amides, imides, lactams, etc.), derivatives of phosphoric acid (esters and amides), carbamates, epoxides, ethers, polysaccharides, and nucleosides. The key factors that have impact on the intrinsic susceptibility of an organic compound to hydrolytic degradation are the steric and electronic factors surrounding the hydrolyzable moieties. While steric hindrance makes hydrolysis substrates of all types more stable, the presence of electron-withdrawing groups adjacent to the carbonyl groups of the hydrolysis substrates renders these carboxylic derivatives more susceptible to hydrolytic degradation [7]. From a different perspective, any factor destabilizing the transition states of hydrolytic degradation renders the hydrolysis substrates more resistant toward hydrolytic degradation [9, 10], while those (derivatives of both carboxylic and phosphoric acid) containing an easy-to-leave group are more susceptible to hydrolytic degradation [11, 12].

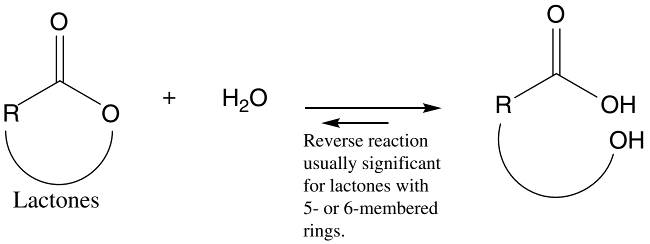
Carboxylic Esters and Lactones

Carboxylic esters, especially those without steric hindrance surrounding the ester linkage, are usually quite susceptible to hydrolytic degradation to produce carboxylic acids and alcohols, particularly under alkaline conditions (Scheme 1).

With increasing steric hindrance on either R_1 or R_2 group adjacent to the carbonyl group, carboxylic esters become more resistant toward hydrolysis. For example, while ethyl acetate only has a reported *Ea* value of 10 kcal/mol, hexyl acetate and *t*-butyl acetate have reported *Ea* values of 11.4 and 27 kcal/mol, respectively [13]. Aromatic carboxylic esters appear to be somewhat more stable than their alkyl counterparts, for example, the $\Delta H^{\neq}$ for hydrolysis of ethyl benzoate in a 0.1 M NaOH solution was reported to be approximately 82–84 kJ/mol (~20 kcal/mol) in the temperature range of 20–40°C, while the *Ea* of ethyl acetate was reported to be only 11.56 kcal/mol in a solution of 0.02 M NaOH [14, 15]. Lactones are cyclized esters, and particularly for lactones with a five- and six-membered lactone ring, their hydrolytic degradation under acidic conditions would usually reach an equilibrium point because the reverse reaction



Scheme 1 Hydrolysis of carboxylic esters.



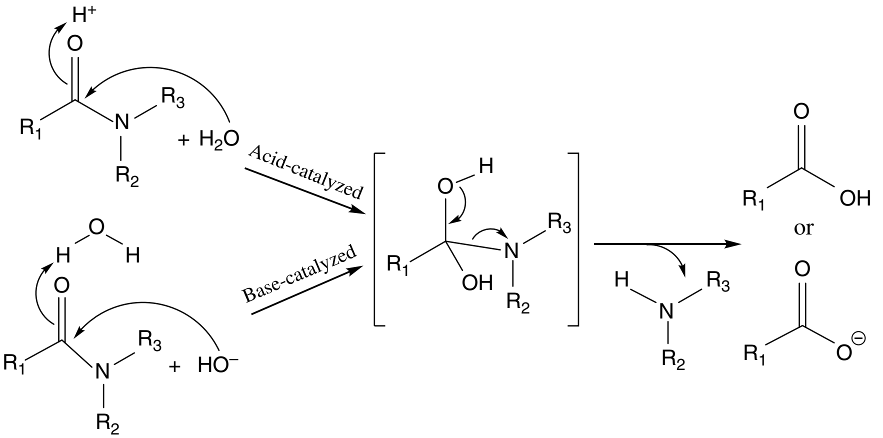
Scheme 2 Hydrolysis of lactones, and the reverse reaction would be significant for lactones with five- or six-membered rings, especially under acidic conditions.

(i.e., re-formation of the lactone ring) becomes increasingly significant as the hydrolysis goes on (Scheme 2) [16].

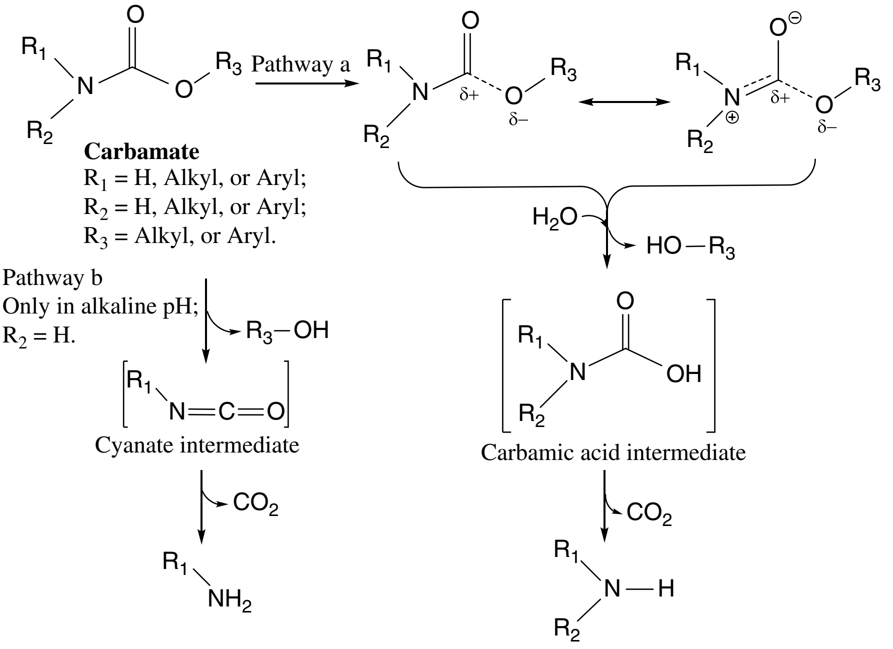
Carboxylic Amides and Lactams

Carboxylic amides and lactams are usually significantly more stable than the corresponding esters and lactones with regard to hydrolytic degradation, despite the fact that their hydrolysis mechanisms are similar to those of carboxylic esters and lactones, respectively (Scheme 3).

Consistently, typical hydrolysis activation energies of carboxylic amides and lactams are meaningfully higher, usually ~20 kcal/mol or higher. Nevertheless, if an electron-withdrawing group is present at the position alpha to the carbonyl, the amide becomes more susceptible to hydrolysis, because the carbonyl is more electron-deficient and thus more susceptible to attack by water or hydroxide ion. For example, the hydrolysis rate of trifluoroacetamide was reported to be several orders of magnitude higher than that of acetamide [17]. For this reason, the trifluoroacetyl group can be used as a protection group for amines in organic syntheses [18].



Scheme 3 Hydrolysis of carboxylic amides. R_1 = alkyl or aryl; R_2 = H, alkyl, or aryl; R_3 = H, alkyl, or aryl.



Scheme 4 Hydrolysis of carbamates.

Carbamates

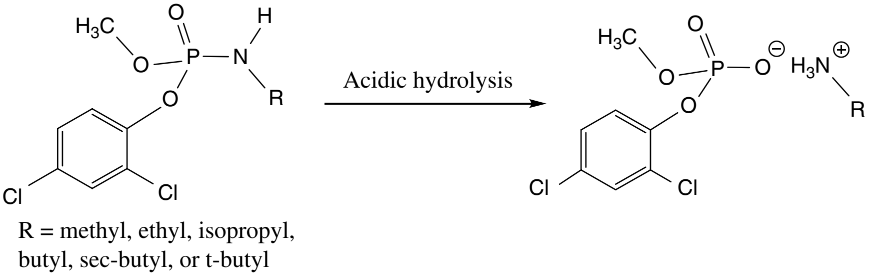
Carbamate is another carbonyl-based functionality often utilized in drug design [19]; structurally, it can be viewed as a hybrid of carbonyl ester and amide. A study by Dittert and Higuchi seemed to be indicated that the hydrolysis of a carbamate in alkaline solution could proceed via two mechanisms: the first one would start with the removal of the ester moiety, resulting in the formation of the unstable intermediate, carbamic acid, which quickly decomposes to an amine degradant and carbon dioxide (Pathway a, Scheme 4). The second one would involve the removal of the amide hydrogen, resulting in the formation of the isocyanate intermediate (Pathway b, Scheme 4) [20]. According to a hydrolytic stability study by Chapman in which *N*-phenyl-carbamates and *N*-cyclohexyl-carbamates were compared to their respective amide analogs, the carbamates were significantly more hydrolytically stable than the amides under acidic conditions, while the carbamates were slightly less stable than the amides under neutral conditions [21]. In a hydrolytic stability study of estramustine, an anticancer drug based on estradiol with a carbamate linkage to a nitrogen mustard, the hydrolytic enthalpy of activation was found to be 89.3 kJ/mol (21.3 kcal/mol) in the pH range of 1–9 [22]. This result is consistent with those obtained by Chapman, suggesting that the hydrolytic stability of carbamates is at least comparable to that of amides in general.

Phosphoryl Esters and Phosphoramidates

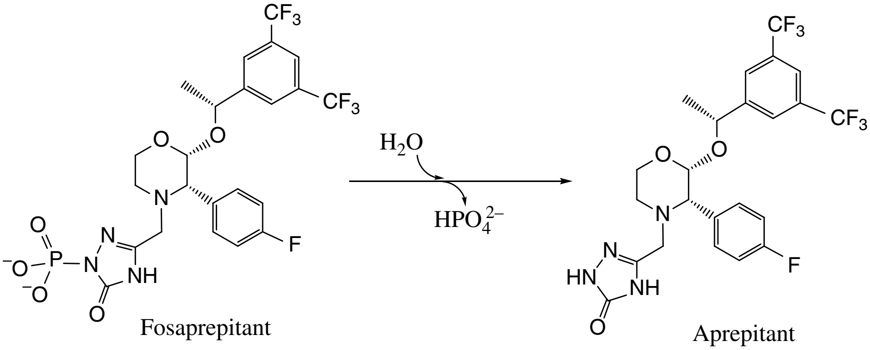
The phosphoryl functional group is often utilized to impart aqueous solubility to a drug molecule that contains either a hydroxyl or an amino group. In the former case, the resulting drug molecule is a phosphoryl monoester, while in the latter case, it is an

N-substituted derivative of phosphoramidic acid (amidophosphoric acid). Usually, the phosphoryl monoesters prepared from alkyl alcohols are quite stable hydrolytically. The hydrolysis activation energies of such phosphoryl monoesters are close to ~30 kcal/mol [23, 24]. On the other hand, *N*-substituted derivatives of phosphoramidic acid, or more commonly *N*-substituted phosphoramidates, are generally less hydrolytically stable than the structurally similar phosphates. For example, in a hydrolytic stability study of six 2,4-dichlorophenyl methyl *N*-alkylphosphoramidates, the activation energies for the hydrolysis of the *N*-alkyl moiety (Scheme 5) in 0.19 M HCl solutions were found to be in the range of 10.1–13.2 kcal/mol [25]. Another example is fosaprepitant dimeglumine, which is the phosphoramidic pro-drug of aprepitant (Scheme 6). The activation energy for the hydrolysis of the phosphorylamidic linkage was determined to be 91 kJ/mol (22 kcal/mol) [26]. The above activation energies for the hydrolysis of the phosphorylamidic linkage are significantly lower than the activation energies of typical alkyl phosphoryl esters.

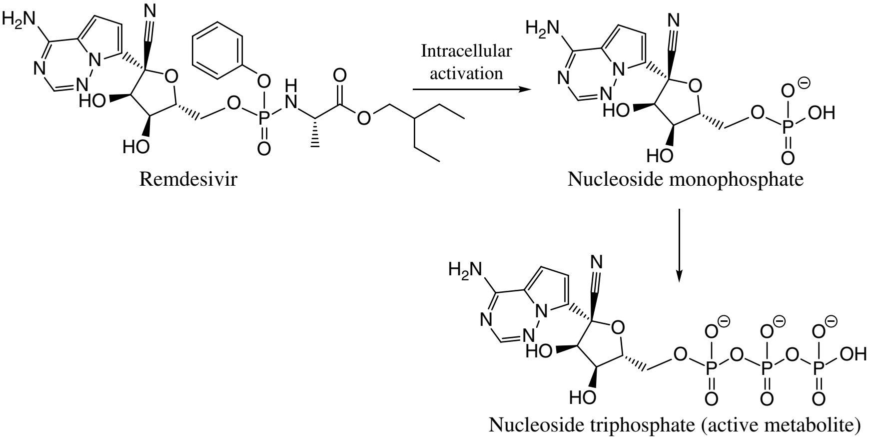
Nevertheless, the phosphoryl esters prepared from phenol or its analogs are not as stable hydrolytically as their alkyl counterparts, because phenol and its analogs are good leaving groups. For this reason, the phenyl phosphoryl functional group is usually used in the design of prodrugs where the free phosphate functionality needs to be released in vivo, such as in the case of anti-viral drug remdesivir (Scheme 7) [27].



Scheme 5 Hydrolysis of 2,4-dichlorophenyl methyl *N*-alkylphosphoramidates.



Scheme 6 Hydrolysis of fosaprepitant. The counter ion, dimegluminium, is not shown for clarity.



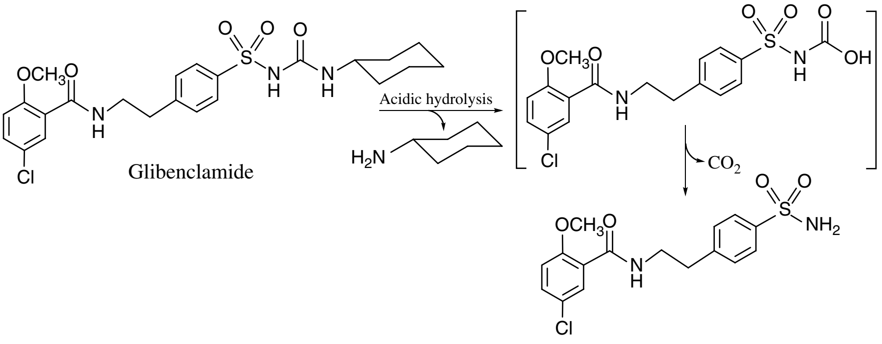
Scheme 7 Bio-activation of remdesivir, a prodrug containing phenyl phosphoramidate moiety.

Sulfonamides and Urea Derivatives

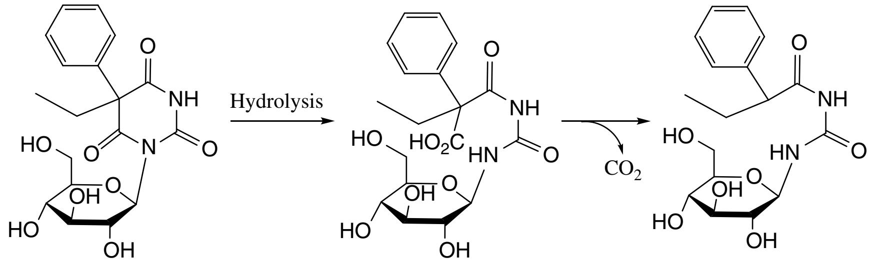
Theoretically, sulfonamide linkage is susceptible to hydrolysis; nevertheless, it is usually extremely stable under both acidic and alkaline hydrolytic degradation conditions, even under some very harsh conditions. The activation energy for a typical sulfonamide was estimated at greater than 30 kcal/mol [28]. Urea and its derivatives are generally resistant toward hydrolytic degradation, as evidenced by the fact that urea, the parent compound, was reported to have hydrolysis activation energies at approximately 30 kcal/mol in water and weakly to modestly acidic solutions [29]. Urea displayed pH-independent hydrolytic behavior under these conditions.

Imides and Sulfonylureas

Imides, particularly cyclic imides, are among the common building blocks utilized in drug design. The activation energies for hydrolysis of cyclic imides, such as phenobarbital and its derivatives (Scheme 8), were reported to be ~20 kcal/mol in



Scheme 8 Hydrolysis of glibenclamide under acidic conditions.



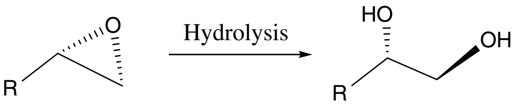
Phenobarbital *N*-Glucoside

Scheme 9 Hydrolysis of phenobarbital *N*-glucoside.

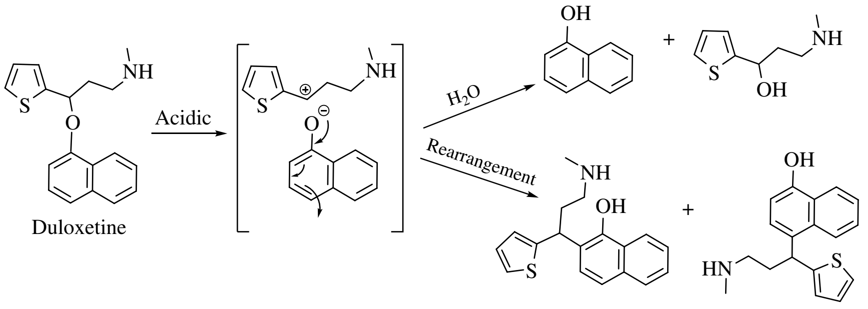
solutions of neutral pH [30], suggesting that they would be reasonably stable with regard to hydrolytic degradation under neutral conditions. On the other hand, sulfonylureas, such as glibenclamide, may be viewed as a class of hybrid imides. Due to the presence of the sulfonyl group, a strong electron-withdrawing group, the urea functionality becomes less resistant toward hydrolysis, particularly under acidic conditions (Scheme 9) [31]. Structurally similar sulfamylureas are also quite susceptible to acidic hydrolysis [32].

Epoxides and Ethers

Epoxides are electrophiles owing to the constrained tricyclic ether ring, which can be hydrolyzed or hydrated, particularly under acidic or alkaline catalysis, giving rise to *trans*-1,2-diol products (Scheme 10). The hydrolytic stability of epoxides depends upon the substituents surrounding the epoxide group [33]. For propylene oxide, a simple epoxide model compound, the hydrolysis activation energy was reported to be ~19.0–19.5 kcal/mol [34], suggesting it would be reasonably stable under uncatalyzed conditions. Ethers of other types are usually hydrolytically stable, except in cases where a relatively good leaving group coincides with a stabilizable transition state of the hydrolysis in the same molecular entity. The hydrolysis of duloxetine under acidic conditions is such a case in which 1-naphthalenol is a relatively good leaving group, while the resulting carbocation transition state can be stabilized by the neighboring thiophene group through conjugation; in addition to the straight hydrolytic products, the other two main degradants were isomers of duloxetine formed via rearrangement (Scheme 11) [35].



Scheme 10 Hydrolysis of epoxides.



Scheme 11 Degradation of duloxetine under acidic conditions.

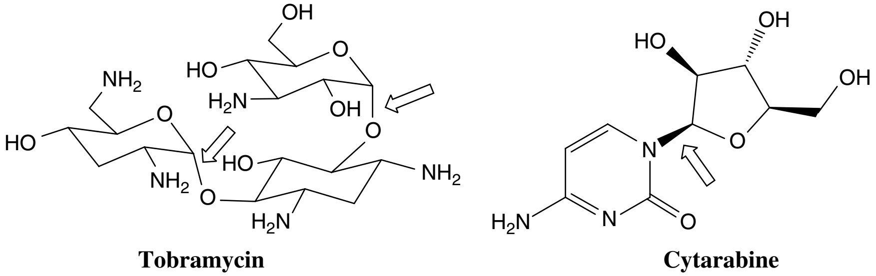


Figure 1.1 Structures of tobramycin and cytarabine. While the two glycosidic bonds in tobramycin may be susceptible to hydrolysis, the glycosyl bond in cytarabine is more resistant than the amino group toward hydrolysis.

Oligosaccharides/Polysaccharides and Nucleoside Analogs

The key linkage in oligosaccharides/polysaccharides and nucleosides is the glycosidic and glycosyl bond, respectively, with the glycosidic bond connecting the monosaccharide units of polysaccharides and glycosyl bond connecting the nucleobase (or its analog) and sugar moiety (Figure 1.1). The glycosidic and glycosyl bonds are usually quite stable hydrolytically; these bonds become less stable under alkaline conditions. For example, the hydrolysis activation energies for the glycosyl bond of tobramycin were reported to be 32 and 15 kcal/mol in 1 N HCl and 1 N NaOH solutions, respectively [36]. It needs to be pointed out that the glycosyl bonds of nucleosides are sometimes incorrectly referred to as glycosidic bonds.

Types of Functional Groups or Structural Moieties That Are Susceptible to Different Types of Oxidative Degradation

Oxidative degradation is another very common degradation pathway. The ultimate "oxidizing reagent" for drug oxidation originates from molecular oxygen, which constitutes ~21% of the atmosphere. The molecular oxygen at ground state is a diradical, which is a triplet species. Usually, the reaction between the triplet molecular oxygen and the vast majority of drug molecules, which are singlet species, is a kinetically forbidden process due to violation of the spin conservation rule [37]. The activation of molecular oxygen is usually mediated by redox reactive transition metal ions, most notably ferrous ions (Scheme 12), during which process three reactive oxidative species (ROS), i.e., superoxide anion radical, hydrogen peroxide, and hydroxyl radical, are generated.

Nucleophilic Oxidation

Tertiary amines and thioethers, particularly the alkyl ones, are capable of reacting with hydroperoxide species (including the prototype hydrogen peroxide; Scheme 13), which are often present in polymeric excipients such as polyethylene glycol (PEG) and polysorbate (e.g., Twin 80), as a result of auto-oxidation [38].

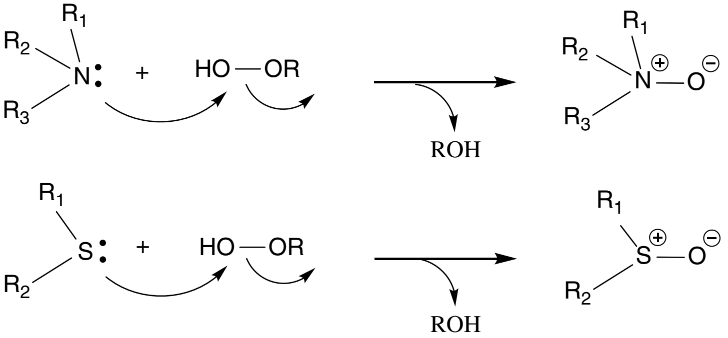
Certain compounds containing electron-rich carbon–carbon double bonds can also undergo analogous nucleophilic oxidation (Scheme 14); this type of compound

$$O_2 + Fe^{2+} \xrightleftharpoons{\text{Electron transfer}} \boxed{O_2^{\bullet-}} + Fe^{3+}$$

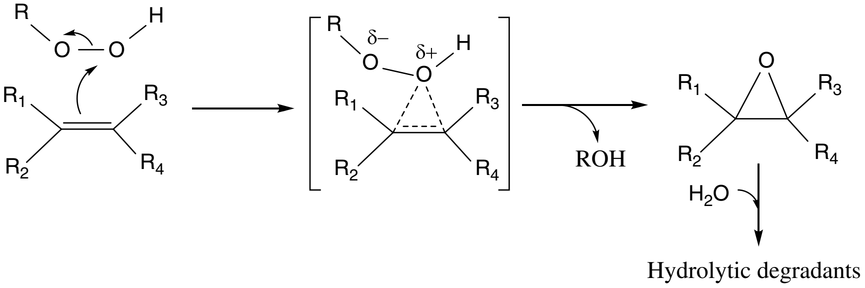
$$O_2^{\bullet-} + 2\,H^+ \longrightarrow O_2 + \boxed{H_2O_2}$$

$$H_2O_2 + Fe^{2+} + H^+ \longrightarrow \boxed{HO^\bullet} + Fe^{3+}$$

Scheme 12 Activation of molecular oxygen by ferrous ion Fe^{2+}, a redox reactive transition metal ion.



Scheme 13 Nucleophilic oxidation of tertiary amines and thioethers.



Scheme 14 Nucleophilic oxidation of compounds containing electron-rich carbon–carbon double bonds.

includes those that possess an indole ring, which can be viewed as an enamine (*N*-substituted carbon–carbon double bond).

Free-Radical-Mediated Oxidation

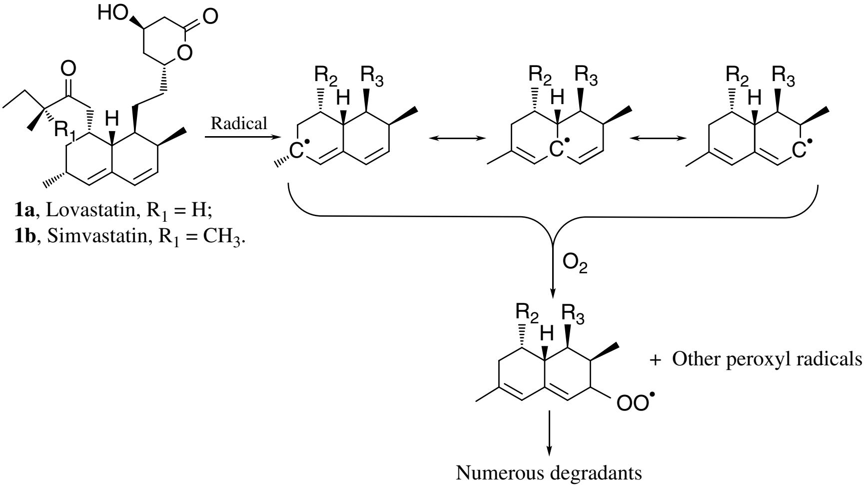
Free-radical-mediated oxidation is probably the most common and yet most complicated type of oxidation. The structural moieties that are susceptible to this type of oxidation include the CH or CH_2 positions that are alpha to carbon–carbon double bonds (particularly to conjugated carbon–carbon double bonds), CH or CH_2 positions that are alpha to a carbonyl group, and benzylic positions. An example is given for the facile free-radical-mediated oxidation of the butadiene moiety that is embedded in the core structures of lovastatin and simvastatin (Scheme 15) [39].

Base-Catalyzed Oxidation

This type of oxidation, also known as carbanion/enolate-mediated auto-oxidation, typically occurs in a group of compounds that contain somewhat "acidic" CH or CH_2 moieties. A classic example is the oxidation of rofecoxib (Scheme 16). The mechanism of the oxidation was originally proposed as free-radical mediated, but was later found to be mediated by carbanion/enolate [40, 41]. The rate of the base-catalyzed oxidation would be two orders of magnitude higher than that mediated by free radicals.

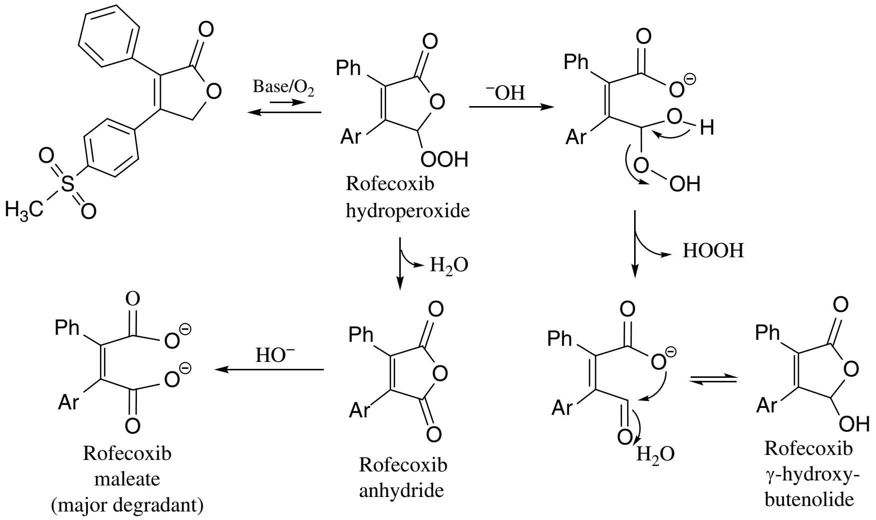
Oxidation Via the Udenfriend Reaction

The Udenfriend reaction is an oxidative degradation pathway that can occur in formulated drug products containing both a metal ion chelator and a reducing agent [38]. The role of the chelator, which is typically EDTA or citrate, is to form a chelating complex with the reduced form of a redox-reactive transition metal ion, most commonly ferrous ion (Fe^{2+}). The resulting complex, Fe^{2+}\{EDTA\},



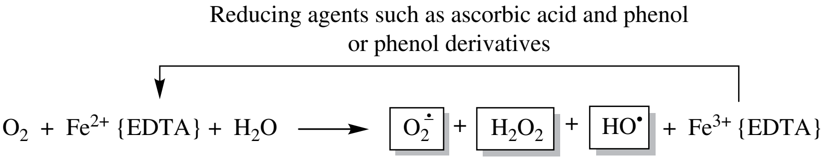
1a, Lovastatin, $R_1 = H$;
1b, Simvastatin, $R_1 = CH_3$.

Scheme 15 Free-radical-mediated oxidation of the butadiene moiety embedded in the core structures of lovastatin and simvastatin.



Scheme 16 Base-catalyzed oxidation of rofecoxib.

would be easier to transfer an electron to molecular oxygen than the unchelated Fe^{2+}, resulting in the formation of superoxide anion radical. The latter species can form hydrogen peroxide via disproportionation, and hydrogen peroxide can then, in turn, be transformed to hydroxyl radical via the Fenton reaction. During this process, which is the activation of molecular oxygen, two equivalents of Fe^{2+}\{EDTA\} are converted into the oxidized form of Fe^{3+}\{EDTA\}. In the presence of a reducing agent, such as ascorbic acid or a phenolic compound, Fe^{3+}\{EDTA\} can be reduced back to Fe^{2+}\{EDTA\}, causing the activation of molecular oxygen and subsequent oxidative degradation to continue until all the reducing agent is consumed. This whole process of the Udenfriend reaction can be illustrated in Scheme 17, which indicates that a reducing agent could actually promote oxidative degradation of a drug product if it also contains a chelating agent in its formulation [42–46].

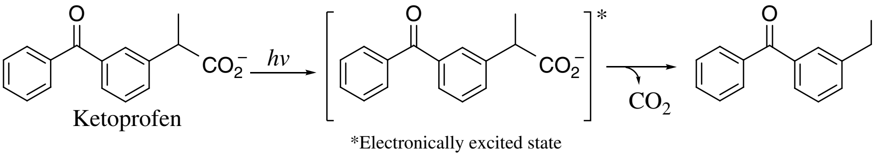


Scheme 17 Schematic presentation of the Udenfriend reaction in which molecular oxygen is activated continuously in the presence of a metal ion chelator and a reducing agent, resulting in the formation of three reactive oxygen species: superoxide anion radical, hydrogen peroxide, and hydroxyl radical.

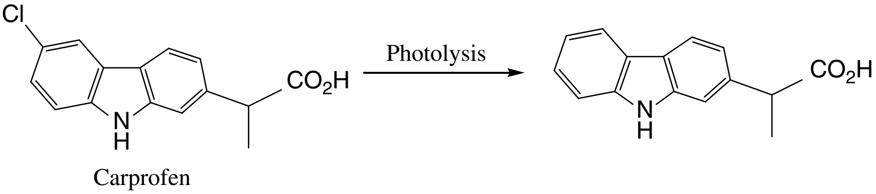
Types of Functional Groups or Structural Moieties That Are Susceptible to Photochemical Degradation

Photochemical reactions are those that take place at electronically excited states. Hence, the prerequisite for a drug molecule to undergo photochemical degradation is that it needs to have a chromophore that would have adequate absorption under the photolytic conditions, for the molecule to be elevated into electronically excited states. The excited states will return or relax to the ground state via several passages, one of which is a chemical transformation, i.e., a photochemical reaction, while others are physical processes such as light emission and collision-induced relaxation [47]. Some compounds lack appropriate chromophores but nevertheless can form chromogenic complexes with metal ions, thus making themselves photochemically vulnerable [44]. Some of the most common moieties that are susceptible to photochemical degradation include benzophenone and its analogs (any two aryl groups connected by a carbonyl), halogenated aryl compounds, and substituted alkenes (e.g., stilbene and its analogs). Several nonsteroidal anti-inflammatory drug (NSAID) molecules contain benzophenone or its analogous moieties in conjunction with a nearby carboxylic group; the resulting 2-arylpropionic acid moieties can usually undergo photochemical decarboxylation (Scheme 18) [48, 49].

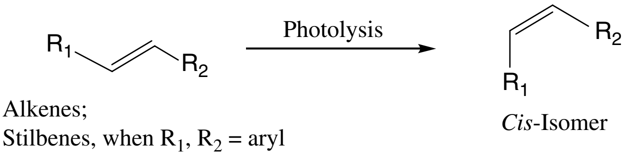
Halogenated aryl drugs, such as carprofen, are susceptible to photochemical dehalogenation (Scheme 19); nevertheless, such dehalogenation tends to occur in solution photolysis and usually would not take place in the solid state, except for iodoaryl compounds. In solution photolysis, if protic solvents such as water and methanol are used, the corresponding hydroxyl- and methoxyl-substituted photodegradants may also be formed. In such cases, the methoxyl-substituted photodegradants are artificial degradants as methanol is usually not a pharmaceutical excipient.



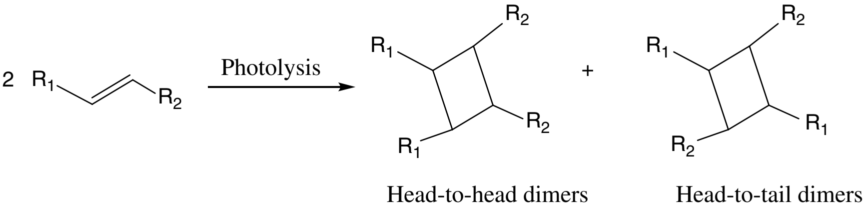
Scheme 18 Photodecarboxylation of ketoprofen, a 2-arylpropionic acid derivative of NSAID.



Scheme 19 Photo dehalogenation of carprofen.



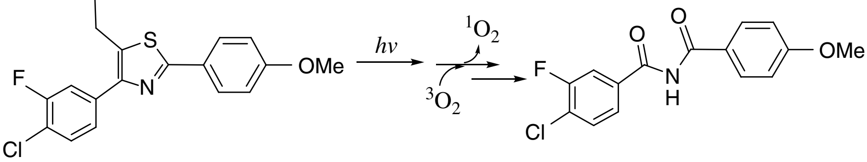
Scheme 20 Photoisomerization of alkenes, including stilbenes and analogs where R_1 and R_2 are aryl groups.



Scheme 21 2 + 2 Photodimerization of alkenes.

The carbon–carbon double bonds in alkenes can undergo photoisomerization (Scheme 20) and such isomerization becomes significant when the substituents of the double bonds render the resulting alkenes to have adequate absorption in UV and even visible regions. Analogous carbon–heteroatom double bonds can also be susceptible to similar photoisomeric degradation. Another important photodegradation pathway for alkenes is the 2 + 2 photodimerization (Scheme 21).

Drug molecules containing multiple aryl groups, either connected or fused, tend to undergo photosensitization during which process molecular oxygen, a triplet molecule, is excited to become very reactive singlet oxygen through collisional energy exchange with the excited state of the aryl rings. The singlet oxygen generated can easily inflict oxidative degradation on the drug molecule itself, such as in the example of an experimental drug containing a substituted thiazole ring (Scheme 22); this photodegradation pathway mediated via singlet oxygen is referred to as type II photosensitized oxidation [50].



Scheme 22 Photosensitized oxidation of an experimental drug containing an aryl-substituted thiazole ring.

Degradation Due to API-Excipient Interactions

The Maillard Reaction

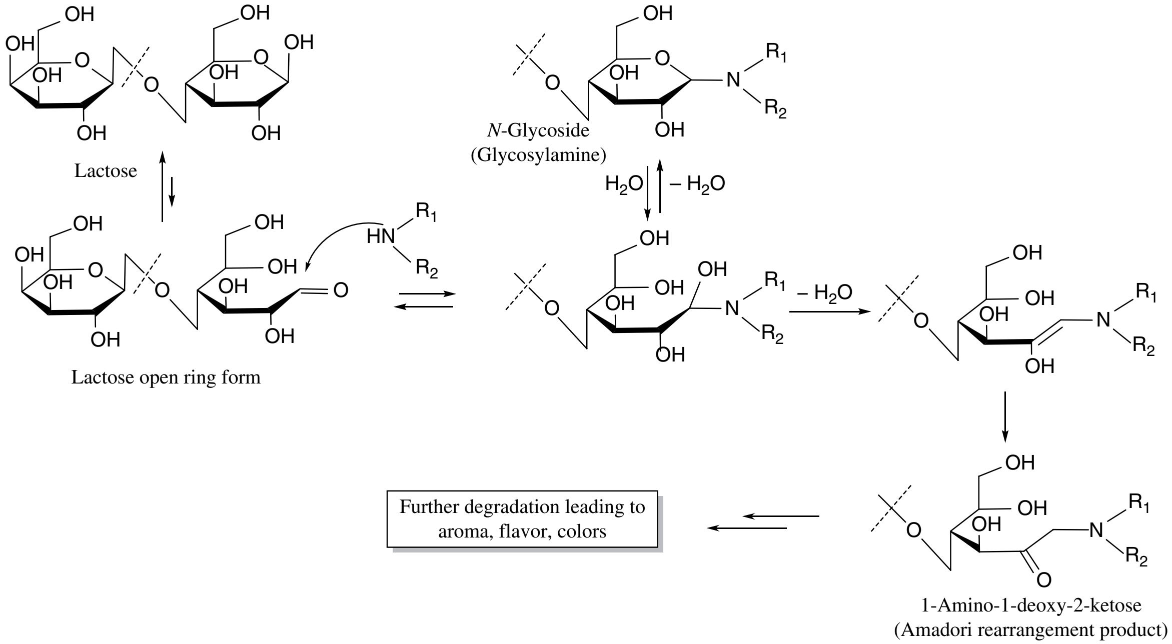
The most common interaction that occurs between active pharmaceutical ingredients (APIs) and excipients is probably the one between primary and secondary amine API molecules with reducing sugars such as lactose, which is known as the Maillard reaction. The Maillard reaction is a very complicated process consisting of hundreds of reactions and its pharmaceutical relevance is primarily limited to the first few steps leading to the formation of the so-called Amadori rearrangement products (Scheme 23) [51].

Degradation Due to Impurities in Excipients – Formaldehyde, Peroxides, Formic Acid, etc.

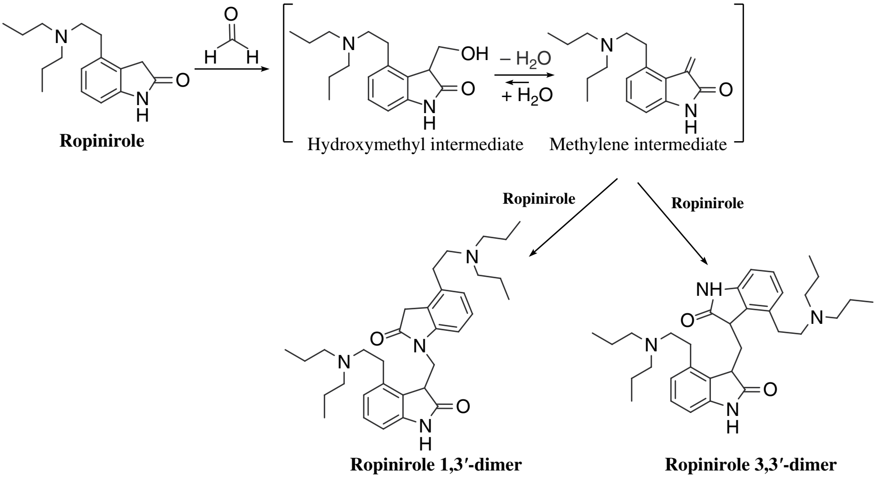
Pharmaceutical excipients contain impurities, some of which are of chemical reactivity and thus capable of reacting with API. The frequently seen impurities in excipients are formaldehyde, peroxides (including hydrogen peroxide), and formic acid, which result from auto-oxidative degradation of certain polymeric excipients such as PEG, polysorbate, and PVP (povidone) [51]. Formaldehyde is an electrophile that can react with API capable of nucleophilicity to form hydroxymethyl intermediates and then dimeric degradants, as in the case of two dimeric degradants of ropinirole (Scheme 24) [52]. Peroxides mainly inflict nucleophilic oxidative degradation of tertiary amines and thioethers (refer to Scheme 13). Formic acid can react with primary and secondary amine API to form *N*-formyl degradants, which is a partial source for this type of degradant, with the other source being from further degradation products of the Amadori rearrangement [53]. Formic acid appears to be an agent that may cause rare reductive degradation as in the case of *N*-methylvarenicline, a degradant of varenicline (Scheme 25) [54].

Formation of *N*-nitrosamines Due to the Reaction of Secondary Amine APIs or API-Related Fragments with Residual Nitrites in Excipients

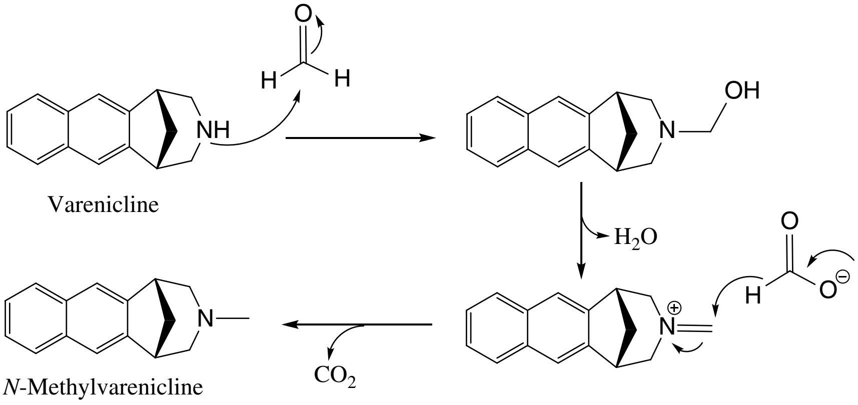
In mid-2018, it was reported to the US FDA and other regulatory agencies that *N*-nitrosodimethylamine (NDMA), an animal carcinogen, was present in valsartan in trace amounts by a manufacturer of the API [55]. Subsequently, NDMA and other low molecular weight *N*-nitrosamines (also referred to as small molecular weight *N*-nitrosamines), e.g., *N*-nitrosodiethylamine (NDEA) and *N*-nitroso-4-(methylamino) butyric acid (NMBA), were found in valsartan and other ARB drugs (angiotensin receptor blockers) [56]. These events prompted the regulatory agencies and the industry to look deeper into the root causes for the occurrence of *N*-nitrosamine impurities in drug products. Starting from 2019, NDMA was found to be present in drug classes other than ARBs, e.g., ranitidine, nizatidine, and metformin [57]. While NDMA is typically a process impurity for ARB drugs, it appears to be a degradant for the latter three drug products. In July 2021, it was announced that varenicline, a secondary amine smoking-cessation drug, contained *N*-nitroso varenicline by the original innovator of the drug [58]. Since that moment, it has become evident that the drugs with secondary amine APIs would be most



Scheme 23 The Maillard reaction between primary and secondary amine API with lactose, a very common reducing sugar excipient.



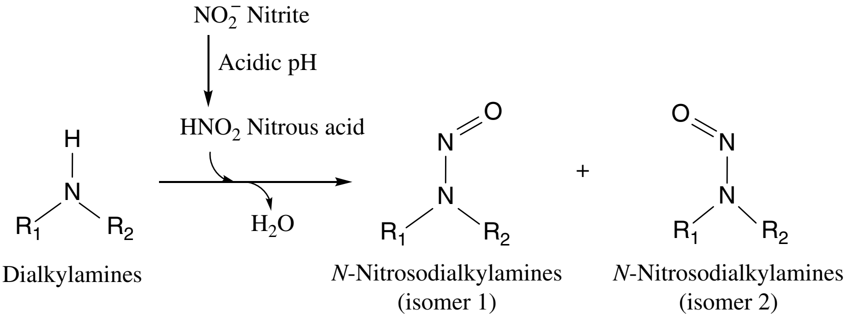
Scheme 24 Proposed mechanism for the formation of two ropinirole dimeric degradants [52].



Scheme 25 Formation of *N*-methylvarenicline.

vulnerable towards the formation of *N*-nitrosamine impurities, particularly for the drugs formulated with excipients that contain uncontrolled amounts of residual nitrites. This is because secondary amine APIs can readily react with residual nitrites, particularly in a somewhat acidic microenvironment of the formulations to form API-related *N*-nitrosamines (Scheme 26).

The *N*-nitrosamines of the secondary amine APIs and their secondary amine intermediates are now categorized as part of the "nitrosamine drug substance-related impurities" (NDSRIs), with the remaining part being *N*-nitrosamines of secondary amine fragments of the APIs, which are typically process impurities but could be degradants as well in certain cases. It is obvious that the highest risk for the



Scheme 26 Reaction of dialkylamines with nitrous acid to form *N*-nitrosodialkylamines. When $R_1 \neq R_2$, *E/Z* isomerism occurs. If R_1 or R_2 is replaced by an aryl substituent, the reactivity toward nitrous acid would be significantly reduced.

formation of NDSRIs comes from the secondary amine APIs, and therefore, as long as the secondary amine API-derived NDSRIs are controlled, the risk from their intermediates and fragments would be negligible since the amounts of the intermediates and fragments are usually three orders of magnitude lower than their APIs and all of them should have similar reactivity toward nitrous acid due to structural similarity. On the other hand, certain reducing agents, such as ascorbic acid and caffeic acid, can inhibit the formation of NDSRIs in drug products with secondary amine APIs [59]. For drug products with tertiary amine APIs, the main risk usually originates from their secondary amine impurities, despite the fact that the tertiary amine APIs could react with nitrous acid to eventually produce *N*-nitrosamines via a few intermediary steps. It seems that such multiplicity in the reaction mechanism would dramatically reduce the efficiency for the tertiary amine APIs to react with nitrous acid, which was reported to be only approximately 1/1,000 of the structurally similar secondary amines [60]. In the laboratories of the present author, we compared the efficiencies of triethylamine and diethylamine to react with nitrous acid, respectively, under the same conditions and found that triethylamine was only able to produce approximately 1/50 to 1/200 quantities of NDEA as compared to those by diethylamine [61]. An exception to the sluggishness of the tertiary amine reactivity toward *N*-nitrosamine formation was observed with those tertiary amines containing an electron-rich benzylic-type substituent [62].

Forced Degradation Study and Prediction of Real-Life Degradation Pathways

Forced degradation is also referred to as stress testing or simply stress. According to the ICH guidance document Q1(R2), *Stability Testing of New Drug Substances and Products*, “Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved" [5]. This statement clearly defines the purpose and significance of stress testing. Due to the complexity of the vast variety of drug molecules, ICH guidance only provides general principles for the stress testing of drug substances, except for the photolytic stress in solid state, where the light sources and duration for the exposure are clearly defined in Q1B [5]. At the high level, Q1A(R2) requires that the effect of temperature (typically in 10°C increments above that for the accelerated stability study), humidity (typically at 75%RH or higher), oxidation, and photolysis be evaluated on a drug substance. Furthermore, the susceptibility of the drug substance to hydrolysis under a wide range of pH also needs to be assessed.

In industry practice, there appears to be a wide variety of ways for performing stress testing or forced degradation [63, 64]. For the selection of hydrolytic conditions, although hydrochloric acid (HCl) and sodium hydroxide (NaOH) are usually the choices of selection for acidic and alkaline stress, respectively, the strengths of the acid and base, temperature, and duration of the hydrolysis can vary significantly without clear justification. With regard to oxidative stress, most studies seem to use hydrogen peroxide as the oxidizing reagent; nevertheless, hydrogen peroxide can usually only help predict nucleophilic oxidation of a drug molecule that contains tertiary amines, thioethers, or other structural moieties that are susceptible to nucleophilic oxidation, as explained above.

There have been efforts in trying to come up with best practices for performing forced degradation studies to maximize the predicting capability of stress testing, while minimizing the production of artificial degradation [65–68]. Such best practices focus on purposefully designed or mechanism-based stress conditions [68], which rely on the perceived degradation mechanism of the target drug molecule, as well as on the consideration of its degradation kinetics that would be correlated to the long-term and accelerated stability studies. Specifically, for the oxidative stress, not only hydrogen peroxide (typically not exceeding 3% concentration) needs to be used, but a free radical initiator such as AIBN or other initiators should also be employed for those drug molecules containing structural moieties or functional groups that are susceptible to free radical-mediated oxidation (e.g., benzylic positions, methylene positions alpha to double bonds and conjugated double bonds) [69]. Reynolds et al. reported the use of *N*-methylpyrrolidone (NMP)-water-air-heat system as a stress tool that can generate degradants from free radical-mediated and nucleophilic oxidation as well as hydrolytic degradation [70]. In the laboratories of the present author, benzoyl peroxide was also found to be capable of predicting oxidative degradants resulting from both free radical-mediated and nucleophilic oxidation [71]. For certain drug molecules, redox-active transition metal ion-mediated oxidation may be a significant pathway, although it was considered not relevant on a routine basis by certain researchers [68]. In those relevant cases, metal ions such as Fe^{2+}/Fe^{3+} may be used. As a matter of fact, metal-ion-mediated stress is a requirement by the Brazilian regulatory authority ANVISA [72]. For certain drug molecules containing "acidic CH or CH_2," it may be necessary to examine their susceptibility to base-catalyzed oxidation, which would be more favored in alkaline solutions prepared with a majority of aprotic solvents. The recommended conditions for oxidative stress studies are

Table 1.5 Recommended conditions for oxidative stress studies.

Type of oxidation	Recommended conditions	Notes
Nucleophilic	0.1%–3% H_2O_2, room temperature, 24 hours, protected from light	Higher temperature or under light may induce irrelevant degradation
Free radical-mediated	AIBN or benzoyl peroxide	For a mostly aqueous system, 4,4′-azobis(4-cyanovaleric acid) (ACVA), a water-soluble analog of AIBN, can be used [63]
Base-catalyzed	Aprotic solvent–water system with 1% of 1 M NaOH solution in methanol	Most common aprotic solvent: acetonitrile
Metal-ion-catalyzed	5 mM $FeSO_4$ and/or $FeCl_3$, 30°C, 2 weeks, protected from light	A combination of Fe^{2+}/Fe^{3+} may be used

Table 1.6 Recommended conditions for hydrolytic stress studies.

Type of stress	Recommended conditions	Notes
Acidic	0.1 N HCl, 60°C, 2 days or 1.0 N HCl, 60°C, 2 hours	H_2SO_4 may be used in place of HCl
Alkaline	0.1 N NaOH, 60°C, 2 days or 1.0 N NaOH, 60°C, 2 hours	KOH may be used in place of NaOH

summarized in Table 1.5, based on the recommendations reported in the literature as well as on the experience in the laboratories of the present author.

For the reagents of hydrolysis, 0.1 N HCl and 0.1 N NaOH solutions are generally used as the starting point [63]. While the temperature and duration of the hydrolytic stress can vary dramatically, the recommended conditions based on the literature as well as the experience at the laboratories of the present author are provided in Table 1.6; these conditions are usually strong enough to hydrolyze a typical amide bond (*Ea* ~20–25 kcal/mol) within the desirable range of ~5–20%.

For the stress of solid drug substances under dry heat or heat/moisture conditions, 60°C for 3 weeks would be approximately equivalent to 40°C for 6 months, assuming the type of degradation follows the Arrhenius equation with an activation energy (*Ea*) of ~25 kcal/mol. For the photo stress of solid drug substances, the photolysis conditions recommended by ICH Q1B are generally followed. In cases where photo stress study in solution state may be needed, the photolysis may be conducted with laboratory UV lighting or sunlight (Table 1.7).

There is no specific guidance for the stress testing of the finished drug products other than the photo stress testing, which is outlined in ICH Q1B. The heat and heat/moisture stress conditions typically used for API (e.g., those listed in

Table 1.7 Recommended conditions for stress studies in solid state.

Type of stress	Recommended conditions	Notes
Dry heat	60°C, ambient moisture, 3 weeks	[a]
Heat/high moisture	60°C, minimum 75% RH, 3 weeks	[a]
Heat/low moisture	60°C, 30% RH, 3 weeks	[a]This stress may be adequate for certain molecules susceptible to dehydration
Photolysis	Per conditions outlined in ICH Q1B	With and without packaging

[a] Assuming *Ea* of ~25 kcal/mol.

Table 1.7) can generally be directly applicable to the finished drug products. On the other hand, stress in solutions is generally not quite relevant to the finished drug products unless the dosage form is in liquid formulation. Preferably, the forced degradation study for solid dosage forms can be combined with the compatibility study that is typically conducted during the formulation development stage. The compatibility study is usually performed on a one-to-one basis between the API and each excipient. Sometimes, compatibility study on a one-to-two or more basis may be warranted.

Overall, the practices in stress or forced degradation studies vary significantly among the practicing scientists, despite the efforts to streamline a best practice that would be applicable in the vast majority cases. Nevertheless, it is now generally agreed that the level of the degradation should be controlled in ~5–20% range in order to avoid excessive degradation, which typically would result in irrelevant degradation profiles. While artificial degradants usually cannot be avoided completely, preferably the degradation profiles obtained under stress conditions will include the real degradation profiles, the ones obtained under the long-term stability conditions. At the same time, the practicing scientists should be able to distinguish the artificial degradants, such as the ones derived from the involvement of the organic cosolvents used to solubilize the drug substances in solution stress studies, from the real degradants.

Concluding Remarks

This introduction is intended to provide a quick overview of the most common drug degradation chemistry and pathways. For an in-depth knowledge in this regard, readers are encouraged to refer to the relevant chapters of *Organic Chemistry of Drug Degradation*. The 300+ drug molecules covered in the monographs of the current book, *Compendium of Drug Degradation Pathways*, would still represent a tiny fraction of all the drug products on the market. The present author hopes that readers can extrapolate from the examples in the monographs to other drug molecules based upon structural similarity and analogy of degradation chemistry.

References

1 Jamrógiewicz, M. and Merchel, M. (2018). A history of the physical and chemical stability of pharmaceuticals – a review. *Acta Pol. Pharm. Drug Res.* 75: 297–304.

2 Connors, K.A., Amidon, G.L., and Stella, V.J. (1986). *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists*, 2e. New York: Wiley-Interscience.

3 Yasmeen, A. and Sofi, G. (2019). A review of regulatory guidelines on stability studies. *J. Phytopharmacol.* 8: 147–151.

4 ICH website. https://www.ich.org/page/history (accessed 28 November 2022).

5 ICH website https://www.ich.org/page/quality-guidelines (accessed 28 November 2022).

6 WHO guidelines on stability testing (30 September 2018). Annex 10, WHO Technical Report Series 1010, 2018. https://www.who.int/publications/m/item/trs1010-annex10 (accessed 11 June 2025).

7 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

8 Tian, Y., Lin, J., Chen, F. et al. (2020). Structure elucidation and formation mechanistic study of a methylene-bridged pregabalin dimeric degradant in pregabalin extended-release tablets. *Int. J. Pharm.* 575: 118910.

9 Marquez, V.E., Tseng, C.K.-H., Kelley, J.A. et al. (1987). 2′,3′-Dideoxy-2′-fluoro-ara-A. An acid-stable purine nucleoside active against human immunodeficiency virus (HIV). *Biochem. Pharmco.* 36: 2719–2722.

10 Namchuk, M.N., McCarter, J.D., Becalski, A. et al. (2000). The role of sugar substituents in glycoside hydrolysis. *J. Am. Chem. Soc.* 122: 1270–1277.

11 Waterman, K.C., Adami, R.C., Alsante, K.M. et al. (2002). Hydrolysis in pharmaceutical formulations. *Pharm. Dev. Technol.* 7: 113–146.

12 Bruice, T.C. and Mayahi, M.F. (1960). The influence of the leaving tendency of the phenoxy group on the ammonolysis and hydrolysis of substituted phenyl acetates. *J. Am. Chem. Soc.* 82: 3067–3071.

13 Robinson, B.A. and Tester, J.W. (1990). Kinetics of alkaline hydrolysis of organic esters and amides in neutrally-buffered solution. *Int. J. Chem. Kinet.* 22: 431–448.

14 Singer, A.K. (2019). Activation parameter and solvent effect on solvolysis of ethyl benzoate in aquo-organic solvent system. https://ajrconline.org/HTML_Papers/Asian. *J. Res. Chem.* 12: 99. accessed 27 October 2022.

15 Tsujikawa, H. and Inoue, H. (1966). The reaction rate of the alkaline hydrolysis of ethyl acetate. *Bull. Chem. Soc. Jpn.* 39: 187–1842.

16 Kaufman, M.J. (1990). Rate and equilibrium constants for acid-catalyzed lactone hydrolysis of HMG-CoA reductase inhibitors. *Int. J. Pharm.* 66: 97–106.

17 Meresaar, U. and Bratt, L. (1974). Hydrolysis of amides. Alkaline and general acid catalyzed alkaline hydrolysis of some substituted acetamides and benzamides. *Acta Chem. Scand. A.* 28: 715–722.

18 King, A.P. and Krespan, C.G. (1974). Secondary amines from trifluoroacetamides. *J. Organomet. Chem.* 39: 1315–1316.

19 Ghosh, A.K. and Brindisi, M. (2015). Organic carbamates in drug design and medicinal chemistry. *J. Med. Chem.* 58: 2895–2940.

20 Dittert, L.W. and Higuchi, T. (1962). Rates of hydrolysis of carbamate and carbonate esters in alkaline solution. *J. Pharm. Sci.* 52: 852–857.

21 Chapman, T.M. (1989). Models for polyurethane hydrolysis under moderately acidic conditions: a comparative study of hydrolysis rates of urethanes, ureas, and amides. *J. Polymer Sci. A: Polymer Chem.* 27: 1993–2005.

22 Loftsson, T., Olafsdottir, B.J., and Baldvinsdottir, J. (1992). Estramustine: hydrolysis, solubilization, and stabilization in aqueous solutions. *J. Int. J. Pharm.* 79: 107–112.

23 Stroud, N., Richardson, N.E., Davies, D.J.G., and Norton, D.A. (1980). Quality control of prednisolone sodium phosphate. *Analyst* 105: 455–461.

24 Flynn, G.L. and Lamb, D.J. (1970). Factors influencing solvolysis of corticosteroid-21-phosphate esters. *J. Pharm. Sci.* 59: 1433–1438.

25 Garrison, A.W. and Boozer, C.E. (1968). The acid-catalyzed hydrolysis of a series of phosphoramidates. *J. Am. Chem. Soc.* 90: 3486–3494.

26 Skrdla, P., Abrahim, A., and Wu, Y. (2006). An HPLC chromatographic reactor approach for investigating the hydrolytic stability of a pharmaceutical compound. *J. Pharm. Biomed. Anal.* 41: 883–890.

27 Malin, J.J., Suárez, I., Priesner, V. et al. (2021). Remdesivir against COVID-19 and other viral diseases. *Clin. Microbiol. Rev.* 34: e00162–e00120.

28 Searles, S. and Nukina, S. (1959). Cleavage and rearrangement of sulfonamides. *Chem. Rev.* 59: 1077–1103.

29 Shaw, W.H.R. and Bordeaux, J.J. (1955). The decomposition of urea in aqueous media. *J. Am. Chem. Soc.* 77: 4729–4733.

30 Garrett, E.R., Bojarski, J.T., and Yakatan, G.J. (1971). Kinetics of hydrolysis of barbituric acid derivatives. *J. Pharm. Sci.* 60: 1145–1154.

31 Bansal, G., Singh, M., Jindal, K.C., and Singh, S. (2008). Ultraviolet-Photodiode array and high-performance liquid chromatographic/mass spectrometric studies on forced degradation behavior of glibenclamide and development of a validated stability-indicating method. *J. AOAC Inter.* 91: 709–719.

32 Wiseman, E.H., Chiaini, J., and Pinson, R. Jr. (1964). Determination of sulfamylurea hypoglycemic agents and their metabolites in biological fluids. *J. Pharm. Sci.* 53: 766–769.

33 Pritchard, J.G. and Long, F.A. (1956). Kinetics and mechanism of the acid-catalyzed hydrolysis of substituted ethylene oxides. *J. Am. Chem. Soc.* 78: 2667–2670.

34 Koskikallio, J. and Whalley, E. (1959). Effect of pressure on the spontaneous and the base-catalyzed hydrolysis of epoxides. *Can. J. Chem.* 37: 783–787.

35 Arava, V.R., Siripalli, U.B.R., and Bandatmakuru, S.R. (2007). Novel acid catalysed rearrangement of duloxetine. *Indian J. Chem. Sect. B* 46B: 1695–1698.

36 Brandl, M. and Gu, L. (1992). Degradation of tobramycin in aqueous solution. *Drug Dev. Ind. Pharm.* 18: 1423–1436.

37 Miller, D.M., Buettner, G.R., and Aust, S.D. (1990). Transition metals as catalysts of "autoxidation" reactions. *Free Radic. Biol. Med.* 8: 95–108.

38 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*. Cambridge: Royal Society of Chemistry.

39 Smith, G.B., DiMichele, L., Colwell, L.F. Jr. et al. (1993). Autooxidation of simvastatin. *Tetrahedron Lett.* 49: 4447–4462.

40 Mao, B., Abrahim, A., Ge, Z. et al. (2002). Examination of rofecoxib solution decomposition under alkaline and photolytic stress conditions. *J. Pharm. Biomed. Anal.* 28: 1101–1113.

41 Harmon, P.A., Biffar, S., Pitzenberger, S.M., and Reed, R.A. (2005). Mechanism of the solution oxidation of rofecoxib under alkaline conditions. *Pharm. Res.* 22: 1716–1726.

42 Hong, J., Lee, E., Carter, J.C. et al. (2004). Antioxidant-accelerated oxidative degradation: a case study of transition metal ion catalyzed oxidation in formulation. *Pharm. Dev. Technol.* 9: 171–179.

43 Wu, S., Waugh, W., and Stella, V.J. (2000). Degradation pathways of a peptide boronic acid derivative, 2-Pyz-(CO)-Phe-Leu-B$(OH)_2$. *J. Pharm. Sci.* 89: 758–765.

44 Reed, R.A., Harmon, P., Manas, D. et al. (2003). The role of excipients and package components in the photostability of liquid formulations. *PDA J. Pharm. Sci. Technol.* 57: 351–368.

45 Dong, J., Karki, S.B., Parikh, M. et al. (2012). Oxidative degradation studies of an oxazolidinone-derived antibacterial agent, RWJ416457, in aqueous solutions. *Drug Dev. Ind. Pharm.* 38: 1289–1297.

46 Pardo-Andreu, G.L., Delgado, R., Nunez-Selles, A.J., and Vercesi, A.E. (2006). Dual mechanism of mangiferin protection against iron-induced damage to 2-deoxyribose and ascorbate oxidation. *Pharmacol. Res.* 53: 253–260.

47 Li, M. (2012). Photochemical degradation. In: *Organic Chemistry of Drug Degradation*. Cambridge: Royal Society of Chemistry.

48 Borsarelli, C.D., Braslavsky, S.E., Sortino, S. et al. (2000). Photodecarboxylation of ketoprofen in aqueous solution. A time-resolved laser-induced optoacoustic study. *Photochem. Photobiol.* 72: 163–171.

49 Cosa, G.L., Martinez, L., and Scaiano, J.C. (1999). Influence of solvent polarity and base concentration on the photochemistry of ketoprofen: independent singlet and triplet pathways. *Phys. Chem. Chem. Phys.* 1: 3533–3537.

50 Wu, L., Hong, T.Y., and Vogt, F.G. (2007). Structural analysis of photo-degradation in thiazole-containing compounds by LC–MS/MS and NMR. *J. Pharm. Biomed. Anal.* 44: 763–772.

51 Li, M. (2012). Drug–excipient interactions and adduct formation. In: *Organic Chemistry of Drug Degradation*. Cambridge: Royal Society of Chemistry.

52 Kuang, Z., Huang, T., Li, D. et al. (2020). Structure elucidation and mechanistic study of a new dimeric degradant in ropinirole hydrochloride extended-release tablets. *Pharm. Res.* 37: 136. https://doi.org/10.1007/s11095-020-02863-3.

53 Wirth, D.D., Baertschi, S.W., Johnson, R.A. et al. (1998). Maillard reaction of lactose and fluoxetine hydrochloride, a secondary amine. *J. Pharm. Sci.* 87: 31–39.

54 Waterman, K.C., Arikpo, W.B., Fergione, M.B. et al. (2008). N-Methylation and N-formylation of a secondary amine drug (varenicline) in an osmotic tablet. *J. Pharm. Sci.* 97: 1499–1507.

55 US FDA news release on July 13, 2018: FDA announces voluntary recall of several medicines containing valsartan following detection of an impurity.

https://www.fda.gov/news-events/press-announcements/fdaannounces-voluntary-recall-several-medicines-containing-valsartanfollowing-detection-impurity (accessed 12 January 2025).

56 Zhang, J., Selaya, S.D., Shakleya, D. et al. (2023). Rapid quantitation of four nitrosamine impurities in angiotensin receptor blocker drug substances. *J. Pharm. Sci.* 112: 1246–1254.

57 US FDA news release on September 24, 2019: FDA announces voluntary recall of Sandoz ranitidine capsules following detection of an impurity. https://www.fda.gov/news-events/press-announcements/fda-announces-voluntary-recall-sandoz-ranitidine-capsules-following-detection-impurity (accessed 12 January 2025).

58 Company announcement posted by the US FDA on July 19, 2021: Pfizer issues a voluntary nationwide recall for twelve lots of CHANTIX® (Varenicline) tablets due to *N*-nitroso varenicline content. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/pfizer-issues-voluntary-nationwide-recall-twelve-lots-chantixr-varenicline-tablets-due-n-nitroso (accessed 12 January 2025).

59 Shakleya, D., Asmelash, B., Alayoubi, A. et al. (2023). Bumetanide as a model NDSRI substrate: N-nitrosobumetanide impurity formation and its inhibition in bumetanide tablets. *J. Pharm. Sci.* 112: 3075–3087.

60 Ashworth, I.W., Dirat, O., Teasdale, A., and Whiting, M. (2020). Potential for the formation of *N*-nitrosamines during the manufacture of active pharmaceutical ingredients: an assessment of the risk posed by trace nitrite in water. *Org. Process. Res. Dev.* 24: 1629–1646.

61 Dong, P., Li, M. Unpublished results.

62 López-Rodríguez, R., McManus, J.A., Murphy, N.S. et al. (2020). Pathways for *N*-nitroso compound formation: secondary amines and beyond. *Org. Process. Res. Dev.* 24: 1558–1585.

63 Singh, S. and Bakshi, M. (2000). Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm. Tech. On-line. https://api.semanticscholar.org/CorpusID:54644738 (accessed 12 January 2025).

64 Alsante, K.M., Martin, L., and Baertschi, S.W. (2003). A stress testing benchmarking study. *Pharm. Technol.* 27: 60–72.

65 Reynolds, D.W., Facchine, K.L., Mullaney, J.F. et al. (2002). Available guidance and best practices for conducting forced degradation studies. *Pharm. Technol.* 26: 48–56.

66 Nelson, E.D., Harmon, P.A., Szymanik, R.C. et al. (2006). Evaluation of solution oxygenation requirements for azonitrile-based oxidative forced degradation studies of pharmaceutical compounds. *J. Pharm. Sci.* 95: 1527–1539.

67 Alsante, K.M., Ando, A., Brown, R. et al. (2007). The role of degradant profiling in active pharmaceutical ingredients and drug products. *Adv. Drug Deliv. Rev.* 59: 29–37.

68 Maheswaran, R. (2012). Scientific considerations of forced degradation studies in ANDA submissions. *Pharm. Technol.* 36: 73–80.

69 Klick, S., Muijselaar, P.G., Waterval, J. et al. (2005). Toward a generic approach for stress testing of drug substances and drug products. *Pharm. Technol.* 29: 48–66.

70 Reynolds, D.W., Galvani, M., Hicks, S.R. et al. (2012). The use of *N*-methylpyrrolidone as a cosolvent and oxidant in pharmaceutical stress testing. *J. Pharm. Sci.* 101: 761–776.

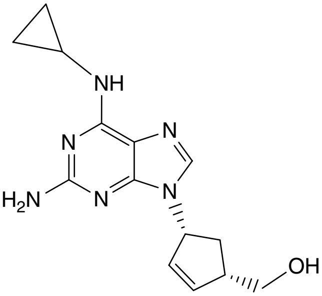
71 Unpublished results from the present author's laboratories.

72 Tattersall, P., Asawasiripong, S., Takenaka, I., and Castoro, J.A. (2016). Impact from the recent issuance of ANVISA resolution RDC-53/2015 on pharmaceutical small molecule forced degradation study requirements. *Am. Pharm. Rev.* https://www.americanpharmaceuticalreview.com/Featured-Articles/184364-Impact-from-the-Recent-Issuance-of-ANVISA-Resolution-RDC-53-2015-on-Pharmaceutical-Small-Molecule-Forced-Degradation-Study-Requirements/ (accessed 11 June 2025).

2

Abacavir to Azithromycin

1: Abacavir



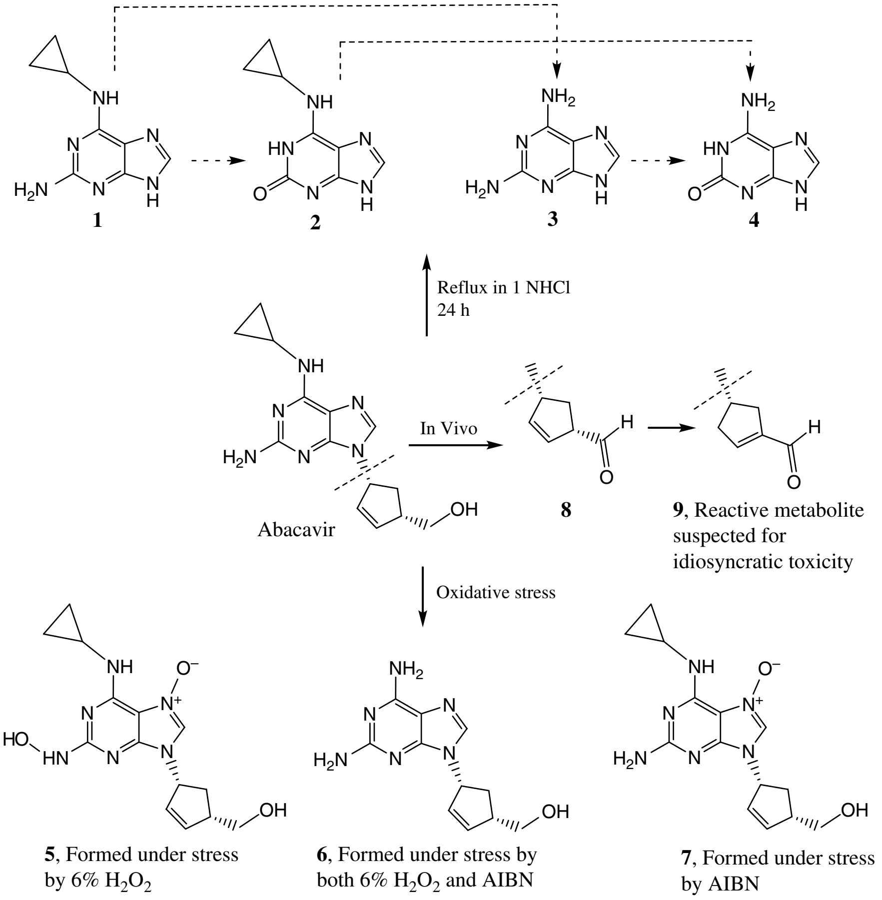
Chemical name: {(1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl] cyclopent-2-en-1-yl}methanol
Brand name: Ziagen
Formula: $C_{14}H_{18}N_6O$
Monoisotopic protonated ion (*m/z*): 287.1615
Molecular weight: 286.34
CAS number: 136470-78-5
Common salt form: Sulfate

Abacavir, a carbocyclic guanosine analog, is available in the form of sulfate salt. Its bicyclic pyrimidine-imidazole core contains three substituents: an amino, cyclopropylamino, and substituted cyclopentenyl group. In the literature, there appears to be no report regarding its degradation chemistry in commercial formulations. On the other hand, a couple of forced degradation studies were reported. In a study reported by Seshachalam et al. [1], abacavir was stable when refluxed in both 0.1 N HCl and 0.1 N NaOH for up to 5 days. In another study, Rao et al. employed 1 N HCl and 1 N NaOH as the acidic and basic stress conditions [2]. After refluxing for 24 hours, abacavir was completely decomposed to form five degradants under the acidic stress, while it remained unchanged under the basic stress. The structures of the

Compendium of Drug Degradation Pathways, First Edition. Min Li.

four major degradants (**1–4**) were proposed based on LC–MS results and MS/MS fragmentation pathways. Two types of degradation, that is, deamination and dealkylation, account for the formation of **1–4**. When a solid sample of abacavir was heated at 100°C for 10 days, no decomposition was observed [2].

Under oxidative stress conditions, abacavir was stable in 3% H_2O_2 at ambient temperature for 7 days [2]. Only at higher concentrations of H_2O_2 (6% and 10%, respectively) [1, 2] was abacavir found to decompose. In the study by Rao et al. [2], abacavir gave rise to degradants **5** and **6**. The same workers also stressed the drug substance using AIBN, and under this radical-initiated stress condition, two degradants, **6** and **7**, were formed. It is not surprising that no oxidation of the hydroxyl group of abacavir was observed, which is consistent with the fact that a typical hydroxyl group is not very susceptible to oxidation under the oxidative stress conditions utilized in the forced degradation studies discussed here [3].



Scheme 1 Degradation pathways of abacavir under stress conditions. Degradant structures were proposed based on only LC–MS results and MS/MS fragmentation pathways. It is likely that all the degradants, except perhaps 6 and 7, may be artificial degradants.

This hydroxyl functionality, however, is susceptible to oxidation in vivo and the aldehyde metabolite formed (**8**) can further degrade to a Michael acceptor (**9**), which is suspected to be responsible for the severe toxicity of the drug in certain patient populations [4, 5].

Under photo stress conditions, abacavir was stable in both solution and solid forms when stressed under UV irradiation at 320 nm for 5 days, although the power of the irradiation source was not disclosed [2]. When stressed under the ICH photo stability condition, however, a low-level degradant was seen, the structure of which was not analyzed [1].

Overall, it appears that abacavir is quite stable. It is likely that the degradants, except perhaps **6** and **7**, observed in the stress studies may be artificial degradants (Scheme 1).

References

1 Seshachalam, U., Haribabu, B., and Chandrasekhar, K.B. (2007). Development and validation of a reverse-phase liquid chromatographic method for assay and related substances of abacavir sulfate. *J. Sep. Sci.* 30: 28–34.

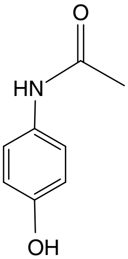
2 Rao, R.N., Vali, R.M., Ramachandra, B., and Raju, S.S. (2011). Separation and characterization of forced degradation products of abacavir sulphate by LC–MS/MS. *J. Pharm. Biomed. Anal.* 54: 279–285.

3 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*, 48–109. Cambridge: Royal Society Chemistry Publishing.

4 Charneira, C., Godinho, A.L.A., Oliveira, M.C. et al. (2011). Reactive aldehyde metabolites from the anti-HIV drug abacavir: amino acid adducts as possible factors in abacavir toxicity. *Chem. Res. Toxicol.* 24: 2129–2141.

5 Charneira, C., Grilo, N.M., Pereira, S.A. et al. (2012). *N*-terminal valine adduct from the anti-HIV drug abacavir in rat haemoglobin as evidence for abacavir metabolism to a reactive aldehyde in vivo. *British J. Pharmacol.* 167: 1353–1361.

2: Acetaminophen (Paracetamol)

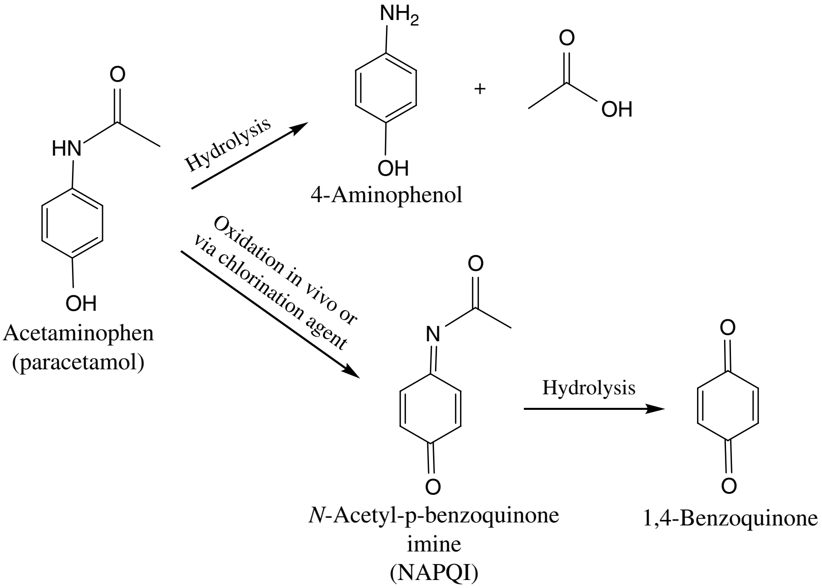


Acetaminophen
(paracetamol)

Chemical name: *N*-Acetyl-4-aminophenol; *N*-(4-hydroxyphenyl)acetamide
Brand name: Tylanol
Formula: $C_8H_9NO_2$
Monoisotopic protonated ion (*m/z*): 152.0706
Molecular weight: 151.17
CAS number: 103-90-2

Acetaminophen is the acetamide of a substituted aniline. The amide linkage of the drug molecule appears to be the only weak spot that may be susceptible to degradation (hydrolysis) in commercial formulations. The activation energy (*Ea*) for the hydrolysis of the amide linkage was determined to be on the order of ~17.5 kcal/mol between pH 2 and 9 [1]. In two more recent studies, the *Ea* for the hydrolysis in 0.5 M HCl was found to be 16.69 and 16.3 kcal/mol, respectively [1, 2]. Hence, although the strength of this particular amide bond is somewhat weaker than a typical aliphatic amide bond, which is on the order of ~20 kcal/mol or higher [3], it is expected that it would still be quite resistant toward hydrolytic degradation. The degradant formed from the hydrolysis is 4-aminophenol (or 4-hydroxyanaline; Scheme 2), which is also a process impurity [4] that has particular toxicological concern [5]. Hence, the pharmacopeia limit for 4-aminophenol in acetaminophen drug substance is a maximum of 0.005% or 50 ppm [6]. In formulated drug products, 4-aminophenol is controlled at maximum 0.15% by major pharmacopeias [7], although internal control by some company was reported to be still at 50 ppm for drug products [8].

One of the critical metabolites of acetaminophen is *N*-acetyl-*p*-benzoquinone imine (NAPQI), which has now been confirmed as the culprit for causing liver toxicity and failure in cases of acetaminophen overdose [9, 10]. There has been no report of this chemical entity in pharmaceutical preparations of acetaminophen. On the other hand, acetaminophen could be oxidized to NAPQI and its subsequent hydrolytic degradant, 1,4-benzoquinone, by residual chlorine in drinking water [11–13].



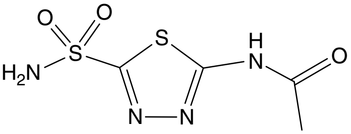
Scheme 2 In vitro and in vivo degradation of acetaminophen.

References

1. Koshy, K.T. and Lach, J.L. (1961). Stability of aqueous solutions of *N*-acetyl-*p*-aminophenol. *J. Pharm. Sci.* 50: 113–118.
2. Chen, G., Ye, J., Bao, H., and Yang, P. (2002). Determination of the rate constants and activation energy of acetaminophen hydrolysis by capillary electrophoresis. *J. Pharm. Biomed. Anal.* 29: 843–850.
3. Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 19. Cambridge, Royal Society Chemistry Publishing.
4. Fairbrother, J.F. (1974). *Analytical profile of drug substances*, vol. 3 (ed. K. Florey), 1–109. New York: Academic Press.
5. Prescott, L.F. (1996). *Paracetamol (Acetaminophen): A Critical Bibliographic Review*. London: Taylor & Francis.
6. USP Monograph Official Acetaminophen. 10.31003/USPNF_M150_04_01 (accessed 1 May 2020).
7. USP Monograph Acetaminophen tablets. 10.31003/USPNF_M200_05_01 (accessed 1 October 2021).
8. Bloomfield, M.S. (2002). A sensitive and rapid assay for 4-aminophenol in paracetamol drug and tablet formulation, by flow injection analysis with spectrophotometric detection. *Talanta* 580: 1301–1310.

9 Dahlin, D.C. and Nelson, S.D. (1982). Synthesis, decomposition kinetics, and preliminary toxicological studies of pure *N*-acetyl-*p*-benzoquinone imine, a proposed toxic metabolite of acetaminophen. *J. Med. Chem.* 25: 885–886.
10 Kalgutkar, A.S., Gardner, I., Obach, R.S. et al. (2005). A comprehensive listing of bioactivation pathways of organic functional groups. *Curr. Drug Metab.* 6: 161–225.
11 Bedner, M. and Maccrehan, W.A. (2006). Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and *N*-acetyl-*p*-benzoquinone imine. *Environ. Sci. Technol.* 40: 516–522.
12 Gibs, J., Stackelberg, P.E., Furlong, E.T. et al. (2007). Persistence of pharmaceuticals and other organic compounds in chlorinated drinking water as a function of time. *Sci. Total Environ.* 373: 240–249.
13 Xagoraraki, I., Hullman, R., Song, W. et al. (2008). Effect of pH on degradation of acetaminophen and production of 1,4-benzoquinone in water chlorination. *J. Water Supply: Res. Tech. – AQUA* 57: 381–390.

3: Acetazolamide



Chemical name: 5-Acetamido-1,3,4-thiadiazo-2-yl-sulfonamide
Brand name: Diamox
Formula: $C_4H_6N_4O_3S_2$
Monoisotopic protonated ion (*m/z*): 222.9954
Molecular weight: 223.24
CAS number: 59-66-5

Acetazolamide, a carbonic anhydrase inhibitor, has a five-membered thiadiazo ring that is connected to a sulfonamide group at the 2-position and an acetamide at the 5-position. According to a few stress studies, the drug molecule is most susceptible to hydrolytic degradation at the acetamide bond to yield degradant **1** (Scheme 3) [1–4]. This is consistent with the fact that a sulfonamide bond is far more stable than an acetamide bond [5]. Parasrampuria and Gupta studied the hydrolytic stability of acetazolamide in phosphate and citrate buffers, respectively, throughout the pH range [1]. It was found that acetazolamide was most stable at pH 4 with an activation energy (*Ea*) of 16.6 kcal/mol. The hydrolytic degradation was determined to be mediated via a specific acid–base catalysis. The rate constants under the acidic and basic catalysis, K_H and K_{OH}, were estimated to be 0.23 and 1.56/d, respectively. The pH stability profile of acetazolamide was confirmed by a study performed by Khamis et al. [2]. In addition, the latter study also determined that the *Ea* for the hydrolysis of acetazolamide in 0.01 N NaOH (pH 12) was 19.6 kcal/mol.[1]

In an oxidative stress of acetazolamide in 30% H_2O_2 at room temperature for 24 hours, an oxidative degradant was found [3]. There was no further structure elucidation by the original workers other than the HPTLC study with UV detection and scanning. However, based on its UV spectrum, the oxidative degradant could be the *S*-oxide (**2**) of acetazolamide, as the UV profile of the latter is dramatically different from that of acetazolamide. The conjugation to the newly formed sulfonyl group should be responsible for the significant shift of UV absorption to longer wavelengths observed. Since the oxidative stress appears to be excessive (in 30% H_2O_2), it is uncertain if this degradant would occur in commercial formulations under long-term stability conditions.

It has been reported that acetazolamide shows phototoxicity clinically. In a photochemical study by Vargas et al. [6], acetazolamide was found to undergo decomposition under both UV-A and UV-B irradiation. Two degradants (**3** and **4**, Scheme 3) were observed under aerobic irradiation, while only **3** was formed under anaerobic irradiation. The two degradants were also produced under a photosensitized

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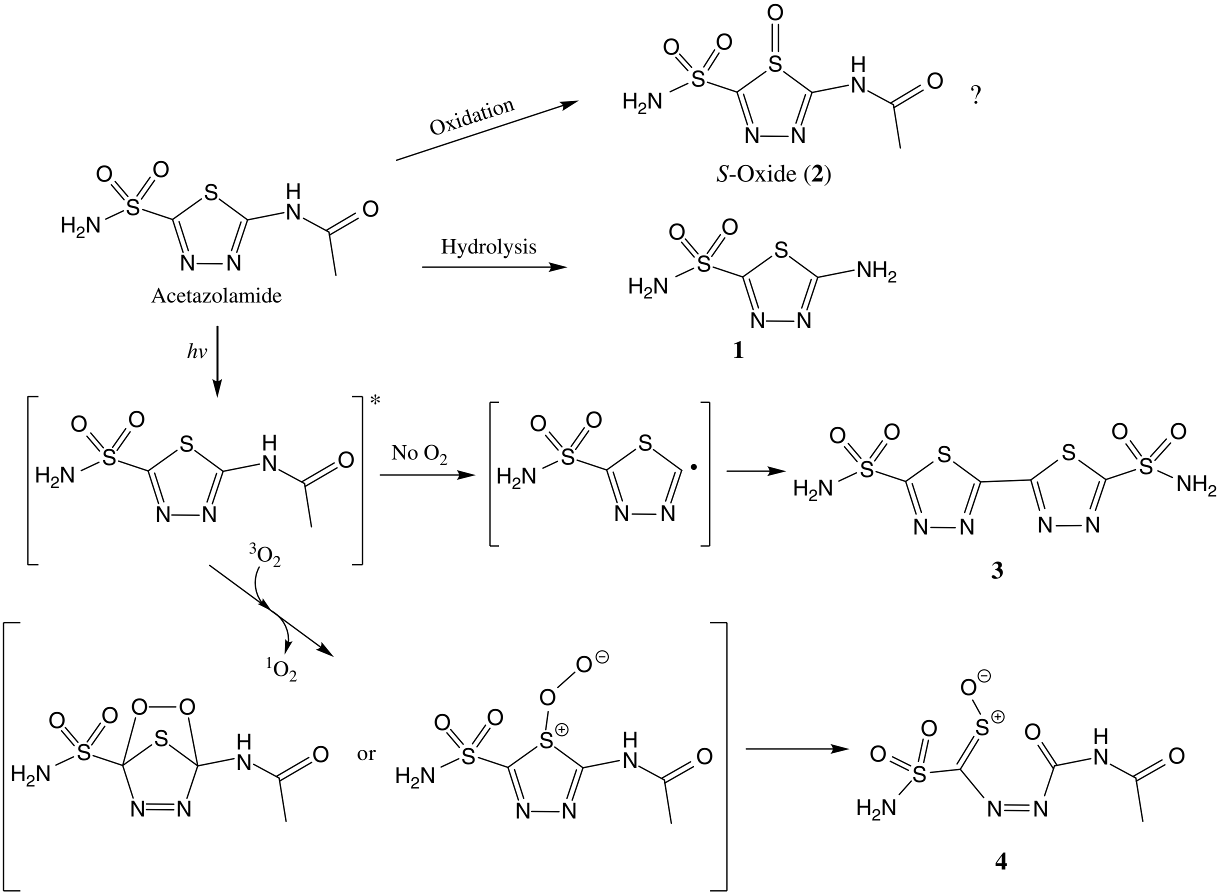
[1] The *Ea* was incorrectly reported as 19.6 kJ/mol in Ref. [2]. It should be 19.6 kcal/mol based on the raw data reported in Ref. [2] as calculated by the current author.



Scheme 3 Degradation pathways of acetazolamide under stress conditions. The structure of degradant 2 was tentative based on its reported UV spectrum in Reference [3].

condition where singlet oxygen ($^1O_2$) was generated via rose Bengal. Therefore, acetazolamide can be both a $^1O_2$ sensitizer and quencher. In a more recent study, Valencia et al. [7] found that acetazolamide is an efficient $^1O_2$ quencher but a poor $^1O_2$ sensitizer (quantum yields 0.015–0.097 in organic protic and aprotic solvents). Based on this finding, it was suggested that the phototoxicity of acetazolamide might involve species other than $^1O_2$ as originally thought.

References

1 Parasrampuria, J. and Gupta, V.D. (1989). Preformulation studies of acetazolamide: effect of pH, two buffer species, ionic strength, and temperature on its stability. *J. Pharm. Sci.* 78: 855–857.

2 Khamis, E.F., Abdel-Hamid, M., Hassan, E.M. et al. (1993). A stability-indicating first-derivative spectrophotometric assay of acetazolamide and its use in dissolution and kinetic studies. *J. Clin. Pharm. Ther.* 18: 97–101.

3 Ramesh, B., Srimannaraya, P., Reddy, A.S., and Sitadevi, P. (2011). Densitometric evaluation of stability-indicating HPTLC method for the analysis of acetazolamide and a study of degradation kinetics. *J. Pharm. Res.* 4: 429–433.

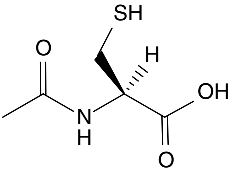
4 Srinivasu, P., Subbarao, D.V., Vegesnaa, R.V.K., and Babu, K.S. (2010). A validated stability-indicating LC method for acetazolamide in the presence of degradation products and its process-related impurities. *J. Pharm. Biomed. Anal.* 52: 142–148.

5 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 16–47. Cambridge: Royal Society Chemistry Publishing.

6 Vargas, F., Hisbeth, M.V., and Rojas, J.K. (1998). Photolysis and photosensitized degradation of the diuretic drug acetazolamide. *J. Photochem. Photobiol. A Chem.* 118: 19–23.

7 Valencia, C., Tobon, E., and Castano, C. (2013). Acetazolamide as a singlet molecular oxygen quencher. *J. Photochem. Photobiol. A Chem.* 251: 113–117.

4: Acetylcysteine



Chemical name: *N*-Acetyl-L-cysteine; 2-Acetamido-3-sulfanylpropanoic acid
Brand name: Mucolysin, Acetadote
Formula: $C_5H_9NO_3S$
Monoisotopic protonated ion (*m/z*): 164.0376
Molecular weight: 163.19
CAS number: 616-91-1

Acetylcysteine is an amino acid derivative. Its pharmacological effects and degradation behavior are primarily attributable to the sulfhydryl or thiol group of the cysteine moiety. The thiol group is a reducing agent as well as a nucleophile. As a reducing agent, acetylcysteine is used as a mucolytic drug because it can liquefy mucus by reducing the disulfide bonds of mucous proteins. As a nucleophile, acetylcysteine is employed as an antidote for liver toxicity caused by acetaminophen overdose. In that capacity, acetylcysteine is capable of intercepting *N*-acetyl-*p*-benzoquinone imine (NAPQI), a toxic, highly electrophilic metabolite of acetaminophen,[2] in a way similar to the action of glutathione, the body's natural defense agent against electrophilic metabolites.

Farquhar et al. studied the solution stability of acetylcysteine in aqueous formulations [1]. Under aerobic conditions and within a pH range of 5–8, *N,N′*-diacetylcystine is the predominant degradant, resulting from the oxidative coupling of the API. The formation of a disulfide bridge, such as the one in this case, is generally catalyzed by trace amounts of redox-active transition metal ions [2]. This catalytic process can be inhibited by the use of a chelating agent, usually EDTA. For this reason, a majority of commercial aqueous formulations of acetylcysteine use EDTA as the stabilizing chelating agent [3]. Since the ultimate oxidizing agent is molecular oxygen or O_2, complete removal of O_2 was shown to be effective in shutting down the oxidative coupling pathway of acetylcysteine. The effectiveness of this approach achieved through deaeration and nitrogen purging has also been demonstrated in kilogram-scale production [3]. Under anaerobic conditions, cysteine was found to be the main degradant within a pH range of 6.5–7 [3]. In the original study of Farquhar et al., cysteine was observed along with *N,S*-diacetylcysteine and *S*-acetylcysteine in pH 2 aqueous solutions under anaerobic conditions. The formation of *N,S*-diacetylcysteine is apparently via an intermolecular transacylation mechanism, during which process cysteine is also formed. On the other hand, the formation of *S*-acetylcysteine would be more likely mediated through an intramolecular transacylation

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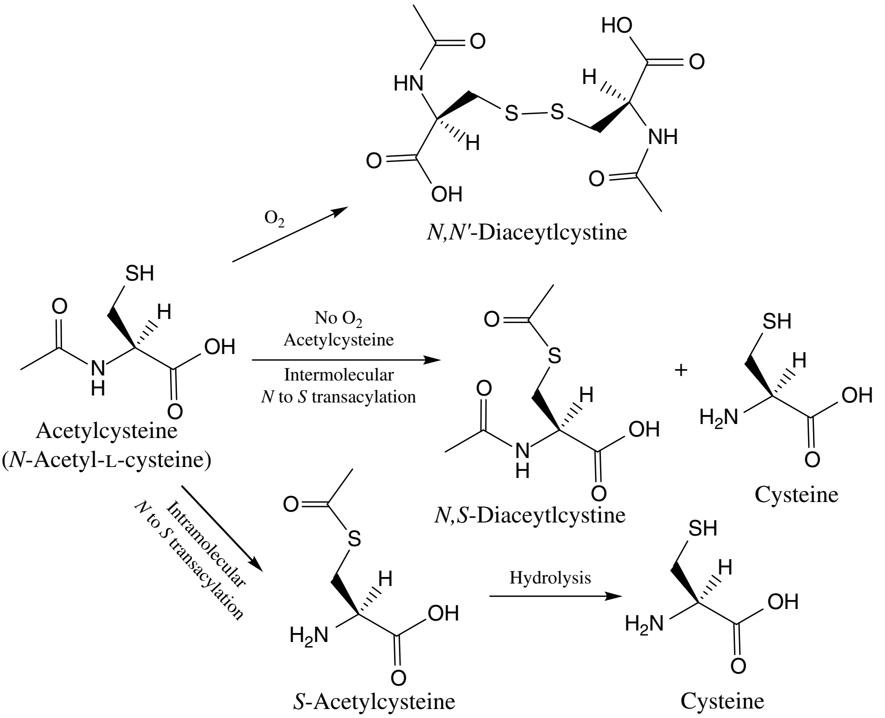
[2] Refer to the monograph of acetaminophen and the relevant references cited therein.



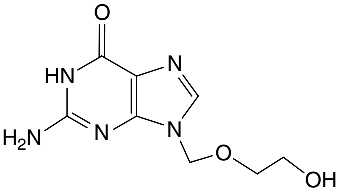
Scheme 4 Degradation pathways of acetylcysteine.

mechanism. The resulting degradant, *S*-acetylcysteine, should be susceptible to hydrolysis to yield cysteine, particularly in the pH range near neutrality and above. The degradation pathways of acetylcysteine are summarized in Scheme 4.

References

1. Farquhar, J., Finlay, G., Ford, P.A., and Martin-Smith, M. (1985). A reversed-phase high-performance liquid chromatographic assay for the determination of *N*-acetylcysteine in aqueous formulations. *J. Pharm. Biomed. Anal.* 3: 279–285.
2. Tomazic, S.J. and Klibanov, A.M. (1988). Mechanisms of irreversible thermal inactivation of Bacillus alpha-amylases. *J. Biol. Chem.* 263: 3086–3091.
3. Pavliv, L. Acetylcysteine composition and uses thereof. United States Patent Application, Pub. No. US2013/0165516 A1. Patent filed 01 February 2013.

5: Acyclovir

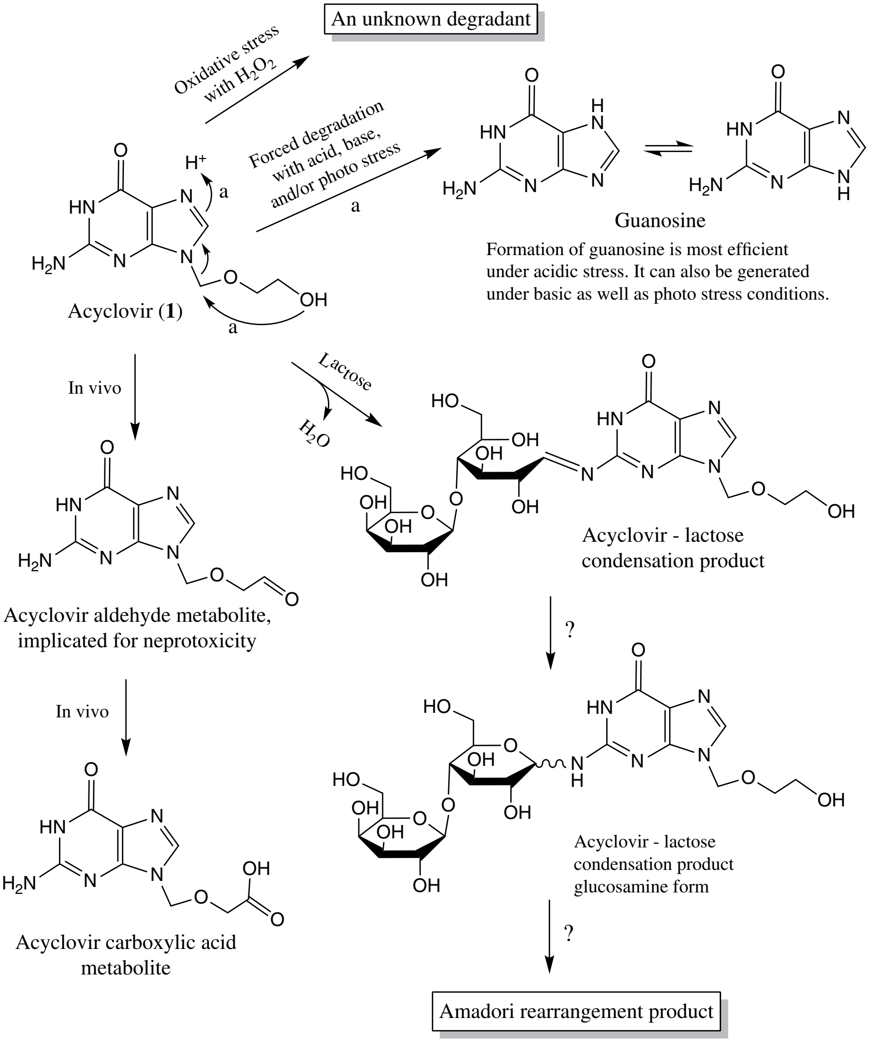


Chemical names: 9-Hyroxyethoxymethylguanine; 2-Amino-9-[(2-hydroxyethoxy) methyl]-1,9-dihydro-6*H*-purin-6-one
Brand name: Zovirax
Formula: $C_8H_{11}N_5O_3$
Monoisotopic protonated ion (*m/z*): 226.0935
Molecular weight: 225.21
CAS number: 59277-89-3

Acyclovir, also spelled as aciclovir, is one of the widely prescribed antiviral drugs for the treatment of many types of herpes virus infections such as cold sores, chickenpox, and shingles. It is a nucleoside (guanosine) analogue with the nucleoside sugar unit being replaced by an acyclic, functionalized alkyl group that is a residual of the sugar unit. In other words, acyclovir can be considered a ^9N-alkylated guanine derivative (**1**, Scheme 5). The removal of this alkyl group constitutes its main degradation pathway under acidic, basic, and photo stress conditions, during which process guanine is formed [1, 2]. Under both the photochemical and non-photochemical stress in solutions, the degradation is more significant when an acid is present. It was suggested that the dealkylation of acyclovir may be facilitated by a nucleophilic attack of the side chain hydroxyl group (Scheme 5) [3]. This mechanism is consistent with the observation that a substituted benzoate prodrug of acyclovir is three times more stable than the parent drug under acidic stress, because the ester oxygen in the prodrug is much less nucleophilic than the hydroxyl oxygen in the parent drug. Despite the fact that guanine, which is also a starting material of acyclovir, can be readily generated, particularly under acidic stress conditions, it appears that it is not a significant degradant in commercial as well as several experimental solid dosage forms [4–6].

Oxidative stress of acyclovir was performed in various concentrations of H_2O_2 and under different temperatures [1, 2], during which processes only a single, unidentified degradant was generated. This stress-generated degradant does not appear to be meaningful in formulated acyclovir products. For the side chain hydroxyl group, it is not expected to be susceptible to oxidation under regular or accelerated stability conditions for the same reason discussed in the monograph of abacavir. Nevertheless, this hydroxyl group is oxidized in vivo to form the major metabolite, carboxyl, via the intermediacy of aldehyde. The intermediary aldehyde was hypothesized to be the culprit for nephrotoxicity observed in some pediatric patients [7].

Since the drug molecule contains a primary amino group, its compatibility with lactose, a reducing sugar, was evaluated by Monajjemzadeh et al. [8].



Formation of guanosine is most efficient under acidic stress. It can also be generated under basic as well as photo stress conditions.

Scheme 5 In vitro and in vivo degradation pathways of acyclovir. None of the in vitro degradants has been reported to be significant in commercial formulations.

A condensation product was formed between the drug molecule and lactose based on LC–MS evidence. This degradant was proposed to be a Schiff base, the first degradant in the Maillard degradation chemistry. Nevertheless, it could also be the isomeric glycosamine or Amadori rearranged product [9]. Monajjemzadeh et al. also analyzed three batches of commercial acyclovir tablets that contain lactose and found no detectable level of the condensation product. Overall, it appears that acyclovir is quite stable in solid dosage forms, and all in vitro degradants discussed in this monograph become significant only when the drug is subjected to forced degradation under various stress conditions.

References

1 Sinha, V.R., Trehan, M.A., Kumar, M. et al. (2007). Stress studies on acyclovir. *J. Chromatogr. Sci.* 45: 319–324.

2 Zacharis, C.K., Verdoukas, A., Tzanavaras, P.D., and Themelis, D.G. (2009). Automated sample preparation coupled to sequential injection chromatography: on-line filtration and dilution protocols prior to separation. *J. Pharm. Biomed. Anal.* 49: 726–732.

3 Jensen, E. and Bundgaard, H. (1991). Kinetics of the acid-catalyzed hydrolysis of acyclovir and an ester prodrug in aqueous solution. *Acta Pharm. Nord.* 3: 147–150.

4 Shafaati, A.R. and Clark, B.J. (2003). Determination of aciclovir and its related substance guanine in bulk drug and tablet preparation by capillary electrophoresis. *Iran. J. Pharm. Res.* 2: 71–75.

5 Vinodbhai, P.K., Gohel, M.C., Parikh, R.K. et al. (2011). Sustained release floating microspheres of acyclovir: formulation, optimization, characterization and in vitro evaluation. *Int. J. Drug Dev. Res.* 3: 242–251.

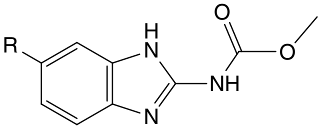
6 Singh, A.V. and Nath, L.K. (2010). Development and evaluation of acyclovir mucoadhesive tablet using a novel starch based platform. *Lat. Am. J. Pharm.* 29: 279–283.

7 Gunness, P., Aleksa, K., Bend, J., and Koren, G. (2011). Acyclovir-induced nephrotoxicity: the role of the acyclovir aldehyde metabolite. *Transl. Res.* 158: 290–301.

8 Monajjemzadeh, F., Hassanzadeh, D., Valizadeh, H. et al. (2009). Compatibility studies of acyclovir and lactose in physical mixtures and commercial tablets. *Eur. J. Pharm. Biopharm.* 73: 404–413.

9 Li, M. (2012). Drug-excipient interactions and adduct formation. In: *Organic Chemistry of Drug Degradation*, 150–164. Cambridge: Royal Society Chemistry Publishing.

6–8: Albendazole, Fenbendazole, Mebendazole



Albendazole (**1**), R = C_3H_7S–;
Fenbendazole (**2**), R = PhS–;
Mebendazole (**3**), R = PhC(O)–.

6: Albendazole

Chemical name: Methyl [5-(propylsulfanyl)-1*H*-benzimidazol-2-yl]carbamate
Brand name: Albenza; Eskazole
Formula: $C_{12}H_{15}N_3O_2S$
Monoisotopic protonated ion (*m/z*): 266.0958
Molecular weight: 265.33
CAS number: 54965-21-8

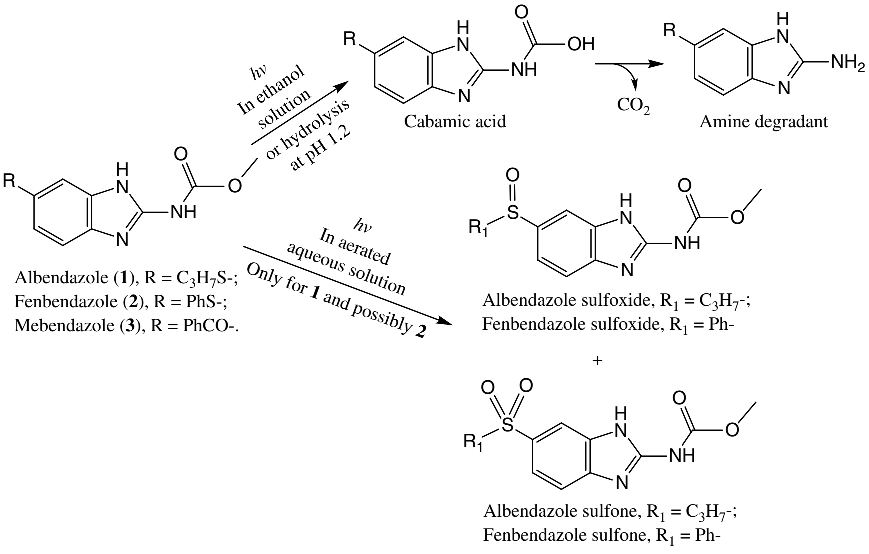
7: Fenbendazole

Chemical name: Methyl *N*-(6-phenylsulfanyl-1*H*-benzoimidazol-2-yl)carbamate
Brand name: Panacur
Formula: $C_{15}H_{13}N_3O_2S$
Monoisotopic protonated ion (*m/z*): 300.0801
Molecular weight: 299.35
CAS number: 43210-67-9

8: Mebendazole

Chemical name: Methyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate
Brand name: Emverm; Vermox
Formula: $C_{16}H_{13}N_3O_3$
Monoisotopic protonated ion (*m/z*): 296.1030
Molecular weight: 295.30
CAS number: 31431-39-7

Albendazole, fenbendazole, and mebendazole are three benzimidazole-based anthelmintic drugs that all contain the same methyl carbamyl group at the two-position and a different substituent at the five-position of the benzimidazole ring. Ragno et al. performed forced degradation studies of the three drugs under thermo as well as photo stress conditions [1]. By heating at 50°C for up to 8 hours, the three drugs were found to be stable in both solid and ethanol solutions. On the other hand, when the ethanol solutions of the three drugs were subjected to photo stress with a xenon lamp under the ICH photo stability conditions, all three drugs were found to decompose in significant quantities with the formation of an intermediary



Scheme 6–8 Degradation pathways of albendazole, fenbendazole, and mebendazole.

degradant and the final amine degradant. The intermediary degradant was proposed to be the carbamic acid, resulting from the initial photo-hydrolysis of each drug (Scheme 6–8) based on the GC–MS analysis of the isolated degradant. This is somewhat surprising as carbamic acids can be present in solutions but are usually too unstable to be isolated due to their facile decomposition to the corresponding amines [2].

In another photochemical degradation study, Weerasinghe et al. evaluated the photostability of albendazole in aerated aqueous solutions between pH 7 and 9 [3]. Albendazole was found to undergo stepwise photooxidation, giving rise to albendazole sulfoxide and sulfone, respectively, in addition to a major unknown photodegradant that was found to be very polar in nature. It is worthwhile to mention that this polar degradant is not the amine degradant as the latter was another compound evaluated in the same study. It needs to be pointed out that the Weerasinghe study used all aqueous solutions while the Ragno study used pure ethanol as the solvent. Consequently, the concentrations of the solutions in the Weerasinghe study were much more dilute than those in the Ragno study due to the poor solubility of albendazole in aqueous solutions. These differences are probably the main reason for the different photodegradation patterns observed in the two studies. Since fenbendazole contains a phenylsulfanyl at the 5-position of the benzimidazole ring, it may also be susceptible to the same photo oxidation as albendazole. The latter has a propylsulfanyl in the 5-position.

Torrado et al. conducted a formulation study of albendazole [4]. Over a pH range of 1.2–7.5, the solubility of albendazole was the highest at the lowest pH 1.2. In an experimental aqueous formulation containing 40% transcutol [2-(2-ethoxyethoxy) ethanol)] with a nominal pH of 1.2, albendazole was found to decompose rather quickly. The authors did not specify what degradants were observed. Nevertheless, it appears to be very likely that the degradation of albendazole was due to the hydrolysis of the carbamyl linkage. A carbamyl bond is usually quite stable as the activation energy for its hydrolysis is typically ≥20 kcal/mol [2]. However, the carbamyl linkage of albendazole became quite unstable at pH 1.2 as the activation energy for its hydrolysis is only 9.26 kcal/mol, which is calculated based on the Arrhenius plot determined by the original researchers [4]. Owing to the fact that the same methyl carbamate is also present in both fenbendazole and mebendazole, the latter two drugs should display very similar hydrolytic kinetics.

References

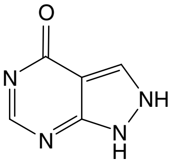
1 Ragno, G., Risoli, A., Ioele, G., and De Luca, M. (2006). Photo- and thermal-stability studies on benzimidazole anthelmintics by HPLC and GC–MS. *Chem. Pharm. Bull.* 54: 802–806.

2 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 16–47. Cambridge: Royal Society Chemistry Publishing.

3 Weerasinghe, C.A., Lewis, D.O., Mathews, J.M. et al. (1992). Aquatic photodegradation of albendazole and its major metabolites. 1. Photolysis rate and half-life for reactions in a tube. *J. Agric. Food Chem.* 40: 1413–1418.

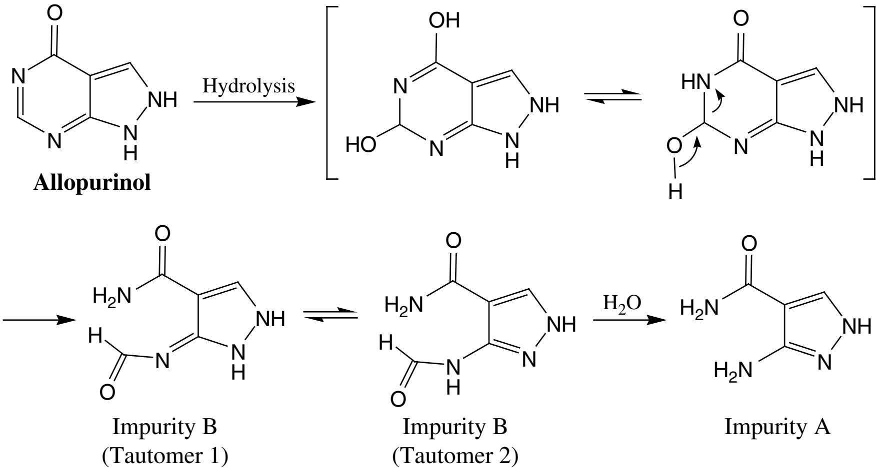
4 Torrado, S., Torrado, S., Cadorniga, R., and Torrado, J.J. (1996). Formulation parameters of albendazole solution. *Int. J. Pharm.* 140: 45–50.

9: Allopurinol [WHO List of Essential Medicines]



Chemical name: 1*H*-pyrazolo[3,4-*d*]pyrimidin-4(2*H*)-one
Brand name: Zyloprim
Formula: $C_5H_4N_4O$
Monoisotopic protonated ion (*m/z*): 137.0458
Molecular weight: 136.11
CAS number: 315-30-0

Allopurinol is a xanthine oxidase inhibitor, which is used primarily for the treatment of gout. Structurally, it is an isomer of hypoxanthine, a naturally occurring purine compound. Its main degradation is of hydrolytic nature, and the point of the hydrolytic attack is the carbon connecting the two nitrogen atoms in the fused six-membered ring [1]. The initial degradation product should be Impurity B, which would mostly further degrade to Impurity A under strong acid or base catalysis [1–3] (Scheme 9).



Scheme 9 Hydrolytic degradation of allopurinol.

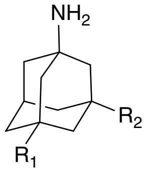
References

1 Gressel, P.D. and Gallelli, J. (1968). Quantitative analysis and alkaline stability studies of allopurinol. *J. Pharm. Sci.* 57: 335–338.

2 Singh, S. and Gadhawala, Z. (2013). Development of a stability indicating RP-RRLC method for determination of allopurinol and its degradation products in solid oral dosage. *Int. J. PharmTech. Res.* 5: 44–53.

3 Matzko-Hollenbach, K., Jira, T., and Mezey, G. (1987). Zur stabilität von allopurinol und lyophilisiertem allopurinol-natrium. *Arch. Pharm.* 320: 282–284.

10–11: Amantadine, Memantine



Amantadine: R_1=R_2=H;
Memantine: R_1=R_2=Me

10: Amantadine

Chemical name: Adamantan-1-amine
Brand name: Gocovri; Osmolex
Formula: $C_{10}H_{17}N$
Monoisotopic protonated ion (*m/z*): 152.1434
Molecular weight: 151.25
CAS number: 768-94-5
Common salt form: HCl salt

11: Memantine

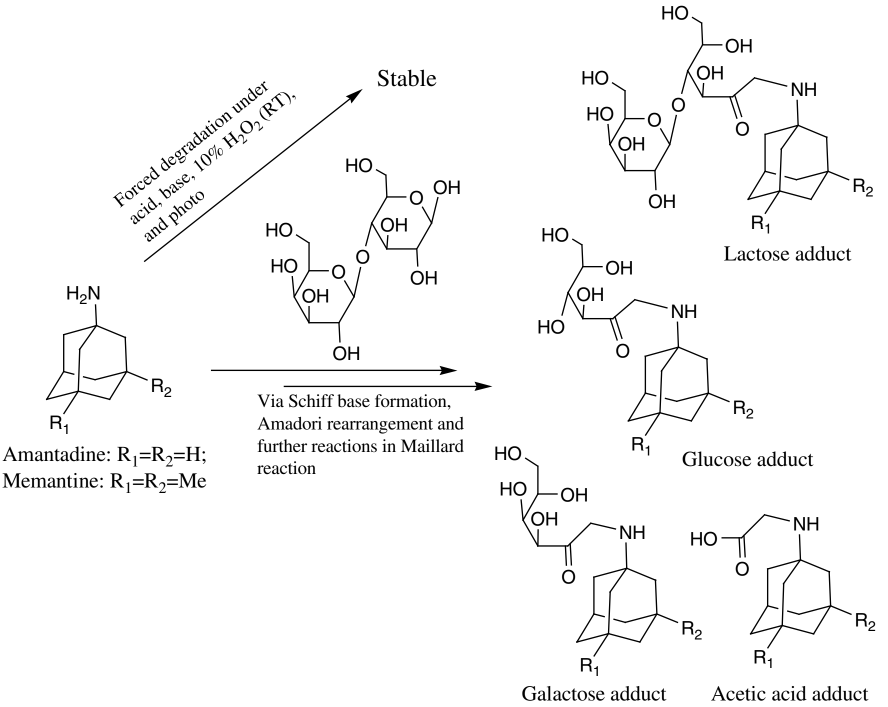
Chemical name: 3,5-Dimethyladamantan-1-amine
Brand name: Axura; Ebixa; Marixino
Formula: $C_{12}H_{21}N$
Monoisotopic protonated ion (*m/z*): 180.1747
Molecular weight: 179.31
CAS number: 19982-08-2
Common salt form: HCl salt

Amantadine and memantine are both derivatives of adamantine that contain a primary amino group. Based on their structures, they should be quite stable compounds. In a stress study with amantadine hydrochloride, the drug substance was found to be stable under the stress conditions of boiling, acid, base, and UV irradiation [1]. The photostability is expected as both compounds have no UV chromophore at all. In another forced degradation study where much harsher stress conditions (except base stress) were used, degradation of amantadine was observed [2]. In the latter study where a derivatization assay method was employed, the base stress was milder than the one as reported in Reference [1]; yet, amantadine was found to retain only 77% assay value after being treated in 1 N NaOH solution for 5 minutes at room temperature. In this case, the missing 23% of amantadine could be due to lower derivatization yield, rather than real degradation.

In the development of a stability-indicating GC method for memantine hydrochloride, stress of the drug substance under harsh acid and base conditions, e.g., refluxing in 5 M NaOH solution for 36 hours, did not yield any degradation product [3]. Only in the oxidative stress in 30% hydrogen peroxide at room temperature for 24 hours, an appreciable amount of degradation was observed; such a harsh oxidative condition tends to yield artificial degradants.

In formulation studies, memantine was found to undergo Maillard reaction with reducing end sugars such as lactose under the long-term stability condition of 25C/60%RH [4]. The observed degradation products were sugar adducts resulting from the Amadori rearrangement, after the initial sequential formation of the corresponding Schiff bases and glycosylamines. In addition to the formation of memantine-lactose adduct as expected, memantine also formed adducts in comparable amounts with galactose and glucose, the two components of lactose. Also formed in comparable quantity was an acetic acid adduct (referred to as memantine-dimethylamino glycine or DMAG adduct in Reference [4]). The latter adduct could result from further degradation of all three sugar adducts.

Due to their highly similar structures, it is reasonable to assume that amantadine and memantine would display very similar chemical stability and degradation behaviors (Scheme 10–11).

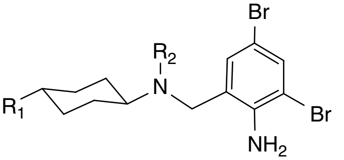


Scheme 10–11 Degradation pathways of amantadine and memantine.

References

1. Askal, H.F., Khedr, A.S., Darwish, I.A., and Mahmoud, R.M. (2008). Quantitative thin-layer chromatographic method for determination of amantadine hydrochloride. *Int. J. Biomed. Sci.* 4: 155–160.
2. Narola, B., Singh, A.S., Santhakumar, P.R., and Chandrashekhar, T.G. (2010). A validated stability-indicating reverse phase HPLC assay method for the determination of memantine hydrochloride drug substance with UV-detection using precolumn derivatization technique. *Anal. Chem. Insights* 5: 37–45. https://doi.org/10.4137/ACI.S3936.
3. Jadhav, S.A., Landge, S.B., Niphade, N.C. et al. (2012). Development and validation of stability-indicating GC-FID method for the quantitation of memantine hydrochloride and its nonchromophoric impurities in bulk and pharmaceutical dosages. *Chromtogr. Res. Inter.* 2012 (1): 806068. https://doi.org/10.1155/2012/806068.
4. Rystov, L., Chadwick, R., Krock, K., and Wang, T. (2011). Simultaneous determination of Maillard reaction impurities in memantine tablets using HPLC with charged aerosol detector. *J. Pharm. Biomed. Anal.* 56: 887–894.

12–13: Ambroxol, Bromhexine



Ambroxol, R_1 = –OH, R_2 = –H;
Bromhexine, R_1 = –H, R_2 = –Me;.

12: Ambroxol

Chemical name: *trans*-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol
Brand name: Ambrolex
Formula: $C_{13}H_{18}Br_2N_2O$
Monoisotopic protonated ion (*m/z*): 376.9859
Molecular weight: 378.11
CAS number: 18683-91-5
Common salt form: HCl salt

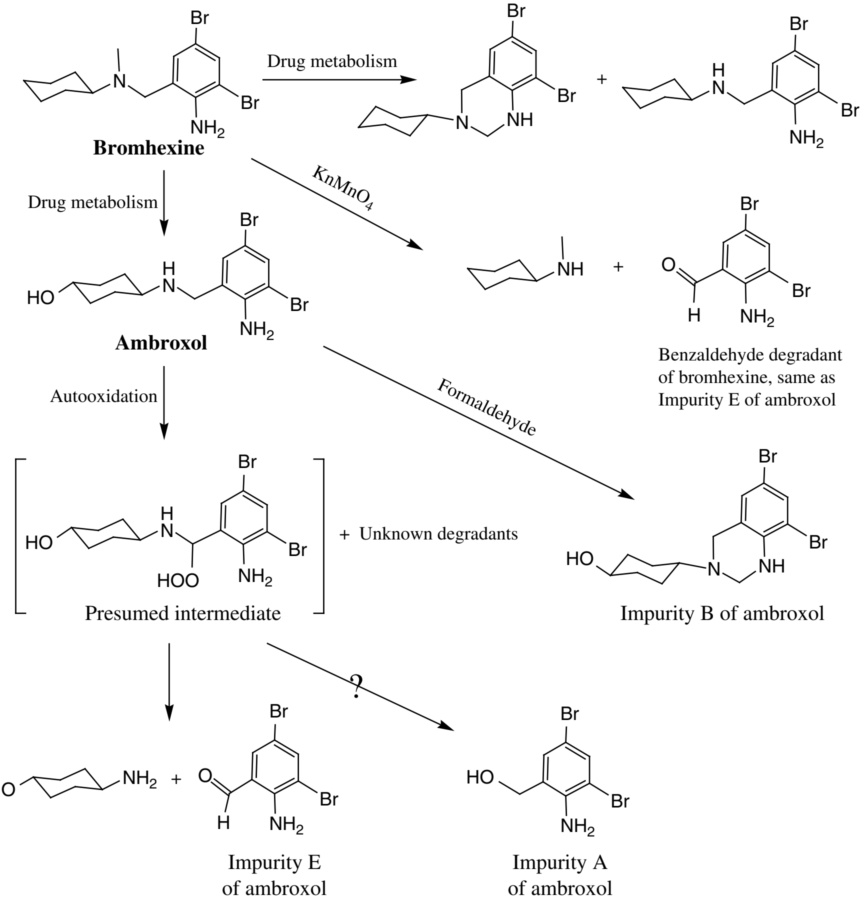
13: Bromhexine

Chemical name: 2,4-Dibromo-6-{[cyclohexyl(methyl)amino]methyl}aniline
Brand names: Amiorel; many others
Formula: $C_{14}H_{20}Br_2N_2$
Monoisotopic protonated ion (*m/z*): 360.9910
Molecular weight: 362.11
CAS number: 3572-43-8
Common salt form: HCl salt

Bromhexine is a mucolytic drug sold over the counter in many cough remedies. It was introduced by Boehringer Ingelheim in 1963 and is still widely used today. Ambroxol is an active metabolite of bromhexine, resulting from demethylation of the tertiary amine and hydroxylation of the cyclohexane ring. Ambroxol was also developed into a drug in 1978 and is an active ingredient in many drug products with different formulations. In a European patent [1], a stabilization study of the liquid formulation of ambroxol was disclosed. Three degradation products were mentioned in this patent and all of them are listed in the European Pharmacopoeia (EP) [2]. The first degradant is the commonly observed EP Impurity B, which is trans-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2*H*)-yl)cyclohexanol. The methylene bridging the two nitrogen atoms in Impurity B originates from excipients that contain formaldehyde impurity, e.g., PEG. Impurity B is also a metabolite of bromhexine; however, in the latter case, the bridging methylene originates from the methyl group on the tertiary amine. The metabolic formation of Impurity B is an alternative pathway to metabolic demethylation [3].

The other two degradants of ambroxol disclosed in the patent were EP Impurity A and Impurity E, which are (2-amino-3,5-dibromophenyl)methanol and 2-amino-3,5-dibromobenzaldeyde, respectively. According to the patent, both impurities are oxidative degradants and can be controlled by operating under a nitrogen atmosphere. The formation of Impurity E, the benzaldehyde degradant, can be easily rationalized via a radical-mediated auto-oxidation pathway. The benzaldehyde degradant can be generated through oxidation of bromhexine electrochemically [4] or by potassium permanganate [5]. On the other hand, it would be difficult to postulate a reasonable mechanism for the formation of Impurity A from the oxidation of ambroxol.

The same European patent also revealed that unknown degradants were in certain liquid formulations that were projected to increase more than the three known degradants, i.e., Impurities A, B, and E (Scheme 12–13).



Scheme 12–13 Degradation pathways of ambroxol and metabolism of bromhexine leading to the formation of ambroxol.

References

1 Giorgioc, P. (2003). Concentrated aqueous solution of ambroxol. European Patent 1543826. Patent filed 09 November 1967. Patent granted 25 October 1968.

2 European Pharmacopoeia, 10th ed. (official as of January 2017) Ambroxol hydrochloride. Council of Europe, Strasbourg, France. pp. 1809–1810.

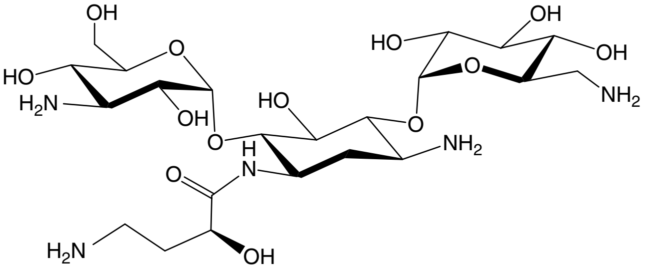
3 Meijer, L.A., Verstegen, J.C.M., Bull, S., and Fink-Gremmels, J. (2004). Metabolism of bromhexine in pig hepatocyte cultures. *J. Vet. Pharm. Col. Therap.* 27: 219–225.

4 Turchan, M., Jara-Ulloa, P., Bollo, S. et al. (2007). Voltammetric behaviour of bromhexine and its determination in pharmaceuticals. *Talanta* 73: 913–919.

5 Susmitha, K., Thirumalachary, M., and Venkateshwarlu, G. (2013). Spectrophotometric determination of bromhexine HCl in pure and pharmaceutical forms. *Int. Sch. Res. Notices* 2013 (1): 861851. http://dx.doi.org/10.1155/2013/861851.

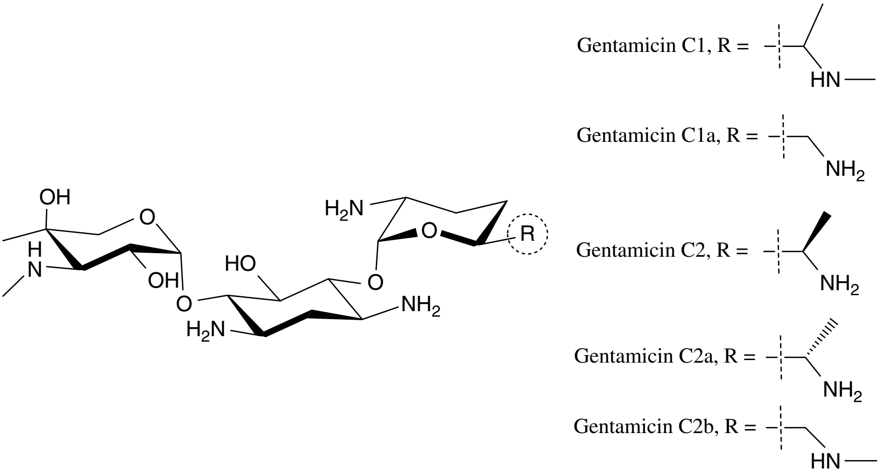
14–16: Aminoglycoside Antibiotics

14: Amikacin [WHO Essential List of Medicines]



Chemical name: (2*S*)-4-Amino-*N*-{(1*R*,2*S*,3*S*,4*R*,5*S*)-5-amino-2-[(3-amino-3-deoxy-α-D-glucopyranosyl)oxy]-4-[(6-amino-6-deoxy-α-D-glucopyranosyl)oxy]-3-hydroxycyclohexyl}-2 -hydroxybutanamide
Brand name: Amikin, Amiglyde-V, Arikayce
Formula: $C_{22}H_{43}N_5O_{13}$
Monoisotopic protonated ion (*m/z*): 586.2930
Molecular weight: 585.60
CAS number: 37517-28-5
Common salt form: Sulfate

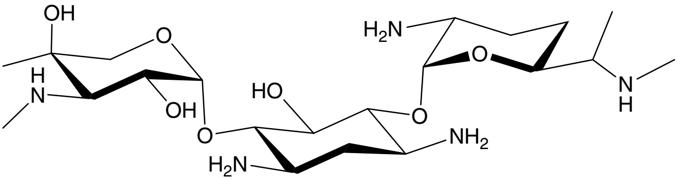
15: Gentamicin



Gentamicin is a mixture of several structural analogs. Its main components include gentamicin C1, C1a, C2, C2a, and C2b. Gentamicin is available in both neutral base form and sulfate salt form.

Brand name: Cidomycin, Genticyn, Garamycin

15a: Gentamicin C1



Chemical name: (1*S*,2*R*,3*S*,4*S*,6*S*)-4,6-Diamino-3-({(2*S*,3*S*,6*S*)-3-amino-6-[(1*S*)-1-(methylamino)ethyl]tetrahydro-2*H*-pyran-2-yl}oxy)-2-hydroxycyclohexyl 3-deoxy-4-C-methyl-3-(methylamino)-β-L-lyxopyranoside

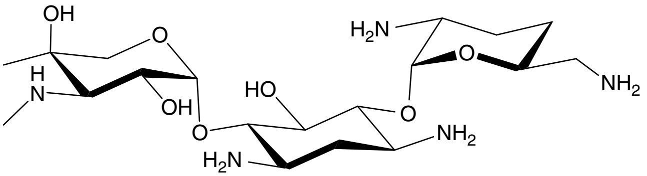
Formula: $C_{21}H_{43}N_5O_7$

Monoisotopic protonated ion (*m/z*): 478.3235

Molecular weight: 477.60

CAS number: 1403-66-3

15b: Gentamicin C1a



Chemical name: (1*R*,2*S*,3*S*,4*R*,6*S*)-4,6-Diamino-3-{[3-deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranosyl]oxy}-2-hydroxycyclohexyl 2,6-diamino-2,3,4,6-tetradeoxy-α-D-erythro-hexopyranoside; (2*R*,3*R*,4*R*,5*R*)-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diamino-3-[(2*R*,3*R*,6*S*)-3-amino-6-(aminomethyl)oxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-5-methyl-4-(methylamino)oxane-3,5 -diol

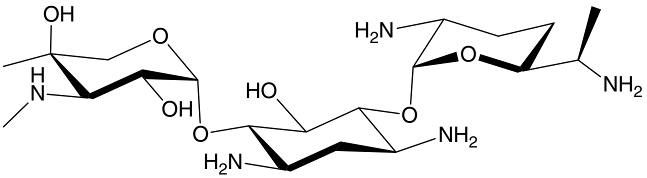
Formula: $C_{19}H_{39}N_5O_7$

Monoisotopic protonated ion (*m/z*): 450.2922

Molecular weight: 449.54

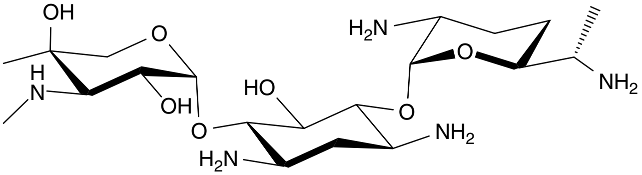
CAS number: 26098-04-4

15c: Gentamicin C2



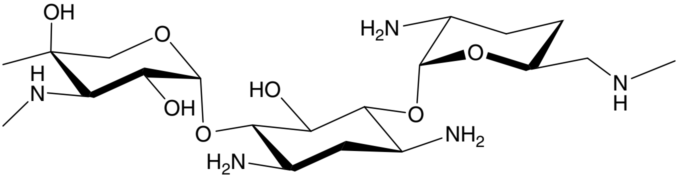
Chemical name: (1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diamino-3-({(2*R*,3*R*,6*S*)-3-amino-6-[(1*R*)-1-aminoethyl]tetrahydro-2*H*-pyran-2-yl}oxy)-2-hydroxycyclohexyl-3-deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranoside
Formula: $C_{20}H_{41}N_5O_7$
Monoisotopic protonated ion (*m/z*): 464.3079
Molecular weight: 463.57
CAS number: 25876-11-3

15d: Gentamicin C2a



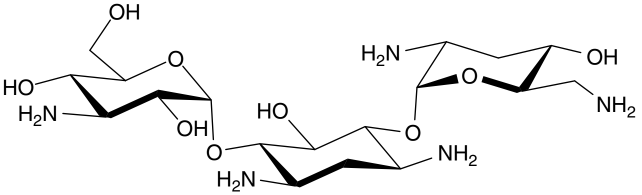
Chemical name: (1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diamino-3-{[(2*R*,3*R*,6*S*)-3-amino-6-[(1*S*)-1-aminoethyl]tetrahydro-2*H*-pyran-2-yl]oxy}-2-hydroxycyclohexyl-3-deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranoside
Formula: $C_{20}H_{41}N_5O_7$
Monoisotopic protonated ion (*m/z*): 464.3079
Molecular weight: 463.57
CAS number: 59751-72-3

15e: Gentamicin C2b (Micronomicin)



Chemical name: (1*R*,2*S*,3*S*,4*R*,6*S*)-4,6-Diamino-3-{[3-deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranosyl]oxy}-2-hydroxycyclohexyl-2-amino-2,3,4,6-tetradeoxy-6-(methylamino)-α-D-erythro-hexopyranoside
Formula: $C_{20}H_{41}N_5O_7$
Monoisotopic protonated ion (*m/z*): 464.3079
Molecular weight: 463.57
CAS number: 52093-21-7

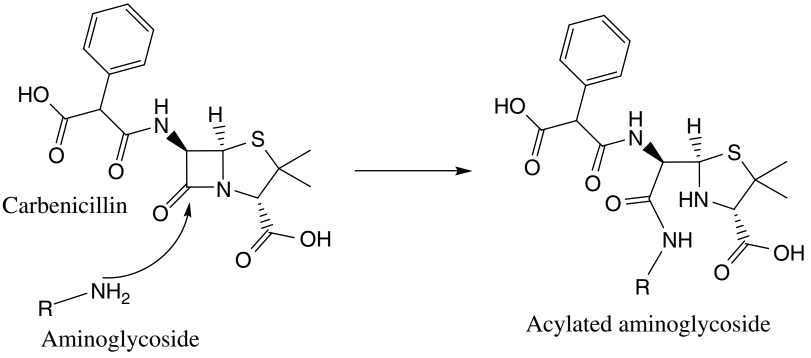
16: Tobramycin [WHO List of Essential Medicines]



Chemical name: (1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diamino-3-[(2,6-diamino-2,3,6-trideoxy-a-D-ribo-hexopyranosyl)oxy]-2-hydroxycyclohexyl 3-amino-3-deoxy-a-D-glucopyranoside
Brand name: Bethkis, Kitabis, Tobi,
Formula: $C_{18}H_{37}N_5O_9$
Monoisotopic protonated ion (*m/z*): 468.2664
Molecular weight: 467.52
CAS number: 32986-56-4
Common salt form: Sulfate

Aminoglycoside antibiotics were first discovered in the 1940s, among which streptomycin is the first medication that can cure tuberculosis [1]. Despite their side effects, they are still among the most prescribed antibiotics worldwide for several diseases caused by infection with Gram-negative pathogens [2, 3]. Aminoglycoside antibiotics are usually quite stable compounds, particularly in their usual sulfate salt forms. The functional groups of aminoglycosides that are potentially susceptible to degradation are the glycosidic linkages and amino groups. The degradation of glycosidic linkages usually would not take place under accelerated or long-term stability conditions. Only under high-temperature and strong acidic or basic conditions would hydrolysis of the glycosidic bonds occur. For the amino moieties, they may undergo the Maillard reaction if their formulations contain excipients possessing reducing sugars [4–6]. In this monograph, we discuss amikacin, gentamicin, and tobramycin as representatives in this important class of drugs.

While amikacin has a somewhat different structure, gentamicin and tobramycin are typical aminoglycoside antibiotics in terms of their structures. Gentamicin has three structural moieties, namely purpurosamine, 2-deoxystreptamine, and garosamine, which are linked together by two glycosidic bonds. The heterogeneity of methylation and/or stereochemistry on the purpurosamine moiety renders the drug substance a heterogeneous mixture that contains five major components: C1, C1a, C2, C2a, and C2b [7]. In addition, numerous minor components or impurities, most notably garamin, sisomicin, and deoxystreptamine, have been identified in gentamicin drug substance, although not all of them may be present at the same time in a single batch [8]. Various long-term stability studies have demonstrated that gentamicin is quite stable and none of the identified impurities have been found to increase over the periods of the studies. For example, a stability study of gentamicin drug substance for at least 5 years was reported [9]. For a number of gentamicin products that were formulated in polymeric excipients such as polymethylmethacrylate (PMMA), the drug was found to be stable under both long-term (25°C/60%RH, 24 months) and accelerated stability conditions (40°C/75%RH, 6 months) [9]. When gentamicin was formulated in dextrose solutions, incompatibility of gentamicin with the reducing sugar was observed as reflected in the development of a yellow color and loss of potency [4]. The compatibility study indicated that several degradation products were quickly formed; although the structures of these degradants were not elucidated, it would be most likely that the degradation was mainly due to the Maillard reaction [6]. Furthermore, when the aminoglycosides were co-formulated with β-lactam antibiotics, the amino groups of the aminoglycosides would be capable of attacking the lactam moieties of the β-lactam

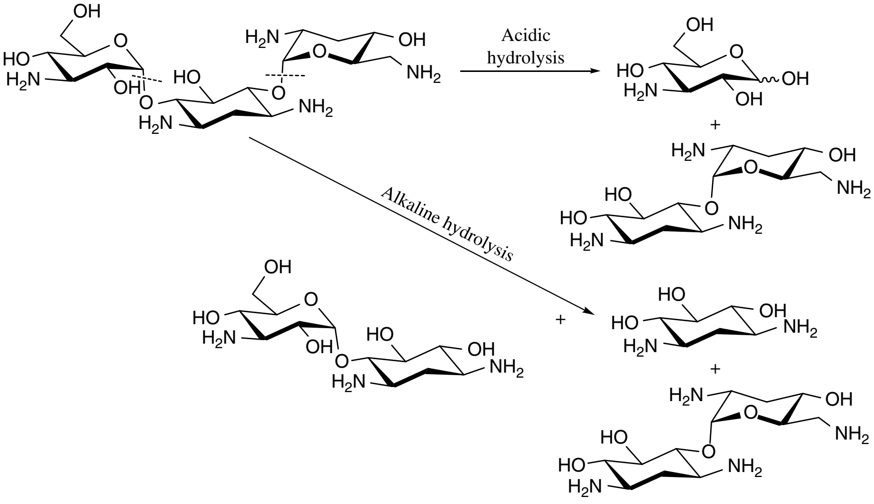


Scheme 14-16-1 Incompatibility between aminoglycosides and carbenicillin, a semi-synthetic penicillin antibiotic, via acylation of the amino groups of aminoglycosides.

antibiotics, resulting in acylation of the amino groups. Such degradation was demonstrated in the case of amikacin acylation by carbenicillin (Scheme 14–16-1) [10].

On the other hand, forced degradation under acidic or basic conditions at elevated temperatures could decompose gentamicin through the cleavage of the glycosidic bonds, which would be expected to be similar to the case of tobramycin as discussed in the next paragraph.

The structure of tobramycin is similar to that of gentamicin, particularly the glycosidic linkages. Brandl and Gu performed a thorough forced degradation study of tobramycin at different pH and temperatures (Scheme 14–16-2) [11]. In 1 N HCl solutions in the temperature range of 60–100°C for up to ~160 hours, only one



Scheme 14-16-2 Hydrolysis of tobramycin under acidic and alkaline conditions. The dash lines indicate the glycosidic cleavage sites. *Source:* Brand and Gu [11]/Taylor & Francis Group.

glycosidic bond was cleaved, and the activation energy was found to be 30 kcal/mol. At the other end of the extreme pH, both glycosidic bonds were found to be cleaved in a 1 N NaOH in the temperature range of 60–100°C. The activation energy for the hydrolysis under the alkaline conditions was determined to be 15 kcal/mol.

In solutions of neutral pH at temperature range of 60–100°C, cleavage of tobramycin was extremely slow in the absence of oxygen; on the other hand, the degradation became rapid in ambient air, giving similar degradation products under alkaline hydrolytic conditions [11], suggesting the cleavage of the glycosidic bonds may be mediated by auto-oxidation [12]. Nevertheless, the implication of the oxidative degradation pathway toward the stability of tobramycin under long-term (25°C) or accelerated stability (40°C) conditions would be difficult to evaluate. This is because auto-oxidation usually involves free radical intermediates and its degradation mechanism and kinetics may change dramatically as temperature decreases significantly.

In a study of gentamicin and tobramycin in concentrated dextrose solution, tobramycin was found to be less stable than gentamicin [13]. Although the study did not elaborate on the degradation mechanisms, it would be quite likely that the underlying degradation would be due to the Maillard reaction.

Amikacin is somewhat structurally different from gentamicin and tobramycin in that it contains an acyl group in one of the amino groups. There appear to be very few reports on its stability study [14]. Among the few studies reported, one studied the chemical compatibility between amikacin and four β-lactam antibiotics over a period of 3 weeks in temperatures ranging from −70 to 25°C [15]. While amikacin demonstrated no loss of activity when combined in solutions with two penicillin antibiotics, carbenicillin and piperacillin, respectively, gentamicin and tobramycin showed significant loss of activities 8–48 hours after admixing with the two penicillins at both 25°C and 4°C. The inactivation of the aminoglycosides by the penicillins was believed to involve nucleophilic attack by the amino groups of aminoglycosides on the 4-membered betalactam rings. The degradation is similar to the case of amikacin acylation by carbenicillin as mentioned above [10].

References

1 Aminoglycoside. Wikipedia.org (accessed 13 May 2022).

2 Forge, A. and Schacht, J. (2000). Aminoglycoside antibiotics. *Audiol. Neurootol.* 5: 3–22.

3 McWilliam, S.J., Antoine, D.J., Smyth, R.L., and Pirmohamed, M. (2017). Aminoglycoside-induced nephrotoxicity in children. *Pediatr. Nephrol.* 32: 2015–2025.

4 Graham, A.E., Speicher, E., and Williamson, B. (1997). Analysis of gentamicin sulfate and a study of its degradation in dextrose solution. *J. Pharm. Biomed. Anal.* 15: 537–543.

5 Kumar, V. and Banker, G.S. (1998). Maillard reaction and drug stability. In: *Maillard Reactions in Chemistry, Food and Health* (ed. T.P. Labuza, G.A. Reineccius, V.M. Monnier, et al.). Woodhead Publishing.

6 Li, M. (2012). Drug-excipient interactions and adduct formation. In: *Organic chemistry of drug degradation*. Cambridge, UK: RSC Publishing.

7 USP (official as of 1 May 2020) Gentamicin sulfate. https://doi.org/10.310.3/USPNF_M34850_04_01 (accessed 13 May 2022).

8 Grahek, R. and Zupancic-Kralj, L. (2009). Identification of gentamicin impurities by liquid chromatography tandem mass spectrometry. *J. Pharm. Biomed. Anal.* 50: 1037–1043.

9 Kühn, K.-D., Weber, C., Kreis, S., and Holzgrabe, U. (2008). Evaluation of the stability of gentamicin in different antibiotic carriers using a validated MEKC method. *J. Pharm. Biomed. Anal.* 48: 612–618.

10 Iyengar, B.S., Kumar, V., Wunz, T.P., and Remers, W.A. (1986). Aminoglycoside antibiotics. 6. Chemical reactions of aminoglycosides with disodium carbenicillin. *J. Med. Chem.* 29: 611–614.

11 Brand, M. and Gu, L. (1992). Degradation of tobramycin in aqueous solution. *Drug Dev. Ind. Pharm.* 18: 1423–1436.

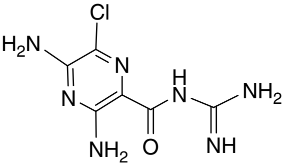
12 Hodges, N.A. and Singh, J. (1978). Enhancement of neomycin stability using propylene-glycol and sodium metabisulfite. *J. Pharm. Pharmacol.* 30: 737–739.

13 Nance, K.S. and Matzke, G.R. (1984). Stability of gentamicin and tobramycin in concentrate solutions for automated peritoneal dialysis. *Am. J. Nephrol.* 4: 240–243.

14 Zhang, Y. and Trissel, L.A. (2003). Stability of amikacin sulfate in AutoDose Infusion System bags. *Inter. J. Pharma. Comp.* 7: 230–232.

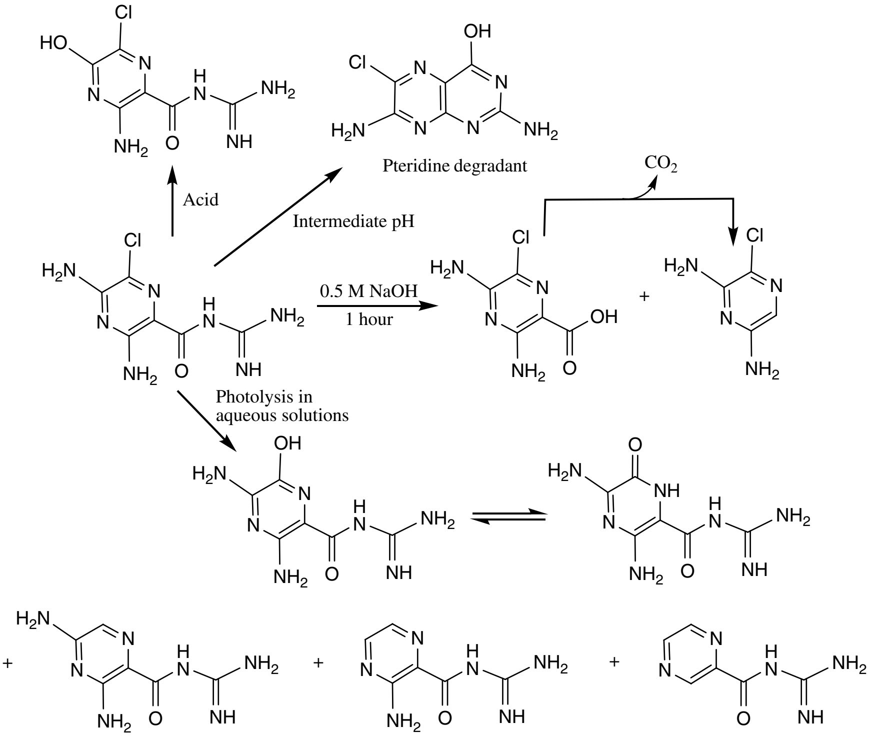
15 Glew, R.H. and Pavuk, R.A. (1983). Stability of gentamicin, tobramycin, and amikacin in combination with four β-lactam antibiotics. *Antimicrob. Agents Chemother.* 24: 474–477.

17: Amiloride



Chemical name: 3,5-Diamino-6-chloro-*N*-(diaminomethylidene)pyrazine-2-carboxamide
Brand name: Midamor
Formula: $C_6H_8ClN_7O$
Monoisotopic protonated ion (*m/z*): 230.0552
Molecular weight: 229.63
CAS number: 2609-46-3
Common salt form: HCl salt

Amiloride is a diuretic with a substituted pyrazine ring and an acylated guanidinyl side chain. In a forced degradation study [1], the drug was found to be unstable under hydrolytic degradations at elevated temperatures. The degradation products



Scheme 17 Photodegradation of amiloride in aqueous solutions with different pH.

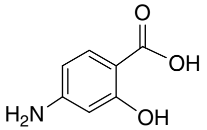
were found to be dependent on the pH of the stress conditions. Under acidic stress, one of the amino groups on the pyrazine ring was displaced by a hydroxyl group. In the intermediate pH range, the guanidine group underwent cyclization to form a pteridine ring. In 0.5 N NaOH solution at 100°C for 1 hour, ~50% of amiloride was decomposed to form two major degradants (A and B) [1]. Degradant A apparently results from the cleavage of the acyl-guanidine linkage, while Degradant B appears to stem from decarboxylation of Degradant A.

Amiloride is a known photosensitizer and its photodegradation chemistry has been studied [2–4]. The chromophore of amiloride is pH dependent, and as such its photochemical stability is also pH dependent. In a photodegradation study in aqueous anaerobic solutions of pH 4.5–11, the only photodegradant of amiloride isolated with a quantum yield of 0.23 was a photosubstitution product in which the chlorine is replaced by hydroxyl. At high pH range, the neutral molecule is ~3 times more photochemically reactive than its protonated form at low pH [2]. In another pH-dependent photodegradation study, four photodegradants were identified with LC–MS (Scheme 17).

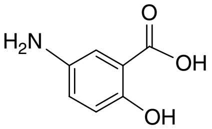
References

1. Lakshmi Narasimham, Y.S. and Barhate, V.D. (2010). Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms. *J. Chem. Metrol.* 4: 1–20.
2. Nian, Y., Li, B., Moore, D.E., and Tattam, B.N. (1999). Photodegradation of amiloride in aqueous solution. *Int. J. Pharm.* 109–116.
3. Calza, P., Massolino, C., Monaco, G. et al. (2008). Study of the photolytic and photocatalytic transformation of amiloride in water. *J. Pharm. Biomed. Anal.* 48: 315–320.
4. De Luca, M., Ioele, G., Mas, S. et al. (2012). A study of pH-dependent photodegradation of amiloride by a multivariate curve resolution approach to combined kinetic and acid–base titration UV data. *Analyst* 137: 5428–5435.

18–19: 4-Aminosalicylic Acid, 5-Aminosalicyclic Acid



4-Aminosalicylic acid



5-Aminosalicylic acid

18: 4-Aminosalicylic Acid

Chemical name: 4-Amino-2-hydroxybenzoic acid
Brand name: Granupas
Formula: $C_7H_7NO_3$
Monoisotopic protonated ion (m/z): 154.0499
Molecular weight: 153.14
CAS number: 65-49-6
Common salt form: Calcium and sodium salts

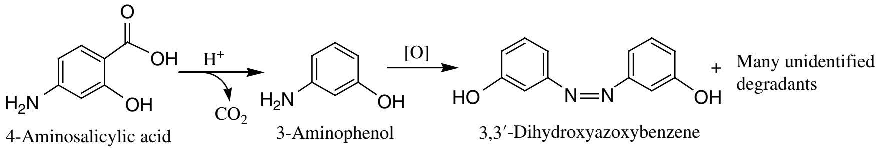
19: 5-Aminosalicyclic Acid (Mesalamine)

Chemical name: 5-Amino-2-hydroxybenzoic acid
Brand name: Asacol, Pentasa
Formula: $C_7H_7NO_3$
Monoisotopic protonated ion (m/z): 154.0499
Molecular weight: 153.14
CAS number: 89-57-6

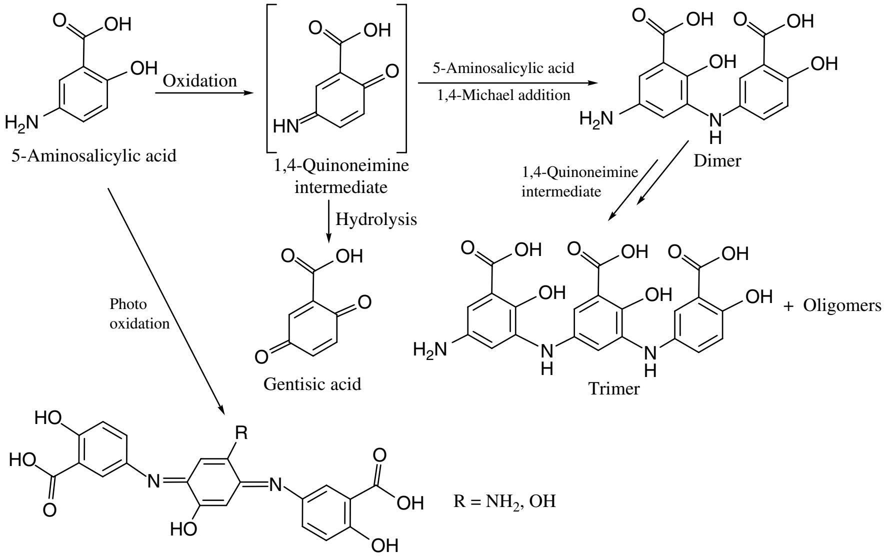
4-Aminosalicylic acid is an antitubercular drug, while 5-aminosalicylic acid (more commonly known as mesalamine) is an anti-inflammatory drug. The main degradation pathways of both drugs are decarboxylation and oxidation, respectively. Sodium, potassium, and calcium salts of 4-aminosalicylic acid are also employed therapeutically.

The decarboxylated degradant of 4-aminosalicylic acid, 3-aminophenol, is of particular toxicological concern, due to the very high therapeutic doses of the drug, which can be as high as 10–12 g/d. The decarboxylation takes place much more easily in acidic solutions than in neutral ones [1]. Hence, it would not be surprising that 4-aminosalicylic acid underwent appreciable decarboxylation in simulated gastric fluid according to the original research by Matsunaka, which was cited by Wesolowski[1]. To overcome the problem, enteric-coated tablets of the drug were developed [2]. In a formulation study, surfactants were found to significantly reduce the rate of decarboxylation [3].

The decarboxylated degradant, 3-aminophenol, is a highly electron-rich species and thus is susceptible to auto-oxidation, giving rise to numerous further degradants including polymeric degradants. Of the many further degradants mentioned in the



Scheme 18–19-1 Degradation pathway of 4-aminosalicylic acid.



Scheme 18–19-2 Degradation pathways of 5-aminosalicylic acid.

literature, only one was structurally identified: 3,3′-dihydroxyazoxybenzene, which is a degradant resulting from oxidative coupling of 3-aminophenol (Scheme 18–19-1) [4].

According to a study by Palsmeier et al. [5], although 5-aminosalicylic acid can undergo decarboxylation, its degradation in aqueous solutions is mostly of oxidative nature. The oxidation of 5-aminosalicylic acid is mediated through a 1,4-quinoneimine intermediate (Scheme 18–19-2). Hydrolysis of this intermediate leads to the formation of gentisic acid. On the other hand, the reaction of the intermediate with 5-aminosalicylic acid itself causes the formation of dimeric and oligomeric degradants. 5-Aminosalicylic acid can also undergo photooxidation; some trimers were among the photodegradants identified [6].

References

1 Wesolowski, M. (1977). The decarboxylation and thermal stability of *p*-aminosalicylic acid and its salts. *Thermochim. Acta* 21: 243–253.

2 Verma, A., Islam, S., Mishra, A.K., and Pandit, J.K. (2012). Formulation, optimization and evaluation of enteric coated tablets of para-amino salicylate sodium. *J. Sci. Ind. Res.* 71: 667–677.

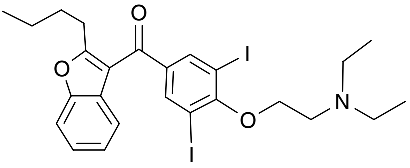
3 Plamondon, J.E. and Nairn, J.G. (1997). The effect of surfactants on the rate of decarboxylation of *p*-aminosalicylic acid in acidic aqueous solutions. *J. Pharm. Sci.* 86: 205–208.

4 Shih, I.K. (1971). Aminosalicylic acid, identity of a major chromogen in *p*-aminosalicylic acid and sodium *p*-aminosalicylate dosage forms. *J. Pharm. Sci.* 60: 1886–1887.

5 Palsmeier, R.K., Radzik, D.M., and Lunte, C.E. (1992). Investigation of the degradation mechanism of 5-aminosalicylic acid in aqueous solution. *Pharm. Res.* 9: 933–938.

6 Jensen, J., Cornett, C., Olsen, C.E. et al. (1992). Identification of major degradation products of 5-aminosalicylic acid formed in aqueous solutions and in pharmaceuticals. *Int. J. Pharm.* 88: 177–187.

20: Amiodarone



Chemical name: (2-Butyl-1-benzofuran-3-yl)-[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone

Brand name: Nexterone, Pacerone

Formula: $C_{25}H_{29}I_2NO_3$

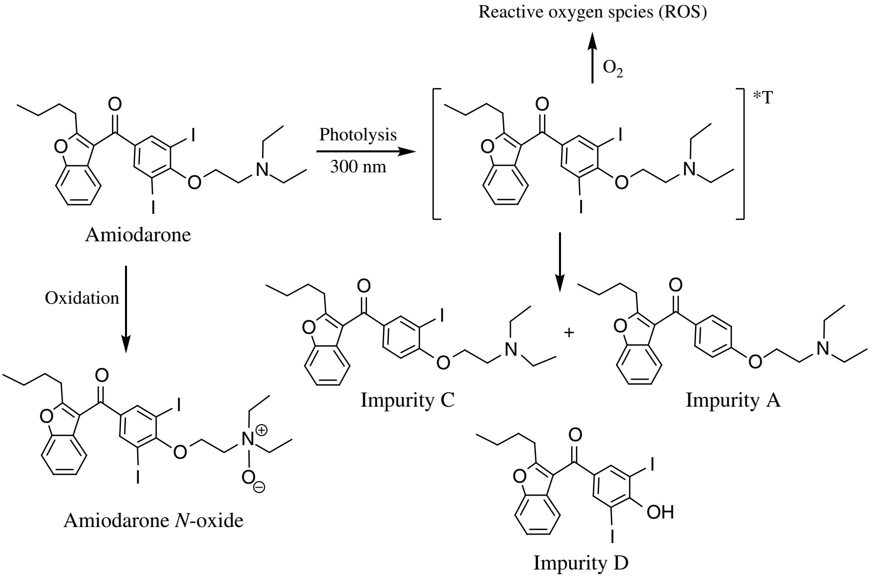
Monoisotopic protonated ion m/z: 646.0310

Molecular weight: 645.31

Common salt form: HCl salt

CAS number: 1951-25-3

Amiodarone is a class III antiarrhythmic drug. Its di-arylketone moiety is susceptible to photochemical degradation. According to the study by Martine and Verrierhe [1], photo-excited triplet state of the drug can sensitize oxygen, leading to the formation of reactive oxygen species. This process was suspected to be a contributing factor to the phototoxicity of the drug [2]. The photo-excited state of the drug can also lead to dehalogenation degradation products (Impurity A and C, Scheme 20) [1]. Further photodegradants include Impurity D, resulting from the



Scheme 20 Degradation pathways of amiodarone.

cleavage of the tertiary amine side chain. Since the drug also contains a tertiary amine moiety, it could potentially form the *N*-oxide degradant; nevertheless, the *N*-oxide would be difficult to form in its HCl salt form [3], as the nucleophilicity of the tertiary amine is blocked by protonation.

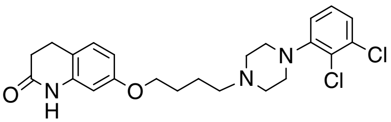
References

1 Paillous, N. and Verrier, M. (1988). Photolysis of amiodarone, an antiarrhythmic drug. *Photochem. Photobiol.* 47: 337–343.

2 Henry, B., Foti, C., and Alsante, K. (2009). Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? *J. Photochem. Photobio. B: Biol.* 96: 57–62.

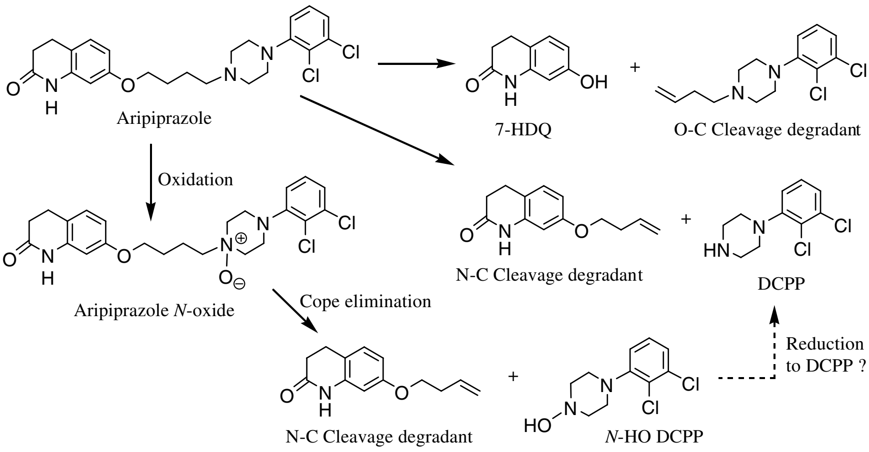
3 Khan, M.A., Kumar, S., Jayachandran, J. et al. (2005). Validation of a stability indicating LC method for amiodarone HCL and related substances. *Chromatographia* 61: 599–607.

21: Aripiprazole



Chemical name: 7-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy]-3,4-dihydro-1*H*-quinolin-2-one
Brand name: Abilify, Aripiprex
Formula: $C_{23}H_{27}Cl_2N_3O_2$
Monoisotopic protonated ion (*m/z*): 448.1553
Molecular weight: 448.39
CAS number: 129722-12-9

Aripiprazole is a novel drug used for the treatment of schizophrenia. Reddy et al. performed a forced degradation study on aripiprazole tablets [1]. After powdered aripiprazole tablets were stressed under dry heat at 105°C for 3 days, three major degradants were formed. Structure elucidation by LC–MS suggested that one of the degradants is the *N*-oxide, while the other two would result from the O–C and N–C cleavage, respectively (Scheme 21). The formation of the O–C cleavage degradant should proceed through the elimination of 7-HDQ, which is rather unusual. Cleavage of an O–C linkage of this nature would usually be preceded by oxidation. In a similar vein, the cleavage of the N–C linkage, leading to the formation of the N–C cleavage degradant, would likely proceed through the *N*-oxide intermediate, which would be followed by the Cope elimination [2]. In the latter scenario, the other cleavage product would be *N*-hydroxyl DCPP, which may be reduced to DCPP by reductive components of the formulation. The *N*-oxide was also generated via stress with hydrogen peroxide in the same forced degradation study by Reddy et al. [1].



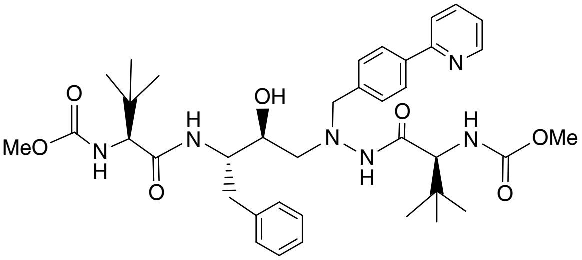
Scheme 21 Degradation pathways of aripiprazole.

References

1 Reddy, G.V.R., Kumarb, A.P., Reddy, B.V. et al. (2010). Identification of degradation products in Aripiprazole tablets by LC-QToF mass spectrometry. *Eur. J. Chem.* 1: 20–27.

2 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*, 73–75. Cambridge: Royal Society Chemistry Publishing.

22: Atazanavir



Chemical name: Methyl *N*-[(2*S*)-1-[2-[(2*S*,3*S*)-2-hydroxy-3-[[(2*S*)-2-(methoxycarbonylamino)-3,3-dimethylbutanoyl]amino]-4-phenylbutyl]-2-[(4-pyridin-2-ylphenyl)methyl]hydrazinyl]-3,3-dimethyl-1-oxobutan-2-yl]carbamate

Brand name: Reyataz

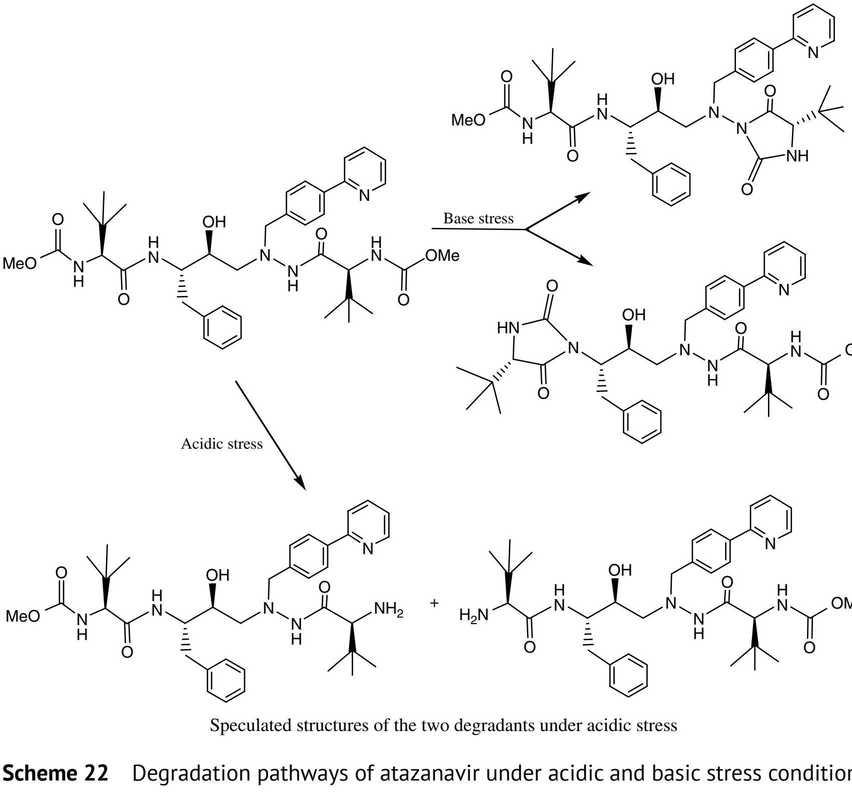
Formula: $C_{38}H_{52}N_6O_7$

Monoisotopic protonated ion (*m/z*): 705.3970

Molecular weight: 704.86

CAS number: 198904-31-3

Common salt form: Sulfate



Speculated structures of the two degradants under acidic stress

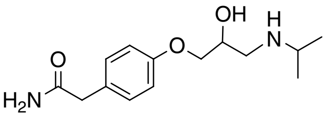
Scheme 22 Degradation pathways of atazanavir under acidic and basic stress conditions.

Atazanavir is an HIV protease inhibitor. Its structure is somewhat symmetric with both ends of the molecule containing a methyl carbamate moiety. Chitturi et al. carried out a comprehensive forced degradation study [1]. Two major base-stress degradants were identified via LC–MS/MS, NMR, and IR. Both degradants stem from the cyclization of the carbamate moieties (Scheme 22). Acidic stress yielded two degradants at 2.91% and 0.68%, but their structures were not identified in the study. Based on the structure of the drug molecule, the two acid-stress degradants could be due to the hydrolysis of the two carbamate moieties, respectively.

Reference

1 Chitturi, S.R., Somannavar, Y.S., Peruri, B.G. et al. (2011). Gradient RP-HPLC method for the determination of potential impurities in atazanavir sulfate. *J. Pharm. Biomed. Anal.* 55: 31–47.

23: Atenolol



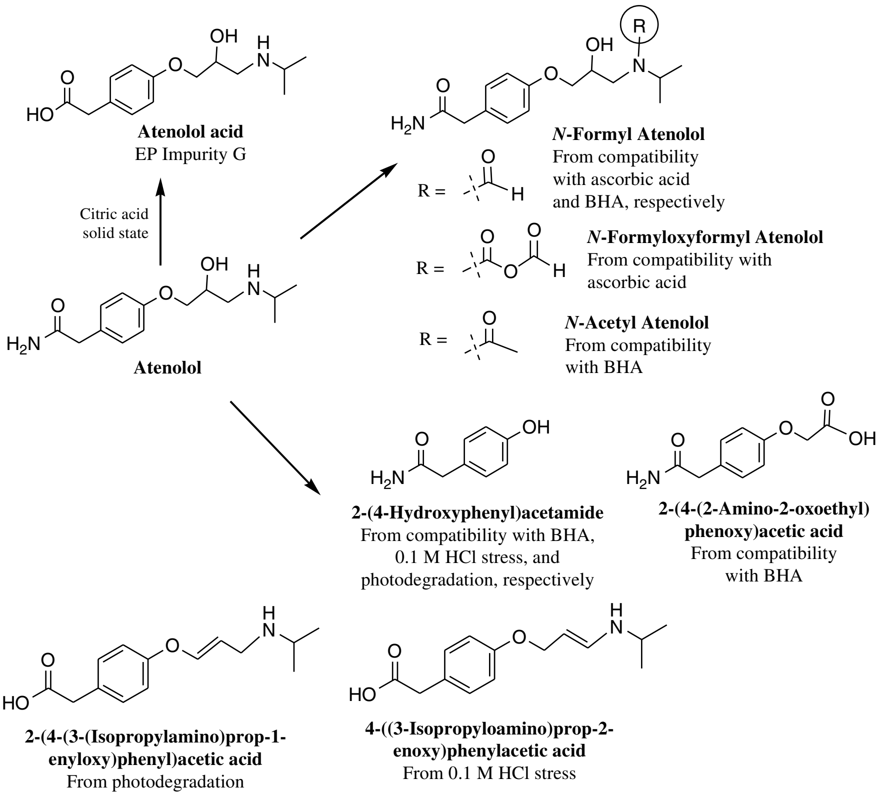
Chemical name: 2-[4-[2-Hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide
Brand names: Tenoretic, Tenormin
Formula: $C_{14}H_{22}N_2O_3$
Monoisotopic protonated ion (*m/z*): 267.1703
Molecular weight: 266.34
CAS number: 29122-68-7

Atenolol is a beta-blocker used as a treatment for hypertension and coronary heart disease. It contains a primary amide and a secondary amine moiety. As a secondary amine, this drug may not be compatible with excipients containing reducing-end sugar such as lactose. In addition, this moiety can interact with impurities of certain excipients that contain reactive keto functionalities. Vijay Kumar et al. performed a series of compatibility studies of atenolol with citric acid, ascorbic acid, and butylated hydroxyanisole (BHA) [1]; solid atenolol was mixed with equal weights of three excipients, respectively and then stressed under the ICH accelerated condition for 1 month. Various degradants were formed and the structures of the degradants were elucidated by LC–MS. In the compatibility study with citric acid, the degradant was the hydrolytic degradant, EP impurity E, resulting from the hydrolysis of the amide linkage. In the compatibility study with ascorbic acid, EP impurity G was also formed. Furthermore, two unknown degradants were observed as well. Accurate MS and MS/MS analysis suggested that these two unknown degradants are *N*-formyl and *N*-formyloxyformyl adducts of atenolol (the latter degradant was referred to as carbamic formic anhydride of atenolol by the original researchers). The *N*-formyl and *N*-formyloxyformyl groups would most likely originate from ascorbic acid, since the latter is not very stable and decomposes to many degradation products under elevated temperature and moisture [2]. Although the structure assignments in the paper seem reasonable, it appears that the majority of the proposed formation mechanisms need additional supporting evidence. In the compatibility study with BHA, four degradants were produced: *N*-formyl atenolol, *N*-acetyl atenolol, 2-(4-hydroxyphenyl)acetamide, and 2-(4-[2-amino-2-oxoethyl]phenoxy)acetic acid. The formation of the latter two degradants apparently resulted from oxidative cleavage of the main backbone of the drug molecule. On the other hand, the formation of the first two degradants was proposed to stem from the reactions of the drug molecule with formyl and acetyl donors, which themselves are products of the oxidative cleavage, respectively. It seems to be paradoxical that the antioxidant, BHA, would cause oxidation of atenolol in this compatibility study when it was present in overwhelming amount as compared to a typical amount that is usually in the range of single digit percentage or less.

In an acidic hydrolytic stress study with 0.1 M HCl at 90°C for 1 hour, in addition to the degradant 2-(4-hydroxyphenyl)acetamide, which was also observed in the oxidative cleavage described above, a dehydrated degradant, 4-([3-isopropyloamino]-2-propenoxy)phenylacetic acid, was also produced [3]. The position of the double bond resulting from the dehydration was confirmed by ^{1}H NMR. The UV spectrum of the dehydrated degradant is very similar to that of atenolol, which would be consistent with the double bond position assignment.

In a photodegradation study of atenolol with UVA–UVB radiation, two photodegradants were found [4]. At pH 7.4, the main degradant was identified as 2-(4-hydroxyphenyl)acetamide based on ^{1}H NMR and UV data and the minor one was identified as a dehydrated degradant based on GC–MS and UV results. However, this dehydrated degradant is different from the dehydrated degradant that was formed in the acidic stress at 90°C as described above. The difference lies in the double bond position of the two degradants.

The degradation products from the aforementioned studies [1–4] are summarized in Scheme 23.

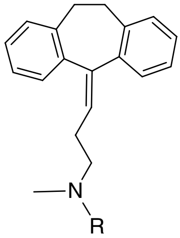


Scheme 23 Degradation pathways of atenolol.

References

1. Kumar, V., Shah, R.P., Malik, S., and Singh, S. (2009). Compatibility of atenolol with excipients: LC–MS/TOF characterization of degradation/interaction products, and mechanisms of their formation. *J. Pharm. Biomed. Anal.* 49: 880–888.
2. Shephard, A.B., Nichols, S.C., and Braithwaite, A. (1999). Moisture induced solid phase degradation of l-ascorbic acid. Part 2, separation and characterization of the major degradation products. *Talanta* 48: 595–606.
3. Krzek, J. and Kwiecien, A. (2006). Stability of atenolol, acebutolol and propranolol in acidic environment depending on its diversified polarity. *Pharm. Dev. Technol.* 11: 409–416.
4. Andrisano, V., Gotti, R., Leoni, A., and Cavrini, V. (1999). Photodegradation studies on atenolol by liquid chromatography. *J. Pharm. Biomed. Anal.* 21: 851–857.

24–25: Amitriptyline, Nortriptyline



Amitriptyline R=Me;
Nortriptyline R=H.

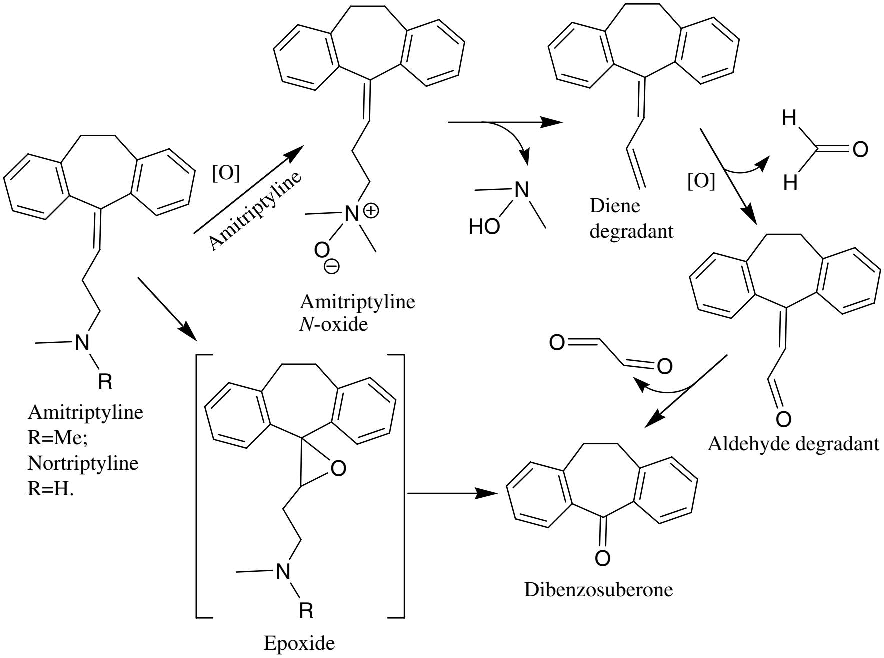
24: Amitriptyline

Chemical name: *N,N*-Dimethyl-3-(2-tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaenylidene)propan-1-amine
Brand names: Elavil
Formula: $C_{20}H_{23}N$
Monoisotopic protonated ion (*m/z*): 278.1903
Molecular weight: 277.4041
CAS number: 50-48-6
Common salt form: HCl salt

25: Nortriptyline

Chemical name: *N*-Methyl-3-(2-tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaenylidene) propan-1-amine
Brand names: Aventyl, Pamelor
Formula: $C_{19}H_{21}N$
Monoisotopic protonated ion (*m/z*): 264.1747
Molecular weight: 263.38
CAS number: 72-69-5
Common salt form: HCl salt

Amitriptyline is a classic tricyclic antidepressant. Nortriptyline is an active metabolite of amitriptyline, which itself is also a drug. In a stability study of amitriptyline in pH 6.8 buffered solution under autoclaving conditions, three degradants, “diene,” “aldehyde,” and dibenzosubrone, were formed (Scheme 24–25) [1]. The formation of the “diene” can be rationalized by the Cope elimination of the *N*-oxide intermediate. The *N*-oxide, a metabolite of amitriptyline, was not detected in this particular study. It is possible that once formed, the *N*-oxide would immediately undergo the Cope elimination under the autoclaving temperature of 115–116°C. Dibenzosubrone appears to be a terminal degradant, which is also an oxidative degradant of nortriptyline [2].



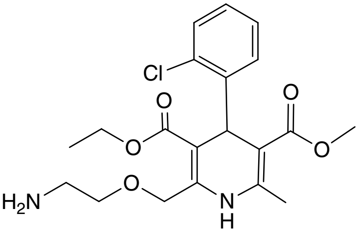
Scheme 24–25 Oxidative degradation pathways of amitriptyline.

References

1 Enever, R.P., Po, A.L.W., Millard, B.J., and Shotton, E. (1975). Decomposition of amitriptyline hydrochloride in aqueous solution: identification of decomposition products. *J. Pharm. Sci.* 64: 1497–1499.

2 Misiuk, W. and Tykocka, A. (2007). Sensitive extractive spectrophotometric methods for the determination of nortriptyline hydrochloride in pharmaceutical formulations. *Chem. Pharm. Bull. (Tokyo)* 55: 1655–1661.

26: Amlodipine

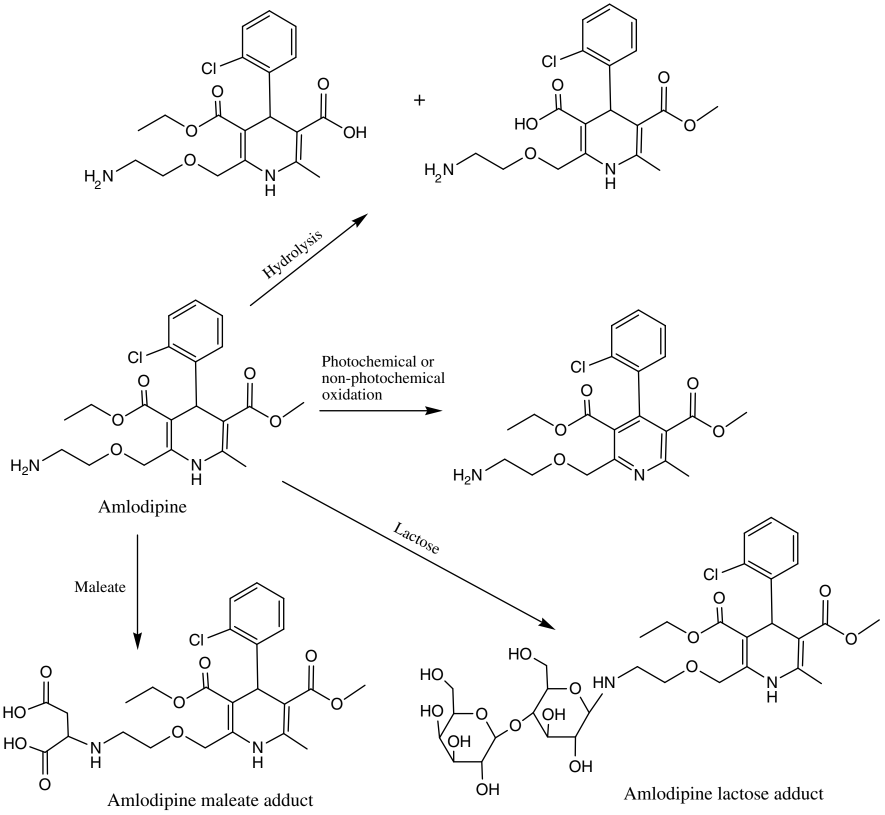


Chemical name: 3-*O*-ethyl 5-*O*-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl) -6-methyl-1,4-dihydropyridine-3,5-dicarboxylate
Brand names: Norvasc (for Amlodipine Besylate)
Formula: $C_{20}H_{25}ClN_2O_5$
Monoisotopic protonated ion (*m/z*): 409.1525
Molecular weight: 408.88
CAS number: 88150-42-9
Common salt form: Besylate, maleate

Amlodipine is a calcium channel blocker used for the treatment of hypertension and angina. Amlodipine besylate, which was originally introduced by Pfizer, is one of the most widely prescribed cardiovascular drugs. Amlodipine has a 1,4-dihydropyridine core structure that is very susceptible to oxidation, leading to the formation of a more stable, aromatic pyridine ring (Scheme 26). This oxidative aromatization can take place both photochemically and non-photochemically. Photochemical aromatization of amlodipine can occur in both UV and natural light environment; in the UV region, irradiation at 366 nm was found to be more effective than at 254 nm [1]. Other drugs in the 1,4-dihydropyridine family, such as nifedipine and nisoldipine, suffer from the same photooxidative degradation pathway [1–3]. To prevent or minimize this main degradation pathway, Ragno et al. performed formulation studies with supramolecular systems such as liposomes, cyclodextrins, and microspheres and found that these matrices could significantly reduce the photochemical oxidation process through physical and chemical barriers [4].

Amlodipine also contains two ester functional groups, which are susceptible to hydrolytic stress, particularly under base stress conditions [5–7].

Lastly, amlodipine has a primary amine group that has been found to be incompatible toward excipients containing reducing sugar components due to the Maillard reaction [8, 9]. The presence of basic excipients like magnesium stearate would accelerate this degradation process due to its ability to neutralize the acid component of the drug. For amlodipine maleate, its amine moiety was found to react with maleate via Michael addition in commercial formulations [9].



Scheme 26 Degradation pathways of amlodipine.

References

1 Fasani, E., Albini, A., and Gemme, S. (2008). Mechanism of the photochemical degradation of amlodipine. *Int. J. Pharm.* 352: 197–201.

2 Ragno, G., Garofalo, A., and Vetuschi, C. (2002). Photodegradation monitoring of amlodipine by derivative spectrophotometry. *J. Pharm. Biomed. Anal.* 27: 19–24.

3 Fasani, E., Dondi, D., Ricci, A., and Albini, A. (2006). Photochemistry of 4-(2-nitrophenyl)-1,4-dihydropyridines. Evidence for electron transfer and formation of an intermediate. *Photochem. Photobiol.* 82: 225–230.

4 Ragno, G., Cione, E., Garofalo, A. et al. (2003). Design and monitoring of photostability systems for amlodipine dosage forms. *Int. J. Pharm.* 265: 125–132.

5 Mohammadi, A., Rezanour, N., Ansari, D.M. et al. (2007). RP-LC Simultaneous determination of nebivolol hydrochloride and amlodipine besilate in Bi-Layer Tablets. *J. Chromatogr. B.* 846: 215–221.

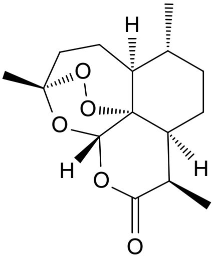
6 Naidu, K.R., Kale, U.N., and Shingare, M.S. (2005). Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benezepril hydrochloride from their combination drug product. *J. Pharm. Biomed. Anal.* 39: 147–155.

7 Chaudhari, B.G., Patel, N.M., and Shah, P.B. (2007). Stability indicating RP-HPLC method for simultaneous determination of atorvastatin and amlodipine from their combination drug products. *Chem. Pharm. Bull.* 55: 241–246.

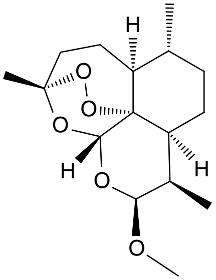
8 Abdoh, A., Al-Omari, M.M., Badwan, A.A., and Jaber, A.M.Y. (2004). Amlodipine besylate-excipients interaction in solid dosage form. *Pharm. Dev. Technol.* 9: 15–24.

9 Murakami, T., Fukutsu, N., Kondo, J. et al. (2008). Application of liquid chromatography-two-dimensional nuclear magnetic resonance spectroscopy using pre-concentration column trapping and liquid chromatography–mass spectrometry for the identification of degradation products in stressed commercial amlodipine maleate tablets. *J. Chromatogr. A.* 1181: 67–76.

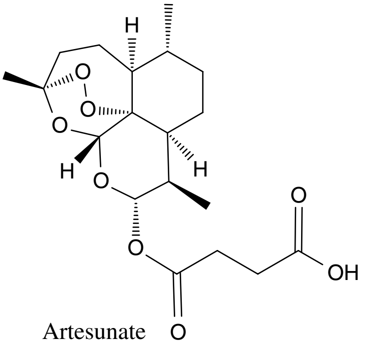
27–29: Artemisinin, Artemether, Artesunate



Artemisinin



Artemether



Artesunate

27: Artemisinin

Chemical name: (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin-10(3*H*)-one; (1*R*,4*S*,5*R*,8*S*,9*R*,12*S*,13*R*)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.$0^{4,13}$.$0^{8,13}$]hexadecan-10-one
Brand names: N/A
Formula: $C_{15}H_{22}O_5$
Monoisotopic protonated ion (*m/z*): 283.1540
Molecular weight: 282.33
CAS number: 63968-64-9

28: Artemether

Chemical name: (1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*R*,13*R*)-10-Methoxy-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.$0^{4,13}$.$0^{8,13}$]hexadecane
Brand names: Coartem (in combination with Lumefantrine)
Formula: $C_{16}H_{26}O_5$
Monoisotopic protonated ion (*m/z*): 299.1853
Molecular weight: 298.38
CAS number: 71963-77-4

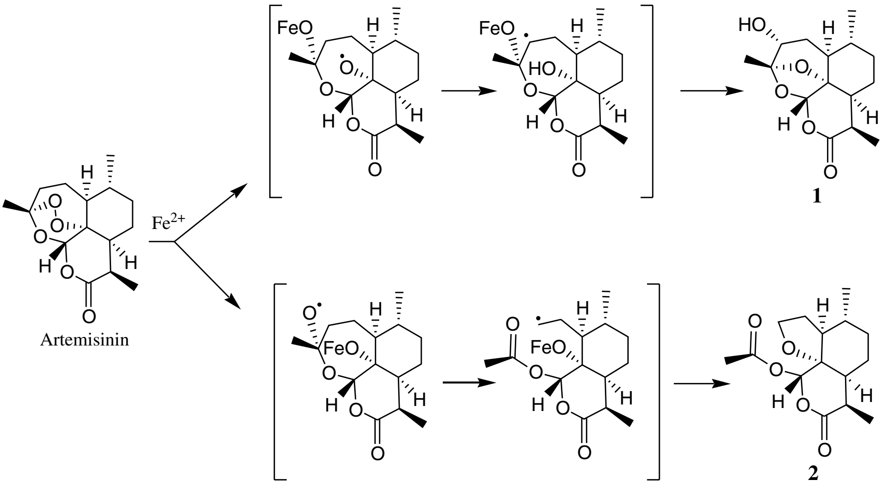
29: Artesunate

Chemical name: 4-oxo-4-[[(1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*R*,13*R*)-1,5,9-Trimethyl-11,14,15,16 -tetraoxatetracyclo[10.3.1.^{04,13}.$0^{8,13}$]hexadecan-10-yl]oxy]butanoic acid
Brand names: Artesunate-Amivas
Formula: $C_{19}H_{28}O_8$
Monoisotopic protonated ion (*m/z*): 385.1857
Molecular weight: 384.42
CAS number: 88495-63-0
Common salt form: Sodium salt

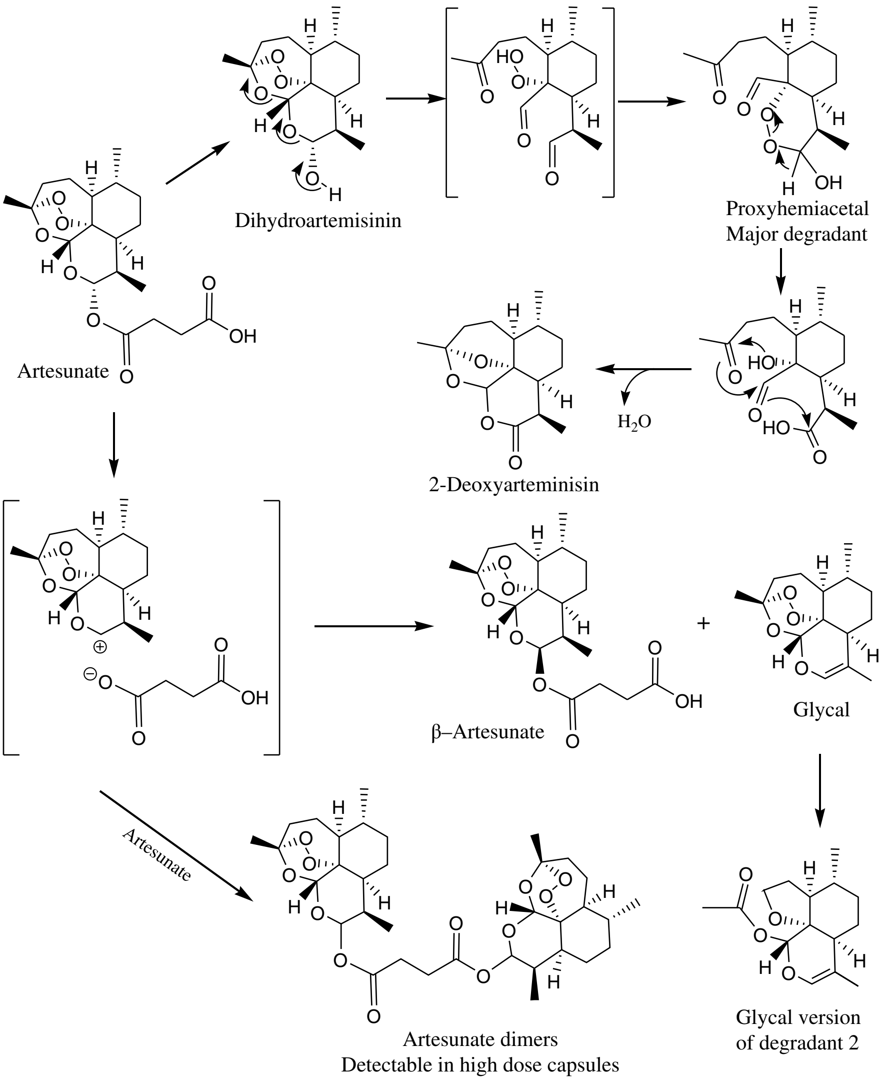
Artemisinin is a potent antimalarial drug that was isolated in the 1970s by Chinese scientists from *Artemisia annua*, a plant native to China. This plant is called Qinhao in Chinese, and artemisinin is known as Qinhaosu in China, meaning the element or essence of Qinhao. Artemisinin has a unique sesquiterpine endoperoxide moiety which is essential to the activity of the drug. Upon chelation with reduced iron ion (Fe^{2+}), the endoperoxide linkage breaks down via reductive scission to form oxy free radicals. The latter can rearrange to form a more stable carbon-based radical [1]. Under in vivo conditions where the reduced iron is mainly in heme, the carbon-based radical is believed to be the agent that kills the malaria parasite through alkylation [2–5]. This mechanism of action also constitutes a major degradation pathway for artemisinin in the presence of reduced iron ion as illustrated in Scheme 27–29-1 [6]. There were a number of minor degradants whose structures were not identified.

Because of its poor bioavailability, artemisinin itself has become much less utilized clinically. Instead, its two derivatives, artemether and artesunate, are now the main therapeutic forms. In addition, due to the concern of drug resistance, WHO mandates that artemisinin derivatives be used in combination with other antimalaria drugs, such as lumefantrine, amodiaquine, and mefloquine. The water-soluble artesunate has additional degradation pathways owing to the introduction of the succinate moiety. Analysis of artesunate rectal capsules stored under long-term stability conditions revealed the presence of several minor degradants due to the succinate moiety, in addition to the proxyhemiacetal degradant, the major degradant, and 2-deoxyartemisinin (Scheme 27–29-2) [7].

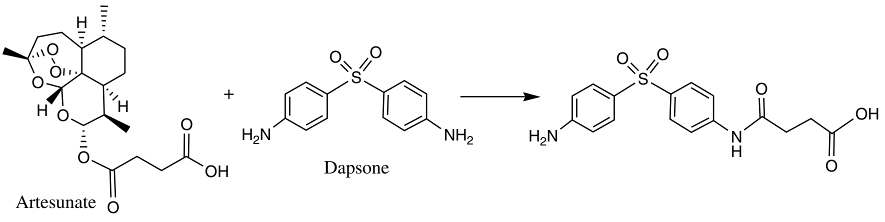
In a fixed dose combination of artesunate and amodiaquine formulated as bi-layer tablets, a glycal version of degradant 2 was observed after the tablets were heated under elevated temperatures [8]. In a triple combination of artesunate, chlorproguanil, and dapsone, a degradant that resulted from the transfer of the succinate moiety from artesunate to dapsone was found in long-term stability samples (Scheme 27–29-3) [9].



Scheme 27–29-1 Degradation of artemisinin in solutions containing spiked Fe^{2+}. A number of unidentified minor degradants were also present.



Scheme 27–29-2 Degradation of artesunate in capsules and combination tablets.



Scheme 27–29-3 Degradation of artesunate in a triple combination product containing dapsone.

References

1 O’Neill, P.M. and Posner, G.H. (2004). A medicinal chemistry perspective on artemisinin and related endo-peroxides. *J. Med. Chem.* 47: 2945–2964.

2 Robert, A., Coppel, Y., and Meunier, B. (2002). Alkylation of heme by the antimalarial drug artemisinin. *Chem. Commun.* 5: 414–415.

3 Wang, D.Y. and Wu, Y.L. (2000). A possible antimalarial action mode of qinghaosu (artemisinin) series compounds. Alkylation of reduced glutathione by C-centered primary radicals produced from anti-malarial compound qinghaosu and 12-(2,4-dime-thoxyphenyl)-12-deoxoqinghaosu. *Chem. Commun.* 22: 2193–2194.

4 Wu, W.M., Yao, Z.J., Wu, Y.L. et al. (1996). Ferrous ion induced cleavage of the peroxy bond in qinghaosu and its derivatives and the DNA damage associated with this process. *Chem. Commun.* 18: 2213–2214.

5 Asawamahasakda, W., Ittarat, I., Pu, Y. et al. (1994). Reaction of antimalarial endo-peroxides with specific parasite proteins. *Antimicrob. Agents Chemother.* 38: 1854–1858.

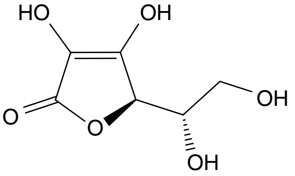
6 Creek, D.J., Chiu Francis, C.K., Prankerd, R.J. et al. (2005). Kinetics of iron-mediated artemisinin degradation: effect of solvent composition and iron salt. *J. Pharm. Sci.* 94: 1820–1829.

7 Haynes, R.K., Chan, H.-W., Lung, C.-M. et al. (2007). Artesunate and dihydroartemisinin (DHA): unusual decomposition products formed under mild conditions and comments on the fitness of DHA as an antimalarial drug. *ChemMedChem.* 2: 1448–1463.

8 Charrier, C., Bertho, G., Petigny, O. et al. (2013). A new derivative detected in accelerated ageing of artesunate-amodiaquine fixed dose combination tablets. *J. Pharm. Biomed. Anal.* 81–82: 20–26.

9 Bardsley, B., Barry, S.J., Drozdz, M.A. et al. (2011). Formation and identification of a degradant in chlorproguanil–dapsone–artesunate (Dacart™) tablets. *J. Pharm. Biomed. Anal.* 54: 610–613.

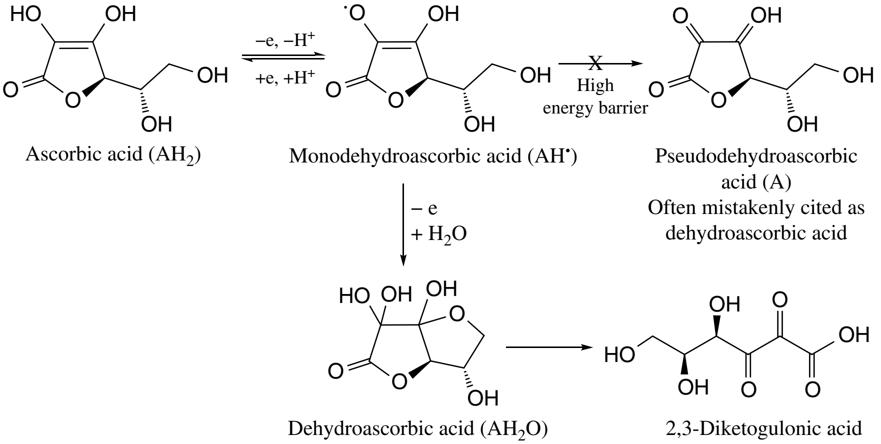
30: Ascorbic Acid [Vitamin C]



Chemical name: L-*threo*-Hex-2-enono-1,4-lactone; (*R*)-3,4-Dihydroxy-5-((*S*)-1,2-dihydroxyethyl)furan-2(5*H*)-one
Brand names: Ascor and many others
Formula: $C_6H_8O_6$
Monoisotopic protonated ion (*m/z*): 177.0394
Molecular weight: 176.12
CAS number: 50-81-7

Ascorbic acid, better known as vitamin C, is a water-soluble essential vitamin. Clinically, it is used to treat vitamin C deficiency and help iron absorption. More commonly it is marketed as a general antioxidant supplement. Furthermore, ascorbic acid is also employed as an antioxidative excipient in the formulation of certain drugs that are labile to oxidative degradation [1].

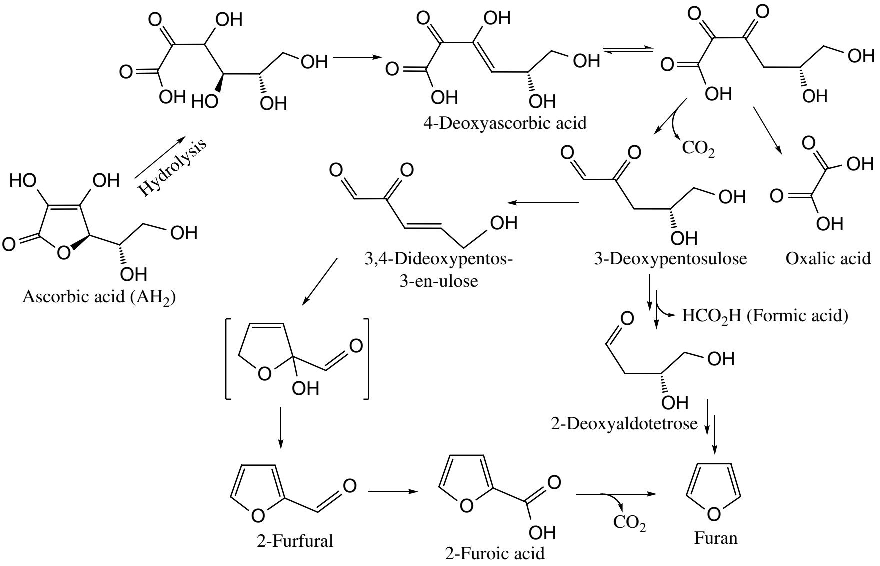
Ascorbic acid (AH_2) displays different degradation pathways in solution and solid states [2, 3]. In solution, its main oxidative degradant is dehydroascorbic acid (DHA) upon exposure to light, transition metal ions (especially cupric and ferric ions), and alkaline pH (Scheme 30-1) [2, 4, 5]. According to a recent paper by Njus et al. [6], the real structure of dehydroascorbic acid is not the commonly cited tricarbonyl structure (A), but rather a bicyclic hemiketal structure (AH_2O). For



Scheme 30-1 Degradation mechanism of ascorbic acid leading to the formation of 2,3-diketogulonic acid.

this reason, Njus et al. suggested that the tricarbonyl structure be called pseudo-dehydroascorbic acid. Ascorbic acid exerts its antioxidative function by affording an equivalent of H (or $H^+ + e^-$) to form monodehydroascorbic acid, which is a free radical and can react with other radical species, hence becoming a terminator of free radical chain reactions. In biological systems, ascorbic acid cycles back and forth with monodehydroascorbic acid. In chemical degradation, monodehydroascorbic acid can further oxidize to dehydroascorbic acid, which can hydrolyze to produce 2,3-diketogulonic acid [4].

Other than the aforementioned degradation pathways, ascorbic acid can also yield furan degradants [7] including hydroxymethylfurfural (HMF), which is a common degradant for several carbohydrates containing six carbons. In a study of ascorbic acid stability in citrus juice concentrates, the accumulation of HMF was highly correlated with the degradation of ascorbic acid [8]. In a study of examining ascorbic acid's decomposition into furan, a Class 2B carcinogen by IARC classification, using model systems to mimic food cooking [7], a number of known ascorbic acid degradants were proposed as the intermediates leading to the formation of furan. These intermediates are 4-deoxyascorbic acid, 3-dexoypentosulose, 3,4-dideoxypentos-3-en-ulose, 2-furfural, 2-furoic acid, and 2-deoxyaldotetrose (Scheme 30-2). During the decomposition process, oxalic acid, formic acid, and carbon dioxide were also formed as elimination products. In a solid-state degradation study of ascorbic acid, carbon dioxide was found to be a degradant formed in 1:1 stoichiometric quantity from ascorbic acid [3].



Scheme 30-2 Degradation of ascorbic acid leading to various degradants.

References

1 Kroselj V., Lakse R., Toporisic R., Kastelic J. Pharmaceutical composition comprising simvastatin and ezetimibe. US Patent 8,921,352 B2. filed 30 June 2006. granted 30 December 2014.

2 Shen, J., Griffiths, P.T., Campbell, S.J. et al. (2021). Ascorbate oxidation by iron, copper and reactive oxygen species: review, model development, and derivation of key rate constants. *Sci. Rep.* 11: 7417. https://doi.org/10.1038/s41598-021-86477-8.

3 Shephard, A.B., Nichols, S.C., and Braithwaite, A. (1999). Moisture induced solid phase degradation of l-ascorbic acid part 3, structural characterisation of the degradation products. *Talanta* 48: 607–622.

4 Yin, X., Chen, K., Cheng, H. et al. (2022). Chemical stability of ascorbic acid integrated into commercial products: a review on bioactivity and delivery technology. *Antioxidants* 11: 153. https://doi.org/10.3390/antiox11010153.

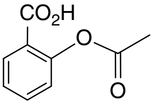
5 Jansson, P.J., Jung, H.R., Lindqvist, C., and Nordström, T. (2004). Oxidative decomposition of vitamin C in drinking water. *Free Radic. Res.* 38: 855–860. https://doi.org/10.1080/10.15760410001700497.

6 Njus, D., Kelley, P.M., Tu, Y.-J., and Schlegel, H.B. (2020). Ascorbic acid: the chemistry underlying its antioxidant properties. *Free Radic. Biol. Med.* 159: 37–43. https://doi.org/10.1016/j.freeradbiomed.2020.07.013.

7 Limacher, A., Kerler, J., Conde-Petit, B., and Blank, I. (2007). Formation of furan and methylfuran from ascorbicacid in model systems and food. *Food Addit. Contam.* 24 (Suppl 1): 122–135. https://doi.org/10.1080/02652030701393112.

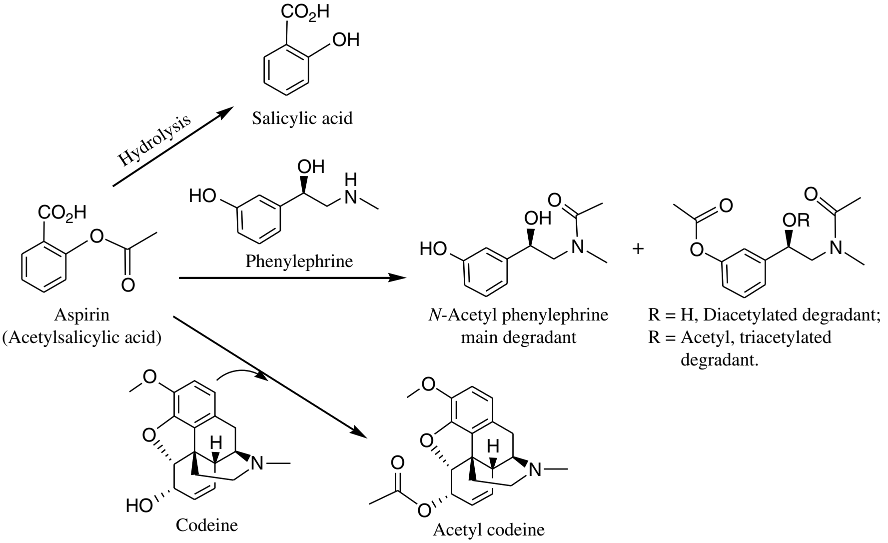
8 Burdurlu, H.S., Koca, N., and Karadeniz, F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. *J. Food Eng.* 74: 211–216.

31: Aspirin



Chemical name: Acetylsalicylic acid; 2-Acetyloxybenzoic acid
Brand name: Aspirin
Formula: $C_9H_8O_4$
Monoisotopic protonated ion (*m/z*): 181.0495
Molecular weight: 180.16
CAS number: 50-78-2

Aspirin, one of the oldest synthetic drugs, is chemically known as acetylsalicylic acid. It remains as one of the most widely used medications. Its ester linkage is quite susceptible to hydrolytic degradation (Scheme 31) [1]. Since aspirin is also co-formulated with other drugs, its acetyl group can transfer to those drugs that contain nucleophilic groups, particularly the amino group. For example, in a tablet formulation of aspirin with phenylephrine, a secondary amine drug molecule, the main degradant was *N*-acetyl phenylephrine, resulting from trans-acetylation of aspirin onto the secondary amino group of phenylephrine [2]. Further degradation produced minor degradants containing two or even three acetyl groups. In the latter case, since the transfer of the second and third acetyl group results in the formation of ester bonds, it can also be considered a type of degradation via trans-esterification. Another case of degradation via trans-esterification was reported in a co-formulation study of aspirin with codeine; acetyl codeine was found to be a detectable degradant even at a low moisture level [3].

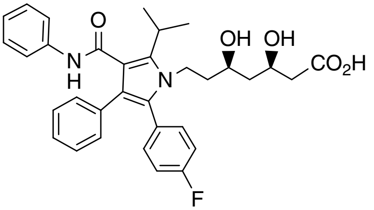


Scheme 31 Degradation pathways of aspirin.

References

1. Garrett, E.R. (1957). The kinetics of solvolysis of acyl esters of salicyclic acid. *J. Am. Chem. Soc.* 79: 3401–3408.
2. Troup, A.E. and Mitchner, H. (1964). Degradation of phenylephrine hydrochloride in tablet formulations containing aspirin. *J. Pharm. Sci.* 53: 375–379.
3. Galante, R.N., Visalli, A.J., and Patel, D.M. (1979). Solid-state acetylation of codeine phosphate by aspirin. *J. Pharm. Sci.* 68: 1494–1498.

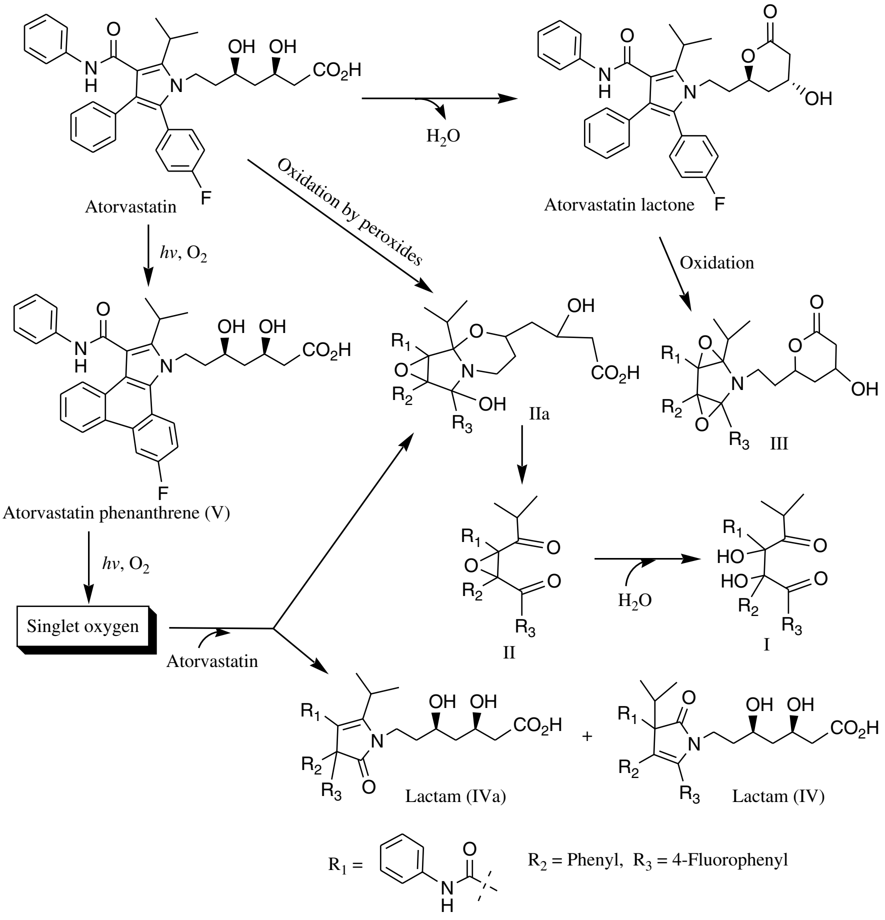
32: Atorvastatin



Chemical name: (3*R*,5*R*)-7-[2-(4-Flluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid
Brand names: Lipitor
Formula: $C_{33}H_{35}FN_2O_5$
Monoisotopic protonated ion (*m/z*): 559.2603
Molecular weight: 558.64
CAS number: 134523-00-5
Common salt form: Calcium salt

Atorvastatin, a top-selling cholesterol-lowering drug, has a fully substituted pyrrole core with a characteristic side chain of 3,5-dihydroxyheptanoic acid. It is usually used as a calcium salt. The most significant degradation pathway of atorvastatin is the cyclization of the 5-hydroxyl group with the carboxyl group of the heptanoic acid side chain to form a six-membered lactone [1]. Oxidative and photodegradation studies showed that the pyrrole ring is susceptible to oxidative degradation, resulting in several degradants [2]. On the other hand, amorphous atorvastatin calcium is much more oxidizable than the crystalline form. In a patent application, antioxidants were added into amorphous atorvastatin calcium to stabilize the drug substance [3]. In various experimental tablet formulations, a total of five oxidative degradants (I to V, Scheme 32) were found. In another patent application, it was found that amorphous atorvastatin calcium might be stabilized by using appropriate antioxidants [3].

In a mechanistic study with regard to the reported phototoxicity of atorvastatin, Montanaro et al. found that it is atorvastatin phenanthrene, rather than atorvastatin itself, that photosensitizes triplet oxygen to produce singlet oxygen [4]. The latter then oxidizes the pyrrole ring via perepoxide [4] and/or endoepoxide [5] intermediates to yield a number of oxidative degradants (Scheme 32). Some of these degradants are not very stable, and their occurrence may vary depending on the conditions of oxidation. The oxidation of the pyrrole ring can also occur nonphotochemically. In an oxidative degradation study of atorvastatin, an oxidative degradant isomeric to IIa was generated by oxidation of atorvastatin with alkaline hydrogen peroxide [5].



Scheme 32 Degradation pathways of atorvastatin.

References

1 Stach, J., Havlíček, J., Plaček, L., and Rádl, S. (2008). Synthesis of some impurities and/or degradation products of atorvastatin. *Collect. Czechoslov. Chem. Commun.* 73: 229–246.

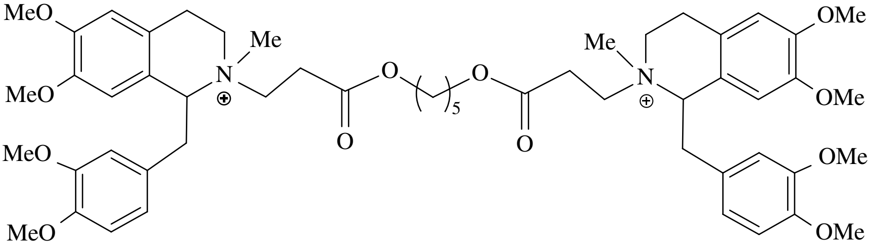
2 Kracun, M., Kocijan, A., Bastarda, A. et al. (2009). Isolation and structure determination of oxidative degradation products of atorvastatin. *J. Pharm. Biomed. Anal.* 50: 729–736.

3 Kumar Y., Kumar S.M.D., and Sathyanarayana S. (2008). Process for the production of atorvastatin calcium in amorphous form. US patent application 20090216029, filed 21 February 2008.

4 Montanaro, S., Lhiaubet-Vallet, V., Iesce, M. et al. (2009). A mechanistic study on the phototoxicity of atorvastatin: singlet oxygen generation by a phenanthrene-like photoproduct. *Chem. Res. Toxicol.* 22: 173–178.

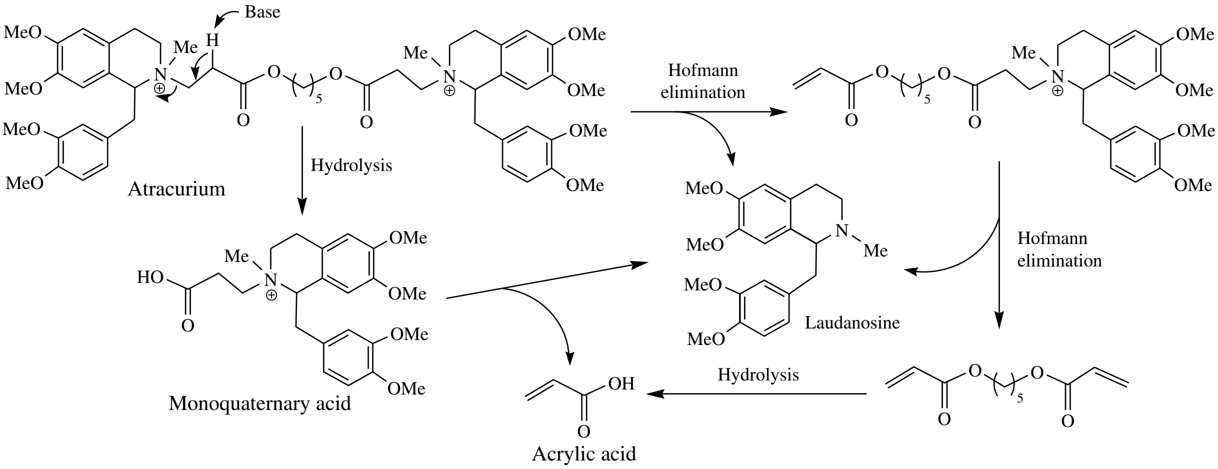
5 Cermola, F., DellaGreca, M., Iesce, M.R. et al. (2006). Photochemical behavior of the drug atorvastatin in water. *Tetrahedron* 62: 7390–7395.

33: Atracurium



Chemical name: 5-[3-[1-[(3,4-Dimethoxyphenyl)methyl]-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-2-ium-2-yl]propanoyloxy]pentyl-3-[1-[(3,4-dimethoxy phenyl)methyl]-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-2-ium-2-yl] propanoate
Brand names: N/A
Formula: $C_{53}H_{72}N_2O_{12}^{+2}$
Monoisotopic cation (*m/z*): 464.2537 (doubly charged ion)
Molecular weight: 1243.5 (as Atracurium Besylate)
CAS number: 64228-79-1
Common salt form: Besylate

Atracurium is a bis-quaternary ion, which is in the form of besylate salt. It has multiple chiral centers, and thus is a mixture of stereoisomers. Cisatracurium is a purified stereoisomer, which accounts for ~15% of the total stereoisomers [1]. Both atracurium and cisatracurium are clinically widely used skeletal muscle relaxants. This molecule is a prodrug made of two identical quaternary salt units that are linked together by a diester spacer. The active drug, laudanosine, is released in vivo from the quaternary salt unit via Hofmann elimination (Scheme 33). Nevertheless, this mechanism of action also constitutes an in vitro degradation pathway of the drug [2]. Since the drug molecule contains two ester bonds, it is also susceptible to hydrolytic degradation. A study in phosphate buffer at the physiological pH of 7.4 indicated that the Hofmann elimination pathway is the rate-determining step in the breakdown of atracurium [3].



Scheme 33 Degradation pathways of atracurium.

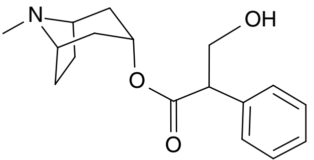
References

1 Mellinghoff, H., Radbruch, L., Diefenbach, C., and Buzello, W. (1996). A Comparison of cisatracurium and atracurium. *Anesth. Analg.* 83: 1072–1075.

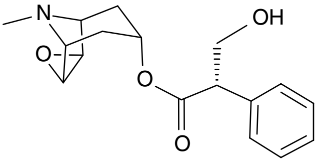
2 Welch, R.M., Brown, A., Ravitch, J., and Dahl, R. (1995). The in vitro degradation of cisatracurium, the R, cis-R′-isomer of atracurium, in human and rat plasma. *Clin. Pharmacol. Ther.* 58: 132–142.

3 Weindlmayr-Goettel, M., Gilly, H., and Kress, H.G. (2002). Does ester hydrolysis change the in vitro degradation rate of cisatracurium and atracurium? *Br. J. Anaesth.* 88: 555–562.

34–35: Atropine, Scopolamine



Atropine



Scopolamine

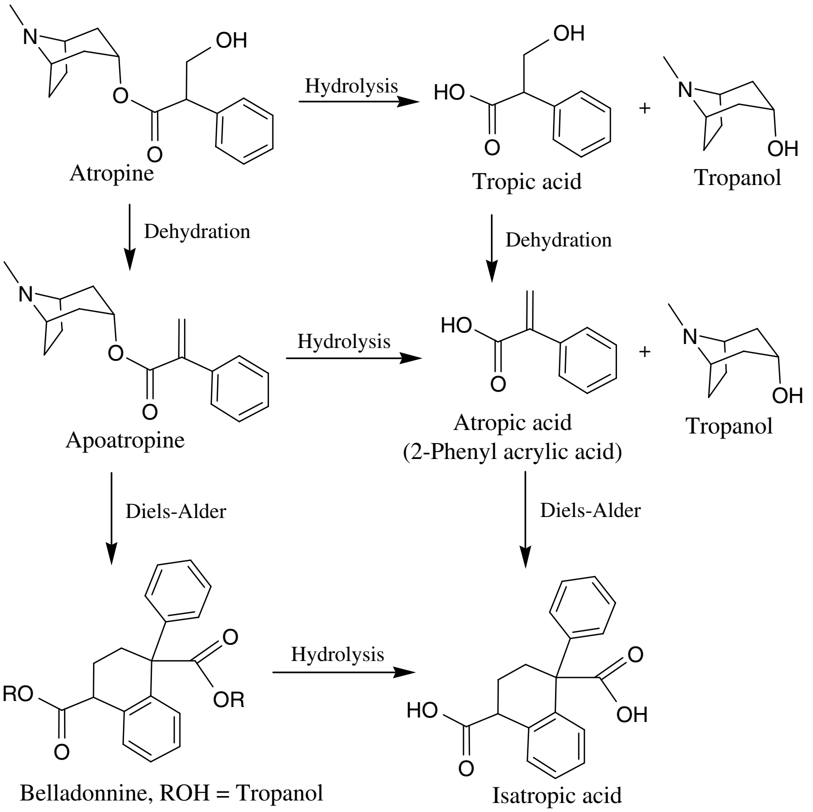
34: Atropine

Chemical: D,L-Hyoscyamine; [(1*S*,5*R*)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl] 3-hydroxy-2-phenylpropanoate
Brand names: Atnaa, Atropen, Busulfex
Formula: $C_{17}H_{23}NO_3$
Monoisotopic protonated ion (*m/z*): 290.1751
Molecular weight: 289.37
CAS number: 51-55-8
Common salt form: Sulfate

35: Scopolamine

Chemical name: Hyoscine; [(1*R*,2*R*,4*S*,5*S*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}] nonan-7-yl]-(2*S*)-3-hydroxy-2-phenylpropanoate
Brand names: Donnatal, Phenohytro, Transderm Scop
Formula: $C_{17}H_{21}NO_4$
Monoisotopic protonated ion (*m/z*): 304.1543
Molecular weight: 303.35
CAS number: 114-49-8
Common salt form: HBr salt

Atropine is a tropane alkaloid that is present in a number of plants of the family *Solanaceae*. Some of these plants have been used medicinally for more than two millennia. Atropine has an ester linkage and, as such, one of its main degradation pathways is hydrolysis, producing tropic acid and tropine (3-tropanol, Scheme 34–35) [1]. Its tropic acid moiety also contains a hydroxyl group that is β to both the carbonyl and phenyl groups. This structural feature renders atropine susceptible to dehydration, yielding apoatropine [2]. The latter can be hydrolyzed to form atropic acid (also known as 2-phenylacrylic acid). Alternately, atropic acid can be formed from the dehydration of tropic acid as well. Hence, the two degradation pathways, i.e., hydrolysis and dehydration (a form of elimination) produced these four main degradants, tropic acid, apoatropine, atropic acid, and tropine, although tropine is not detectable by a regular HPLC UV detector [3]. On the other hand, a GC method with pentafluoropropionic anhydride as a derivatizing reagent can detect tropine [4]. Lund and Waaler performed a thorough kinetic study of atropine degradation in a pH range of



Scheme 34-35 Degradation pathways of atropine.

~1–6 [5]. Atropine displayed a U-shape pH stability curve and at pH 3, it appeared most stable as the overall rate of degradation is the smallest. And at this pH, the rate of dehydration was approximately the same as that of hydrolysis.

The degradant resulting from dehydration, namely apoatropine, could also react with atropine via Diels-Alder reaction to produce belladonnine and then isotropic acid upon further degradation by hydrolysis [2]. These secondary and tertiary degradants would most likely be minor ones. Atropine *N*-oxide is a metabolite of the drug; however, no report has been found to suggest that it would be a meaningful degradant in pharmaceutical preparations.

Scopolamine is another naturally occurring tropane alkaloid that is highly similar to atropine. As such, it may likely have similar degradation pathways.

References

1 Zvirblis, P., Socholitsky, I., and Kondritzer, A.A. (1956). The kinetics of the hydrolysis of atropine. *J. Am. Pharm. Assoc. Sci. Ed.* 45: 450–454.

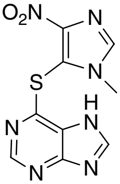
2 Voigtlander, H.-W. and Rosenberg, W. (1959). The constitution of isatropasic esters belladonnin and scopadonnin (Translation from German). *Arch. Pharm.* 292: 632–642.

3 Zimmermann, T., Dimmel, A., Jüttemeyer, S. et al. (2012). Rapid resolution liquid chromatography for monitoring the quality of stockpiled atropine preparations for injection. *Drug Test. Anal.* 4: 222–228.

4 Schier, J.G., Ravikumar, P.R., Nelson, L.S. et al. (2004). Preparing for chemical terrorism: stability of injectable atropine sulfate. *Acad. Emerg. Med.* 11: 329–334.

5 Lund, W. and Waaler, T. (1968). The kinetics of atropine and apoatropine in aqueous solutions. *Acta Chem. Scand.* 22: 3085–3097.

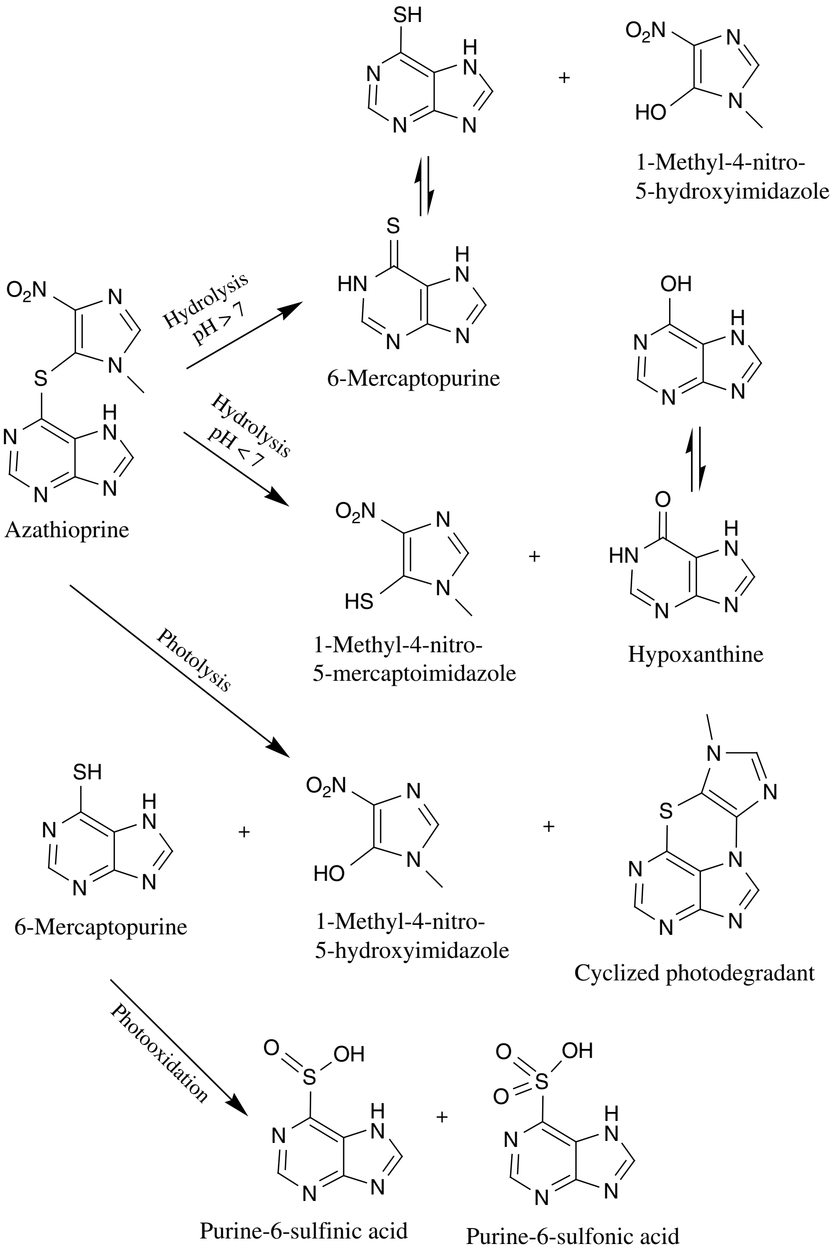
36: Azathioprine



Chemical name: 6-(3-Methyl-5-nitroimidazol-4-yl)sulfanyl-7*H*-purine
Brand names: Azasan, Imuran
Formula: $C_9H_7N_7O_2S$
Monoisotopic protonated ion (*m/z*): 278.0455
Molecular weight: 277.26
CAS number: 446-86-6
Common salt form: Sodium salt

Azathioprine was originally developed as an anticancer drug but it is now mainly used against organ rejection after transplant. It is a prodrug of 6-mercaptopurine (thiopurine). Its main degradation pathway is hydrolysis, and its pH stability profile displayed a U-shape, with it being most stable at ~pH 6 [1]. According to a study by Singh and Gupta [2], azathioprine decomposed to 6-mercaptopurine and 1-methyl-4-nitro-5-hydroxyimidazole at pH greater than 7 (Scheme 36). In acidic pH, it degraded to hypoxanthine and 1-methyl-4-nitro-5-mercaptoimidazole. In either case, the hydrolysis seemed to proceed via a direct nucleophilic attack by either hydroxide ion or water molecule [1].

It was reported that long-term use of azathioprine may cause skin cancer, implying that the drug is photolabile. Photolysis of an azathioprine solution yielded 6-mercaptopurine, 1-methyl-4-nitro-5-hydroxyimidazole, and a cyclized photodegradant [3]. Photolysis of 6-mercaptopurine and its photodegradants, purine-6-sulfinic acid and purine-6-sulfonic acid caused the formation of a variety of free radicals that may be responsible for the phototoxicity of the drug [4].



Scheme 36 Degradation pathways of azathioprine.

References

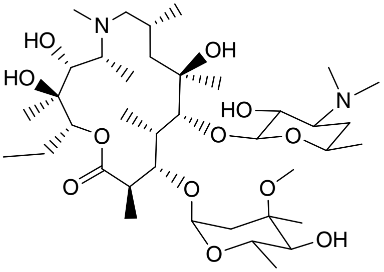
1 Mitra, A.K. and Narurkar, M.M. (1986). Kinetics of azathioprine degradation in aqueous solution. *Int. J. Pharm.* 35: 165–171.

2 Singh, S. and Gupta, R.L. (1988). A critical study on degradation of azathioprine in aqueous solutions. *Int. J. Pharm.* 42: 263–266.

3 Hemmens, V.J. and Moore, D.E. (1986). Photochemical sensitization by azathioprine and its metabolites – II. Azathioprine and nitroimidazole metabolites. *Photochem. Photobiol.* 43: 257–262.

4 Moore, D.E., Sik, R.H., Bilski, P. et al. (1994). Photochemical sensitization by azathioprine and its metabolites. Part 3. A direct EPR and spin-trapping study of light-induced free radicals from 6-mercaptopurine and its oxidation products. *Photochem. Photobiol.* 60: 574–581.

37: Azithromycin



Chemical name: (*2R,3S,4R,5R,8R,10R,11R,12S,13S,14R*)-11-[(*2S,3R,4S,6R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-2-ethyl-3,4,10-trihydroxy-13-[(*2R,4R,5S,6S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one

Brand names: Azasite, Zithromax, Zmax

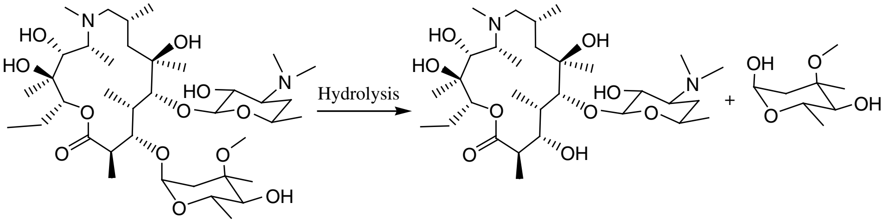
Formula: $C_{38}H_{72}N_2O_{12}$

Monoisotopic protonated ion (*m/z*): 749.5158

Molecular weight: 748.99

CAS number: 83905-01-5

Azithromycin is an antibiotic that is structurally modified based on erythromycin. The 9-keto functionality of erythromycin is replaced by a methyl-substituted nitrogen moiety in azithromycin. This modification makes azithromycin no longer undergo dehydration as opposed to erythromycin. The main degradation pathway of azithromycin, nevertheless, is the hydrolysis of the glycosidic bond that connects to the cladinose sugar unit at the 3-position of the macrocyclic ring (Scheme 37). The pH stability profile of azithromycin showed a V shape with the most stable pH at 6.3. At this pH, the activation energy for the ether hydrolysis was found to be 96.8 kJ/mol (23.1 kcal/mol), and the degradation product is desosaminylazithromycin (des-cladinose azithromycin, DCA) [1]. On the other hand, azithromycin quickly decomposed in pH 1.5 boiled human gastric fluid with a half-life of ~25 minutes [2].[3]



Scheme 37 Hydrolytic degradation pathway of azithromycin.

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[3] The author of reference 2 did not specify the temperature of the gastric fluid study but it appears the temperature should be 37 °C.

Nonetheless, the overall stability of azithromycin in acidic media is greatly improved as compared to erythromycin. For example, the time for 10% decomposition ($T_{1/10}$) for azithromycin at pH 2 was found to be 20.1 minutes, which compares favorably with erythromycin that had a $T_{1/10}$ of only 3.7 seconds at the same pH [3].

Azithromycin does not have a chromophore that absorbs significantly in UV A and UV B regions. Therefore, it would not be expected to be photochemically active. There are some studies on azithromycin and analogous drugs regarding their photochemical degradation in the environment [4, 5]. However, the relevance of the studies with respect to the photostability of these drugs in pharmaceutical dosage forms is unknown.

References

1 Zhang, Y., Liu, X., Cui, Y. et al. (2009). Aspects of degradation kinetics of azithromycin in aqueous solution. *Chromatographia* 70: 67–73.

2 Curatolo, W. (2011). Interdisciplinary science and the design of a single-dose antibiotic therapy. *Pharm. Res.* 28: 2059–2071.

3 Fiese, E.F. and Steffen, S.H. (1990). Comparison of the acidic stability of azithromycin and erythromycin A. *J. Antimicrob. Chemother.* 25: 39–47.

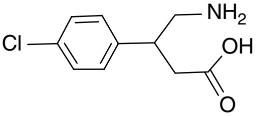
4 Tong, L., Eichhorn, P., Pérez, S. et al. (2011). Photodegradation of azithromycin in various aqueous systems under simulated and natural solar radiation: kinetics and identification of photoproducts. *Chemosphere* 83: 340–348.

5 Vione, D., Feitosa-Felizzola, J., Minero, C., and Chiron, S. (2009). Phototransformation of selected human-used macrolides in surface water: kinetics, model predictions and degradation pathways. *Water Res.* 43: 1959–1967.

3

Baclofen to Butalbital and Related Barbiturates

38: Baclofen



Chemical name: (*R*,*S*)-4-Amino-3-(4-chlorophenyl)butanoic acid
Brand name: Lioresal
Formula: $C_{10}H_{12}ClNO_2$
Monoisotopic protonated ion (*m/z*): 214.0629
Molecular weight: 213.66
CAS number: 1134-47-0

Baclofen is a derivative of γ-aminobutyric acid (GABA). It is a GABA receptor antagonist and used as a treatment for spasticity. As a γ-amino acid, it is prone to intramolecular dehydration, leading to the formation of a five-membered ring lactam, 4-(4-chlorophenyl)-2-pyrrolidone (CPP), which is its main degradation product (Scheme 38) [1, 2]. In contrast, as it contains a primary amino group, it may also undergo the Maillard reaction. A study between baclofen and lactose indeed showed that early-stage Maillard reaction products were formed, the major one of which was proposed as the Schiff base form of the adduct based on IR analysis of a solid mixture of baclofen and lactose [3]. However, in the solution of baclofen and lactose from which the LC-MS analysis was performed, the glucosamine form or even the Amadori rearrangement product may be possible; all three forms have an identical molecular formula [4].

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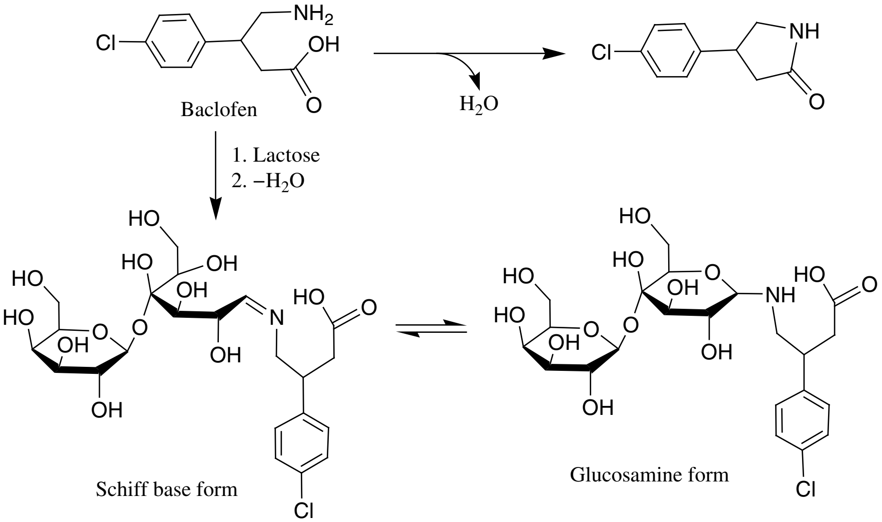


Scheme 38 Degradation of baclofen.

References

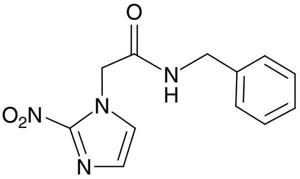
1 Ahuja, S. (1985). Baclofen. In: *Analytical Profiles of Drug Substances*, vol. 14 (ed. K. Florey), 527–548. New York, NY: Academic Press Inc.

2 Cutrignelli, A., Denora, N., Lopedota, A. et al. (2007). Comparative effects of some hydrophilic excipients on the rate of gabapentin and baclofen lactamization in lyophilized formulations. *Int. J. Pharm.* 332: 98–106.

3 Monajjemzadeh, F., Hassanzadeh, D., Valizadeh, H. et al. (2009). Assessment of feasibility of Maillard Reaction between baclofen and lactose by liquid chromatography and tandem mass spectrometry, application to pre formulation studies. *AAPS PharmSciTech* 10: 649–659.

4 Li, M. (2012). Drug–excipient interactions and adduct formation. In: *Organic Chemistry of Drug Degradation*, 150–164. Cambridge: RSC Publishing.

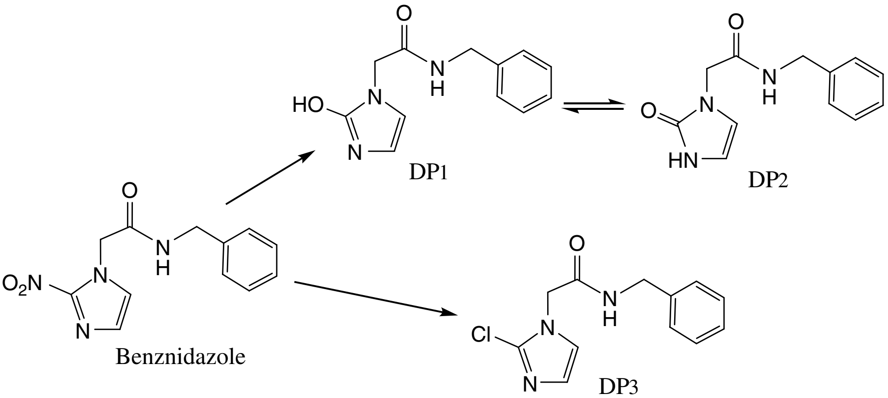
39: Benznidazole [WHO List of Essential Medicines]



Chemical name: *N*-Benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide
Brand name: Rochagan
Formula: $C_{12}H_{12}N_4O_3$
Monoisotopic protonated ion (*m/z*): 261.0982
Molecular weight: 260.2530
CAS number: 22994-85-0

Benznidazole is used for the treatment of Chagas disease, a tropical disease that is caused by the parasite *Trypanosoma cruzi*. According to the study by Kawaguchi et al., the main degradation pathway of the drug is the substitution of the nitro group by either hydroxide under alkaline conditions or chloride under acidic conditions (in 2 M HCl at 60°C for 96 hours), rather than the hydrolysis of the amide linkage (Scheme 39) [1].

The hydroxyl-substituted degradant was present in "enol and ketone" forms, based on the LC-MS analysis that showed two isomeric peaks [1]. It should be noted that the chloro-substituted degradant is certainly an artificial degradant under the forced degradation conditions. On the other hand, the drug appeared to be stable under other stress conditions: oxidative (3% H_2O_2 at room temperature for 48 hours), photostress (in solution), and heat and moisture (70°C, 65% RH, 60 days) conditions.

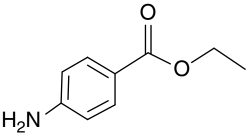


Scheme 39 Degradation of benznidazole under acidic stress conditions.

Reference

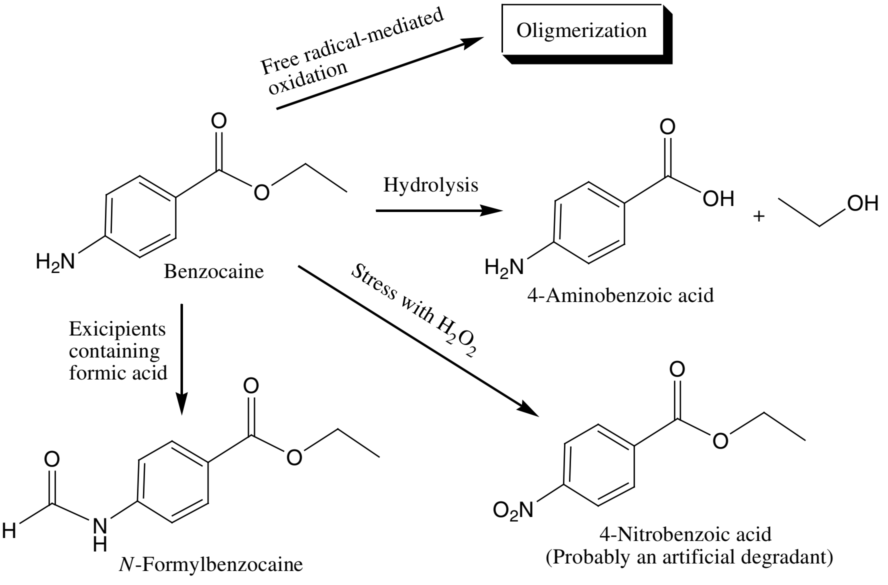
1 Kawaguchi, W.H., Fachi, M.M., Cerqueira, L.B. et al. (2020). Stability indicating method for determination of benznidazole and its degradation products in active pharmaceutical ingredient. *J. Braz. Chem. Soc.* 31: 1194–1202.

40: Benzocaine



Chemical name: Ethyl 4-aminobenzoate
Brand name: Anbesol, Lanacane, Orajel
Formula: $C_9H_{11}NO_2$
Monoisotopic protonated ion (*m/z*): 166.0863
Molecular weight: 165.19
CAS number: 94-09-7
Common salt form: HCl salt

Benzocaine, chemically known as ethyl 4-aminobenzoate, is a local anesthetic. Its ester functionality is susceptible to hydrolytic degradation (Scheme 40); the activation energy (*E*a) of acid-catalyzed hydrolysis was reported to be ~18.6 kcal/mol [1]. However, the amino group of the aniline moiety is incompatible with excipients containing carbonyl functional groups. For example, *N*-formylbenzocaine was observed in a liquid formulation containing polyvinylpyrrolidone [2] and an orally disintegrating tablet formulation containing silicified microcrystalline cellulose [3]. In a forced degradation study with H_2O_2, the aniline amino group was oxidized to



Scheme 40 Degradation pathways of benzocaine under various conditions including forced degradation.

nitro group [4]. Such oxidation probably would not occur under long-term stability storage conditions. On the other hand, free radical-mediated oxidation of the aniline moiety may cause the oligomerization of the drug [5].

References

1 Marcus, A.D. and Baron, S. (1959). A comparison of the kinetics of the acid catalyzed hydrolyses of procainamide, procaine, and benzocaine. *J. Am. Pharm. Assoc.* 48: 85–90.

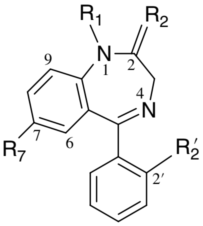
2 Prasad, A. and Langley, N. *The Effect of Secondary Oxidation Impurities in PVP on API Stability*. Tarrytown, NY: BASF Corporation https://www.scribd.com/document/378037462/The-Effect-of-Secondary-Oxidation-Impurities (accessed 26 February 2022).

3 Köllmer, M., Popescu, C., Manda, P. et al. (2013). Stability of benzocaine formulated in commercial oral disintegrating tablet platforms. *AAPS PharmSciTech* 14: 1333–1340.

4 Perez-Lozano, P., Garcia-Montoya, E., Orriols, A. et al. (2005). A new validated method for the simultaneous determination of benzocaine, propylparaben and benzyl alcohol in a bioadhesive gel by HPLC. *J. Pharm. Biomed. Anal.* 39: 920–927.

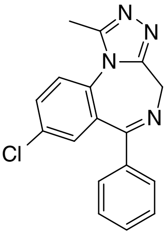
5 Marjanović, B., Juranić, I., Ćirić-Marjanović, G. et al. (2011). Chemical oxidative polymerization of benzocaine. *React. Funct. Polym.* 71: 704–712.

41–50: Benzodiazepines



General structures of 1,4-benzodiazepines

41: Alprazolam



Chemical name: 8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-a][1,4] benzodiazepine

Brand name: Xanax, Xanor, Niravam

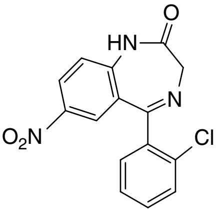
Formula: $C_{17}H_{13}ClN_4$

Monoisotopic protonated ion (*m/z*): 309.0902

Molecular weight: 308.77

CAS number: 28981-97-7

42: Clonazepam



Chemical name: 5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepin-2-one

Brand name: Klonopin, Rivotril

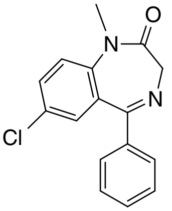
Formula: $C_{15}H_{10}ClN_3O_3$

Monoisotopic protonated ion (*m/z*): 316.0483

Molecular weight: 315.71

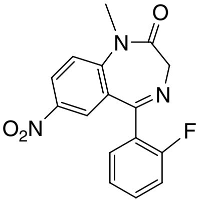
CAS number: 1622-61-3

43: Diazepam [WHO Lists of Essential Medicines]



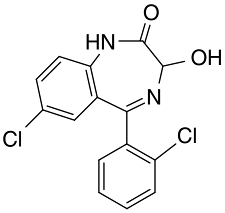
Chemical name: 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-one
Brand name: Valium, Vazepam, Valtoco
Formula: $C_{16}H_{13}ClN_2O$
Monoisotopic protonated ion (*m/z*): 285.0789
Molecular weight: 284.74
CAS number: 439-14-5

44: Flunitrazepam



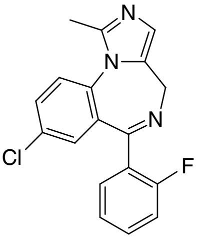
Chemical name: 5-(2-Fluorophenyl)-1-methyl-7-nitro-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one
Brand name: Rohypnol
Formula: $C_{16}H_{12}FN_3O_3$
Monoisotopic protonated ion (*m/z*): 314.0935
Molecular weight: 313.29
CAS number: 1622-62-4

45: Lorazepam



Chemical name: 7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-1,4-benzodiazepin-2-one
Brand name: Ativan, Tavor, Temesta
Formula: $C_{15}H_{10}Cl_2N_2O_2$
Monoisotopic protonated ion (*m/z*): 321.0192
Molecular weight: 321.16
CAS number: 846-49-1

46: Midazolam [WHO Lists of Essential Medicines]



Chemical name: 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-a][1,4] benzodiazepine

Brand name: Dormicum, Hypnovel, Versed

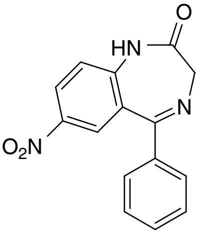
Formula: $C_{18}H_{13}ClFN_3$

Monoisotopic protonated ion (*m/z*): 326.0855

Molecular weight: 325.77

CAS number: 59467-70-8

47: Nitrazepam



Chemical name: 7-Nitro-5-phenyl-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one

Brand name: Alodorm, Apodorm, Arem

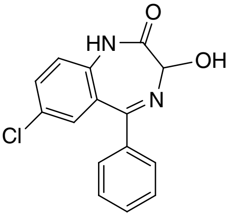
Formula: $C_{15}H_{11}N_3O_3$

Monoisotopic protonated ion (*m/z*): 282.0873

Molecular weight: 281.27

CAS number: 146-22-5

48: Oxazepam



Chemical name: 7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one

Brand name: Serax, Alepam

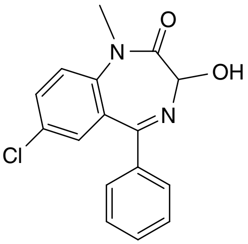
Formula: $C_{15}H_{11}ClN_2O_2$

Monoisotopic protonated ion (*m/z*): 287.0582

Molecular weight: 286.72

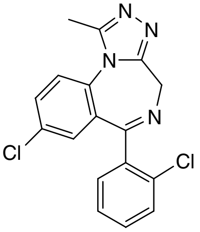
CAS number: 604-75-1

49: Temazepam



Chemical name: 7-Chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-1,4-benzodiazepin-2-one
Brand name: Restoril, Normison, Nortem
Formula: $C_{16}H_{13}ClN_2O_2$
Monoisotopic protonated ion (*m/z*): 301.0738
Molecular weight: 300.74
CAS number: 846-50-4

50: Triazolam

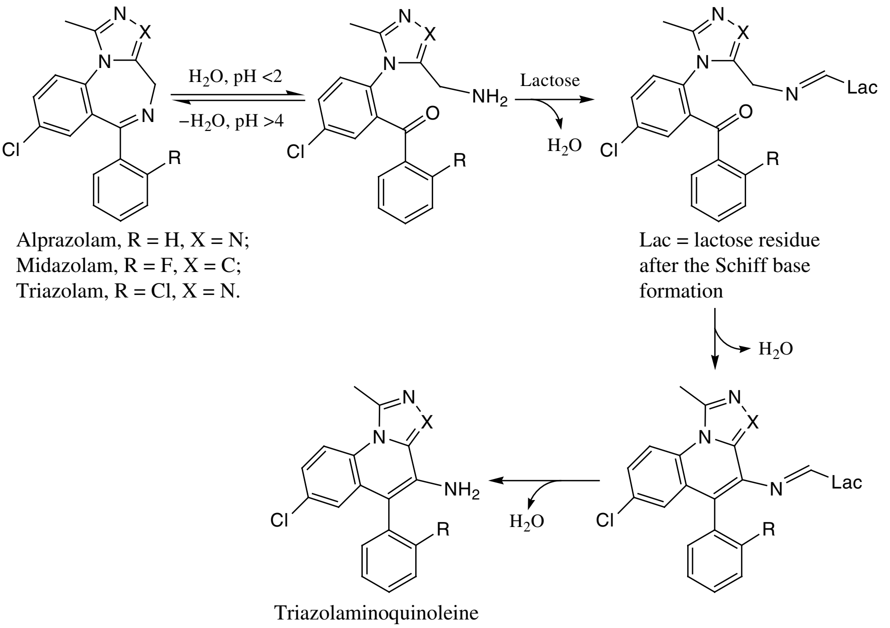


Chemical name: 8-Chloro-6-(2-chlorophenyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
Brand name: Halcion
Formula: $C_{17}H_{12}Cl_2N_4$
Monoisotopic protonated ion (*m/z*): 343.0512
Molecular weight: 343.21
CAS number: 28911-01-5

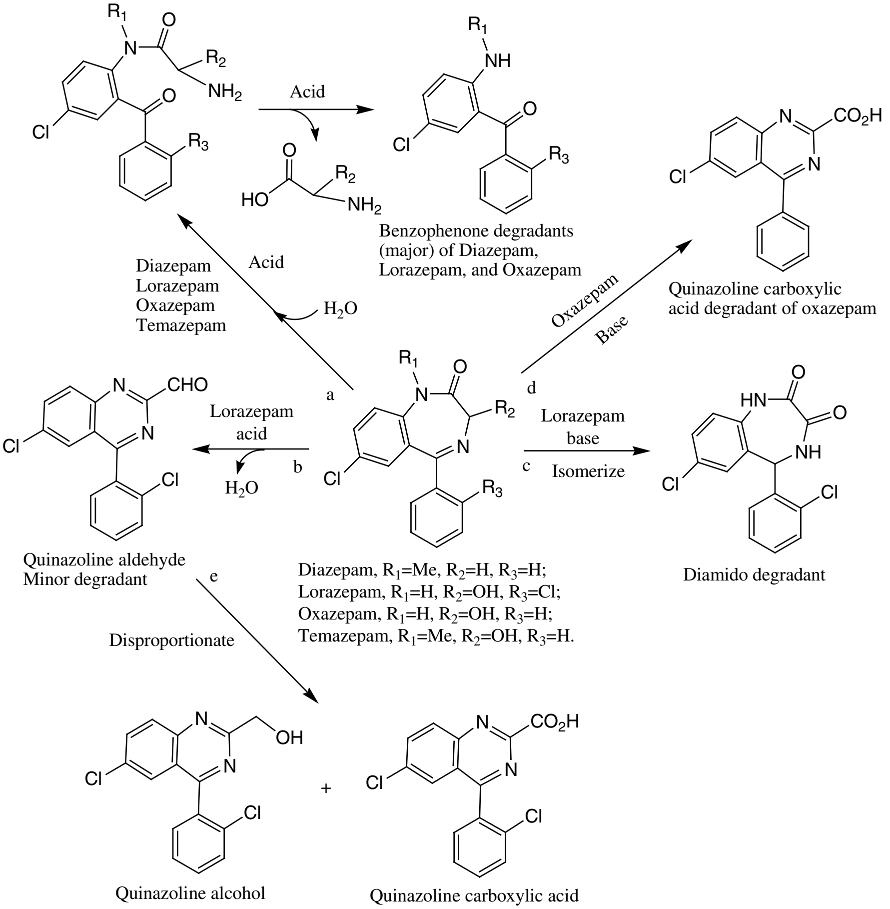
Benzodiazepines, or more specifically 1,4-benzodiazepines, are a class of psychoactive drugs widely used for the treatment of anxiety, insomnia, seizure, and muscle spasms, as well as for inducing sedative effects. The family of benzodiazepines consists of more than a dozen drugs, and the first one, chlordiazepoxide, was synthesized by Leo Sternbach serendipitously in 1955 while working for Hoffmann-La Roch, and chlordiazepoxide was approved for clinical use in 1960 [1]. Generally, the core structure of benzodiazepines can be viewed as deriving from 2-aminobenzophenone upon which a glycine first forms an amide linkage with the amine group and then condenses with the carbonyl group of benzophenone, resulting in the formation of the seven-membered diazepine ring via an intramolecular imine bond. Both the amide and imine bonds are susceptible to hydrolytic degradation, with the imine being more vulnerable, particularly under acidic conditions.

The embedded imine moiety in the diazepine part of the benzodiazepine core structure is sensitive to pH. A study of alprazolam, midazolam, and triazolam showed that at pH below 2, the open ring form was favored, while at pH above 4, the ring-closed form was favored (Scheme 41–50-1) [2]. Although the ring-open form may be considered a hydrolytic degradant, the ring opening and closure are reversible in solution. Hence, for an IV formulation of midazolam, the open form can be considered a prodrug as the ring would be completely closed at the physiological pH of 7.4 [2, 3]. On the contrary, as the ring-open form is a primary amine, it can undergo the Maillard reaction if the excipients contain reducing sugars. In a tablet formulation of alprazolam containing lactose, the Maillard degradant was found to be quickly increasing under the accelerated stability conditions (Scheme 41–50-1) [4].

Other benzodiazepine drugs should follow similar ring open-closure pathways as outlined in Scheme 41–50-1, except for the following differences. First, under very strong acidic conditions such as acid-catalyzed forced degradation, the open form of other benzodiazepines, e.g., diazepam and temazepam, that possess a 2,3-amide bond can be further hydrolyzed to form 2-aminobenzophenone degradants (Pathway a, Scheme 41–50-2) [5, 6]. Second, those benzodiazepines containing a 3-hydroxyl group on the diazepine ring behave differently under acidic and basic conditions; such drugs include lorazepam and temazepam. Under acidic conditions, lorazepam degraded to 2-aminobenzophenone derivative and quinazoline carboxaldehyde (Pathways a and b, Scheme 41–50-2) [7, 8]. The latter aldehyde could disproportionate to yield the corresponding alcohol and carboxylic acid [8]. Under basic conditions, lorazepam



Scheme 41–50-1 Photodegradation of alprazolam, midazolam, and triazolam in the presence of lactose, a reducing sugar excipient.

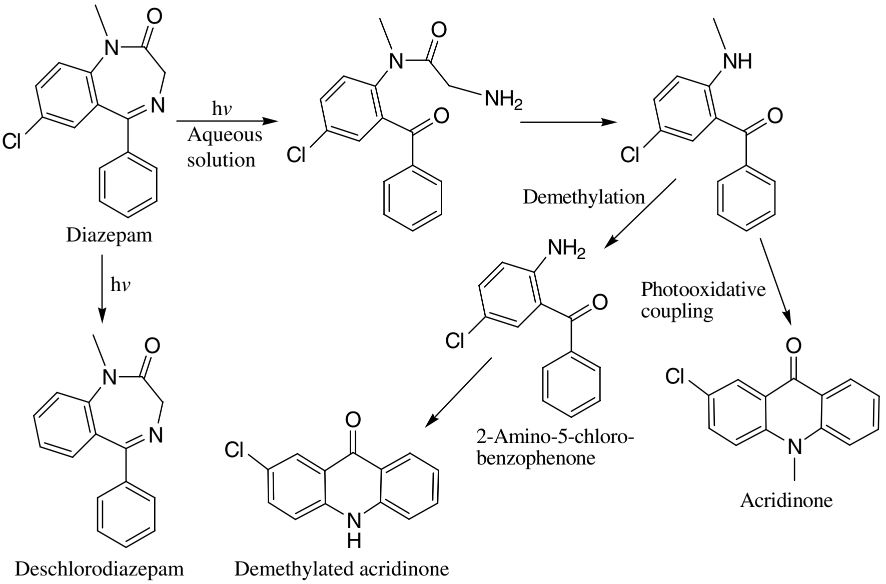


Scheme 41–50-2 Degradation pathways of diazepam, lorazepam, oxazepam, and temazepam.

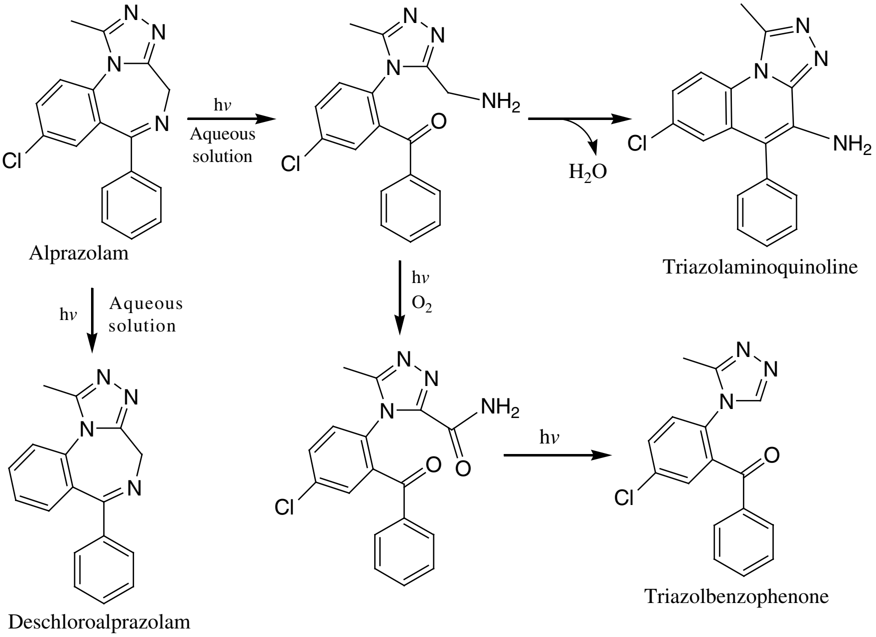
rearranged to an isomeric diamido degradant (Pathway c, Scheme 41–50-2). Surprisingly, the degradation pathways of oxazepam, which is structurally very similar to lorazepam, are somewhat different from those of lorazepam (Pathway d, Scheme 41–50-2).

Benzodiazepines can also undergo photochemical degradation. For those that do not have a nitro group, the photochemical transformation occurs mainly on the diazepine ring (Scheme 41–50-3). For example, photostress of diazepam under UV and simulated sunlight produced a number of degradants, the vast majority of which are derivatives of benzophenone, acridinone and quinazoline [5, 9]. The first step of the photodegradation is most likely the dissociation of the imine bond [5], which also occurs under acidic stress.

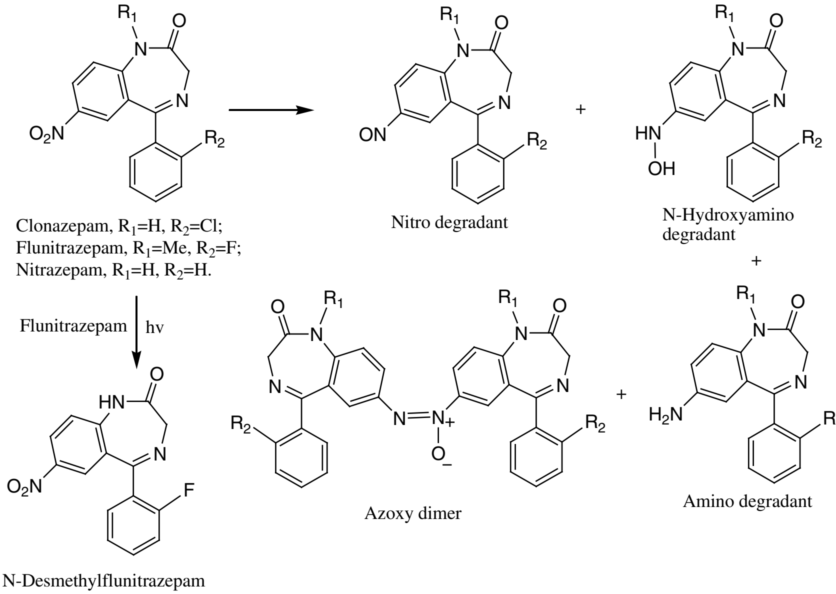
The photochemical degradation of alprazolam (Scheme 41–50-4) is somewhat different from that of simpler benzodiazepines, such as diazepam (Scheme 41–50-3),



Scheme 41–50-3 Photochemical degradation of diazepam.



Scheme 41–50-4 Photochemical degradation of alprazolam.



Scheme 41–50-5 Photodegradation pathways of clonazepam, flunitrzepam, and nitrazepam.

due to the presence of the triazilo group, which prevents the formation of an acridinone-type degradant.

For benzodiazepines with a nitro substituent, such as clonazepam, flunitrazepam, and nitrazepam, their photochemical degradation under anaerobic conditions occurs on the nitro group, which is reduced to 7-nitroso, hydroxylamino, and amino groups sequentially (Scheme 41–50-5) [10, 11]. Moreover, during the photoreductive process, an azoxy dimer was also observed [11]. Under aerobic conditions, these nitrobenzodiazepines appeared relatively more stable, probably because their photochemically excited states are quenched by molecular oxygen to produce singlet oxygen. The latter is believed to be the most responsible for the phototoxicity of these drugs. For flunitrazepam containing a 1-methyl group on the diazepine ring, *N*-desmethylflunitrazepam, the demethylated degradant, was found to be the major photodegradant under the aerobic condition [12].

References

1 Benzodiazepines, Wikipedia, https://en.wikipedia.org/wiki/Benzodiazepine (accessed 16 April 2022).

2 Loftsson, T., Guðmundsdottir, H., Sigurjonsdottir, J.F. et al. (2001). Cyclodextrin solubilization of benzodiazepines: formulation of midazolam nasal spray. *Int. J. Pharm.* 212: 29–40.

3 Gerecke, M. (1983). Chemical structure and properties of mida-zolam compared with other benzodiazepines. *Br. J. Clin. Pharmacol.* 16 (Suppl. 1): 11S–16S.

4 Huidobro, A.L. and Barbas, C. (2009). Analytical study proving alprazolam degradation to its main impurity triazolaminoquinoleine through Maillard reaction. *Anal. Bioanal. Chem.* 394: 1349–1359.

5 Cabrera, C.G., Goldberg de Waisbaum, R., and Nudelman, N.S. (2005). Kinetic and mechanistic studies on the hydrolysis and photodegradation of diazepam and alprazolam. *J. Phys. Org. Chem.* 18: 156–161.

6 Fatmi, A.A. and Hickson, E.A. (1988). Determination of temazepam and related compounds in capsules by high-performance liquid chromatography. *J. Pharm. Sci.* 77: 87–89.

7 Siddegowda, M.S., Yathirajan, H.S., and Ramakrishna, R.A. (2012). Facile thermal rearrangement of lorazepam and oxazepam. *Indian J. Chem.* 51B: 1628–1632.

8 Rutgers, J.G. and Shearer, C.M. (1981). Lorazepam. *Anal. Prof. Drug Sub.* 9: 397–426.

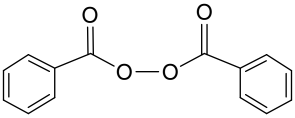
9 West, C.E. and Rowland, S.J. (2012). Aqueous phototransformation of diazepam and related human metabolites under simulated sunlight. *Environ. Sci. Technol.* 46: 4749–4756.

10 Cornelissen, P.J.G., Beijersbergen van Henegouwen, G.M.J., and Mohn, G.R. (1981). Relationship between structure and photobiological activity of 7-nitro-1,4-benzodiazepines. *Photochem. Photobiol.* 34: 345–350.

11 Cornelissen, P.J.G. and Beijersbergen Van Henegouwen, G.M.J. (1981). Photochemical activity of 7-nitro-1,4-benzodiazepines. *Pharm. Weekbl.* 3: 800–809.

12 Busker, R.W., Beijersbergen van Henegouwen, G.M.J., Kwee, B.M.C., and Winkens, J.H.M. (1987). Photobinding of flunitrazepam and its major photo-decomposition product N-desmethylflunitrazepam. *Int. J. Pharm.* 36: 113–120.

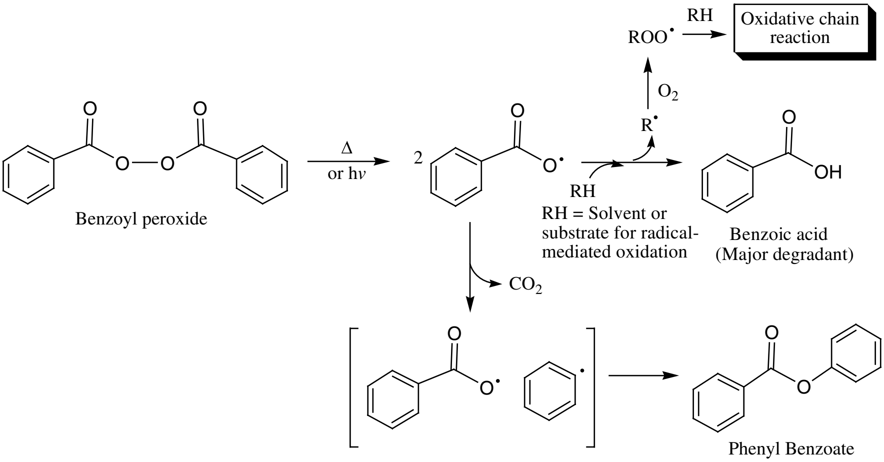
51: Benzoyl Peroxide



Chemical name: Benzoic peroxyanhydride
Brand name: Benzac
Formula: $C_{14}H_{10}O_4$
Monoisotopic protonated ion (*m/z*): 243.0652
Molecular weight: 242.23
CAS number: 94-36-0

Benzoyl peroxide is widely used in topical formulations for treating acne. Its peroxyl O-O bond is weak, as evidenced by a relatively small bond dissociation energy of 30 kcal/mol [1]. Hence, it can dissociate homolytically into two benzoate radicals under somewhat elevated temperature, particularly in solution (Scheme 51). The homolytic dissociation can also proceed photochemically. The benzoate radical is capable of abstracting a proton from a weak C—H bond of either the solvent of the solution or a substance in the solution to produce benzoic acid, which is the major degradant of benzoyl peroxide [2]. Benzoate radical can also undergo decarboxylation to form phenyl radical, which can combine with another benzoate radical to yield phenyl benzoate.

The stability of benzoyl peroxide in pharmaceutical formulations depends on the nature of solvents used and the solubility of benzoyl peroxide in these solvents [3]. In general, benzoyl peroxide tends to be more stable in media where it may exist as a suspension or emulsion rather than a true solution [4].



Scheme 51 Degradation of benzoyl peroxide.

References

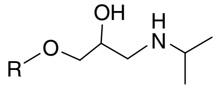
1 Walling, C. (1995). *Fifty Years of Free Radicals*. New York, NY: Wiley.

2 Cass, W.E. (1947). Products of the reaction of benzoyl peroxide with certain ethers. *J. Am. Chem. Soc.* 69: 500–503.

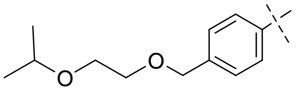
3 Nielloud, F., Mestres, J.P., and Mestres, G.M. (2002). Consideration on the formulation of benzoyl peroxide at ambient temperature: choice of non-polar solvent and preparation of submicron emulsion gels. *Drug Dev. Ind. Pharm.* 28: 863–870.

4 Chellquist, E.M. and Gorman, W.G. (1992). Benzoyl peroxide solubility and stability in hydric solvents. *Pharm. Res.* 9: 1341–1346.

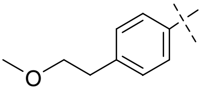
52–54: Bisoprolol, Metoprolol, Propranolol



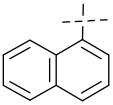
Bisoprolol, R =



Metoprolol, R =



Propranolol, R =



52: Bisoprolol

Chemical name: (*R*,*S*)-1-{4-[(2-Isopropoxyethoxy)methyl]phenoxy}-3-(isopropylamino)propan-2-ol
Brand name: Zebeta, Concor, Selecta
Formula: $C_{18}H_{31}NO_4$
Monoisotopic protonated ion (*m/z*): 326.2326
Molecular weight: 325.449
CAS number: 66722-44-9
Common salt form: Fumarate

53: Metoprolol

Chemical name: (*R*,*S*)-1-[4-(2-Methoxyethyl)phenoxy]-3-[(propan-2-yl)amino] propan-2-ol
Brand name: Lopressor, Metolar XR, Toprol XL
Formula: $C_{15}H_{25}NO_3$
Monoisotopic protonated ion (*m/z*): 268.1907
Molecular weight: 267.37
CAS number: 51384-51-1
Common salt form: Succinate

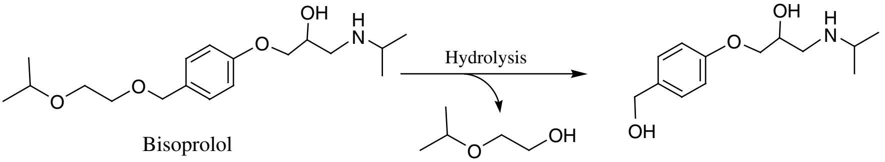
54: Propranolol [WHO List of Essential Medicines]

Chemical name: (*R*,*S*)-1-(1-methylethylamino)-3-(1-naphthyloxy)propan-2-ol
Brand name: Inderal
Formula: $C_{16}H_{21}NO_2$
Monoisotopic protonated ion (*m/z*): 260.1645
Molecular weight: 259.35
CAS number: 525-66-6
Common salt form: HCl salt

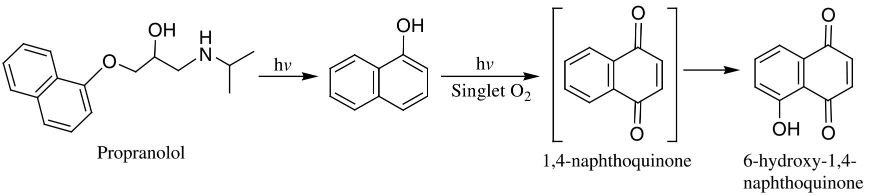
Bisoprolol, metoprolol, and propranolol all belong to the family of β-blockers, which are a group of therapeutic agents widely used for the treatment of hypertension. All these three drugs contain a 3-(isopropylamino)propylene glycol moiety. The latter moiety is also present in atenolol, another β-blocker whose degradation chemistry is discussed previously in this book. Therefore, for the degradation chemistry resulting from this moiety, please also refer to that monograph.

Under acidic and basic stress conditions, the main degradant for bisoprolol is the hydrolysis of the benzylic ether bond, one of its two ether bonds (Scheme 52–54-1) [1]. A formulation study with a solid dosage form of bisoprolol indicated that bisoprolol is not stable in a low pH environment [2]. A kinetic study showed that the half-life of bisoprolol under stress by 1 M HCl at 333.2K was only 0.12 hour [3]. Under the same conditions, the half-life of propranolol was found to be 385 hours. A forced degradation study of metoprolol was performed, and it was found that extensive degradation occurred under basic and thermal stress conditions [4]. The degradation probably took place in the 3-(isopropylamino)propylene glycol side chain of the drug molecule.

For propranolol, as it contains a naphthalene group, it would be expected to be photochemically active (Scheme 52–54-2). A laser-flash photolysis study of propranolol found that it is a good photosensitizer with a quantum yield of 0.24 for sensitizing singlet oxygen formation [5]. Depending on the condition of the photolysis, the initial photodegradant can be naphthol [6]. In the presence of oxygen, propranolol can be oxidized by singlet oxygen, which is formed during the photosensitization process, to give 6-hydroxy-1,4-naphthoquinone via the highly photolabile intermediate 1,4-naphthoquinone [5]. A liquid formulation study with β-cyclodextrin indicated that the rate of photodecomposition of propranolol could be reduced by ~50% [7].



Scheme 52–54-1 Hydrolytic degradation of bisoprolol.



Scheme 52–54-2 Photochemical degradation of propranolol.

References

1 Joshi, S.J., Karbhari, P.A., Bhoir, S.I. et al. (2010). RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. *J. Pharm. Biomed. Anal.* 52: 362–371.

2 Dulin, W.A. (1995). Degradation of bisoprolol fumarate in tablets formulated with dicalcium phosphate. *Drug Dev. Ind. Pharm.* 21: 393–409.

3 Szalka, M., Rokaszewski, E., and Kaczmarski, K. (2013). Kinetics of hydrolysis of bisoprolol hemifumarate in aqueous acidic solutions. *Int. J. Chem. Kinet.* 45: 744–754.

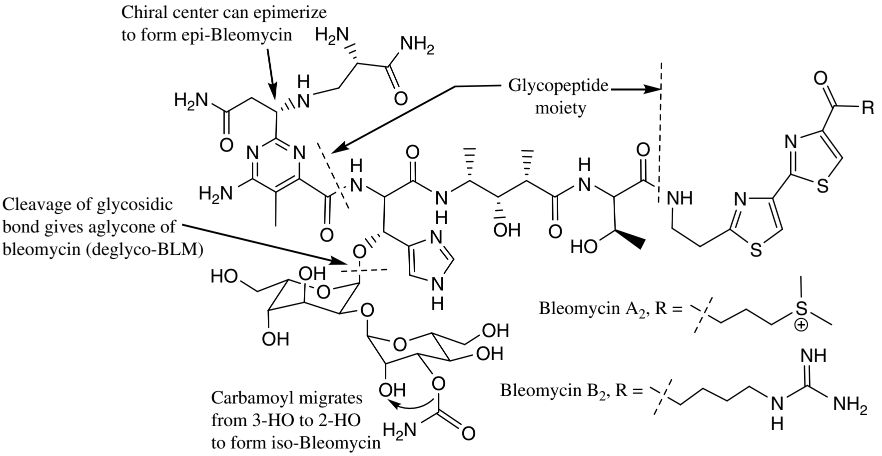
4 Phale, M.D. and Hamrapurkar, P.D. (2010). Optimization and establishment of a validated stability-indicating HPLC method for study of the stress degradation behavior of metoprolol succinate. *J. AOAC Int.* 93: 911–916.

5 Sortino, S., Petralia, S., Boscà, F., and Miranda, M.A. (2002). Irreversible photo-oxidation of propranolol triggered by self-photogenerated singlet molecular oxygen. *Photochem. Photobiol. Sci.* 1: 136–140.

6 Uwai, K., Tani, M., Ohtake, Y. et al. (2005). Photodegradation products of propranolol: the structures and pharmacological studies. *Life Sci.* 78: 357–365.

7 Ansolin, T.L., Bariccatti, R.A., Ferreira da Rosa, M., and Lindino, C.A. (2014). The effect of β-cyclodextrin in the photochemical stability of propranolol hydrochloride in aqueous solution. *Acta Sci. Technol.* 36: 337–340.

55: Bleomycin [WHO List of Essential Medicines]



Chiral center can epimerize to form epi-Bleomycin

Glycopeptide moiety

Cleavage of glycosidic bond gives aglycone of bleomycin (deglyco-BLM)

Carbamoyl migrates from 3-HO to 2-HO to form iso-Bleomycin

Bleomycin A2, R =

Bleomycin B2, R =

55a: Bleomycin A$_2$

Chemical name: 3-[[2-[2-[2-[[(2*S*,3*R*)-2-[[(2*S*,3*S*,4*R*)-4-[[(2*S*,3*R*)-2-[[6-Amino-2-[(1*S*)-3-amino-1-[[(2*S*)-2,3-diamino-3-oxopropyl]amino]-3-oxopropyl]-5-methylpyrimidine-4-carbonyl]amino]-3-[(2*R*,3*S*,4*S*,5*S*,6*S*)-3-[(2*R*,3*S*,4*S*,5*R*,6*R*)-4-carbamoyloxy-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3-(1*H*-imidazol-5-yl)propanoyl]amino]-3-hydroxy-2-methylpentanoyl]amino]-3-hydroxybutanoyl]amino]ethyl]-1,3-thiazol-4-yl]-1,3-thiazole-4-carbonyl]amino]propyl-dimethylsulfanium

Brand name: N/A

Formula: $C_{55}H_{84}N_{17}O_{21}S_3$

Monoisotopic protonated ion (*m/z*): 1414.5184

Molecular weight: 1415.55

CAS number: 11116-31-7; 11056-06-7 (Sulfate salt)

55b: Bleomycin B$_2$

Chemical name: [(2*R*,3*S*,4*S*,5*R*,6*R*)-2-[(2*R*,3*S*,4*S*,5*S*,6*S*)-2-[(1*R*,2*S*)-2-[[6-Amino-2-[(1*S*)-3-amino-1-[[(2*S*)-2,3-diamino-3-oxopropyl]amino]-3-oxopropyl]-5-methylpyrimidine-4-carbonyl]amino]-3-[[(2*R*,3*S*,4*S*)-5-[[(2*S*,3*R*)-1-[2-[4-[4-[4-(diaminomethylideneamino)butylcarbamoyl]-1,3-thiazol-2-yl]-1,3-thiazol-2-yl] ethylamino]-3-hydroxy-1-oxobutan-2-yl]amino]-3-hydroxy-4-methyl-5-oxopentan-2-yl]amino]-1-(1*H*-imidazol-5-yl)-3-oxopropoxy]-4,5-dihydroxy-6-(hydroxymethyl) oxan-3-yl]oxy-3,5-dihydroxy-6-(hydroxymethyl)oxan-4-yl] carbamate

Formula: $C_{55}H_{84}N_{20}O_{21}S_2$

Monoisotopic protonated ion (*m/z*): 1424.5562

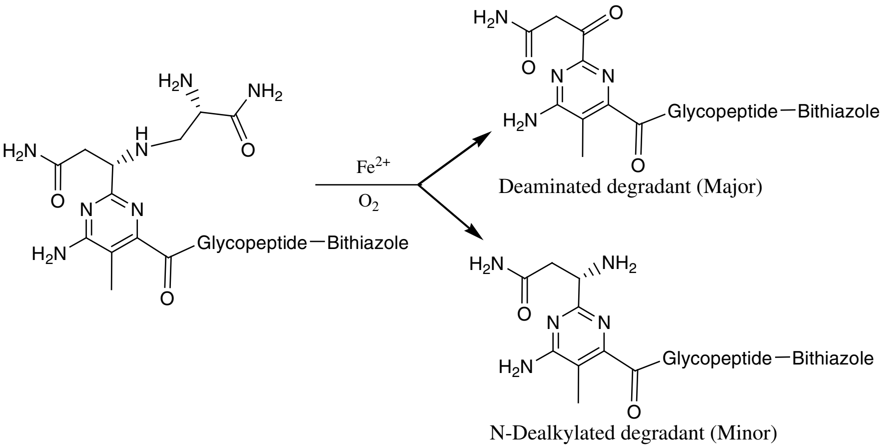
Molecular weight: 1425.51

CAS number: 9060-10-0

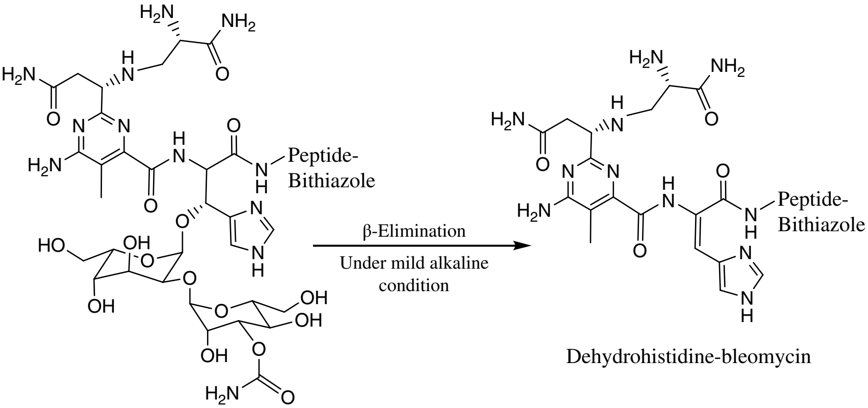
Bleomycin is a widely used anticancer antibiotic containing at least 10 components, and the major ones are bleomycin A_2 (*N1*-[3-(dimethylsulfonio)propyl]-bleomy cinamide) and B_2 (*N1*-[4-[(aminoiminomethyl)amino]butyl]-bleomycinamide) [1]. Structurally, the main skeleton of the molecule is an unusual glycopeptide, which is connected to a substituted pyrimidine moiety on the left and to a substituted bithiazole moiety on the right. The pyrimidine moiety is capable of chelating with transition metal ions with five nitrogens in the pyrimidine moiety as the ligands, while the positively charged bithiazole moiety can intercalate into DNA [2]. When the chelating metal ion is ferrous ion, bleomycin is activated upon binding by molecular oxygen onto the sixth coordination site to the chelated metal ion. This form of bleomycin is referred to as activated bleomycin, which is capable of cleaving DNA, and in the absence of DNA, it undergoes auto-oxidation. The latter oxidation occurs on the β-aminoalanine side chain of the pyrimidine moiety, resulting in the formation of a deaminated bleomycin as the major degradant and an *N*-dealkylated one as a minor degradant in aqueous solutions (Scheme 55-1) [3].

The research group of Umezawa, who discovered bleomycin [4, 5], performed a series of forced degradation studies. In aqueous alcoholic solutions containing weak bases such as triethylamine, the carbamoyl group of metal-free bleomycin can migrate from the 3-hydroxyl to 2-hydroxyl of the mannose moiety to form iso-bleomycin (refer to the structure of bleomycin above) [6]. Under the same conditions, copper-chelated bleomycin undergoes epimerization at the chiral position α to the pyrimidine ring to produce epi-bleomycin.

Under acidic hydrolytic conditions, all the amide, the glycosidic, and carbamoyl linkages are susceptible to hydrolysis; nevertheless, the glycosidic linkage is among the most acid-sensitive bonds in bleomycin, and its cleavage leads to the formation of aglycone of bleomycin (also known as deglyco-BLM) [7]. Treatment under mild alkaline conditions can cause the cleavage of the sugar moiety via β-elimination, forming dehydrohistidine-bleomycin (Scheme 55-2) [8]. Under elevated temperature, the β-aminoalanine can be eliminated via β-elimination as well.



Scheme 55-1 Oxidative degradation of bleomycin in the presence of ferrous ions.



Scheme 55-2 Degradation of bleomycin under mild alkaline condition.

References

1 Hecht, S.M. (2000). Bleomycin: new perspectives on the mechanism of action. *J. Nat. Prod.* 63: 158–168.

2 Stubbe, J. and Kozarich, J.W. (1987). Mechanisms of bleomycin-induced DNA degradation. *Chem. Rev.* 87: 1107–1136.

3 Sam, J.W., Tang, X.-J., Magliozzo, R.S., and Peisachts, J. (1995). Electrospray mass spectrometry of iron bleomycin II: investigation of the reaction of Fe(III)-bleomycin with iodosylbenzene. *J. Am. Chem. Soc.* 117: 1012–1018.

4 Umezawa, H., Maeda, K., Takeuchi, T., and Okami, Y. (1966). New antibiotics, bleomycin A and B. *J. Antibiot.* 19: 200–209.

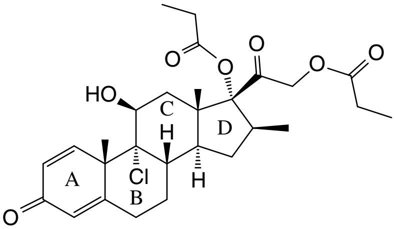
5 Takita, T., Muraoka, Y., Nakatani, T. et al. (1978). Chemistry of bleomycin. XIX. Revised structures of bleomycin and phleomycin. *J. Antibiot.* 31: 801–804.

6 Muraoka, Y., Kobayashi, H., Fujii, A. et al. (1976). Chemistry of bleomycin. XVI Epi-Bleomycin. *J. Antibiot.* 29: 853–856.

7 Muraoka, Y., Suzuki, M., Fujii, A. et al. (1981). Chemistry of bleomycin. XXVIII preparation of deglycobleomycin by mild acid hydrolysis of bleomycin. *J. Antibiot.* 34: 353–357.

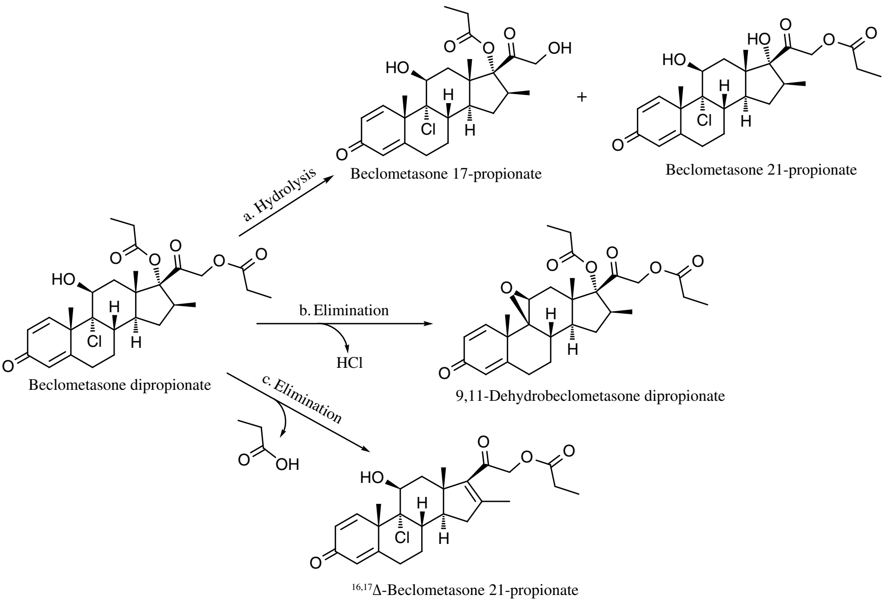
8 Omoto, S., Takita, T., Maeda, K. et al. (1972). The chemistry of bleomycin. VIII The structure of the sugar moiety of bleomycin A_2. *J. Antibiot.* 25: 752–754.

56: Beclometasone Dipropionate



Chemical name: (11β,16β)-9-Chloro-11-hydroxy-16-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate
Brand name: Qvar
Formula: $C_{28}H_{37}ClO_7$
Monoisotopic protonated ion (*m/z*): 521.2301
Molecular weight: 521.05
CAS number: 5534-09-8

Beclometasone dipropionate is a second-generation synthetic corticosteroid, which is a prodrug and its active metabolites, beclometasone 17-dipropionate and beclomethasone, are effective anti-inflammatory agents. Clinically, beclometasone dipropionate is primarily used for the treatment of asthma, allergic rhinitis, dermatitis, and psoriasis, typically in the dosage forms of a pressurized metered-dose inhaler, dry powder inhaler, aqueous nasal spray, and cream. Structurally, beclometasone dipropionate can be regarded as a chloro analog of betamethasone dipropionate in which the 9-fluoro of the latter is replaced by a chloro substituent. Thus, the degradation chemistry of beclometasone 17-propionate would be very similar to that of betamethasone dipropionate, which will be described in the Monograph of Betamethasone 21-acetate, Betamethasone 17-valerate, Betamethasone 17,21-dipropionate, and Betamethasone 21-phosphate. Indeed, in an accelerated stability study of beclometasone dipropionate, both hydrolytic degradants, i.e., beclometasone 17-propionate and beclometasone 21-propionate, were observed (Scheme 56) [1]. The difference in their degradation behavior between beclometasone dipropionate and betamethasone dipropionate appears to lie in the presence of the 9-chloro substituent in the former drug molecule versus the 9-fluoro one in the latter. For betamethasone and its analogs, the A-ring is susceptible to isomerization under normal laboratory fluorescent lighting. In contrast, beclometasone dipropionate and its analogs (i.e., those containing the 9-chloro substituent) appear to be resistant toward this type of photodegradation, due to the "heavy atom effect" of the 9-chloro substituent that shortens the triplet lifetime of the photochemically excited state [2]. In contrast, beclometasone dipropionate and its analogs tend to form the corresponding 9,11-epoxide degradants via elimination of HCl more easily than betamethasone dipropionate and its analogs via elimination of HF. This is probably because the chloro is a better leaving group than the fluoro. In the beclometasone dipropionate cream accelerated stability study, beclomethasone 9,11-epoxide



Scheme 56 Degradation pathways of beclomethasone dipropionate.

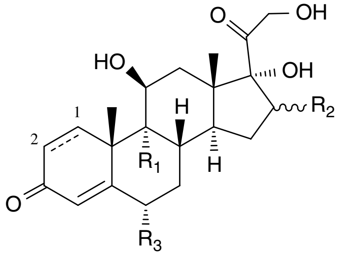
dipropionate was observed among the degradants [1]. In the same stability study, the major degradant was found to be another elimination degradant, $^{16,17}\Delta$-beclometasone 21-propionate; for comparison purpose, it should be mentioned that $^{16,17}\Delta$-betamethasone (16,17-dehydrobetamethasone) was observed in the forced degradation of betamethasone with HCl (refer to the next Monograph).

References

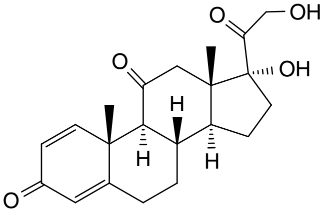
1 Sambandan, E., Kathavarayan, T., Sellappan, S. et al. (2019). Identification and characterization of unknown degradation impurities in beclomethasone dipropionate cream formulation using HPLC, ESI-MS and NMR. *J. Pharm. Biomed. Anal.* 167: 123–131.

2 Ricci, A., Fasani, E., Mella, M., and Albini, A. (2003). General patterns in the photochemistry of pregna-1,4-dien-3,20-diones. *J. Org. Chem.* 68: 4361–4366.

57–61: Betamethasone, Dexamethasone, Prednisolone, Methylprednisolone, Prednisone



Betamethasone, 1-2 double bond,
R_1 = F, R_2 = CH_3 at β-position, R_3 = H;
Dexamethasone, 1-2 double bond,
R_1 = F, R_2 = CH_3 at α-position, R_3 = H;
Prednisolone, 1-2 double bond,
R_1 = H, R_2 = H, R_3 = H;
Methylprednisolone, 1-2 single double bond,
R_1 = H, R_2 = H, R_3 = CH_3.



Prednisone

57: Betamethasone

Chemical name: (11β,16β)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione
Brand name: Celestone, Eleuphrat
Formula: $C_{22}HFO_5$
Monoisotopic protonated ion (*m/z*): 393.2072
Molecular weight: 392.46
CAS number: 378-44-9

58: Dexamethasone

Chemical name: (11β,16α)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione
Brand name: Decadron, Ozurdex, Dexycu
Formula: $C_{22}HFO_5$
Monoisotopic protonated ion (*m/z*): 393.2072
Molecular weight: 392.46
CAS number: 50-02-2

59: Prednisolone

Chemical name: (11β)-11,17,21-Trihydroxypregna-1,4-diene-3,20-dione
Brand name: Orapred, PediaPred, Millipred
Formula: $C_{21}H_{28}O_5$

Monoisotopic protonated ion (*m/z*): 361.2010
Molecular weight: 360.45
CAS number: 50-24-8

60: Methylprednisolone

Chemical name: (6α)-11,17,21-Trihydroxy-6-methylpregna-1,4-diene-3,20-dione
Brand name: Medrol
Formula: $C_{22}H_{30}O_5$
Monoisotopic protonated ion (*m/z*): 375.2166
Molecular weight: 374.48
CAS number: 83-43-2

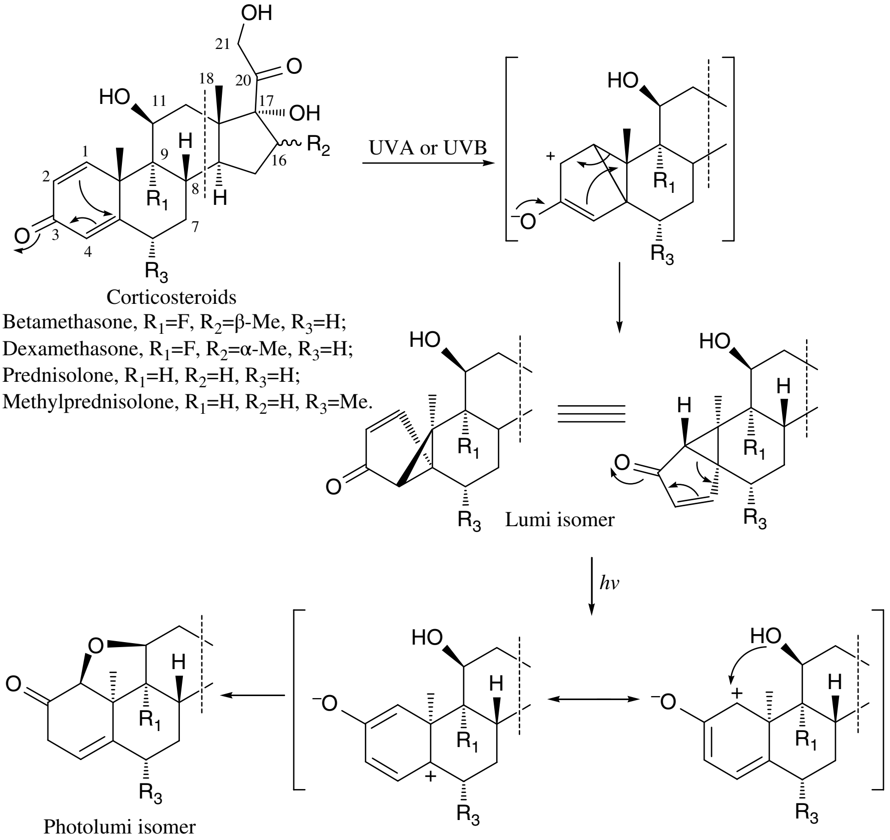
61: Prednisone

Chemical name: 17,21-Dihydroxypregna-1,4-diene-3,11,20-trione
Brand name: Deltasone
Formula: $C_{21}H_{26}O_5$
Monoisotopic protonated ion (*m/z*): 359.1853
Molecular weight: 358.43
CAS number: 53-03-2

Betamethasone and related corticosteroids such as dexamethasone, prednisolone, methylprednisolone, and prednisone are effective anti-inflammatory therapeutic agents that have been used for many decades. The structural moieties that are susceptible to degradation are mainly the A and D rings of the steroid core.

As a derivative of cyclohexa-2,5-dienone, the A ring undergoes facile photoisomerization particularly in solutions (Scheme 57–61-1), giving rise to lumi-isomers of the steroids; in the case of betamethasone and its esters, lumi-betamethasone and its esters are formed [1, 2]. The lumi-degradants can be observed in solutions of the corticosteroids when exposed to the fluorescent lighting in regular analytical laboratories [2]. Further photolysis can lead to many more photodegradants including photolumi degradants [3, 4].

However, the degradation chemistry on the D ring and its 1,3-dihydroxyacetone side chain is much more abundant than the A ring chemistry. For simplicity, we will use betamethasone as the prototype for our discussion and illustration of the degradation pathways. The degradation chemistry of the D ring comprises primarily two types: dehydration by the 17-hydroxyl group and oxidation of the 21-hydroxyl group. The dehydration can take place via two routes: one proceeds through the enolization of the 20-carbonyl group, which is referred to as the Mattox rearrangement [5], and the other can take place directly along with a hydrogen on the 16-position (Scheme 57–61-2). In formulated drug products, the former dehydration, which yields the *E*- and *Z*-isomers of enol aldehyde, is more frequently observed than the latter one. Both acidic stress and basic stress can catalyze the dehydration. When stressed by diluted sulfuric acid, only the enol aldehydes were

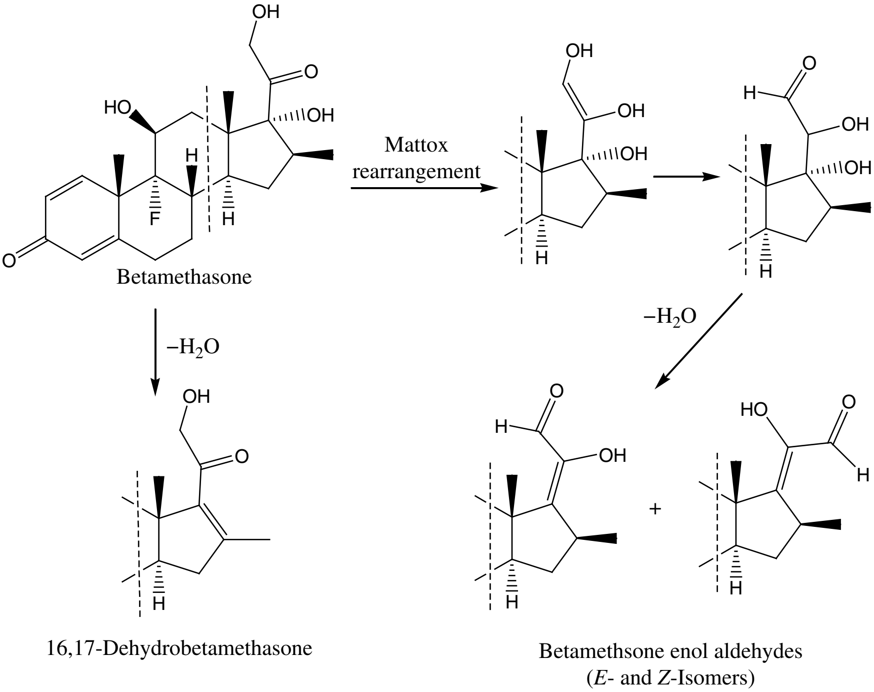


Scheme 57–61-1 Photochemical degradation pathways of betamethasone and related corticosteroids.

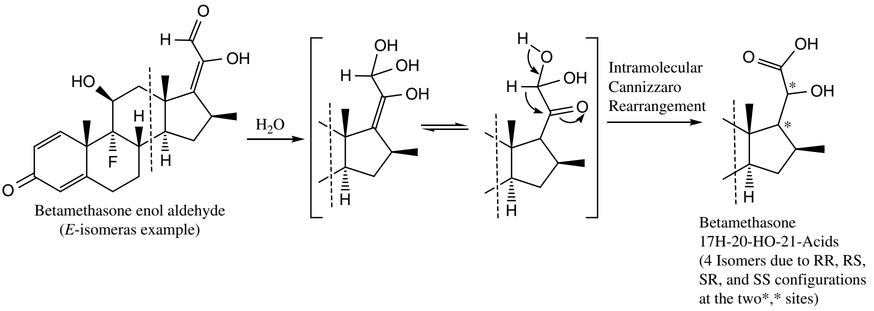
observed among the dehydrated products of betamethasone [6]. On the other hand, when stressed by hydrochloric acid, dehydration of betamethasone produces a significant amount of the 16,17-dehydro degradant in addition to the two isomers of enol aldehyde [1].

The enol aldehydes formed can undergo hydration on the aldehyde group, thanks to the activation by the neighboring keto group rendering the aldehyde group much more electrophilic. The hydrated intermediate, observable by LC-MS, can then isomerize to four acidic degradants of 17*H*-20-hydroxy-21-acids via intramolecular Cannizzaro rearrangement (Scheme 57–61-3) [7, 8].

The methylene at the 21-position is connected to both a keto and a hydroxyl group. Structure moiety of this type may be susceptible to oxidation via the carbanion/enolate auto-oxidation mechanism, especially in liquid formulations [8, 9]. The feasibility for such a mechanism (Scheme 57–61-4) is demonstrated in a base stress study of betamethasone [8]. The key intermediate is the Cregee type peroxide anion, which can have several routes of rearrangement, resulting in the formation of various degradants. Route a of Scheme 57–61-4 yields 21-aldehyde degradant, which is also

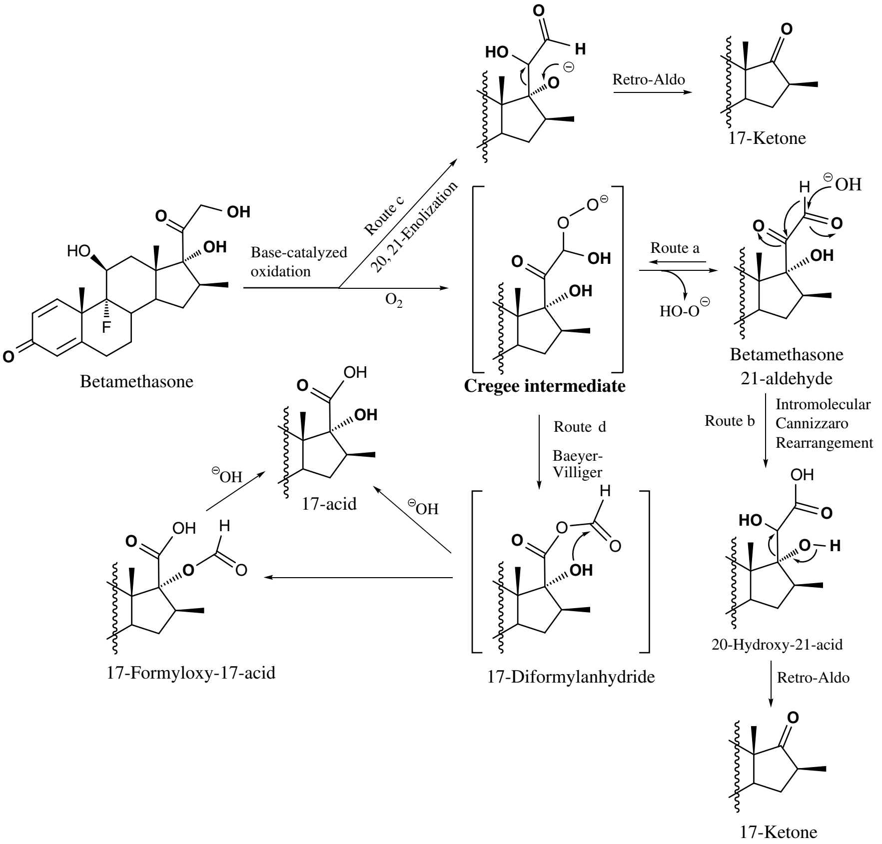


Scheme 57–61-2 Degradation of betamethasone via two different routes of dehydration.



Scheme 57–61-3 Further degradation of betamethasone enol aldehyde, a relatively stable intermediate degradant of betamethasone, leading to the formation of four acidic degradants of betamethasone.

an α-keto aldehyde just like the enol aldehydes. Hence, the aldehyde group is susceptible to hydration. In reality, 21-aldehyde is more readily hydrated than the enol aldehydes, which is evidenced by the fact that it is exclusively present as the hydrated form once dissolved in aqueous solutions based on LC-MS data [8]. The hydrated form can also undergo intramolecular Cannizzaro rearrangement to produce 20-hydroxy-21-acid (Route b), which should be prone to retro-aldo to give 17-ketone degradant. However, it would also be possible that 17-ketone is formed via a direct



Scheme 57–61-4 Base-catalyzed oxidative degradation of betamethasone.

retro-aldo of the rearranged anion (Route c). In such a case, 17-ketone is not a result of oxidative degradation. Route d of Scheme 57–61-4 produces the diformylanhydride intermediate, which readily isomerizes to 17-formyloxy-17-acid. The latter is an ester of formic acid, which is not very stable and is eventually hydrolyzed to 17-acid. 17-Acid can also be formed directly from the 17-diformylanhydride intermediate.

It should be noted that the degradation pathways elucidated from this study may also be feasible for a free radical version of auto-oxidation in which the Cregee intermediate would be a peroxy free radical. The free radical mechanism may be more suitable for the steroids formulated in solid dosage forms.

The degradants discussed in this monograph have been observed in various drug products containing betamethasone or analogous corticosteroids that were stored under either ICH stability or ambient conditions [10–12], increasing the validity of the proposed carbanion/enolate auto-oxidation mechanism. Obviously not all the degradants are observed all the times. The occurrence or distribution of these degradants depends on the formulation and the age of a drug product sample. Certain degradants, e.g., 17-acid, 17-ketone, and enol aldehydes, are more frequently observed than others.

References

1 Hidaka, T., Huruumi, S., Tamaki, S. et al. (1980). Studies on betamethasone: behavior of betamethasone in acid or alkaline medium, photolysis and oxidation (Original in Japanese). *Yakugaku Zasshi* 100: 72–80.

2 Lin, M., Li, M., Buevich, A.V. et al. (2009). Rapid structure elucidation of drug degradation products using mechanism-based stress studies in conjunction with LC–MS^n and NMR spectroscopy: identification of a photodegradation product of betamethasone dipropionate. *J. Pharm. Biomed. Anal.* 50: 275–280.

3 Ricci, A., Fasani, E., Mella, M., and Albini, A. (2003). General patterns in the photochemistry of pregna-1,4-dien-3,20-diones. *J. Org. Chem.* 68: 4361–4366.

4 Miolo, G., Gallocchio, F., Levorato, L. et al. (2009). UVB photolysis of betamethasone and its esters: characterization of photoproducts in solution, in pig skin and in drug formulations. *J. Photochem. Photobiol. B Biol.* 96: 75–81.

5 Mattox, V.R. (1952). Steroids derived from bile acids. XV. The formation of a glyoxal side chain at C-17 from steroids with dihydroxyacetone and Δ^{16}-ketol side chains. *J. Am. Chem. Soc.* 74: 4340–4347.

6 Li, M., Chen, B., Lin, M. et al. (2007). A variation of Mattox rearrangement mechanism under alkaline condition. *Tetrahedron Lett.* 48: 3901–3905.

7 Görög, S. and Horváth, P. (1978). Analysis of steroids. Part XXXI. Mechanism of the tetrazolium reaction of corticosteroids. *Analyst* 103: 305–316.

8 Li, M., Chen, B., Monteiro, S., and Rustum, A.M. (2009). Mechanism of base-catalyzed autooxidation of corticosteroids containing 20-keto-21-hydroxyl side chain. *Tetrahedron Lett.* 50: 4575–4581.

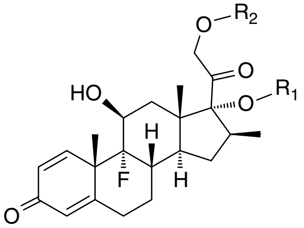
9 Li, M. (2012). Carbanion/enolate-mediated autooxidation (base-catalyzed autooxidation). In: *Organic Chemistry of Drug Degradation*, 61–62. Cambridge: RSC Publishing.

10 Shou, M., Galinada, W.A., Wei, Y.-C. et al. (2009). Development and validation of a stability-indicating HPLC method for simultaneous determination of salicylic acid, betamethasone dipropionate and their related compounds in Diprosalic Lotion®. *J. Pharm. Biomed. Anal.* 50: 356–361.

11 Johnston, S.E., Gill, N.L., Wei, Y.-C. et al. (2010). Development and validation of a stability-indicating RP-HPLC method for simultaneous assay of betamethasone dipropionate, chlorocresol, and for the estimation of betamethasone dipropionate related compounds in a pharmaceutical cream and ointment. *J. Chromatogr. Sci.* 48: 733–741.

12 Matter, B., Ghaffari, A., Bourne, D. et al. (2019). Dexamethasone degradation in aqueous medium and implications for correction of in vitro release from sustained release delivery systems. *AAPS PharmSciTech* 20: 320. https://doi.org/10.1208/s12249-019-1508-7.

62–65: Betamethasone 21-Acetate, Betamethasone 17-Valerate, Betamethasone 17,21-Dipropionate, Betamethasone 21-Phosphate



Betamethasone 21-acetate, R_1=H, R_2=Acetyl;
Betamethasone 17-valerate, R_1=Valeryl, R_2=H;
Betamethasone 17,21-dipropionate, R_1=R_2=Propionyl;
Betamethasone 21-Phosphate, R_1=H, R_2=Phosphoryl.

Esters of Betamethasone

62: Betamethasone 21-Acetate (also Known as Betamethasone Acetate)

Chemical name: (11β,16β)-9-Fluoro-11,17-dihydroxy-16-methyl-3,20-dioxopregna-1, 4-dien-21-yl acetate
Brand name: Celestone Chronodose (injection combo with betamethasone sodium phosphate), Celestone Soluspan (injection combo with betamethasone sodium phosphate)
Formula: $C_{24}H_{31}FO_6$
Monoisotopic protonated ion (*m/z*): 435.2177
Molecular weight: 434.50
CAS number: 987-24-6

63: Betamethasone 17-Valerate (also Known as Betamethasone Valerate)

Chemical name: (11β,16β)-9-Fluoro-11,17-dihydroxy-16-methyl-3,20-dioxopregna-1, 4-dien-21-yl valerate
Brand name: Betnovate, Celestoderm
Formula: $C_{27}H_{37}FO_6$
Monoisotopic protonated ion (*m/z*): 477.2647
Molecular weight: 476.59
CAS number: 2152-44-5

64: Betamethasone 17,21-Dipropionate (also Known as Betamethasone Dipropionate)

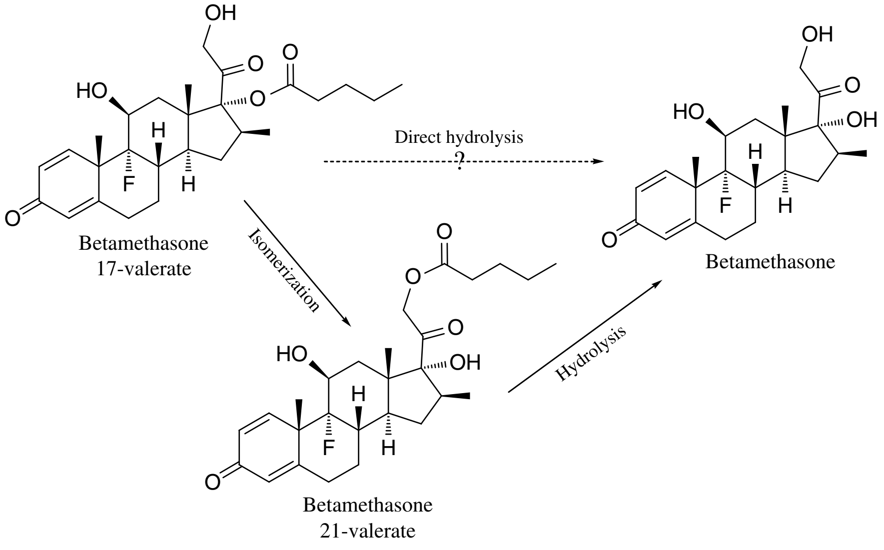
Chemical name: (11β,16β)-9-Fluoro-11-hydroxy-16-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate

Brand name: Diprolene, Diprosone, Sernivo
Formula: $C_{28}H_{37}FO_7$
Monoisotopic protonated ion (*m/z*): 505.2596
Molecular weight: 504.60
CAS number: 5593-20-4

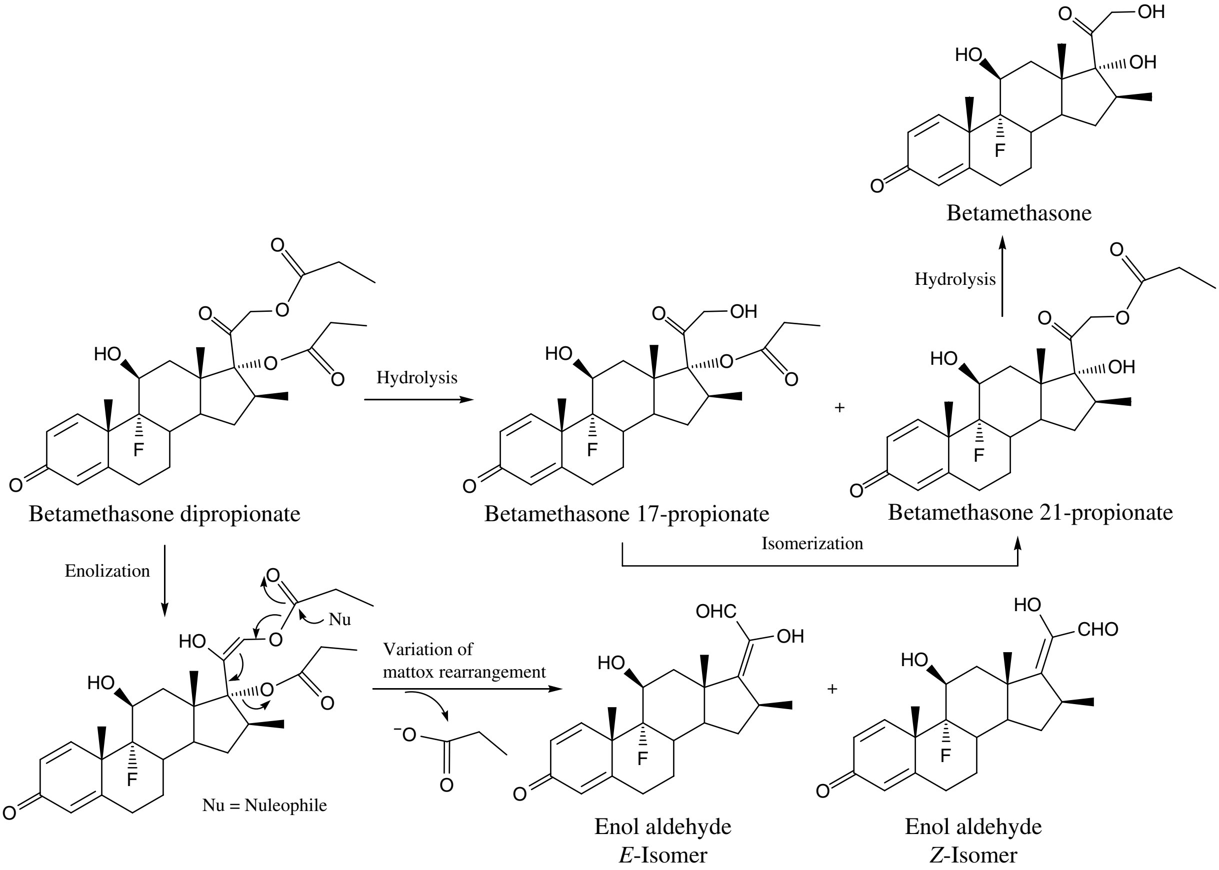
65: Betamethasone 21-Phosphate (also Known as Betamethasone Phosphate)

Chemical name: Disodium (11β,16β)-9-fluoro-11,17-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-21-yl phosphate
Brand name: Celestone Chronodose (injection combo with betamethasone acetate), Celestone Soluspan (injection combo with betamethasone acetate)
Formula: $C_{22}H_{28}FNa_2O_8P$
Monoisotopic protonated ion (*m/z*): 473.1735
Molecular weight: 516.41
CAS number: 360-63-4

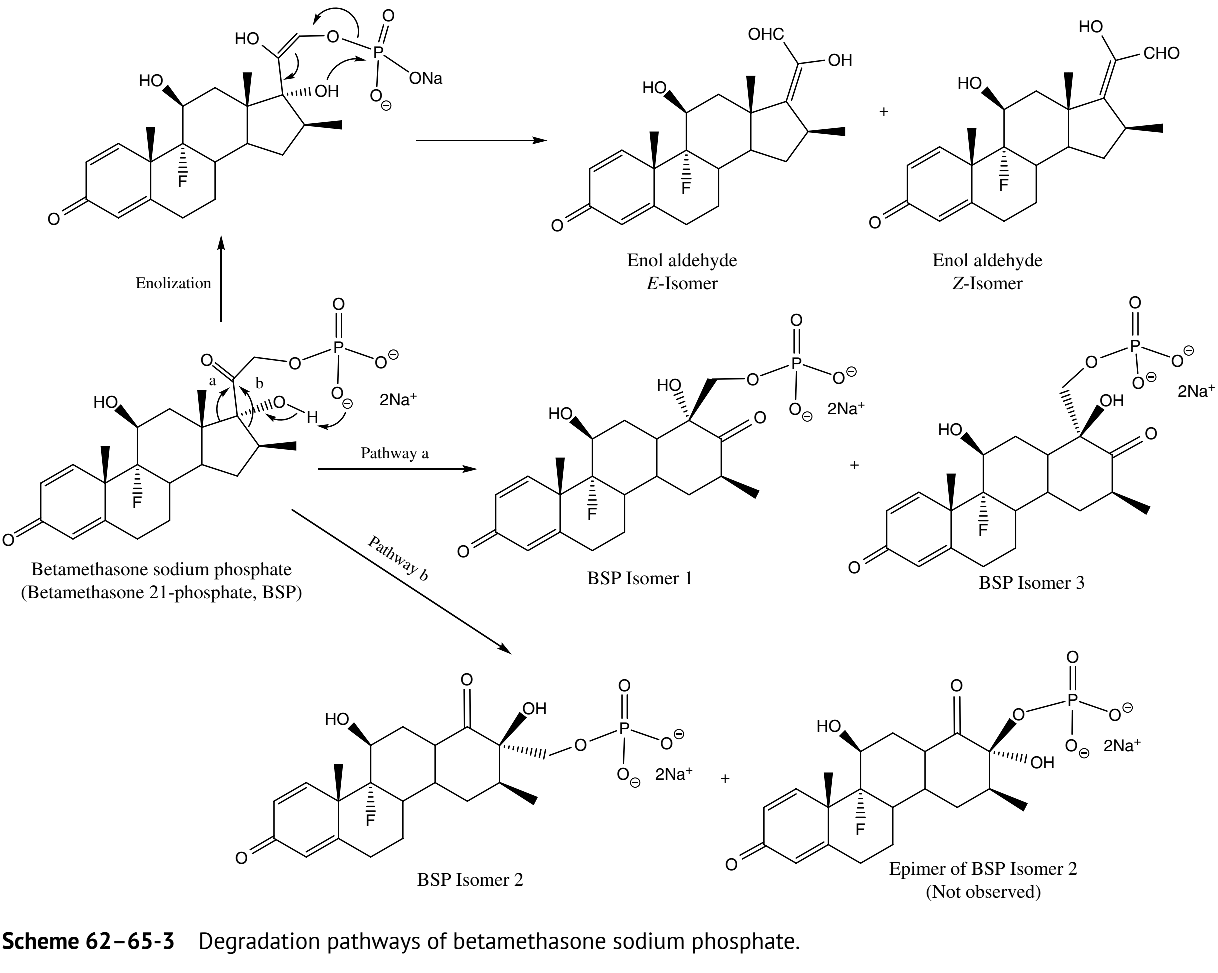
The esters of corticosteroids are also widely used anti-inflammatory therapeutic agents, and they are generally susceptible to hydrolytic degradation. For simplicity, we will use the esters of betamethasone as the prototype for our discussion and illustration of the degradation pathways of analogous corticosteroid esters. Among the esters, 21-acetate would be most susceptible to hydrolysis to yield betamethasone, while 21-phosphate is the least susceptible. This prediction can be made based on the activation energies for hydrolysis of the esters that can be used as model compounds for the ester drugs discussed in this monograph [1]. For example, the



Scheme 62–65-1 Degradation of betamethasone 17-valerate.



Scheme 62–65-2 Degradation pathways of betamethasone dipropionate.



Scheme 62–65-3 Degradation pathways of betamethasone sodium phosphate.

activation energy for hydrolysis of a typical acetate is usually no more than 15 kcal/mol. On the other hand, the activation energy for hydrolysis of a typical phosphate is usually ca. 30 kcal/mol. Such a high energy barrier implies that hydrolysis would be an insignificant degradation pathway for betamethasone 21-phosphate and its formulated products that are stored under long-term stability conditions.

According to a study by Hansen and Bundgaard, betamethasone 17-varylate does not undergo hydrolysis directly [2]. Instead, it isomerizes to 21-varylate, which is then followed by the hydrolysis of the latter (Scheme 62–65-1).

The hydrolysis of betamethasone 17,21-dipropionate produces 17-mono ester and 21-mono ester, respectively. The mono esters can further degrade to betamethasone; the degradation of 17-mono ester would likely proceed through 21-mono ester, in the same fashion as betamethasone 17-valerate. Additionally, betamethasone 17,21-dipropionate can undergo the variation of the Mattox rearrangement to yield the two enol aldehydes directly (Scheme 62–65-2) [3, 4].

Betamethasone 21-phosphate can also degrade to the two enol aldehydes directly, through a mechanism (Scheme 62–65-3) that is somewhat different from the variation of the Mattox rearrangement [5]. The enol aldehydes formed can further degrade to give four 17*H*-20-hydroxy-21-acids as discussed previously. In a solid state, the D-ring of betamethasone 21-phosphate can undergo isomeric ring expansion, a process referred to as D-homoannular ring expansion [5]. During the latter process, four isomeric degradants could be produced; however, in the case of betamethasone sodium phosphate, only three such degradants were observed. The missing one (epimer of BSP Isomer 2, Scheme 62–65-3) could be too difficult to form, probably due to steric hindrance. In all the esters, the A-rings remain intact, and hence they are still susceptible to the photochemical rearrangement as discussed in the previous monograph.

References

1 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 16–47. Cambridge: RSC Publishing.

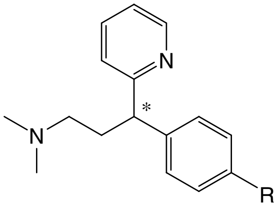
2 Bundgaard, H. and Hansen, J. (1981). Studies on the stability of corticosteroids VI. Kinetics of the rearrangement of betamethasone-17-valerate to the 21-valerate ester in aqueous solution. *Int. J. Pharm.* 7: 197–203.

3 Li, M., Chen, B., Lin, M. et al. (2007). A variation of Mattox rearrangement mechanism under alkaline condition. *Tetrahedron Lett.* 48: 3901–3905.

4 Chen, B., Li, M., Lin, M. et al. (2009). A comparative study of enol aldehyde formation from betamethasone, dexamethasone, beclomethasone and related compounds under acidic and alkaline conditions. *Steroids* 74: 30–41.

5 Li, M., Wang, X., Chen, B. et al. (2009). Forced degradation of betamethasone sodium phosphate under solid state: formation, characterization, and mechanistic study of all four 17,20-diastereomers of betamethasone 17-deoxy-20-hydroxy-21-oic acid. *J. Pharm. Sci.* 98: 894–904.

66–69: Brompheniramine, Dexbrompheniramine, Chlorpheniramine, Pheniramine



Brompheniramine, R = Br, * = racemic,
Dexbrompheniramine, R = Br, * = *S*-isomer,
Chlorpheniramine, R = Cl, * = Racemic
Pheniramine, R = H, * = Racemic.

66: Brompheniramine

Chemical name: (*R*,*S*)-3-(4-Bromophenyl)-*N*,*N*-dimethyl-3-pyridin-2-yl-propan-1-amine
Brand name: Bromfed, Dimetapp, Bromfenex
Formula: $C_{16}H_{19}BrN_2$
Monoisotopic protonated ion (*m/z*): 319.0804
Molecular weight: 319.25
CAS number: 86-22-6
Common salt form: Maleate

67: Dexbrompheniramine

Chemical name: (3*S*)-3-(4-Bromophenyl)-*N*,*N*-dimethyl-3-pyridin-2-yl-propan-1-amine
Brand name: Drixoril, Conex, Dixaphedrine
Formula: $C_{16}H_{19}BrN_2$
Monoisotopic protonated ion (*m/z*): 319.0804
Molecular weight: 319.25
CAS number: 132-21-8
Common salt form: Maleate

68: Chlorpheniramine

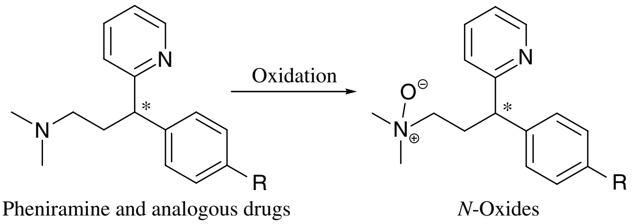
Chemical name: 3-(4-Chlorophenyl)-*N*,*N*-dimethyl-3-(pyridin-2-yl)-propan-1-amine
Brand name: Demazin
Formula: $C_{16}H_{19}ClN_2$
Monoisotopic protonated ion (*m/z*): 275.1310
Molecular weight: 274.79
CAS number: 132-22-9
Common salt form: Maleate, HCl salt

69: Pheniramine

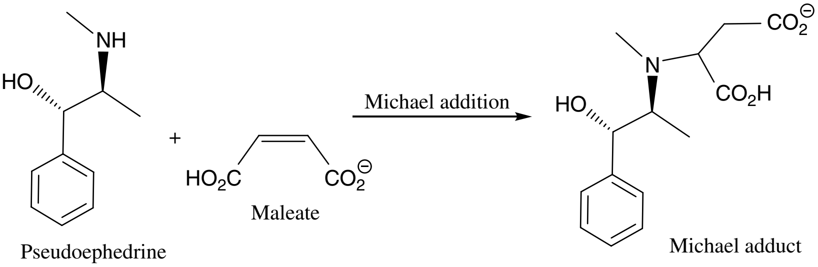
Chemical name: *N,N*-Dimethyl-3-phenyl-3-pyridin-2-yl-propan-1-amine
Brand name: Under various brand names in many OTC combo medicines
Formula: $C_{16}H_{20}N_2$
Monoisotopic protonated ion (*m/z*): 241.1699
Molecular weight: 240.35
CAS number: 86-21-5
Common salt form: Maleate

Brompheniramine, dexbrompheniramine, chlorpheniramine, and pheniramine are first-generation antihistamine medicines, and their therapeutic use can date back to the 1950s. Nowadays, they are mostly utilized in combination with other therapeutic agents as OTC drugs. Despite their long history of medical use, the literature on their degradation chemistry is scarce. On the contrary, based on their structures, it is reasonable to assume that the tertiary amine moiety of this drug family would be susceptible to nucleophilic oxidation to produce *N*-oxide degradants under long-term stability and forced degradation conditions. During a forced degradation study of a cough-cold medicine containing chlorpheniramine, an oxidative degradant was observed, which contains an extra oxygen as compared to chlorpheniramine [1]. Although the original paper did not identify the exact structure of this degradant, it is quite likely the tertiary amine *N*-oxide (Scheme 66–69-1). Chlorpheniramine also has a pyridine group that could form the pyridine *N*-oxide. Nevertheless, the formation of pyridine *N*-oxide would be less likely than the formation of the tertiary amine *N*-oxide, due to the poor nucleophilicity of the pyridine ring.

Chlorpheniramine and dexbrompheniramine are typically present in their maleate salt forms for use as active pharmaceutical ingredients. In several OTC combo drugs, chlorpheniramine maleate and dexbrompheniramine maleate are used in combination with pseudoephedrine, a very common nasal decongestant. In these formulated products, maleate may react with pseudoephedrine, a secondary amine, to form a degradant via Michael addition (Scheme 66–69-2) [2, 3].



Scheme 66–69-1 Oxidative degradation of pheniramine and analogous drugs.



Scheme 66–69-2 Degradation of pseudoephedrine via Michael addition.

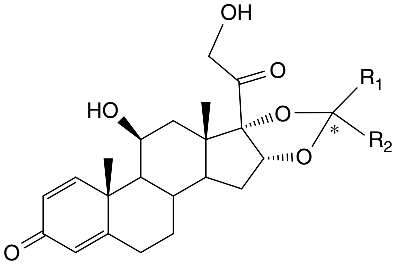
References

1 Marin, A. and Barbas, C. (2004). LC/MS for the degradation profiling of cough–cold products under forced conditions. *J. Pharm. Biomed. Anal.* 35: 1035–1045.

2 Wong, J., Wiseman, L., Al-Mamoon, S. et al. (2006). Major degradation product identified in several pharmaceutical formulations against the common cold. *Anal. Chem.* 78: 7891–7895.

3 Chan, T.-M., Kong, J., McNamara, P., and Wong, J.K. (2008). Syntheses of potential degradation products of phenylephrine in OTC products. *Synth. Commun.* 38: 2252–2260.

70–71: Budesonide, Desonide



Budesonide, R_1 = Propyl, R_2 = H, * = racemic;
Desonide, $R_1 = R_2$ = Methyl.

70: Budesonide

Chemical name: 11β,21-Dihydroxy-16α,17α-[butane-1,1-diylbis(oxy)]pregna-1,4-diene-3,20-dione
Brand name: Pulmicort, Rhinocort, Entocort
Formula: $C_{25}H_{34}O_6$
Monoisotopic protonated ion (*m/z*): 431.2428
Molecular weight: 430.54
CAS number: 51333-22-3

71: Desonide

Chemical name: 11β,21-Dihydroxy-16α,17α-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione
Brand name: Desowen
Formula: $C_{24}H_{32}O_6$
Monoisotopic protonated ion (*m/z*): 417.2272
Molecular weight: 416.51
CAS number: 638-94-8

Budesonide and desonide are acetal derivatives of nonhalogenated glucocorticosteroids that are widely used in the treatment of inflammatory diseases. Budesonide is a condensation product between the steroid core and butyraldehyde; the formation of the five-membered acetal ring creates a new chiral center. Hence, budesonide is a mixture of two epimers, and major pharmacopoeias require a specific range of ratios between the two epimers [1]. On the other hand, desonide is a condensation product between the steroid core and acetone; the formation of its acetal ring does not create any new chiral center due to the presence of the two identical methyl groups.

Ferraboschi et al. performed a detailed study of budesonide tablets that were put on stability at 25°C/60%RH for 3 years [2]. Five major degradants were identified based on structural characterization data and/or synthesis of the degradants that were not included in the European Pharmacopoeia. Although the authors described the general pathways for each major degradant, detailed mechanisms were lacking particularly for the formation of the D-homoannual ring expansion product (EP impurity C) and budesonide 16-butyroyl-17-ketone. The current author attempted to propose possible mechanisms for the degradants (Scheme 70–71-1). Apparently, budesonide 11-ketone results from the oxidation of the 11-hydroxyl group. Notably, the oxidation of the 11-hydroxyl group is usually not a significant degradation pathway for fluoro-containing corticosteroids such as betamethasone and its derivatives under long-term stability conditions. The D-ring annual ring expansion likely involves a concerted rearrangement as depicted in Scheme 70–71-1. The formation of budesonide 16-butyroyl-17-ketone probably starts with the oxidation on the acetal carbon: a free-radical mediated oxidation would first give a peroxyl intermediate, which can decompose to the hydroxyl intermediate with concurrent enolization of the hydroxyacetone side chain (Scheme 70–71-1) [3]. A retro-aldo process of the hydroxyl intermediate should produce the budesonide 16-butyroyl-17-ketone [4]. The oxidation on the 21-carbon (of the hydroxyacetone side chain) should yield the budesonide 21-aldehyde (EP impurity D) and 17-acid. The mechanism for the oxidation should be analogous to that of betamethasone [4]. In the solid state such as the stability study of budesonide tablets, the oxidation is likely mediated via free radicals. However, in liquid formulations, the oxidation would more likely proceed through the carbanion/enolate auto-oxidation pathway [5]. In a stability study of budesonide in propylene glycol that was continuously being oxygenated and stored at 40°C for 3 months, more than 10% of the API was found to be decomposed and approximately 5 to 7 degradants were observed, of which only the two 21-aldehyde epimers were identified based on MS data [6]. In two patents, it was revealed that ethanolic solutions of budesonide can be stabilized by the addition of organic acids [7, 8]. These reports suggest that this steroid is also likely susceptible to base-catalyzed carbanion/enolate auto-oxidation in liquid formulations [9], similar to the base-catalyzed oxidation of betamethasone [4].

The steroid core of desonide is identical to that of budesonide. Hence, desonide should undergo similar degradation pathways with regard to its steroid core. Indeed, desonide was found to be photochemically unstable primarily due to its A-ring [10],[1] which can be problematic because the drug is frequently utilized in topical applications. Mechanistic study revealed that under irradiation at 254 nm, the main photodegradant was the lumi-degradant of desonide (Scheme 70–71-2). When irradiated at 310 nm under the aerated and nonaerated conditions, the main photodegradants were 17-peroxyl desonide and 17*H*-desonide, respectively. The peroxyl degradant can further degrade to desonide 17-ketone. The photochemical stability of formulated desonide can be enhanced by the use of benzophenone-3 [11]. In a study by Nguyen et al., desonide 17-acid and 17*H*-desonide were observed as degradants in a desonide ointment product [12]. The two degradants were synthesized by treating desonide with alkaline hydrogen peroxide.

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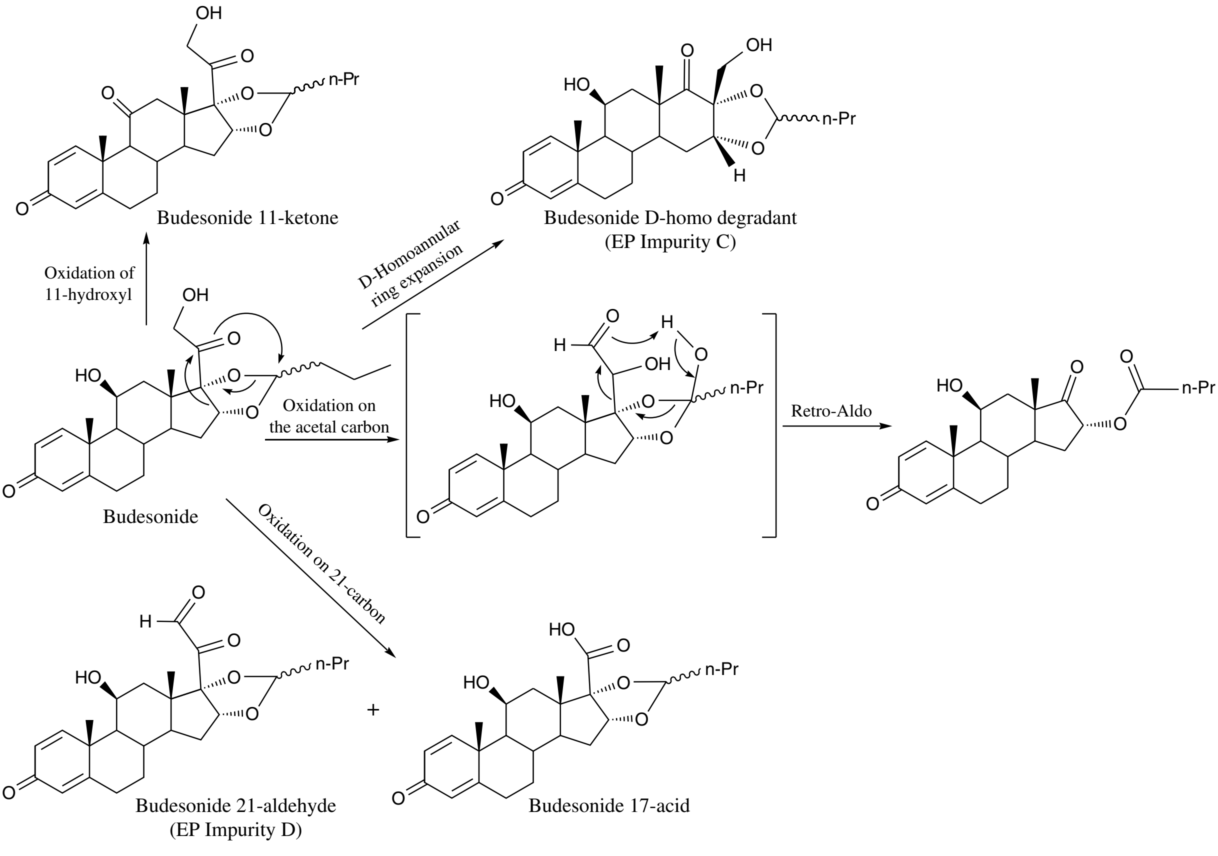
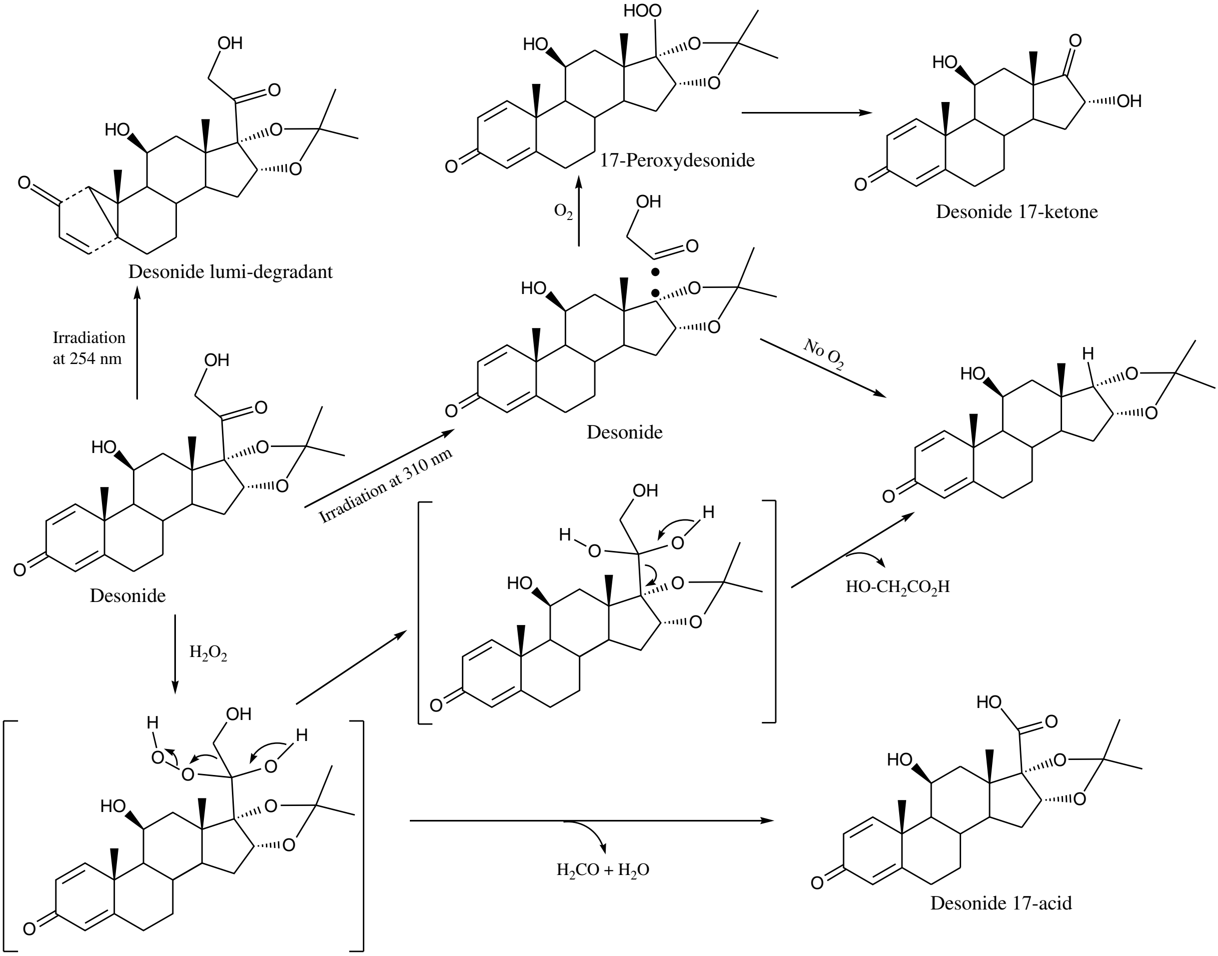
[1] Also refer to the Monograph for betamethasone and its analogous drugs in this book.



Scheme 70–71-1 Degradation pathways of budesonide.



Scheme 70–71-2 Degradation pathways of desonide.

References

1 Budesonide. United States Pharmacopoeia, Official as of 1 July 2021, DOI: 10.31003/USPNF_M10458_04_01.

2 Ferraboschi, P., Bertacche, V., Maccone, I. et al. (2008). Estimation and characterisation of budesonide tablets impurities. *J. Pharm. Biomed. Anal.* 47: 636–640.

3 Li, M., Wang, X., Chen, B. et al. (2009). Forced degradation of betamethasone sodium phosphate under solid state: formation, characterization, and mechanistic study of all four 17,20-diastereomers of betamethasone 17-deoxy-20-hydroxy-21-oic acid. *J. Pharm. Sci.* 98: 894–904.

4 Li, M., Chen, B., Monteiro, S., and Rustum, A.M. (2009). Mechanism of base-catalyzed autooxidation of corticosteroids containing 20-keto-21-hydroxyl side chain. *Tetrahedron Lett.* 50: 4575–4581.

5 Li, M. (2012). Carbanion/enolate-mediated autooxidation (Base-catalyzed autooxidation). In: *Organic Chemistry of Drug Degradation*, 61–62. Cambridge: RSC Publishing.

6 Hou, S., Hindle, M., and Byron, P.R. (2001). A stability-indicating HPLC assay method for budesonide. *J. Pharm. Biomed. Anal.* 24: 371–380.

7 Freund B, Krueger M, Zierenberg B, (2002) Stable pharmaceutical budesonide preparation for producing propellant-free aerosols. US Patent No. 6,491,897 B1.

8 Freund B, Krueger M, Zierenberg B, (2011) Stable pharmaceutical budesonide preparation for producing propellant-free aerosols. US Patent No US 8,062,626 B2.

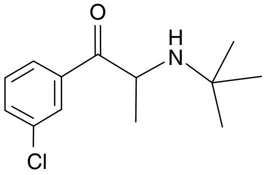
9 Slavíková, B., Kasal, A., and Buděšínský, M. (1999). Autoxidation vs hydrolysis in 16α-acyloxy steroids. *Collect. Czechoslov. Chem. Commun.* 64: 1125–1134.

10 Iqbal, J., Husain, A., and Gupta, A. (2006). Photochemistry of desonide, a non-fluorinated steroidal anti-inflammatory. *Drug Chem. Pharm. Bull.* 54: 836–838.

11 Nguyen, T.T., Kringstad, R., Aasen, A.J., and Rasmussen, K.E. (1988). Identification and analysis of a degradation product of the glucocorticoid desonide in ointment. *Acta Chem. Scand. B* 42: 403–407.

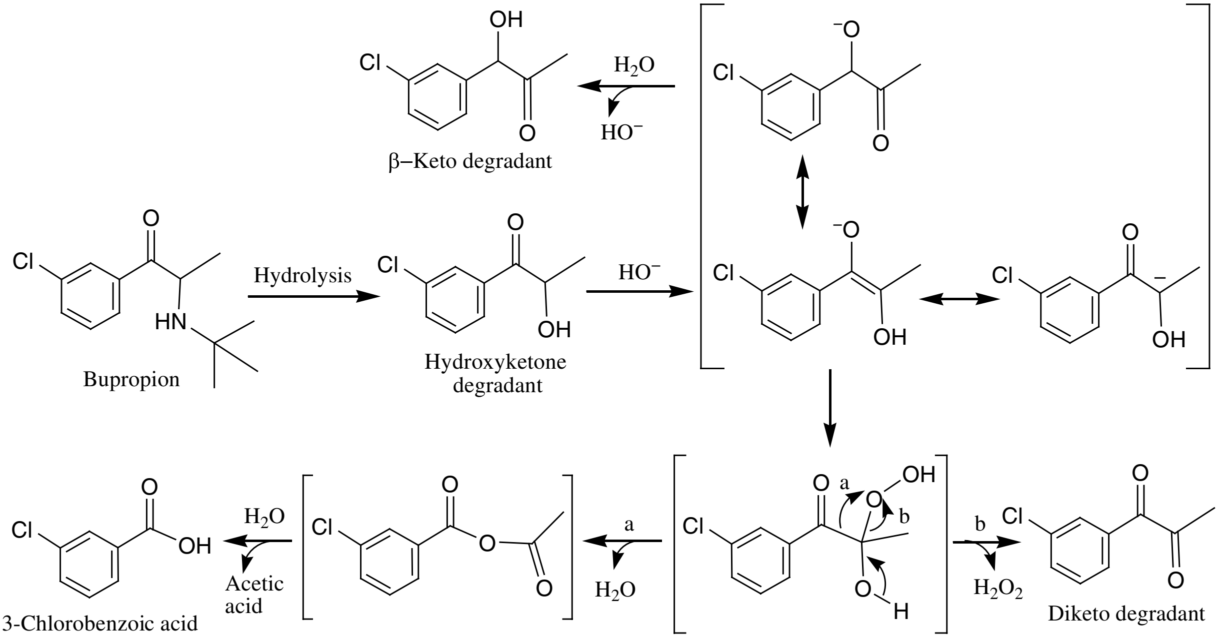
12 Rosa, P., Salla, A.P.S., de Bona da Silva, C. et al. (2014). Investigation of the stabilizing effects of antioxidants and benzophenone-3 on desonide photostability. *AAPS PharmSciTech* 15: 1155–1162.

72: Bupropion



Chemical name: (*R*,*S*)-2-(*tert*-Butylamino)-1-(3-chlorophenyl)propan-1-one
Brand name: Wellbutrin, Zyban, Elontril
Formula: $C_{13}H_{18}ClNO$
Monoisotopic protonated ion (*m/z*): 240.1150
Molecular weight: 239.74
CAS number: 34911-55-2
Common salt form: HCl salt

Bupropion is an aminoketone that has many therapeutic indications, including antidepression and smoking cessation; it is one of the most prescribed antidepressants in the United States. O'Byrne et al. studied the stability of bupropion in aqueous solutions over a pH range of 2–13 [1]. No bupropion decomposition was found at 60°C in a solution of $pH \leq 5$ in which the drug molecule is mostly in its protonated form (the pKa of bupropion is 7.9). In neutral to alkaline solutions, bupropion started to degrade, and the first degradant is the hydroxyketone, which is formed via nucleophilic substitution of the amino moiety by water/hydroxide ion (Scheme 72). The hydroxyketone then undergoes base-catalyzed auto-oxidation as well as enolization to yield other three degradants, i.e., 3-chlorobenzoic acid, the β-keto degradant, and the diketo degradant. There are a number of bupropion formulation patents in which both organic and inorganic acids were used to stabilize the drug products [2–4]. This fact is consistent with the base-catalyzed oxidation mechanism as mentioned above.



Scheme 72 Hydrolytic degradation of bupropion in neutral to alkaline solutions.

References

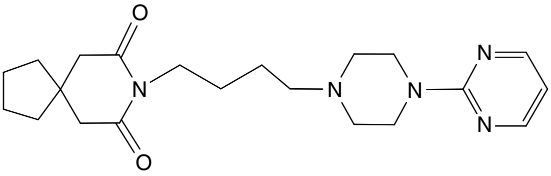
1 O'Byrne, P.M., Williams, R., Walsh, J.J., and Gilmer, J.F. (2010). The aqueous stability of bupropion. *J. Pharm. Biomed. Anal.* 53: 376–381.

2 M.D. Ruff, S.R. Kalidindi, J.E. Sutton Jr., Pharmaceutical composition containing bupropion hydrochloride and a stabilizer, US Patent No. 5,358,970 (1994).

3 A. Maitra, P.S. Kulkarni, B.B. Shah, J.M. Devito, Pharmaceutical composition containing bupropion hydrochloride and an inorganic acid stabilizer, US Patent No. 5,968,553 (1999).

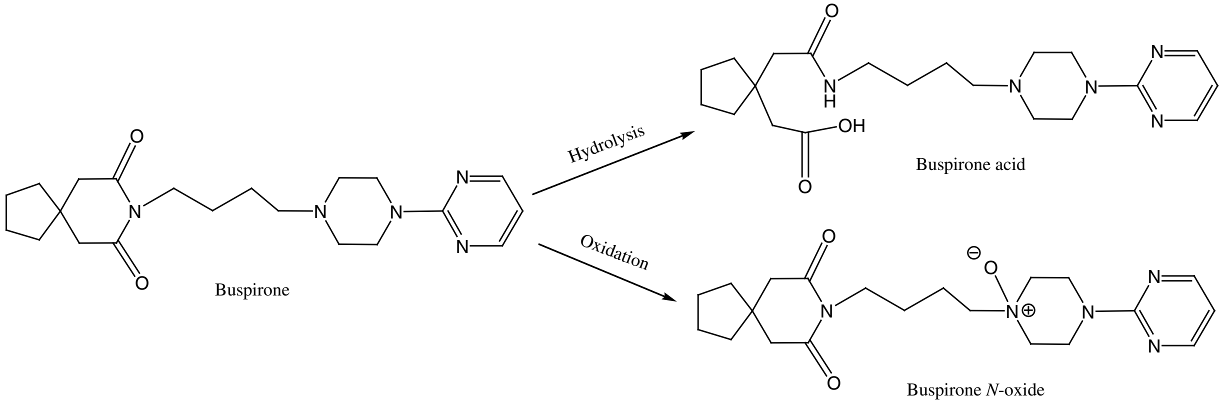
4 C.-H. Han, G. Liaw, Stabilized pharmaceutical compositions containing bupropion hydrochloride, US Patent No. 6,333,332 (2001).

73: Buspirone



Chemical name: 8-{4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl}-8-azaspiro[4.5] decane-7,9-dione
Brand name: Buspar
Formula: $C_{21}H_{31}N_5O_2$
Monoisotopic protonated ion (*m/z*): 386.2551
Molecular weight: 385.51
CAS number: 36505-84-7
Common salt form: HCl salt

Buspirone is a serotonin $5HT_{1A}$ receptor agonist of the arylpiperazine class that is clinically utilized for the treatment of general anxiety [1]. Its structural moieties that are susceptible to degradation include the cyclic imide, piperazine, and pyrimidine ring. The two symmetric imide bonds are equally vulnerable to hydrolytic attack, giving rise to the buspirone acid [2]. The nitrogens of the piperazine ring, particularly the piperazine nitrogen connected to the butyl group, and those of the pyrimidine ring can be oxidized by H_2O_2 to form the piperazine and pyrimidine *N*-oxides, with the piperazine *N*-oxide much preferred over the pyrimidine *N*-oxide [3]. The degradation pathways of buspirone are summarized in Scheme 73.

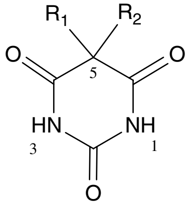


Scheme 73 Degradation pathways of buspirone.

References

1. Bockaert, J., Dumuis, A., Bouhelal, R. et al. (1987). Piperazine derivatives including the putative anxiolytic drugs, buspirone and ipsapirone, are agonists at 5-HT$_{1A}$ receptors negatively coupled with adenylate cyclase in hippocampal neurons. *Naunyn Schmiedeberg's Arch. Pharmacol.* 335: 588–592.
2. Khedr, A. and Sakr, A. (1999). Stability-indicating high-performance liquid chromatographic assay of buspirone HCl. *J. Chromatogr. Sci.* 37: 462–468.
3. Chen, B.-C. and Stark, D.R. (1996). A facile preparation of buspirone N-oxide using Davis' reagent. *Org. Prep. Proced. Int.* 28: 115–117.

74–78: Butalbital and Related Barbiturates



Butalbital, R_1=1-Isobutyl, R_2=2-Propenyl;
Pentobarbital, R_1=Ethyl, R_2=2-Pentyl;
Phenobarbital, R_1=Ethyl, R_2=Phenyl;
Secobarbital, R_1=2-Pentyl, R_2=2-Propenyl;
Talbital, R_1=1-sec-Butyl, R_2=2-Propenyl.

74: Butalbital

Chemical name: 5-(2-Methylpropyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Brand name: Various brand names for its combination with other drugs.
Formula: $C_{11}H_{16}N_2O_3$
Monoisotopic protonated ion (*m/z*): 225.1234
Molecular weight: 224.26
CAS number: 77-26-9

75: Pentobarbital

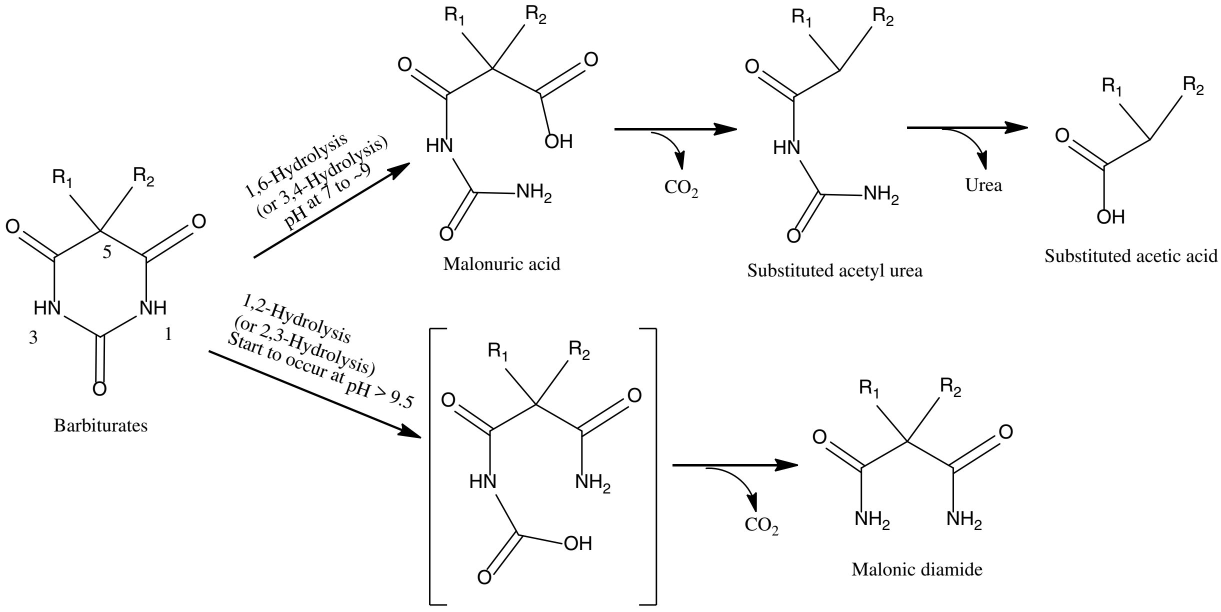
Chemical name: 5-Ethyl-5-(1-methylbutyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Brand name: Nembutal
Formula: $C_{11}H_{18}N_2O_3$
Monoisotopic protonated ion (*m/z*): 227.1390
Molecular weight: 226.28
CAS number: 76-74-4

76: Phenobarbital [WHO List of Essential Medicines]

Chemical name: 5-Ethyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Brand name: Luminal
Formula: $C_{12}H_{12}N_2O_3$
Monoisotopic protonated ion (*m/z*): 233.0921
Molecular weight: 232.24
CAS number: 50-06-6

77: Secobarbital

Chemical name: (*R*,*S*)-5-(1-Methylbutyl)-5-(2-propen-1-yl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Brand name: Seconal



Scheme 74–78 Hydrolytic degradation of barbiturates under different pHs.

Formula: $C_{12}H_{18}N_2O_3$
Monoisotopic protonated ion (*m/z*): 239.1390
Molecular weight: 238.28
CAS number: 76-73-3

78: Talbutal

Chemical name: (*R,S*)-5-Allyl-5-sec-butyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Brand name: Lotusate
Formula: $C_{11}H_{16}N_2O_3$
Monoisotopic protonated ion (*m/z*): 225.1234
Molecular weight: 224.26
CAS number: 115-44-6

Barbiturates are derivatives of barbituric acid with different substituents at the 4-position of the barbituric acid ring. Barbiturates have a long history of medical use as sedatives, hypnotics, and antiepileptic, which dates back to as early as the 1910s [1]. The molecular weights of bartiturates for medical use are relatively small. Their modes of action had not been known until quite recently: they act as positive modulators of $GABA_A$ receptors, and at high concentrations, they become agonists of the receptors [2]. Among the $GABA_A$ receptor-binding sites, barbiturates bind at sites different from those by benzodiazepines, and their use in routine medical practice has largely been replaced by benzodiazepines and other drugs with less side effects or less tendency for dependency. Nevertheless, barbiturates are still used in general anesthesia, epilepsy, and other cases. In pharmaceutical dosage forms, both the free acid form and salt form (such as sodium, calcium, or other salts) are employed. Among the barbiturates, butalbital is usually used in conjunction with other therapeutic agents such as acetaminophen, aspirin, caffeine, and codeine in binary or tertiary combination for the treatment of pain and headache.

The main degradation pathway of the barbiturates is the hydrolysis of the barbituric acid ring, followed by decarboxylation and further hydrolysis (Scheme 74–78) [3–5]. According to a study by Maulding et al., the hydrolytic cleavage site depends on the pH of the media [3]. At neutral pH and a few pH units above, the 1,6-(or 3,4-) cleavage is the predominant pathway. At pH greater than 9.5, the 1,2-(or 2,3-) cleavage plays a role in the overall degradation of the drug. The activation energy for the hydrolytic degradation of phenobarbital was reported to be ~19 kcal/mol [6, 7].

References

1 Barbiturate, Wikipedia. https://en.wikipedia.org/wiki/Barbiturate (accessed 2 May 2022).

2 Löscher, W. and Rogawski, M.A. (2012). How theories evolved concerning the mechanism of action of barbiturates. *Epilepsia* 53: 12–25.

3 Maulding, H.V., Nazareno, J., Polesuk, J., and Michaelis, A. (1972). Investigations of hydrolytic products of butalbital. *J. Pharm. Sci.* 61: 1389–1393.

4 Scott, E.P. (1983). Application of postcolumn ionization in the high-performance liquid chromatographic analysis of butabarbital sodium elixir. *J. Pharm. Sci.* 72: 1089–1091.

5 Gannett, P.M., Daft, J.R., James, D. et al. (2001). In vitro reaction of barbiturates with formaldehyde. *J. Anal. Toxicol.* 25: 443–449.

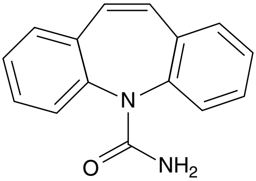
6 Garrett, E.R., Bojarski, J.T., and Yakatan, G.J. (1971). Kinetics of hydrolysis of barbituric acid derivatives. *J. Pharm. Sci.* 60: 1145–1154.

7 Tarsa, M., Zuchowski, G., Stasiewicz-Urban, A., and Bojarski, J. (2009). Hydrolysis of 2,4-dithiophenobarbital. *Acta Pol. Pharm.-Drug Res.* 66: 123–128.

4

Carbamazepine to Decitabine

79: Carbamazepine [WHO List of Essential Medicines]



Chemical name: Benzo[b][1]benzazepine-11-carboxamide
Brand name: Tegretol
Formula: $C_{15}H_{12}N_2O$
Monoisotopic protonated ion (*m/z*): 237.1022
Molecular weight: 236.27
CAS number: 298-46-4

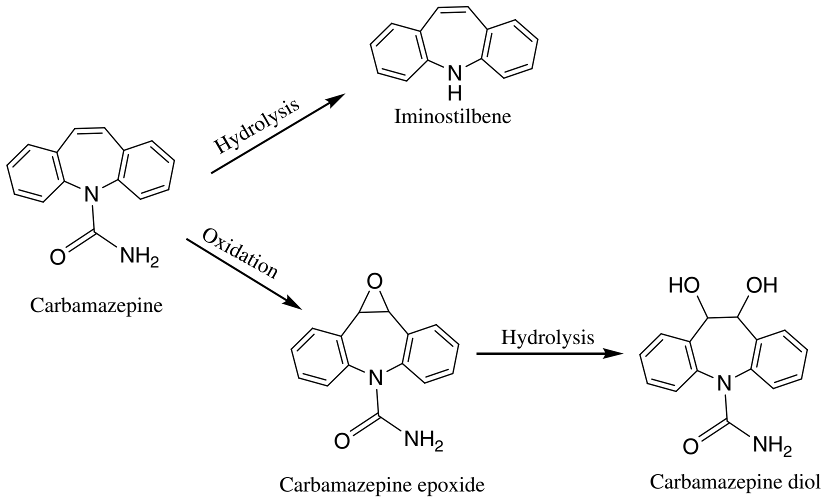
Carbamazepine is one of the most prescribed CNS drugs, which is most commonly used for the treatment of epilepsy. Due to its widespread use, there have been a large number of papers describing its disposal or decomposition in sewage [1–3]. On the other hand, few studies in the literature have reported its degradation under pharmaceutically relevant conditions. Hence, an effort is made here to predict its degradation under pharmaceutically relevant conditions, considering the results reported from the papers regarding its disposal.

Based on the structure of carbamazepine, this drug molecule would appear to be susceptible to oxidative, hydrolytic, and photochemical degradation, due to its carbon–carbon double bond, carbamate, and tricyclic moieties, respectively. First, the oxidation of the carbon–carbon double bond could produce carbamazepine 10,11-epoxide as the initial degradant, which is also a metabolite of carbamazepine [4]. The analogous epoxide was reported in the case of cyclobenzaprine, which has a similar carbon-carbon double bond that is also embedded in a tricyclic conjugated ring system (refer to the Monograph of Cyclobenzaprine and Cyproheptadine of this book). The initially formed epoxide would decompose to produce the

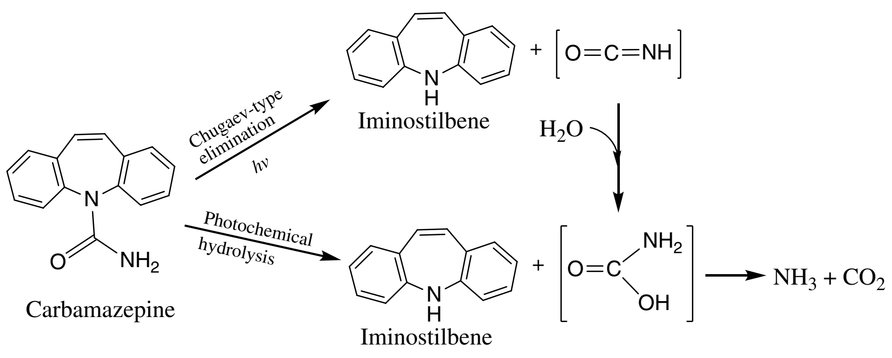
Compendium of Drug Degradation Pathways, First Edition. Min Li.

di-hydroxyl degradant, carbamazepine-diol, upon attack by water. Second, the carbamate moiety is usually stable enough to resist significant hydrolytic degradation under pharmaceutically relevant conditions. Nevertheless, under harsh conditions such as refluxing in 1N HCl or 1N NaOH, the carbamate can be hydrolyzed to yield iminostilbene [5], which was reported to be a metabolite of carbamazepine [6]. The above degradation pathways are illustrated in Scheme 79-1.

Lastly, the tricyclic ring would be likely susceptible to photochemical degradation, including photosensitized oxidation [7]. Indeed, under UV irradiation at 254 nm, iminostilbene was also formed and its formation was proposed via either a Chugaev-type elimination or a photochemical hydrolysis. The mechanisms presented in Scheme 79-2 were based on those proposed by Ali et al. [1], with modification.



Scheme 79-1 Degradation pathways of carbamazepine.



Scheme 79-2 Proposed mechanisms for photochemical degradation of carbamazepine.

References

1 Ali, F., Khan, J.A., Shah, N.S. et al. (2018). Carbamazepine degradation by UV and UV-assisted AOPs: kinetics, mechanism and toxicity investigations. *Proc. Safety Environ. Protec.* 117: 307–314.

2 Dai, C.-M., Zhou, X.-F., Zhang, Y.-L. et al. (2012). Comparative study of the degradation of carbamazepine in water by advanced oxidation processes. *Environ. Technol.* 33: 1101–1109.

3 Bu, L., Zhou, S., Zhu, S. et al. (2018). Insight into carbamazepine degradation by UV/monochloramine: reaction mechanism, oxidation products, and DBPs formation. *Water Res.* 146: 288–297.

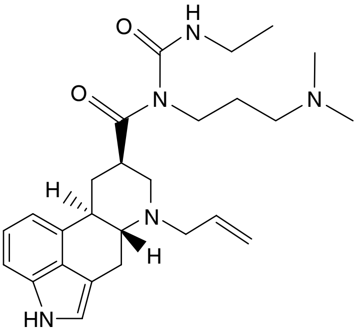
4 Bertilsson, L. (1978). Clinical pharmacokinetics of carbamazepine. *Clin. Pharmacokinet.* 3: 128–143.

5 Naguib, I.A., Elyazeed, N.A., Elroby, F.A., and El-Ghobashy, M.R. (2019). Stability indicating spectrophotometric methods for quantitative determination of carbamazepine and its degradation product, iminostilbene, in pure form and pharmaceutical formulations. *Spectrochim. Acta Part A: Mole. Biomol. Spect.* 214: 21–31.

6 Baselt, R.C. (2004). *Disposition of Toxic Drugs and Chemicals in Man*, 7e, 164–167. Foster City, CA: Biomedical.

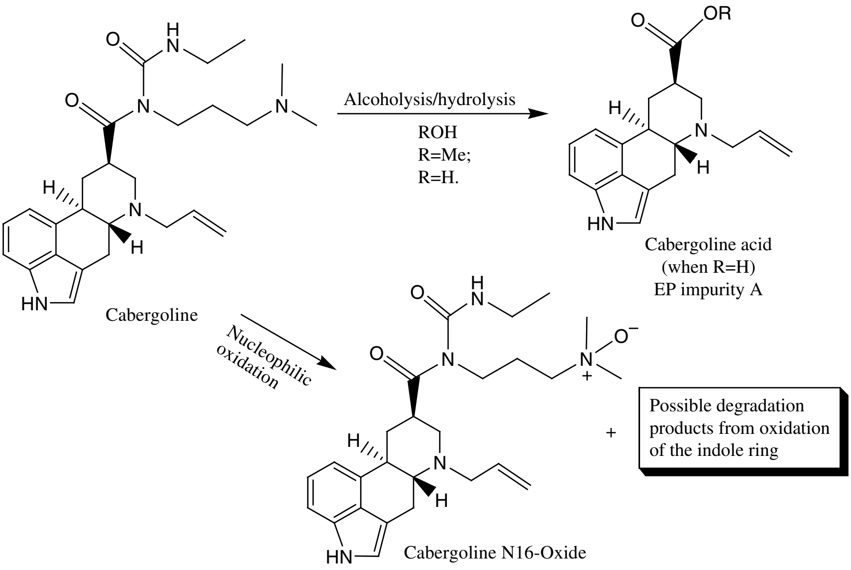
7 Li, M. (2012). Photochemical degradation. In: *Organic Chemistry of Drug Degradation*, 165–297. Cambridge: Royal Society Chemistry Publishing.

80: Cabergoline



Chemical name: (6*aR*,9*R*,10*aR*)-*N*-[3-(Dimethylamino)propyl]-*N*-(ethylcarbamoyl)-7-prop-2-enyl-6,6*a*,8,9,10,10*a*-hexahydro-4*H*-indolo[4,3-fg]quinoline-9-carboxamide
Brand name: Cabaser, Dostinex
Formula: $C_{26}H_{37}N_5O_2$
Monoisotopic protonated ion (*m/z*): 452.3020
Molecular weight: 451.61
CAS number: 81409-90-7

Cabergoline is a natural product-based therapeutic agent. As a dopamine agonist, it is utilized clinically for the treatment of hyperprolactinemic disorders and Parkinson's disease. The functional groups susceptible to degradation include the



Scheme 80 Degradation pathways of cabergoline.

acylated urea functionality, two tertiary amine groups, and the indole ring. There appear to be very few reports on the degradation chemistry of cabergoline. In a study by Paul et al. [1], cabergoline underwent alcoholysis upon extended storage in methanol solution at room temperature, resulting in the substitution of the urea moiety by methanol (Scheme 80). This observation suggests that cabergoline should also be susceptible to hydrolysis, giving rise to cabergoline acid, which is EP impurity A [2, 3] and also a major metabolite of the drug [4]. The two tertiary amine moieties and the indole ring should be prone to oxidation; indeed, "cabergoline *N*-oxide", the *N*-oxide with oxygen on the dimethylamino side chain, is included in the PubChem database [5] and currently available as an impurity reference material per information on many websites [6]. Recently, Lechel et al. found the second *N*-oxide degradant of cabergoline, i.e., the one with oxygen on the *N*-allyl nitrogen, in an injectable formulation product of cabergoline [7]. Önal et al. performed stress studies under hydrolytic, oxidative, thermal, and photochemical conditions [8]. The results showed that the drug was less stable in 0.1 M HCl than in 0.1 M NaOH solution. However, no elucidation of degradants was done.

References

1 Paul, G., Winnik, W., Hughes, N. et al. (2003). Accurate mass measurement at enhanced mass-resolution on a triple quadrupole mass-spectrometer for the identification of a reaction impurity and collisionally-induced fragment ions of cabergoline. *Rapid Commun. Mass Spectrom.* 17: 561–568.

2 (2012). *European Pharmacopoeia, Monograph C*, 7e (7.5), 1535. Strasbourg, France: Council of Europe.

3 Wagger, J., Pozesb, A., and Pozgan, F. (2013). Synthesis of European pharmacopoeial impurities A, B, C, and D of cabergoline. *RSC Adv.* 3: 23146–23156.

4 Wiseman, L.R. and Fitton, A. (1999). Cabergoline, a review of its efficacy in the treatment of Parkinson's disease. *CNS Drugs* 12: 485–497.

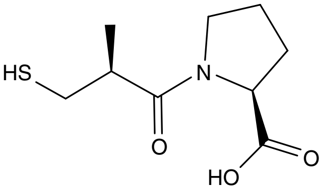
5 Cabergoline N-Oxide. PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/71314472. (accessed 4 July 2025).

6 Cabergoline N-Oxide. Chemical Book. https://www.chemicalbook.com/ChemicalProductProperty_EN_CB12558018.htm. (accessed 4 July 2025).

7 Lechel, T., Otero, R.S., Springer, A. et al. (2024). Identification and structural elucidation of an oxidation product generated during stability studies of cabergoline drug product. *J. Pharm. Biomed. Anal.* 248: 116282.

8 Önal, A., Sağırlı, O., and Sensoy, D. (2007). Selective LC determination of cabergoline in the bulk drug and in tablets: In vitro dissolution studies. *Chromatographia* 65: 561–567.

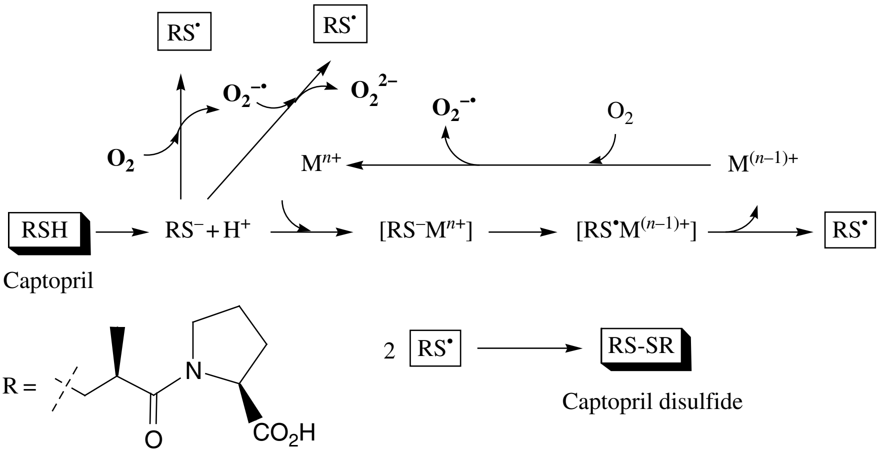
81: Captopril



Chemical name: 1-[2(*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline; (2*S*)-1-[(2*S*)-2-Methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid
Brand name: Capoten
Formula: $C_9H_{15}NO_3S$
Monoisotopic protonated ion (*m/z*): 218.0845
Molecular weight: 217.29
CAS number: 62571-86-2

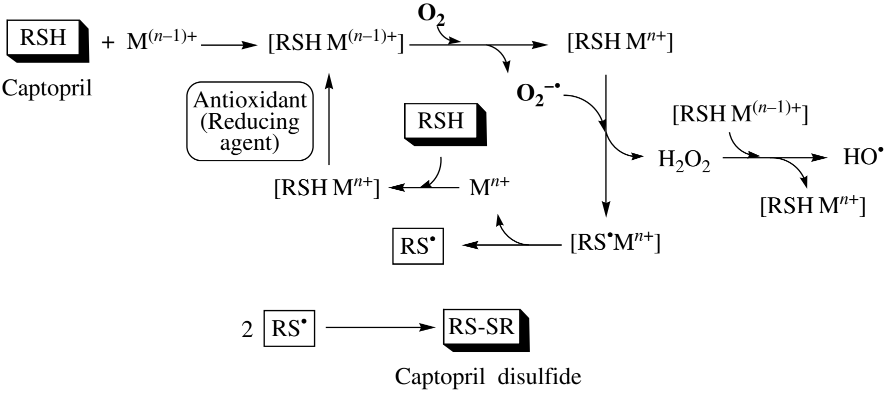
Captopril is the first angiotensin-converting enzyme (ACE) inhibitor developed by Bristol-Myers Squibb for the treatment of hypertension. It is an acylated L-proline with the acylated side chain containing a thiol (sulfhydryl) group. Since the thiol group is very susceptible to auto-oxidation, particularly in aqueous solutions, the predominant degradation pathway of captopril is the oxidation of its thiol group to form captopril disulfide. The oxidation is generally believed to proceed through the ionized thiol group, from which an electron is removed by either a redox transition metal ion (most likely iron(III) ion in vast majority cases) or molecular oxygen directly. According to a study by Lee and Notari using cupric ion as the model redox transition metal ion [1], both pathways should be operative during the auto-oxidation of captopril in the pH range of 6.6–8.0, because the degradation kinetics cannot be well explained by either of the pathways alone. The auto-oxidative degradation mechanism is shown in Scheme 81-1, which is mostly based on the work by Lee and Notari [1] with some modification.

According to the oxidative degradation mechanism shown in Scheme 81-1, control of oxygen content and redox transition metal ions in conjunction with low pH should promote the stability of formulated captopril products. From the formulation perspective, control of residual metal ions (from the API, excipients and/or packaging materials) is usually achieved by the addition of metal chelators such as EDTA. Since the pKa of captopril thiol group is around 9.8 [2], liquid formulations with pH well below the pKa value should also stabilize the formulated drug products. Indeed, many formulation studies demonstrated that the shelf lives were extended significantly by the addition of EDTA as well as by keeping the pH of the formulation in the acidic range [2–4]. The studies by Timmins et al. [2] and Huang et al. [3] showed that the rates of oxidative degradation increased with ascending pH, particularly at pH above 4, from which point the rates of oxidation dramatically accelerated. On the other hand, addition of antioxidants either had no effect (e.g., ascorbic acid and sodium bisulfite) [3] or unexpectedly promoted oxidative degradation of captopril (e.g., propyl gallate and sodium



Scheme 81-1 Proposed mechanism for the auto-oxidation of captopril to form captopril disulfide. The mechanism was based on the work of Lee and Notari with some modification; the oxidation study was performed in the pH range of 6.6–8.0.

metabisulfite) [2]. The latter results were attributed to the residual metal ions in the two antioxidative excipients by the original researchers. In light of the elucidation of the Udenfriend chemistry and its role in the auto-oxidation of drugs [5], both seemingly paradoxical results may be explained by the Udenfriend chemistry. As illustrated in Scheme 81-2, the role of propyl gallate and sodium metabisulfite would likely be to reduce [RSH M^{n+}] to [RSH $M^{(n-1)+}$] so that the latter can continue the cycle during which molecular oxygen is activated (or reduced) to form superoxide anion radical ($O_2^{-•}$). The latter could be further converted to hydroxyl free radical via the intermediacy of hydrogen peroxide; the hydroxyl free radical would be more damaging toward captopril than superoxide anion radical.



Scheme 81-2 Proposed mechanism for antioxidant-promoted oxidation of captopril based on the Udenfriend chemistry [5]. The original work was performed by Timmins et al. [2] and the study was conducted in aqueous solutions with pH of 4. *Source:* Timmins et al. [2] and Li [5].

As an acylated amino acid, the amide linkage of captopril is technically susceptible to hydrolytic degradation. The activation energy for the hydrolysis was found to be 21.4 kcal/mol when the hydrolysis was performed in 0.5 N HCl [2]. This result suggests that hydrolysis would not be a meaningful degradation pathway for captopril unless under harsh forced degradation conditions. Various formulation studies found captopril disulfide as either the sole or predominant degradant [1–4].

References

1 Lee, T.-Y. and Notari, R.E. (1987). Kinetics and mechanism of captopril oxidation in aqueous solution under controlled oxygen partial pressure. *Pharm. Res.* 4: 98–103.

2 Timmins, P., Jackson, I.M., and Wang, Y.J. (1982). Factors affecting captopril stability in aqueous solution. *Int. J. Pharm.* 11: 329–336.

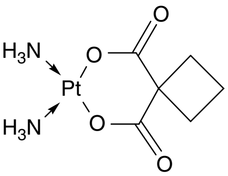
3 Huang, Y.-B., Tsai, Y.-H., Chang, J.-S. et al. (2002). Effect of antioxidants and anti-irritants on the stability, skin irritation and penetration capacity of captopril gel. *Int. J. Pharm.* 241: 345–351.

4 Berger-Gryllaki, M., Podilsky, G., Widmer, N. et al. (2007). The development of a stable oral solution of captopril for paediatric patients. *EJHP Sci.* 13: 27–32.

5 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*, 49–53. Cambridge: Royal Society Chemistry Publishing.

82–83: Carboplatin, Cisplatin

82: Carboplatin



Chemical name: cis-Diammine(cyclobutane-1,1-dicarboxylate-O,O′)platinum (II); [1,1-Cyclobutanedicarboxylato(2-)-κ^2O^1,O^1]platinum diammoniate

Brand name: Paraplatin

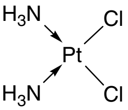
Formula: $C_6H_{12}N_2O_4Pt$

Monoisotopic protonated ion (*m/z*): 372.0518 (for the most abundant ^{195}Pt isotope)

Molecular weight: 371.25

CAS number: 41575-94-4

83: Cisplatin



Chemical name: cis-Diamminedichloroplatinum (II)

Brand name: Transplatin

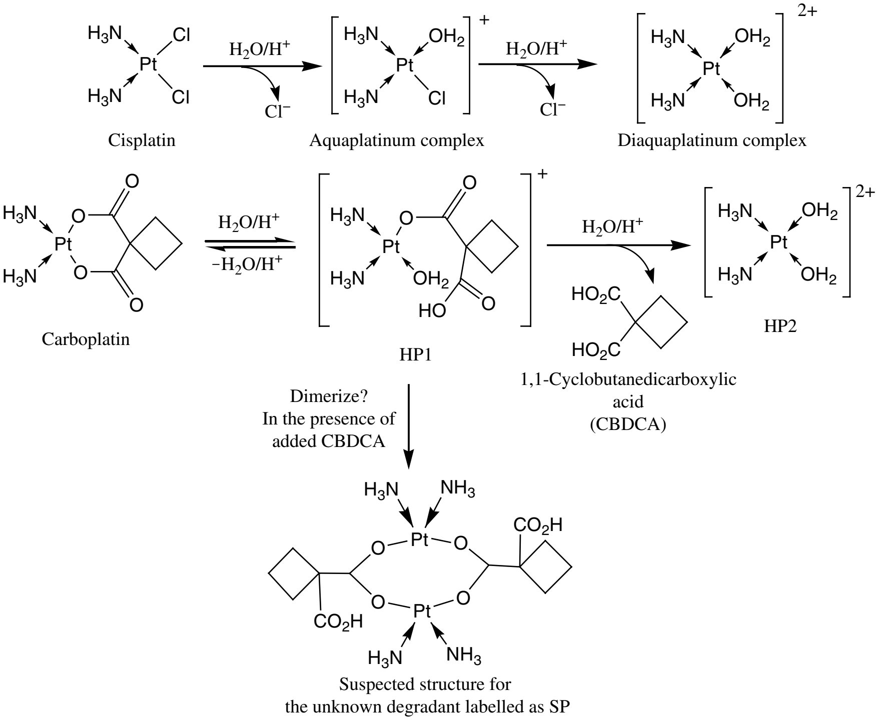
Formula: $Cl_2H_6N_2Pt$

Monoisotopic protonated ion (*m/z*): 299.9629 (for the most abundant ^{195}Pt isotope)

Molecular weight: 300.04

CAS number: 14913-33-8

The degradation pathways of both carboplatin and cisplatin are mostly of hydrolytic nature. Carboplatin is a second generation of this widely used chemotherapeutic family, which was developed to reduce the side effects of cisplatin, the original platinum-containing anticancer drug. Carboplatin is about 100 times more stable in aqueous solutions at 37°C than cisplatin [1] and hence, it can be formulated in liquid dosage forms. The hydrolytic degradation of carboplatin was reported to be catalyzed by acid [2, 3]. However, it was revealed in a patent that the real degradation behavior of carboplatin in acidic pH range may be more complicated: across the pH range between 2.5 and 7.5 achieved by phosphate buffers, carboplatin was found to be more stable in the pH range of 3.5–4.5 than in pH 6.5–7.5 at a concentration of 10 mM [4]. In addition, at pH 3.5 the stability of carboplatin versus its concentration



Scheme 82–83 Plausible mechanisms for the hydrolytic degradation of cisplatin and carboplatin, respectively. The nomenclature for the degradation products of carboplatin follows that of Schnurr et al. [1]. *Source:* Schnurr et al. [1]/Springer Nature.

was studied. It was found that carboplatin became more unstable as its concentration increased. Therefore, the actual degradation mechanism of carboplatin may very well be more complex than what is displayed in Scheme 82–83. In a study by Jin et al., light and UV irradiation were found to accelerate the degradation of carboplatin [5]. Furthermore, in the presence of NaCl, the degradation rate increased, and the main degradant was cisplatin. The latter observation was consistent with that reported by Cheng et al. [6].

References

1 Schnurr, B., Heinrich, H., and Gust, R. (2002). Investigations on the decomposition of carboplatin in infusion solutions II. Effect of 1,1-cyclobutanedicarboxylic acid admixture. *Microchim. Acta* 140: 141–148.

2 Canovese, L., Cattalini, L., Chessa, G., and Tobe, M.L. (1988). Kinetics of the displacement of cyclobutane-1,1-dicarboxylate from diammine(cyclobutane-1,1-dicarboxylato) -platinum(II) in aqueous solution. *J. Chem. Soc. Dalton Trans.* 8: 2135–2140.

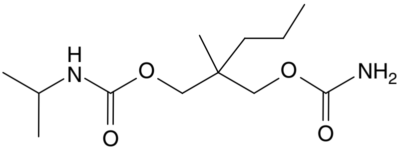
3 Allsopp, M.A., Sewell, G.J., Rowland, C.G. et al. (1991). The degradation of carboplatin in aqueous solutions containing chloride or other selected nucleophiles. *Int. J. Pharm.* 69: 197–210.

4 Nijker, A.J., Vermeer, J.M.P. Carboplatin composition. European patent application 0401896A1. Filed 28 May 1990. Granted 11 August 1993.

5 Jin, Y.-R., Huang, T.-M., Yang, Y.-K. et al. (1994). Stability study of carboplatin by HPLC (original in Chinese). *Chinese J. Pharm.* 25: 69–72.

6 Cheng, Y., Cheung, Y.-W., Cradock, J.C. et al. (1987). Stability of cisplatin, iproplatin, carboplatin, and tetraplatin in commonly used intravenous solutions. *Am. J. Hosp. Pharm.* 44: 124–130.

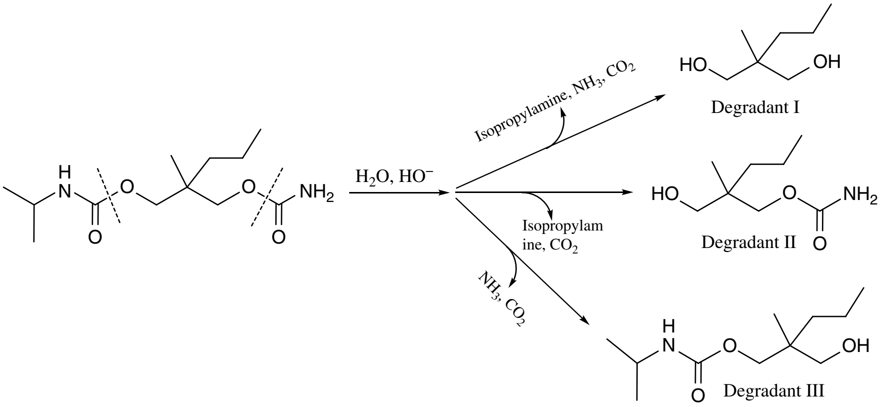
84: Carisoprodol



Chemical name: [2-(carbamoyloxymethyl)-2-methylpentyl] *N*-propan-2-ylcarbamate
Brand name: Soma, Vanadom
Formula: $C_{12}H_{24}N_2O_4$
Monoisotopic protonated ion (*m/z*): 261.1809
Molecular weight: 260.33
CAS number: 78-44-4

Carisoprodol and meprobamate are muscle relaxants, and the latter is a metabolite of carisoprodol, upon which carisoprodol was developed according to Wikipedia [1].

There appear to be very few reported studies for the degradation study of both drugs. The only functional groups that are potentially susceptible to degradation are the two carbamates present in both drugs. For carisoprodol, the unsubstituted carbamate moiety would be somewhat less stable than the substituted one because it is less sterically hindered than the latter. Since the activation energy for the hydrolysis of carbamates is typically greater than 20 kcal/mol [2], both drugs are expected to be reasonably stable, particularly in solid dosage forms. In what appear to be the only two reported forced degradation studies of carisoprodol, this drug molecule was found to be quite stable under very harsh acidic conditions, e.g., refluxing in 3N or 5N HCl solutions [3, 4]. Nevertheless, the results in alkaline stress conditions reported by the two research groups were quite different; while Murali and Rambabu reported that carisoprodol was stable when refluxed in 5 N NaOH solution at 80°C for 8 hours [3], the results of Acharya et al. indicated that when refluxed in 0.1 N NaOH for 90 minutes, sufficient degradation of carisoprodol was produced. Among the degradation products whose structures were proposed by Acharya et al. were those resulting from hydrolytic cleavage of the two carbamate bonds (Scheme 84); it needs to point out that the structure for one of the degradants (referred to as Degradant II) appears to be erroneous in the original paper [4].



Scheme 84 Hydrolytic degradation of carisoprodol under alkaline conditions. Please note that the structure of Degradant II was erroneous in the original paper by Acharya et al. [4]. *Source:* Acharya et al. [4]/with permission of Elsevier.

References

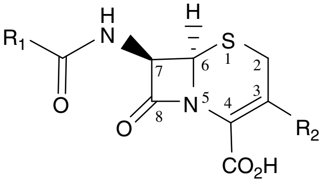
1 Carisoprodol. https://en.wikipedia.org/wiki/Carisoprodol (accessed 29 November 2015).

2 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 16–47. Cambridge: Royal Society Chemistry Publishing.

3 Murali, D. and Rambabu, C. (2016). Stability indicating high performance liquid chromatographic method for the estimation of carisoprodol in bulk and in tablet dosage form. *Int. J. PharmTech Res.* 9: 171–180.

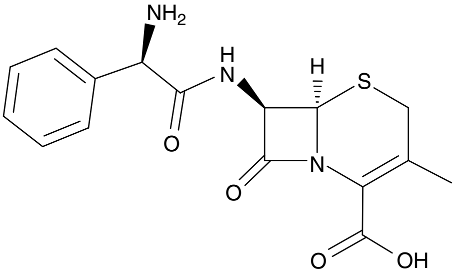
4 Acharya, P.C., Vasi, R., and Suares, D. (2016). FTIR assay method for UV inactive drug carisoprodol and identification of degradants by RP-HPLC and ESI-MS. *J. Chromatogr. B* 1029–1030: 16–21.

85–93: Cephalosporin Antibiotics



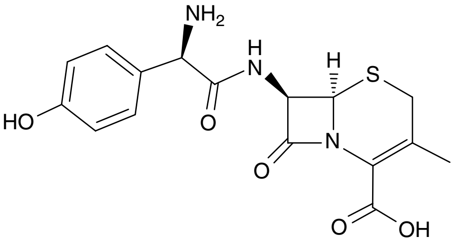
Cephalosporins

85: Cefalexin [Cephalexin]



Chemical name: (6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Brand name: Keflex
Formula: $C_{16}H_{17}N_3O_4S$
Monoisotopic protonated ion (*m/z*): 348.1013
Molecular weight: 347.39
CAS number: 15686-71-2

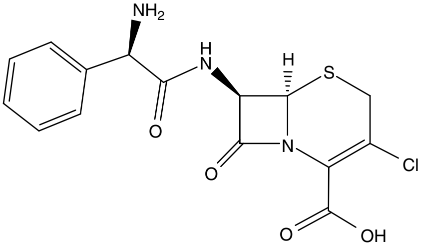
86: Cefadroxil [Cephadroxil]



Chemical name: (6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Brand name: Baxan, Bidocef, Cefamox
Formula: $C_{16}H_{17}N_3O_5S$

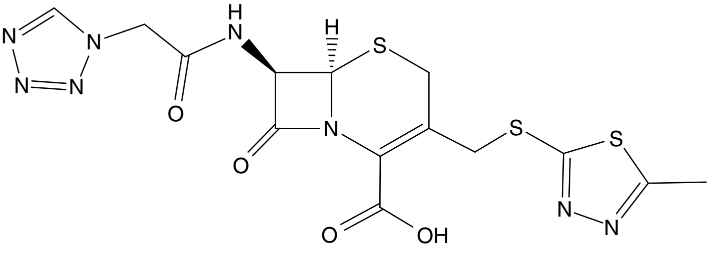
Monoisotopic protonated ion (*m/z*): 364.0962
Molecular weight: 363.39
CAS number: 50370-12-2

87: Cefaclor



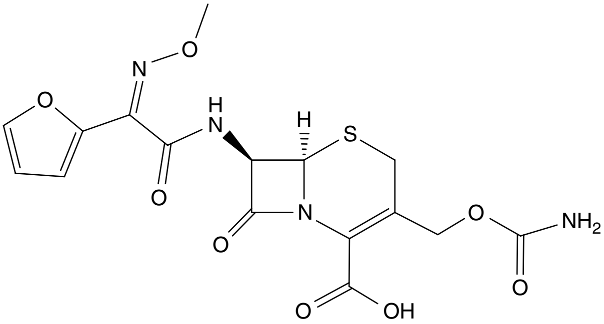
Chemical name: (6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Brand name: Ceclor
Formula: $C_{15}H_{14}ClN_3O_4S$
Monoisotopic protonated ion (*m/z*): 368.0466
Molecular weight: 367.81
CAS number: 53994-73-3

88: Cephazolin [Cefazolin]



Chemical name: (6*R*,7*R*)-3-[(5-Methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Brand name: Elzogram, Zolicef
Formula: $C_{14}H_{14}N_8O_4S_3$
Monoisotopic protonated ion (*m/z*): 455.0373
Molecular weight: 454.51
CAS number: 25953-19-9

89: Cefuroxime



Chemical name: (6*R*,7*R*)-3-(Carbamoyloxymethyl)-7-[[(2*Z*)-2-(furan-2-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Brand name: Ceftin

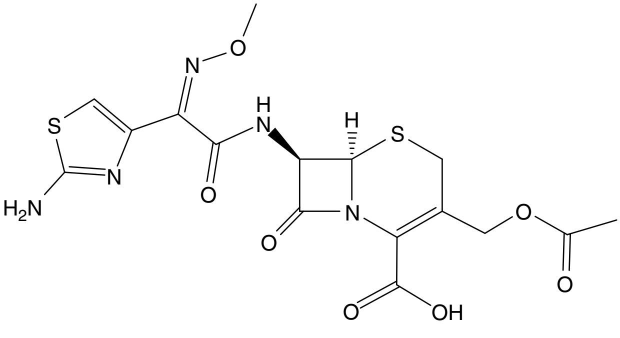
Formula: $C_{16}H_{16}N_4O_8S$

Monoisotopic protonated ion (*m/z*): 425.0762

Molecular weight: 424.39

CAS number: 55268-75-2

90: Cefotaxime



Chemical name: (6*R*,7*R*)-3-(Acetyloxymethyl)-7-[[(2*Z*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Brand name: Claforan

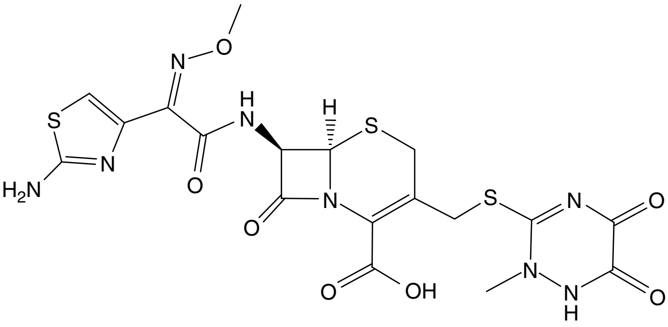
Formula: $C_{16}H_{17}N_5O_7S_2$

Monoisotopic protonated ion (*m/z*): 456.0642

Molecular weight: 455.47

CAS number: 63527-52-6

91: Ceftriaxone



Chemical name: (6*R*,7*R*)-7-[[(2*Z*)-2-(2-Amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(2-methyl-5,6-dioxo-1*H*-1,2,4-triazin-3-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Brand name: Rocephin

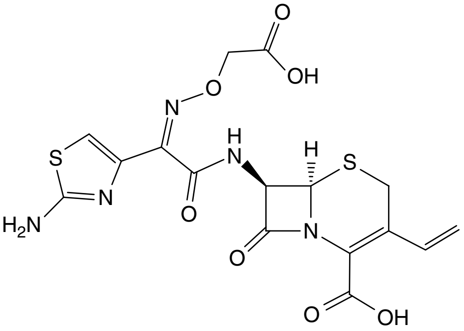
Formula: $C_{18}H_{18}N_8O_7S_3$

Monoisotopic protonated ion (*m/z*): 555.0533

Molecular weight: 554.58

CAS number: 73384-59-5

92: Cefixime



Chemical name: (6*R*,7*R*)-7-[[(2*Z*)-2-(2-Amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Brand name: Suprax

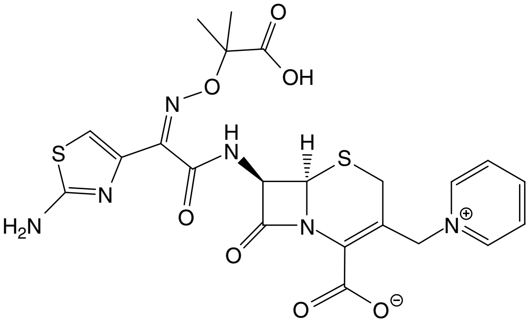
Formula: $C_{16}H_{15}N_5O_7S_2$

Monoisotopic protonated ion (*m/z*): 454.0486

Molecular weight: 453.45

CAS number: 79350-37-1

93: Ceftazidime



Chemical name: (6*R*,7*R*)-7-[[(2*Z*)-2-(2-Amino-1,3-thiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino)acetyl]amino]-8-oxo-3-(pyridin-1-ium-1-ylmethyl)-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate

Brand name: Fortaz, Tazicef

Formula: $C_{22}H_{22}N_6O_7S_2$

Monoisotopic protonated ion (*m/z*): 547.1064

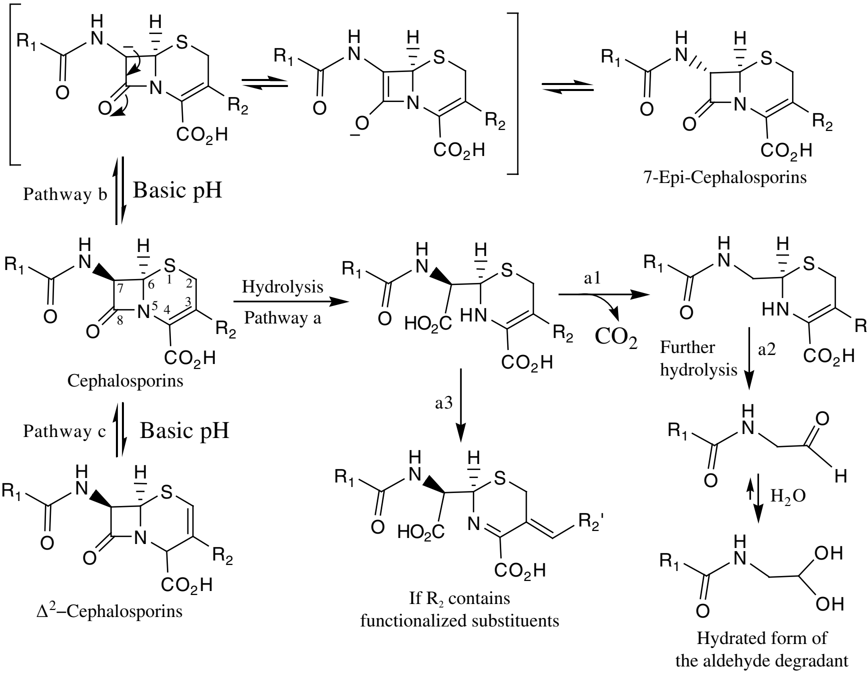
Molecular weight: 546.58

CAS number: 72558-82-8

Cephalosporins are one of the most important classes of antibacterial drugs. A great number of them [1] were brought to market in different generations. Discussed in this monograph are the representative ones, including those in the first-generation (cefalexin, cefadroxil, cefaclor, and cefazolin), second-generation (cefuroxime), and third-generation cephalosporin families (cefotaxime, ceftriaxone, cefixime, and ceftazidime).

Cephalosporins contain an eight-membered nucleus consisting of a four-membered β-lactam ring fused to a dihydrothiazine ring. The main degradation pathway of cephalosporins is the hydrolysis of the β-lactam ring, giving rise to the open ring form (Pathway a, Scheme 85–93-1) [1]. The susceptibility of the β-lactam to hydrolysis depends upon the nature of the substituents on the core, pH, and temperature. The pH profiles of cephalosporins are typically of a U-shape, with their most stable pH range usually in the acidic range [2]. For example, cefaclor was shown to be most stable in the pH range of 2–4 [3], cefepime pH 3–6 [4], cefixime pH 4–7 [5], cefotaxime pH 3–7 [6], and cefuroxime pH 4–7 [7]. The open ring form can undergo decarboxylation (Pathway a1, Scheme 85–93-1) [5, 8]. Upon further hydrolysis of the dihydrothiazine ring, the decarboxylated degradant gives rise to the aldehyde degradant that is usually present in its hydrated form (Pathway a2, Scheme 85–93-1), which is not stable and tends to polymerize [5].

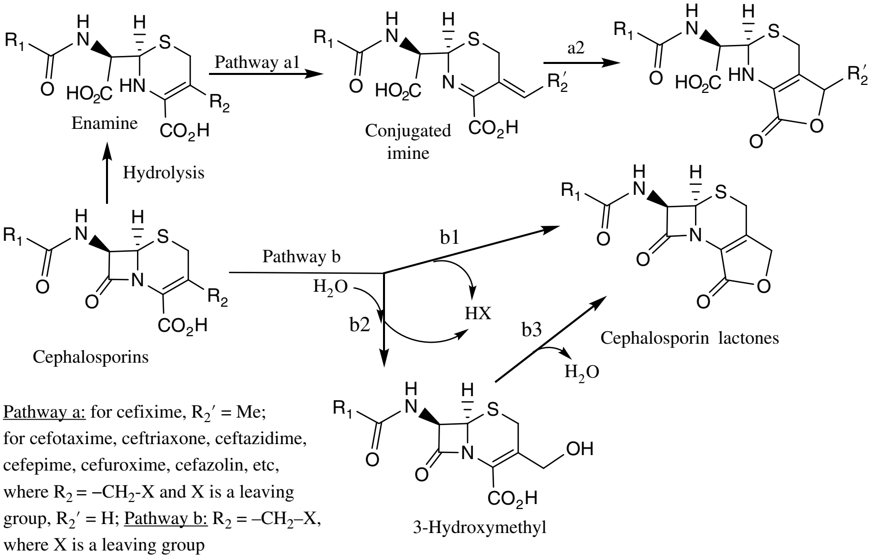
Under alkaline conditions, cephalosporins can undergo two additional decomposition pathways: epimerization at the 7-position to produce 7-epi-cephalosporins (Pathway b, Scheme 85–93-1) and rearrangement to give Δ^2-cephalosporins (Pathway c, Scheme 85–93-1) [5].



Scheme 85–93-1 Degradation pathways of the cephalosporin core.

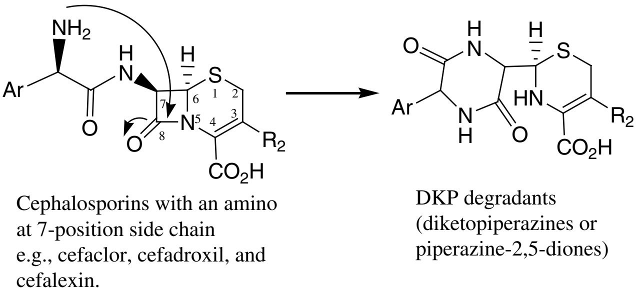
On the other hand, if the R_2 group (in Schemes 85–93-1 and 85–93-2) is an alkyl group that is functionalized at the position α to the dihydrothiazine ring (e.g., a vinyl in cefixime and acetoxylmethyl in cefotaxime), the enamine form of the open ring degradant can rearrange to the conjugated imine form with concurrent formation of a simple or substituted methylene group at the 3-position (Pathway a1, Scheme 85–93-2). For cefixime, R_2' (in Scheme 85–93-2) is a methyl group, while in many others, such as cefotaxime, ceftriaxone, ceftazidime, cefepime, cefuroxime, and cefazolin, it is a hydrogen [7, 8]. The conjugated imines thus formed are electrophilic species and are susceptible to nucleophilic attack by the carboxyl group to yield the lactone degradants (Pathway a2, Scheme 85–93-2), particularly under acidic conditions [5, 8]. Many cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefepime, cefuroxime, and cefazolin) can also directly degrade to the corresponding lactones (Pathway b1, Scheme 85–93-2) or via the intermediacy of 3-hydroxymethyl degradants (Pathway b2 and b3, Scheme 85–93-2) [9].

For those cephalosporins containing an amino group on the 7-position side chain, such as cefalexin, cefadroxil, and cefaclor, this amino group can attack the β-lactam, a process referred to as intramolecular aminolysis, to form diketopiperazine (DKP) degradants (Scheme 85–93-3). The DKP degradants, now containing a dihydrothiazine ring that is no longer fused to the lactam ring, are capable of further degradation, such as rearrangement and lactonization, as illustrated in Scheme 85–93-2.

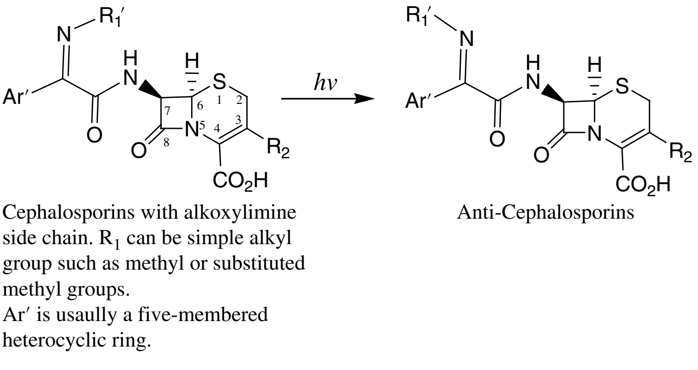


Pathway a: for cefixime, $R_2' = Me$; for cefotaxime, ceftriaxone, ceftazidime, cefepime, cefuroxime, cefazolin, etc, where $R_2 = -CH_2\text{-}X$ and X is a leaving group, $R_2' = H$; Pathway b: $R_2 = -CH_2-X$, where X is a leaving group

Scheme 85-93-2 Rearrangement to the enamine/imine forms and lactone formation.



Scheme 85-93-3 Formation of the diketopiperazine (DKP) degradants.



Cephalosporins with alkoxylimine side chain. R_1 can be simple alkyl group such as methyl or substituted methyl groups.
Ar′ is usaully a five-membered heterocyclic ring.

Scheme 85-93-4 Photoisomerization of cephalosporins with an alkoxylimine side chain.

Many newer generations of cephalosporins, such as cefotaxime, ceftriaxone, cefixime, and ceftazidime, contain an alkoxylimine group on the 7-position side chain; the alkoxylimine double bond is susceptible to photochemical isomerization to yield the corresponding anti-cephalosporins (Scheme 85–93-4) [10].

References

1 Alekseev, V.G. (2010). Drug synthesis methods and manufacturing technology–acid-base properties of penicillins and cephalosporings (a review). *Pharm. Chem. J.* 44: 14–24.

2 Llinas, A., Vilanova, B., Frau, J. et al. (1998). Chemical reactivity of penicillins and cephalosporins. Intramolecular involvement of the acyl-amido side chain. *J. Organomet. Chem.* 63: 9052–9060.

3 Dimitrovska, A., Stojanoski, K., and Dorevski, K. (1995). Kinetics of degradation of cefaclor: I. Effects of temperature, phosphate buffer, pH, and copper (II) ion. *Int. J. Pharm.* 115: 175–182.

4 Fubara, J.O. and Notari, R.E. (1998). Influence of pH, temperature and buffers on cefepime degradation kinetics and stability predictions in aqueous solutions. *J. Pharm. Sci.* 87: 1572–1576.

5 Namiki, Y., Tanabe, T., Kobayashi, T. et al. (1987). Degradation kinetics and mechanism of a new cephalosporin, cefixime, in aqueous solution. *J. Pharm. Sci.* 76: 208–214.

6 Fabre, H., Eddine, N.H., and Berge, G. (1984). Degradation kinetics in aqueous solution of cefotaxime sodium, a third-generation cephalosporin. *J. Pharm. Sci.* 73: 611–618.

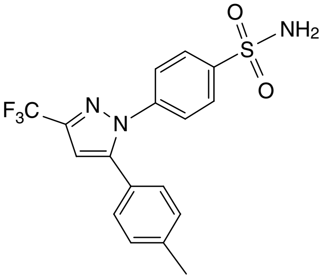
7 Wang, D. and Notari, R.E. (1994). Cefuroxime hydrolysis kinetics and stability predictions in aqueous solution. *J. Pharm. Sci.* 83: 577–581.

8 Sivakumar, B., Parthasarathy, K., Murugan, R. et al. (2013). Isolation and characterisation of degradation impurities in the cefazolin sodium drug substance. *Sci. Pharm.* 81: 933–950.

9 Kukolja, S. (1968). Chemistry of cephalosporin antibiotics. XI. Preparation and properties of desacetylcephaloglycin and its lactone. *J. Med. Chem.* 11: 1067–1069.

10 Nguyen, N.A. (1991). The relationship of diastereomer hydrolysis kinetics to shelf-life predictions for cefuroxime axetil. *Pharm. Res.* 8: 893–898.

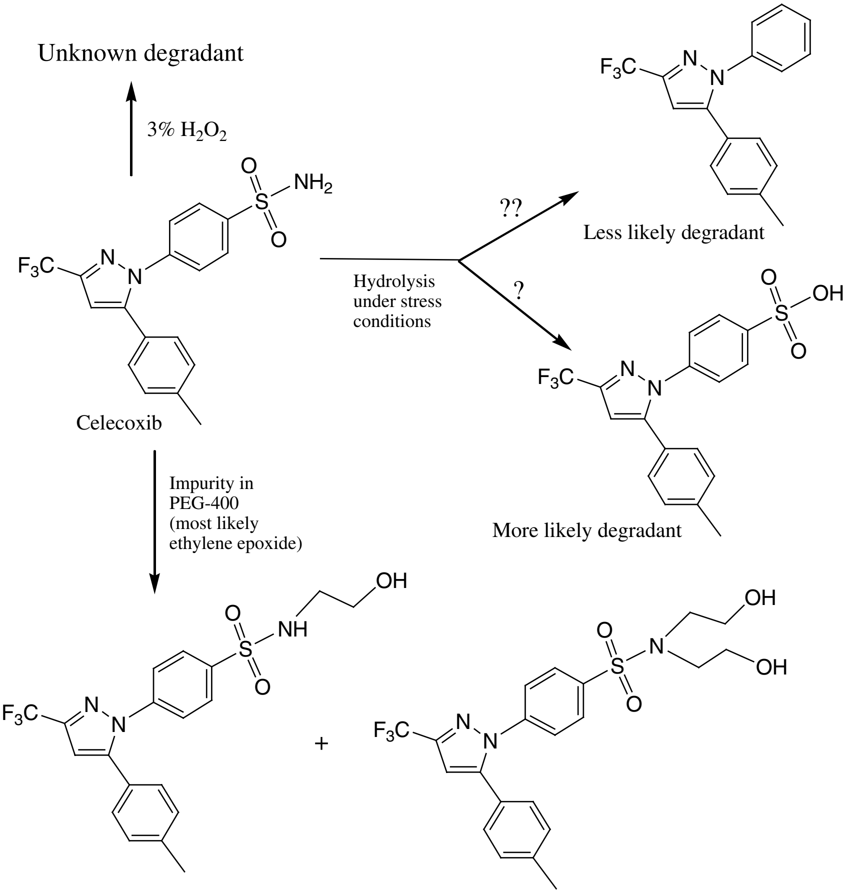
94: Celecoxib



Chemical name: 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide
Brand name: Celebrex
Formula: $C_{17}H_{14}F_3N_3O_2S$
Monoisotopic protonated ion (*m/z*): 382.0832
Molecular weight: 381.37
CAS number: 169590-42-5

Celecoxib is a selective COX-2 inhibitor, which differentiates itself from the vast majority of NSAIDs that display no selectivity in inhibiting COX-1 and COX-2. Other than a few forced degradation studies in which none of the degradation products was fully characterized [1–4], there appear to be very few reports regarding the degradation chemistry of celecoxib. In a forced degradation study by Hashem et al. [1], celecoxib was found to be stable under all stress conditions except UV irradiation (in an acetonitrile solution). In another forced degradation study, hydrolysis under very harsh conditions did generate a degradant, which most likely should result from the hydrolysis of the sulfonamide functional group [2]. The hydrolytic degradant was proposed by the original authors as a desulfonyl derivative of the drug based on infrared spectrometric evidence. Nevertheless, it would be more reasonable that the hydrolytic degradant is the sulfonic acid derivative of the drug, which was consistent with the TLC behavior of the degradant (a very polar compound that did not move on a normal phase TLC plate) as presented by the authors. Since the activation energy for the hydrolysis of a sulfonamide bond is usually about 30 kcal/mol or greater [5], this hydrolysis would not be expected to occur under a long-term stability or even the accelerated stability condition of 40°C/75%RH. In other words, the hydrolysis is unlikely to be a real degradation pathway for celecoxib. In two oxidative stress studies using H_2O_2 [3, 4], significant degradation was observed, although the structure of the oxidative degradant was not determined.

On the other hand, a real issue with the degradation of celecoxib in formulated products may be related to the fact that its sulfonylamino group is still nucleophilic [6] and as such, it is capable of reacting with excipients containing electrophilic moiety and/or electrophilic impurities such as formaldehyde. In a liquid formulation of celecoxib in PEG-400 [7], it was revealed that an impurity from PEG-400, presumably ethylene epoxide, reacted with the nucleophilic sulfonylamino group of celecoxib to produce



Scheme 94 Degradation of celecoxib under forced degradation conditions and in an experimental formulation.

two adducts (Scheme 94); use of antioxidant such as propyl gallate was shown to be effective in suppressing the formation of these two degradants. The latter result suggests that the presumed ethylene epoxide is an oxidative impurity of PEG-400. The nucleophilic sulfonylamino group is also capable of the Maillard reaction if a formulation contains reducing-end sugars such as lactose.

References

1 Hashem, H., Trundelberg, C., and Jira, T. (2010). Chromatographic application on calixarene bonded stationary phases: a stability indicating LC-method for determination of celecoxib in tablet formulation. *Chromatographia* 71: 91–94.

2 Bebawy, L.I., Moustafa, A.A., and Abo-Talib, N.F. (2002). Stability-indicating methods for the determination of doxazosin mezylate and celecoxib. *J. Pharm. Biomed. Anal.* 27: 779–793.

3 Baboota, S., Faiyaz, S., Ahuja, A. et al. (2007). Development and validation of a stability-indicating HPLC method for analysis of celecoxib (CXB) in bulk drug and microemulsion formulations. *Acta Chromatogr.* 18: 116–129.

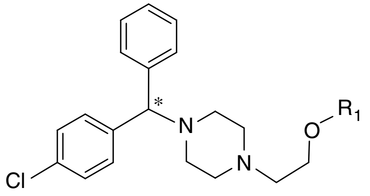
4 Donnelly, R.F., Pascuet, E., Ma, C., and Vaillancourt, R. (2009). Stability of celecoxib oral suspension. *Can. J. Hosp. Pharm.* 62: 464–468.

5 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 30–32. Cambridge: Royal Society Chemistry Publishing.

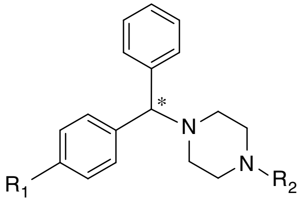
6 Li, M. (2012). Drug–excipient interactions and adduct formation. In: *Organic Chemistry of Drug Degradation*. Cambridge: Royal Society Chemistry Publishing.

7 Gao, P., Huang, T., Robins, R.H. et al. (2002). Orally deliverable pharmaceutical composition comprising an active compound having an aminosulfonyl group (cox-2 inhibitor), a polyethylene glycol and a free-radical scavenging antioxidant. European patent: EP1494666. Filing date: 12 April 2002.

95–100: Cetirizine and Structurally Related Drugs



Ceritizine, * = Racemic; R = $–CH_2CO_2H$
Levoceritizine, * = *R*-configuration; R = $–CH_2CO_2H$
Hydroxyzine, * = Racemic; R = $–CH_2CH_2OH$



Cyclozine, R_1 = H; R_2 = $–CH_3$;
Chlorcyclizine, R_1 = Cl; R_2 = $–CH_3$;
Meclizine, * = Racemic; R_1= Cl; R_2 = $–CH_2Ph$

95: Cetirizine

Chemical name: 2-[2-[4-[(4-Chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy] acetic acid
Brand name: Zyrtec
Formula: $C_{21}H_{25}ClN_2O_3$
Monoisotopic protonated ion (*m/z*): 389.1626
Molecular weight: 388.89
CAS number: 83881-51-0
Common salt form: HCl

96: Levocetirizine

Chemical name: 2-[2-[4-[(*R*)-(4-Chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy]acetic acid
Brand name: Xyzal
Formula: $C_{21}H_{25}ClN_2O_3$
Monoisotopic protonated ion (*m/z*): 389.1626
Molecular weight: 388.89
CAS number: 130018-77-8
Common salt form: 2(HCl)

97: Hydroxyzine

Chemical name: 2-[2-[4-[(4-Chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy] ethanol
Brand name: Atarax, Vistaril
Formula: $C_{21}H_{27}ClN_2O_2$
Monoisotopic protonated ion (*m/z*): 375.1834
Molecular weight: 374.90
CAS number: 68-88-2
Common salt form: HCl

98: Cyclizine

Chemical name: 1-Benzhydryl-4-methylpiperazine
Brand name: Nausicalm
Formula: $C_{18}H_{22}N_2$
Monoisotopic protonated ion (*m/z*): 267.1856
Molecular weight: 266.38
CAS number: 82-92-8
Common salt form: HCl

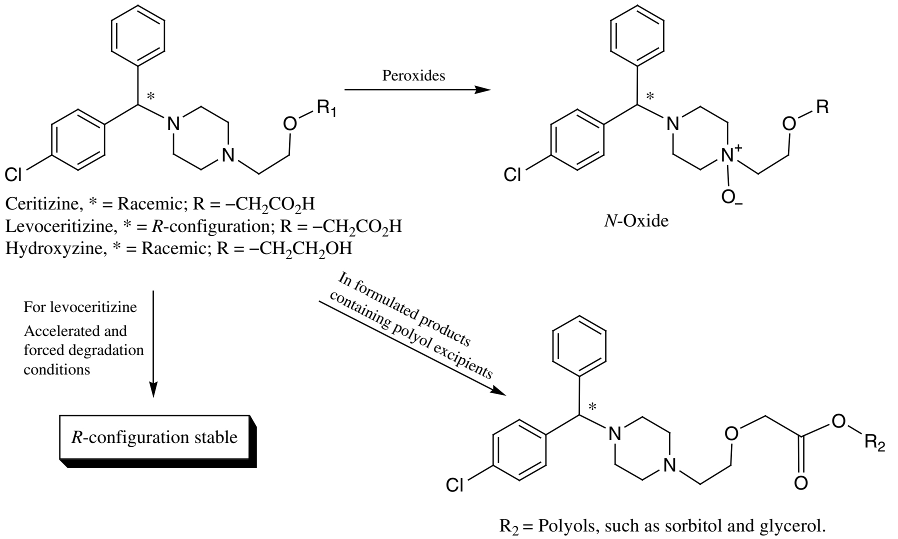
99: Chlorcyclizine

Chemical name: 1-[(4-Chlorophenyl)-phenylmethyl]-4-methylpiperazine
Brand name: Ahist, Biclora
Formula: $C_{18}H_{21}ClN_2$
Monoisotopic protonated ion (*m/z*): 301.1466
Molecular weight: 300.83
CAS number: 82-93-9
Common salt form: HCl

100: Meclizine

Chemical name: 1-[(4-Chlorophenyl)-phenylmethyl]-4-[(3-methylphenyl)methyl] piperazine
Brand name: Antivert, Diphen
Formula: $C_{25}H_{27}ClN_2$
Monoisotopic protonated ion (*m/z*): 391.1936
Molecular weight: 390.9488
CAS number: 163837-49-8
Common salt form: HCl

Cetirizine is a metabolite of hydroxyzine, a first-generation antihistamine, and is categorized as a second-generation antihistamine. It is a racemic mixture, and its *R*-enantiomer is levocetirizine, which is a third-generation antihistamine. All of them are members of the phenylpiperazine family of antihistamine drugs. As tertiary amines, cetirizine, levocetirizine, and hydroxyzine should be susceptible to nucleophilic oxidation to form the corresponding *N*-oxide degradants. Indeed, a study on a formulation containing PEG, which is known to contain residual peroxides, showed that *N*-oxidation occurs on the less sterically hindered nitrogen of the piperazine ring (Scheme 95–100-1) [1]. On the other hand, the carboxyl group of cetirizine and levocetirizine can be reactive toward hydroxyl-containing excipients by forming ester-linked adducts [2]. A study by Yu et al. found that two marketed preparations of cetirizine contained between 0.1% and 0.3% of esters that were apparently formed between the drug substance and polyols excipients such as sorbitol and glycerol [3]. For levocetirizine, its stereochemical stability was evaluated by



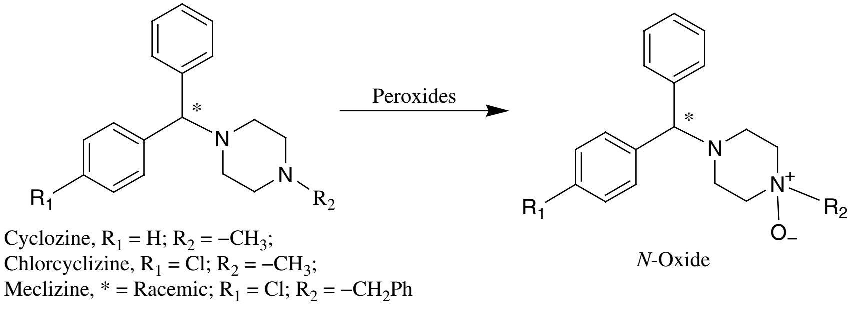
Ceritizine, * = Racemic; R = $-CH_2CO_2H$
Levoceritizine, * = *R*-configuration; R = $-CH_2CO_2H$
Hydroxyzine, * = Racemic; R = $-CH_2CH_2OH$

R_2 = Polyols, such as sorbitol and glycerol.

Scheme 95–100-1 Degradation pathways of cetirizine and structurally related drugs.

Hommoss et al.; no racemization was observed under both accelerated stability as well as forced degradation conditions (acid, base, hydrogen peroxide, thermal, and photolysis) [4]. Since cetirizine does not contain a strong chromophore, it should be photochemically stable. However, under extensive photo stress conditions particularly in solutions in the UV-A region, cetirizine was found to produce two photo degradants [5]; The structures of the two photo degradants were not elucidated; it would be likely that the photo degradation such as dichlorination, may occur with the chlorophenyl moiety [6].

Other members of the phenylpiperazine antihistamines include cyclizine and meclizine. Similarly, the distal tertiary amine moieties of both molecules, which are more reactive due to less steric hindrance and more alkalinity (the distal amines having pKa of 8.05 and 7.65 for cyclizine and meclizine, respectively, versus 2.16 and 2.12 for their proximal amine moieties), should be susceptible to nucleophilic oxidation to give the corresponding *N*-oxides (Scheme 95–100-2) [7]. In addition to the



Cyclozine, R_1 = H; R_2 = $-CH_3$;
Chlorcyclizine, R_1 = Cl; R_2 = $-CH_3$;
Meclizine, * = Racemic; R_1 = Cl; R_2 = $-CH_2Ph$

Scheme 95–100-2 *N*-Oxidation of cyclozine, chlorcyclizine, and meclizine.

N-oxide, other forced degradation products of meclizine were reported; however, the identities of these degradants were established only based upon retention time matching, and the proposed structures of certain degradants do not seem reasonable[1].

References

1 Dyakonov, T., Muir, A., Nasri, H. et al. (2010). Isolation and characterization of cetirizine degradation product: mechanism of cetirizine oxidation. *Pharm. Res.* 27: 1318–1324.

2 Li, M. (2012). Drug–excipient interactions and adduct formation. In: *Organic Chemistry of Drug Degradation*, 150–164. Cambridge: Royal Society Chemistry Publishing.

3 Yu, H., Cornett, C., Larsen, J., and Hansen, S.H. (2010). Reaction between drug substances and pharmaceutical excipients: formation of esters between cetirizine and polyols. *J. Pharm. Biomed. Anal.* 53: 745–750.

4 Hommoss, R., Elzein, H., and Haidar, S. (2011). Determination of levocetirizine configurational stability in tablets using chiral HPLC method. *Int. J. Pharm. Pharm. Sci.* 3: 103–107.

5 Jaber, A.M.Y., Al Sherife, H.A., Al Omari, M.M., and Badwan, A.A. (2004). Determination of cetirizine dihydrochloride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC. *J. Pharm. Biomed. Anal.* 36: 341–350.

6 Li, M. (2012). Photochemical degradation. In: *Organic Chemistry of Drug Degradation*, 165–197. Cambridge: Royal Society of Chemistry.

7 Peraman, R., Manikala, M., Kondreddy, V.K., and Yiragamreddy, P.R. (2015). A stability-indicating RP-HPLC method for the quantitative analysis of meclizine hydrochloride in tablet dosage form. *J. Chromatogr. Sci.* 53: 793–799.

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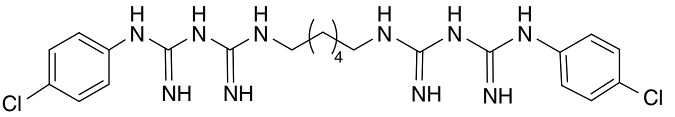
[1] For example, in Reference 7, it was reported that under acidic stress, the only degradant observed was 3-(hydroxymethyl)toluene. One would assume that this degradant should be a product resulting from the breakup of meclizine at the piperazine ring; such a breakup should also result in, concomitantly, the formation of at least another degradant bearing the phenylpiperazine core, such as desmethylchlorcyclizine. Nevertheless, no additional degradant was observed.

101: Chlorhexidine [WHO Essential List of Medicines]



Chemical name: (1*E*)-2-[6-[[Amino-[(*E*)-[amino-(4-chloroanilino)methylidene] amino]methylidene]amino]hexyl]-1-[amino-(4-chloroanilino)methylidene] guanidine

Brand name: Betasept, Hibiclens, Instillagel, etc.

Formula: $C_{22}H_{30}Cl_2N_{10}$

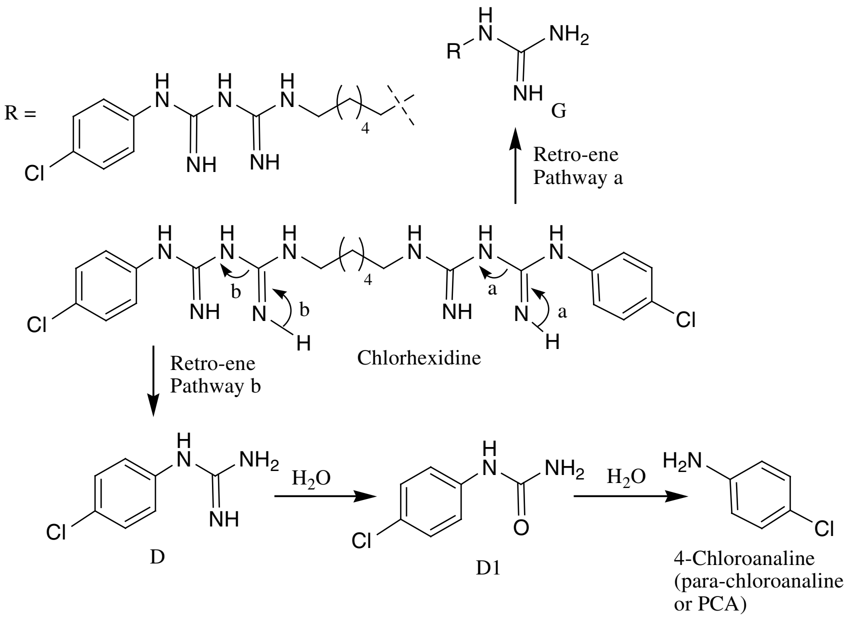
Monoisotopic protonated ion (*m/z*): 505.2105

Molecular weight: 505.45

CAS number: 55-56-1

Common salt form: Diacetate, digluconate, 2(HCl)

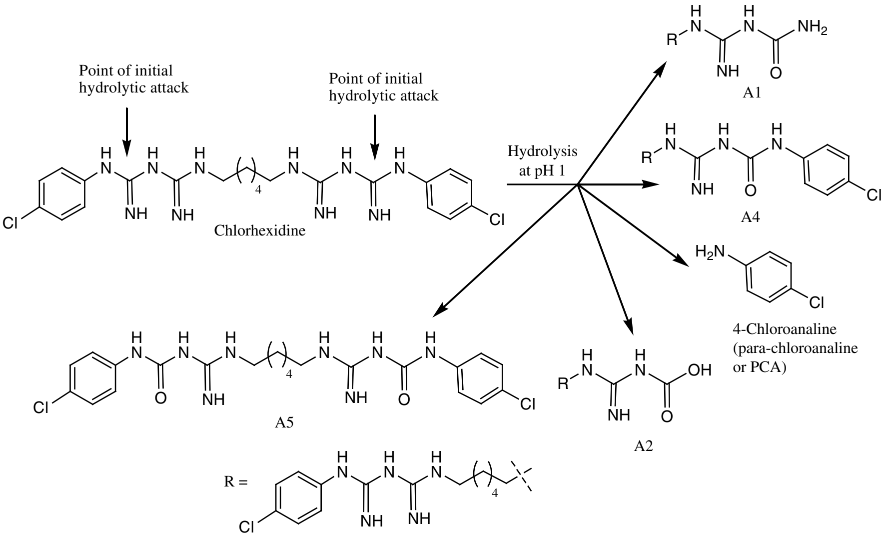
Chlorhexidine is a biguanide, which is a very strong base, and present as a positively charged ion at pH < 11 [1]. It is widely used as an antibacterial agent in pharmaceutical preparations as well as in consumer products such as mouthwash. In aqueous solutions, it is most stable in the pH range of 5–8 [2]. In a forced degradation study of a commercial chlorhexidine digluconate solution, the main degradation pathway was proposed to be a retro-ene mechanism by Revelle et al. [3] (Scheme 101-1), to



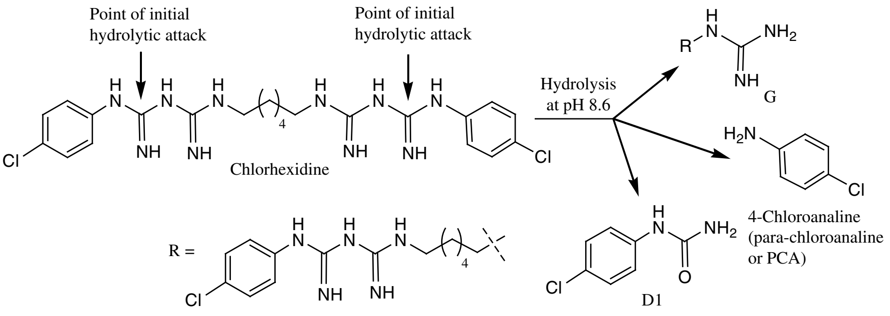
Scheme 101-1 Forced degradation of a commercial chlorhexidine digluconate solution via the proposed retro-ene mechanism. Nomenclature of Revelle et al. for the impurities is adopted [3]. *Source:* Revelle et al. [3]/Springer Nature.

give rise to impurity D and G as the main degradants. It should be noted that these two degradants can also be formed via hydrolytic mechanisms. The degradant D can further degrade to 4-chloroanaline (also referred to as para-chloroanaline or PCA).

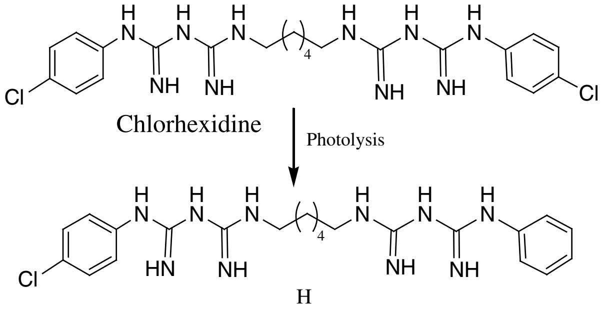
Under both acidic and alkaline stress conditions, the main degradation pathway seems to be of hydrolytic nature. At pH 1.0 at 90°C for 5 hours, chlorhexidine was found to decompose to A1, A2, A4, A5, and 4-chloroanaline (Scheme 101-2) [3]. On the other hand, when stressed at pH 8.6 at 90°C for 24 hours, chlorhexidine degraded to G, D1, and 4-chloroanaline (Scheme 101-3). Among all the degradants observed, 4-chloroaniline (PCA) is of most toxicological concern because it is a known carcinogen, and the USP limit on this impurity is set at 3 ppm [4].



Scheme 101-2 Forced degradation of chlorhexidine at pH 1.0 and 90°C for 5 hours. Nomenclature of Zong and Kirsch [3] for the impurities is adopted. *Source:* Revelle et al. [3] and Zong and Kirsch [4].



Scheme 101-3 Forced degradation of chlorhexidine at pH 8.6 at 90°C for 24 hours. The nomenclature of Revelle et al. for the impurities is adopted [3]. *Source:* Revelle et al. [3]/ Springer Nature.



Scheme 101-4 Photochemical dechlorination of chlorhexidine. Nomenclature of Revelle et al. for the impurities is adopted [3]. *Source:* Revelle et al. [3]/Springer Nature.

When stressed under sunlight for 69 hours, impurity H was observed, which apparently results from photodechlorination of chlorhexidine (Scheme 101-4).

References

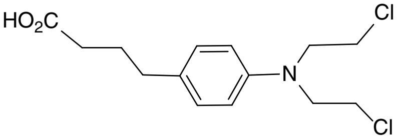
1 Elpern, B. (1968). Chemistry of the biguanides. *Ann. N. Y. Acad. Sci.* 148: 577–586.

2 Nicolay, A., Wolff, E., Vergnes, M.-F. et al. (2011). Rapid HPLC method for determination of parachloroaniline in chlorhexidine antiseptic agent in mouthrinses, ophthalmic and skin solution. *Am. J. Anal. Chem.* 2: 422–428.

3 Revelle, L.K., Doub, W.H., Wilson, R.T. et al. (1993). Identification and isolation of chlorhexidine digluconate impurities. *Pharm. Res.* 10: 1777–1784.

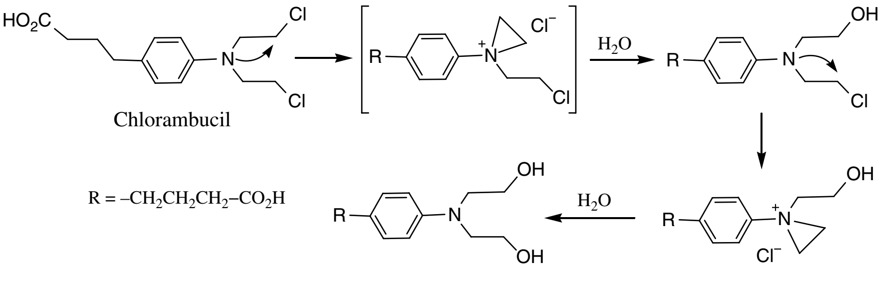
4 Zong, Z. and Kirsch, L.E. (2012). Studies on the instability of chlorhexidine, part I: kinetics and mechanisms. *J. Pharm. Sci.* 101: 2417–2427.

102: Chlorambucil



Chemical name: 4-[4-[Bis(2-chloroethyl)amino]phenyl]butanoic acid
Brand name: Leukeran
Formula: $C_{14}H_{19}Cl_2NO_2$
Monoisotopic protonated ion (*m/z*): 304.0866
Molecular weight: 304.21
CAS number: 305-03-3

Chlorambucil is an analine-based nitrogen mustard, which was introduced in the 1940s as an antitumor agent. Its main degradation pathway is hydrolysis via the aziridium intermediate (Scheme 102); this mechanism is consistent with the fact that chloride stabilizes the drug due to its reacting with the intermediate to regenerate the drug [1]. The aziridium intermediate is also supported by density functional theory (DFT) calculation [2]. Using this calculation, the activation energy for the hydrolysis was determined to be 24.8 kcal/mol, which is consistent with the experimental result [3]. The pKa of chlorambucil was determined to be approximately 3.5 and in the pH range of 3.5–8, the rate of chlorambucil hydrolysis was independent of pH. At pH below 3.5, the hydrolysis rate decreased rapidly, apparently due to the protonation of the aniline nitrogen, which should slow down the formation of the aziridium intermediate. In another study for the stability of chlorambucil in aqueous solution, it was found that the stability of chlorambucil was unaffected in the pH range between 5 and 10, while it became more stable at lower pH [4]. The general trends for the stability of chlorambucil in these studies were consistent, despite some minor differences in the pH range.

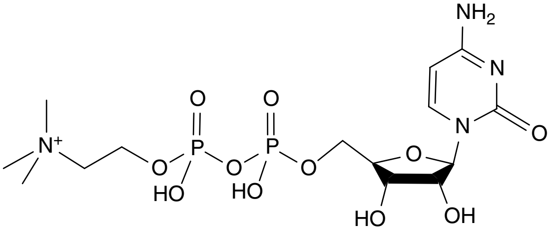


Scheme 102 Hydrolysis of chlorambucil.

References

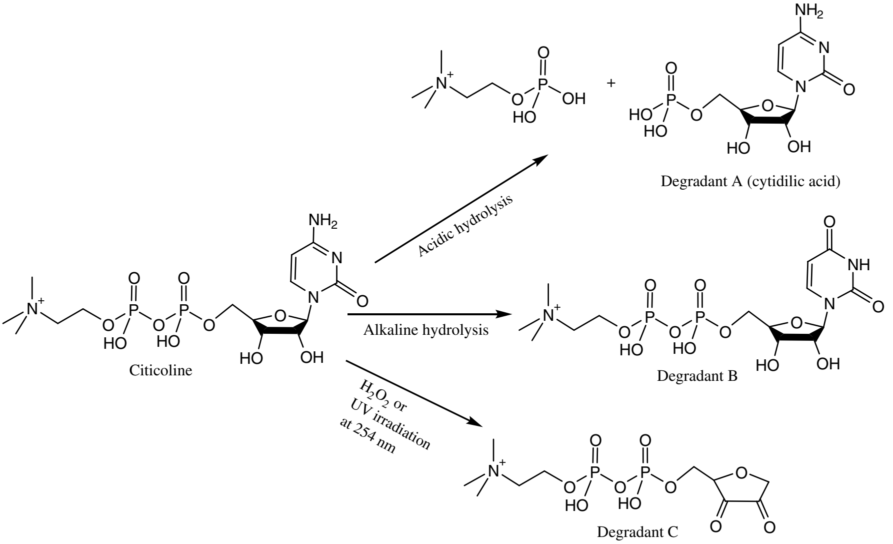
1. Cullis, P.M., Green, R.E., and Malone, M.E. (1995). Mechanism and reactivity of chlorambucil and chlorambucil-spermidine conjugate. *J. Chem. Soc. Perkin Trans. 2*: 1503–1511.
2. Pineda, F.P., Ortega-Castro, J., Alvarez-Idaboy, J.R. et al. (2011). Hydrolysis of a Chlorambucil analogue. A DFT study. *J. Phys. Chem. A* 115: 2359–2366.
3. Gamcsik, M.P., Millis, K.K., and Hamill, T.G. (1997). Kinetics of the conjugation of aniline mustards with glutathione and thiosulfate. *Chem-Bio. Interac.* 105: 35–52.
4. Ehrsson, H., Eksborg, S., Wallin, I., and Nilsson, S.O. (1980). Degradation of chlorambucil in aqueous solution. *J. Pharm. Sci.* 69: 1091–1094.

103: Citicoline



Chemical name: [[(2*R*,3*S*,4*R*,5*R*)-5-(4-Amino-2-oxopyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl] 2-(trimethylazaniumyl)ethyl phosphate
Brand name: CerAxon
Formula: $C_{14}H_{26}N_4NaO_{11}P_2$
Monoisotopic ion (*m/z*): 488.1068 (citicoline is a quaternary ammonium ion)
Molecular weight: 510.31 (for citicoline sodium, $C_{14}H_{25}N_4NaO_{11}P_2$)
CAS number: 987-78-0
Common salt form: Sodium salt

Citicoline is a psychostimulant, and structurally it is a conjugate of cytidine and choline that are linked together by a diphosphate. There are a couple of forced degradation studies of citicoline reported in the literature [1, 2]. Under acidic and alkaline conditions, approximately two to three degradants were observed depending on the harshness of the stress conditions. The main degradant was



Scheme 103 Degradation pathways of citicoline under acidic, alkaline, oxidative, and photo-forced degradation conditions.

identified via LC-MS as cytidilic acid, which is formed via the cleavage of the phosphoryl bond of diphosphate (Scheme 103) [2]. Holbrook and Ouellet reported in 1957 that the activation energy (*E*a) for hydrolysis of adenosine diphosphate, which can be considered an analog of citicoline for the sake of the phosphoryl bond hydrolysis, was about 24 kcal/mol at pH 8 [3]. The *E*a value of this scale suggests that citicoline should be reasonably stable toward hydrolytic degradation under long-term stability conditions at a similar pH range. In two forced degradation studies, when citicoline was stressed in H_2O_2 solutions under elevated temperature [1, 2], one major degradant was observed; however, its structure was not elucidated. In a later forced degradation study performed by Derbouz et al. [4], citicoline was found to produce three degradants (Degradants A, B, and C) under acidic, alkaline, and oxidative (H_2O_2) conditions, respectively. When the solid citicoline sodium was irradiated at 254 nm, Degradant C was also formed.

The formation of Degradants A and B is relatively straightforward: under acidic stress, the diphosphate linkage was cleaved, while under alkaline stress, the cytosine moiety was converted to the uracil moiety in Degradant B via deamination. On the other hand, the formation of Degradant C appears to be more complicated; the formation mechanism provided by Derbouz et al. [4] seems to be vague.

References

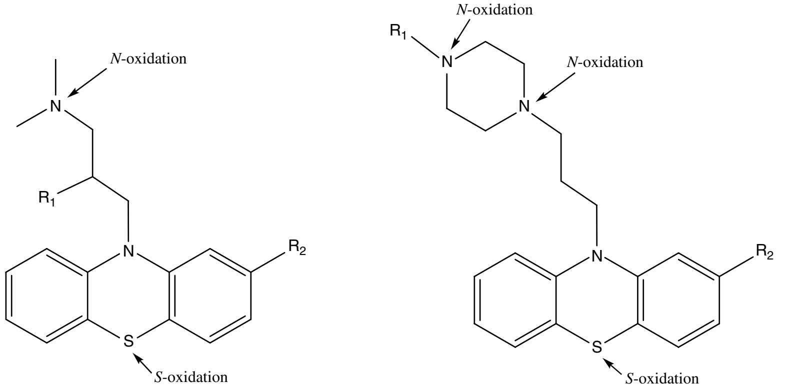
1 Ganduri, R.B., Peddareddigari, J.R., Dasari, N.R., and Saiempu, R.K. (2010). Stability indicating LC method for the determination of citicoline sodium in injection formulation. *Int. J. PharmTech Res.* 2: 427–433.

2 Patel, J.A., Panigrahi, B., Patel, C.N., and Ramalingan, B. (2011). Stress degradation studies on citicoline sodium and development of a validated stability-indicating HPLC assay. *Chron. Young Sci.* 2: 150–154.

3 Holbrook, K.A. and Ouellet, L. (1957). The non-enzymatic hydrolysis of adenosine diphosphate. *Can. J. Chem.* 35: 1496–1503.

4 Derbouz, S., Guermouche, M.H., and Guermouche, S. (2017). Stability-indicating HILIC method for the determination of citicoline and characterization of its degradation products by LC–MS/TOF, 1H and 13C NMR. *Chromatographia* 80: 265–274.

104–116: Chlorpromazine and Structurally Analogous Drugs



Chlorpromazine, R_1 = hydrogen, R_2 = chloro;
Acepromazine, R_1 = hydrogen, R_2 = acetyl;
Methotrimeparzine, R_1 = (*R*)-methyl, R_2 = methoxyl;
Promazine, R_1 = hydrogen, R_2 = hydrogen;
Triflupromazine, R_1 = hydrogen, R_2 = trifluoromethyl;
Trimeprazine, R_1 = methyl, R_2 = hydrogen.

Perphenazine, R_1 = 2-hydroxyethyl, R_2 = chloro;
Carphenazine, R_1 = 2-hydroxyethyl, R_2 = propionyl;
Fluphenazine, R_1 = 2-hydroxyethyl, R_2 = trifluoromethyl;
Prochlorperazine, R_1 = methyl, R_2 = chloro;
Thiethylperazine, R_1 = methyl, R_2 = ethylmercapto;
Trifluoperazine, R_1 = methyl, R_2 = trifluoromethyl;
Thioproperazine, R_1 = methyl, R_2 = dimethylsulfamyl.

Structures of chlorpromazine and its analogous phenothiazine-based drugs

104: Chlorpromazine [List of WHO Essential Medicines]

Chemical name: 3-(2-Chlorophenothiazin-10-yl)-*N*,*N*-dimethylpropan-1-amine
Brand name: Aminazine, Chlorprom
Formula: $C_{17}H_{19}ClN_2S$
Monoisotopic protonated ion (*m/z*): 319.1030
Molecular weight: 318.87
CAS number: 50-53-3
Common salt form: HCl

105: Acepromazine

Chemical name: 1-[10-[3-(Dimethylamino)propyl]phenothiazin-2-yl]ethanone
Brand name: Concentrat vo34
Formula: $C_{19}H_{22}N_2OS$
Monoisotopic protonated ion (*m/z*): 327.1526
Molecular weight: 326.46
CAS number: 61-00-7
Common salt form: Maleate

106: Carphenazine (Carfenazine)

Chemical name: 1-[10-[3-[4-(2-Hydroxyethyl)piperazin-1-yl]propyl]phenothiazin-2-yl]propan-1-one
Brand name: Proketazin, Proketazine
Formula: $C_{24}H_{31}N_3O_2S$
Monoisotopic protonated ion (*m/z*): 426.22097
Molecular weight: 425.59
CAS number: 2622-30-2
Common salt form: Maleate

107: Fluphenazine

Chemical name: 2-[4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]piperazin-1-yl]ethanol
Brand name: Permitil, Prolixin
Formula: $C_{22}H_{26}F_3N_3OS$
Monoisotopic protonated ion (*m/z*): 438.1821
Molecular weight: 437.52
CAS number: 5002-47-1
Common salt form: HCl

108: Levomepromazine (Methotrimeprazine)

Chemical name: (2*R*)-3-(2-Methoxyphenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine
Brand name: Levoprome, Nozinan
Formula: $C_{19}H_{24}N_2OS$
Monoisotopic protonated ion (*m/z*): 329.1682
Molecular weight: 328.47
CAS number: 60-99-1
Common salt form: HCl, Maleate

109: Perphenazine

Chemical name: 2-[4-[3-(2-Chlorophenothiazin-10-yl)propyl]piperazin-1-yl]ethanol
Brand name: Trilafon
Formula: $C_{21}H_{26}ClN_3OS$
Monoisotopic protonated ion (*m/z*): 404.1558
Molecular weight: 403.97
CAS number: 58-39-9

110: Prochlorperazine

Chemical name: 2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine
Brand name: Compazine, Compro
Formula: $C_{20}H_{24}ClN_3S$
Monoisotopic protonated ion (*m/z*): 374.1452
Molecular weight: 373.94
CAS number: 58-38-8

111: Promazine

Chemical name: *N,N*-Dimethyl-3-phenothiazin-10-ylpropan-1-amine
Brand name: Pliva, Talofen
Formula: $C_{17}H_{20}N_2S$
Monoisotopic protonated ion (*m/z*): 285.1420
Molecular weight: 284.42
CAS number: 58-40-2
Common salt form: HCl

112: Thiethylperazine

Chemical name: 2-Ethylsulfanyl-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine
Brand name: Torecan
Formula: $C_{22}H_{29}N_3S_2$
Monoisotopic protonated ion (*m/z*): 400.1876
Molecular weight: 399.62
CAS number: 1420-55-9
Common salt form: Malate, Maleate

113: Thioproperazine

Chemical name: *N,N*-Dimethyl-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine-2-sulfonamide
Brand name: Majeptil
Formula: $C_{22}H_{30}N_4O_2S_2$
Monoisotopic protonated ion (*m/z*): 447.1883
Molecular weight: 446.63
CAS number: 316-81-4

114: Trifluoperazine

Chemical name: 10-[3-(4-Methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)phenothiazine
Brand name: Stelazine

Formula: $C_{21}H_{24}F_3N_3S$
Monoisotopic protonated ion (*m/z*): 408.1716
Molecular weight: 407.50
CAS number: 117-89-5
Common salt form: HCl

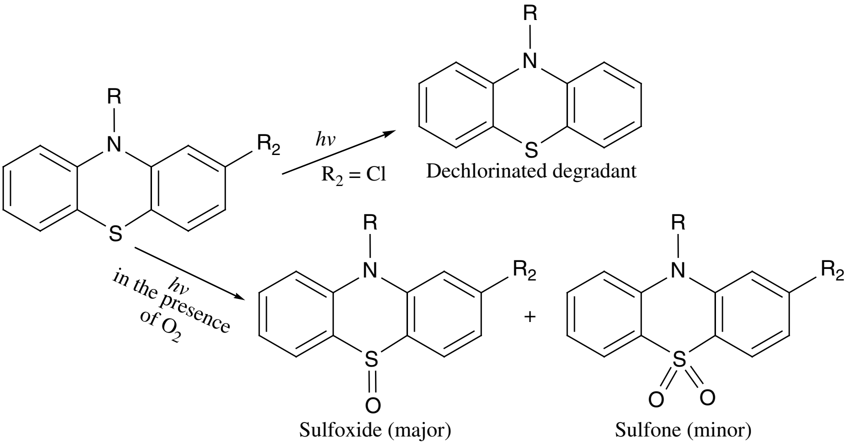
115: Triflupromazine

Chemical name: *N,N*-Dimethyl-3-[2-(trifluoromethyl)phenothiazin-10-yl]propan-1-amine
Brand name: Vesprin
Formula: $C_{18}H_{19}F_3N_2S$
Monoisotopic protonated ion (*m/z*): 353.1294
Molecular weight: 352.42
CAS number: 146-54-3
Common salt form: HCl

116: Trimeprazine

Chemical name: *N,N*,2-Trimethyl-3-phenothiazin-10-ylpropan-1-amine
Brand name: Panectyl
Formula: $C_{18}H_{22}N_2S$
Monoisotopic protonated ion (*m/z*): 299.1576
Molecular weight: 298.45
CAS number: 84-96-8
Common salt form: Tartrate

Chlorpromazine and its analogous drugs are used as antipsychotic medications, except in rare cases, such as trimeprazine (which is an antihistamine). This family of drugs contains a phenothiazine core with one or two substituents on the core (refer to the structures of chlorpromazine and its analogous drugs above). Based on the structure of the *N*-substituent, this family of drugs can be further divided into two subtypes: one has a simple tertiary amine group in the side chain (as represented by chlorpromazine), and the other contains a piperazine ring (as represented by perphenazine). All the tertiary amine functionalities in the side chain, including both tertiary amines in the piperazine ring, are susceptible to nucleophilic oxidation to form the corresponding *N*-oxides [1]. The sulfur in the phenothiazine ring is susceptible to both nucleophilic [2, 3], free-radical mediated oxidation, and photochemical oxidation [4, 5]; usually, the degradation product is limited to sulfoxide [5], but sulfone could be formed [6]. In a forced degradation study of perphenazine in methanol solution containing H_2O_2, the ^{17}N-oxide was the main degradant in the early stage of the stress, in addition to ^{14}N-oxide, perphenazine sulfoxide, and a low level of ^{14}N,^{17}N-dioxide [2]. The solution stress study was in good agreement with a study on solid dosage form of perphenazine in which ^{17}N-oxide was also found as the main oxidative degradant.



Scheme 104–116 Photochemical degradation of phenothiazine-based drugs.

The phenothiazine ring is quite susceptible to photochemical activation, and as such, this class of drugs displays certain levels of phototoxicity [4]. For those phenothiazine-derived drugs that contain a chlorine on the phenothiazine ring, e.g., chlorpromazine and perphenazine, photo dechlorination is a common degradation pathway (Scheme 104–116) [3, 7]. *S*-Oxidation could also occur in the photochemical degradation process.

For fluphenazine, a drug molecule in the phenothiazine-based family that contains a 2-hydroxyethyl side chain, it is also available in the forms of decanoate and enanthate esters with the ester linkages with the 2-hydroxyethyl side chain. The ester APIs are employed in intramuscular or subcutaneous injections for an extended duration of pharmacological effect. The ester linkages would be susceptible to hydrolytic degradation,

References

1 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*, 71–73. Cambridge: Royal Society Chemistry Publishing.

2 Wang, X., Li, M., and Rustum, A.M. (2010). Thermally induced intramolecular oxygen migration of *N*-oxides in atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 24: 2805–2811.

3 Li, X., Blondino, F.E., Hindle, M. et al. (2005). Stability and characterization of perphenazine aerosols generated using the capillary aerosol generator. *Int. J. Pharm.* 303: 113–124.

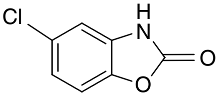
4 Chagonda, L.F.S. and Millership, J.S. (1989). The determination of chlorpromazine, related impurities and degradation products in pharmaceutical dosage forms. *J. Pharm. Biomed. Anal.* 7: 271–278.

5 Chagonda, L.F.S. and Millership, J.S. (1988). High-performance liquid chromatographic determination of chlorpromazine and its degradation products in pharmaceutical dosage forms: a stability-indicating assay. *Analyst* 113: 233–237.

6 Takahashi, D.M. (1980). Rapid determination of chlorpromazine hydrochloride and two oxidation products in various pharmaceutical samples using high-performance liquid chromatography and fluorescence detection. *J. Pharrn. Sci.* 69: 184–187.

7 Li, M. (2012). Photochemical degradation. In: *Organic chemistry of drug degradation*, 178–179. Cambridge: Royal Society Chemistry Publishing.

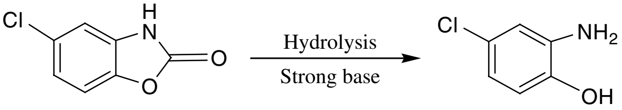
117: Chlorzoxazone



Chemical name: 5-Chloro-3*H*-1,3-benzoxazol-2-one
Brand name: Lorzone, Parafon Forte
Formula: $C_7H_4ClNO_2$
Monoisotopic protonated ion (*m/z*): 168.9931
Molecular weight: 169.57
CAS number: 95-25-0

Chlorzoxazone is a skeletal muscle relaxant. Structurally, it can be viewed as a cyclic carbamate fused into the chlorobenzene ring. Hence, its main degradation pathway is the hydrolysis of the carbamate [1] in which 2-amino-4-chlorophenol is produced (Scheme 117).

The hydrolysis is greatly enhanced under the catalysis of a strong base [2]; according to a forced degradation study, the activation energies for the hydrolysis were found to be 62.8 and 54.4 kJ/mol in 3 and 8 M sodium hydroxide solutions, respectively[2]. This impurity is also a process impurity that has some toxicological concerns [3]. There is no evidence that this impurity is a metabolite of the drug [4].



Scheme 117 Hydrolysis of chlorzoxane under strong basic conditions.

References

1 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 30–32. Cambridge: Royal Society Chemistry Publishing.

2 Stewart, J.T. (1987). *Analytical Profiles of Drug Substances and Excipients*, vol. 16, 119–144. Academic Press.

3 Hazardous Chemicals Fact Sheet, New Jersey Department of Health and Senior Services, 1996.

4 Twele, R. and Spiteller, G. (1982). Identification of chlorzoxazone metabolites in human urine (English translation from German). *Arzneimittelforschung* 32: 759–763.

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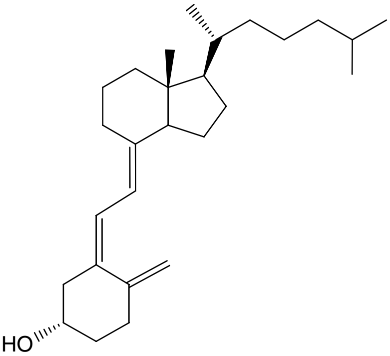
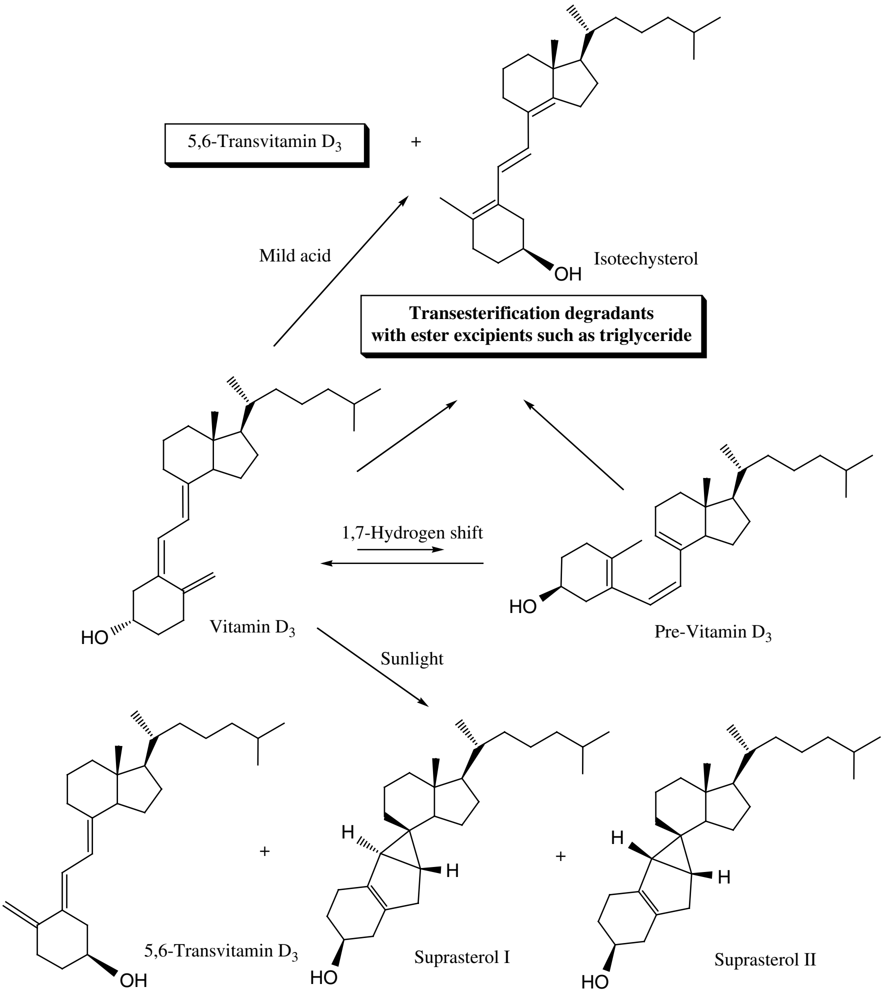
[2] In the original paper by Ellaithy et al. (*Il Farmaco*, 2003, 58, 337–342.), the activation energies in 3 M and 8 M sodium hydroxide solutions were reported to be 62 829.43 and 54 393.2 kcal/mol, respectively, which are apparently erroneous.

118: Cholecalciferol



Cholecalciferol (Vitamin D_3)
Chemical name: (1*S*,3*Z*)-3-[(2*E*)-2-[(1*R*,3a*S*,7a*R*)-7*a*-Methyl-1-[(2*R*)-6-methylheptan-2-yl]-2,3,3*a*,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidene cyclohexan-1-ol
Formula: $C_{27}H_{44}O$
Monoisotopic protonated ion (*m/z*): 385.3465
Molecular weight: 384.64
CAS number: 67-97-0

Cholecalciferol, more commonly known as vitamin D_3, is a fat-soluble vitamin, which is used as a supplement as well as a therapeutic agent particularly for helping calcium absorption. In human body, vitamin D_3 is produced photochemically in skin from 7-dehydrocholesterol via the intermediacy of previtamin D_3 by exposure to sunlight [1]. Previtamin D_3 is thermodynamically less stable than vitamin D_3 and upon heating in solution (but not in solid state) [2], previtamin D_3 is converted to vitamin D_3 [3]. Since this isomerization process is reversible, vitamin D_3 can also convert back to previtamin D_3 through 1,7-H shift [4]; the ratio of vitamin D_3 versus D_2 is a function of temperature, and at 20°C, this ratio is 93:7 [5]. Upon sunlight irradiation, vitamin D_3 can degrade to 5,6-transvitamin D_3, suprasterol I and suprasterol II as its main photo degradants (Scheme 118) [6]. Vitamin D_3 is stable under alkaline conditions but undergoes isomerization to give 5,6-transvitamin D_3 and isotachysterol under mild acidic condition [7]. On the other hand, the hydroxyl group of vitamin D_3 and previtamin D_3 can undergo transesterification if the excipients of a finished product contain an ester component such as triglyceride [8]. Ballard et al. reported four degradants resulting from transesterification between vitamin D_3 and previtamin D_3 with the octanoate and decanoate components of triglycerides in an experimental tablet formulation [9].



Scheme 118 Degradation pathways of cholecalciferol.

References

1 Holick, M.F. (1989). *Vertebrate Endocrinology: Fundamentals and Implications*, vol. 3 (ed. P.K.T. Pang and M., F. Holick), 7–34. Orlando, FL: Academic.

2 Ball, G.F.M. (2006). Vitamin D. In: *Vitamins in Foods, Analysis, Bioavailability and Stability*, 107–118. Boca Raton, FL: CRC Press.

3 Holick, M.F., MacLaughlin, J.A., Clark, M.B. et al. (1980). Photosynthesis of previtamin D_3 in human skin and the physiologic consequences. *Science* 210: 203–205.

4 Brandl, M., Wu, X.Y., Liu, Y. et al. (2003). Chemical reactivity of Ro-26-9228, 1α-fluoro-25-hydroxy-16,23E-diene -26,27-bishomo-20-epi-cholecalciferol in aqueous solution. *J. Pharm. Sci.* 92: 1981–1989.

5 Mulder, F.J., de Vries, E.J., and Borsje, B. (1971). Chemical analysis of vitamin D in concentrates and its problems. XII. Analysis of fat-soluble vitamins. *J. Assoc. Off. Anal. Chem.* 54: 1168–1178.

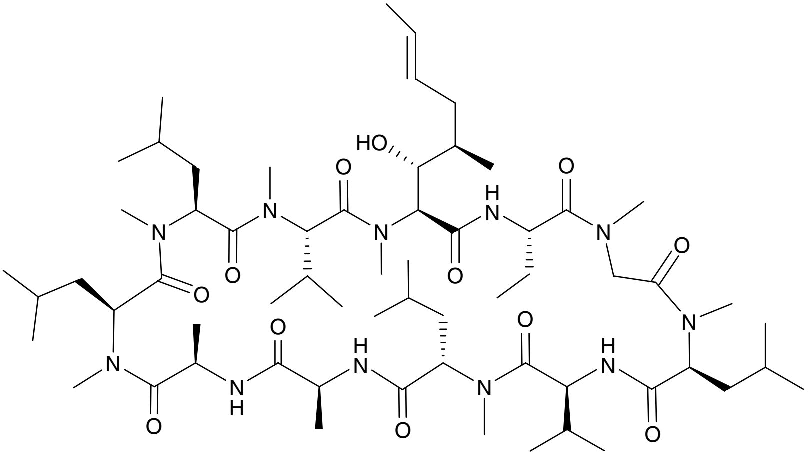
6 Webb, A.R., DeCosta, B.R., and Holick, M.F. (1989). Sunlight regulates the cutaneous production of vitamin D_3 by causing its photodegradation. *J. Clin. Endocrinol. Metab.* 68: 882–887.

7 DeLuca, H.F. (1978). Vitamin D, in vitamin D. In: *Handbook of Lipid Research*, vol. 2 (ed. H.F. DeLuca), 69. New York: Plenum Press.

8 Li, M. (2012). Drug-excipient interactions and adduct formation. In: *Organic Chemistry of Drug Degradation*, 150–164. Cambridge: RSC Publishing.

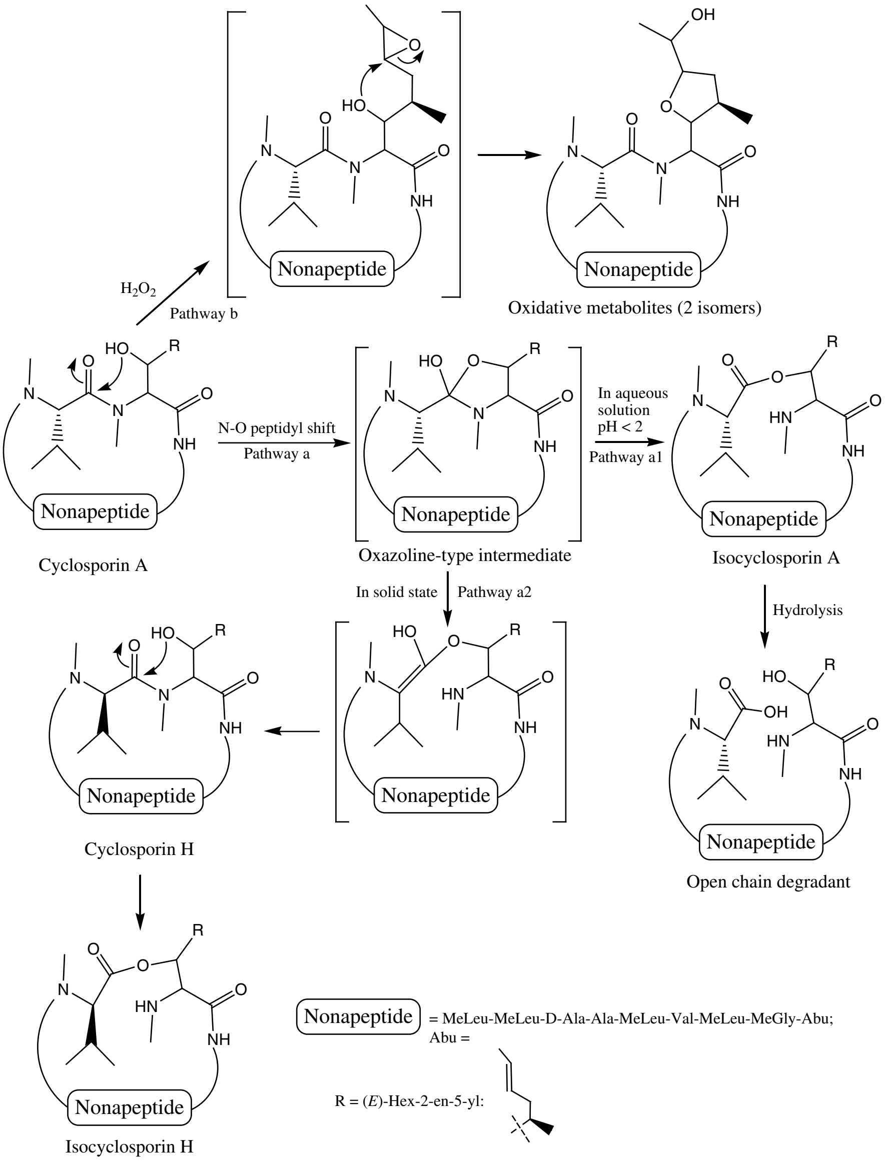
9 Ballard, J.M., Zhu, L., Nelson, E.D., and Seburg, R.A. (2007). Degradation of vitamin D_3 in a stressed formulation: the identification of esters of vitamin D3 formed by a transesterification with triglycerides. *J. Pharm. Biomed. Anal.* 43: 142–150.

119: Cyclosporin A [Ciclosporin]



Chemical name: Cyclo[((2*S*)-2-aminobutyryl)-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-*N*-methyl-(4*R*)-4-[(*E*)-but-2-enyl]-4-methyl-L-threonyl]
Brand name: Cequa, Gengraf, Neoral, SandIMMUNE
Formula: $C_{62}H_{111}N_{11}O_{12}$
Monoisotopic protonated ion (*m/z*): 1202.8486
Molecular weight: 1202.61
CAS number: 59865-13-3

Cyclosporin A, an immunosuppressive drug, is a cyclic undecapeptide, which consists of residues of many methylated amino acids, a D-alanine, and two unusual amino acids. The major degradation of cyclosporin A starts from the N—O peptidyl shift in which the hydroxyl side chain of the unusual amino acid residue attacks the preceding amide bond, forming the oxazoline-type intermediate (Pathway a, Scheme 119) [1]. In a study of degradation kinetics in aqueous solutions of pH 0.1–4.0, isocyclosporin A was found to be the predominant degradant at pH < 2 (Pathway a1) [2]. In a solid state, according to a study by Bonifacio et al. [1], the oxazoline-type intermediate seems to prefer the enolization pathway, which leads to the racemization of the MeVal^{11} residue and produces cyclosporin H (Pathway a2). Bonifacio et al. analyzed commercial batches of cyclosporin A capsules from several manufacturers, which showed that only cyclosporin H may be present as the predominant degradant dependent upon the formulation, storage condition, and age of the sample analyzed [1]. The oxazoline-type intermediate should be susceptible to hydrolysis, yielding the open-chain degradant, which was mentioned as one of the principal degradants in the same study by Bonifacio et al., although it was not detected in commercial samples of cyclosporin A capsules [1]. In a hydrolytic stress study with 3 M HCl at 60°C, two open-chain peptides (a deca- and nona-peptide) were identified among the major degradation products [3]. This forced degradation study seems



Scheme 119 Degradation pathways of cyclosporine A.

to be too harsh to represent the real degradation chemistry of the drug product. In an oxidative degradation study, Liu et al. were able to generate two oxidative metabolites by oxidizing an ether solution of cyclosporin A with 30% hydrogen peroxide through heterogeneous vortexing at 37°C [4]. It is apparent that the cyclized side chain is formed via an epoxide intermediate (Pathway b, Scheme 119). There has been no report to indicate that these two metabolites are also degradation products in finished dosage forms of cyclosporine.

References

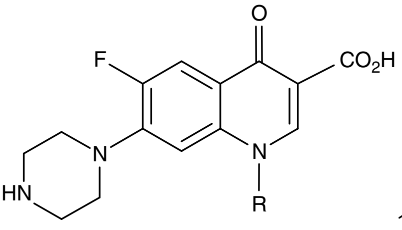
1 Nunes Bonifacio, D.F., Giocanti, M., Reynier, J.P. et al. (2009). Development and validation of HPLC method for the determination of Cyclosporin A and its impurities in Neoral® capsules and its generic versions. *J. Pharm. Biomed. Anal.* 49: 540–546.

2 Friis, G.J. and Bundgaad, H. (1992). Kinetics of degradation of cyclosporin A in acidic aqueous solution and its implication in its oral absorption. *Int. J. Pharm.* 82: 79–83.

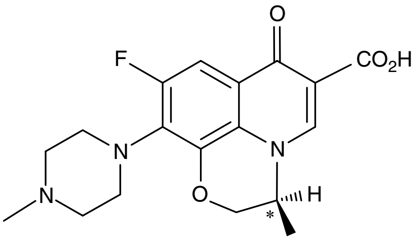
3 Magni, F., Arcelloni, C., Paroni, R. et al. (1994). Open-chain peptides obtained by acidic hydrolytic cleavage of cyclosporin A. *Biol. Mass Spec.* 23: 514–518.

4 Liu, W.T., Marat, K., Ren, Y. et al. (1998). Structural characterization of two novel oxidative derivatives of cyclosporine generated by a chemical method. *Clin. Biochem.* 31: 173–180.

120–123: Ciprofloxacin, Norfloxacin, Ofloxacin, Levofloxacin



Ciprofloxacin, R = Cyclopropyl.
(Norfloxacin, R = Ethyl)



Levofloxacin
(Norfloxacin, * = racemic)

120: Ciprofloxacin

Chemical name: 1-Cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid
Brand name: Cipro
Formula: $C_{17}H_{18}FN_3O_3$
Monoisotopic protonated ion (*m/z*): 332.1405
Molecular weight: 331.34
CAS number: 85721-33-1

121: Levofloxacin

Chemical name: (2*S*)-7-Fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.$0^{5,13}$]trideca-5(13),6,8,11-tetraene-11-carboxylic acid
Brand name: Levaquin, Quinsair
Formula: $C_{18}H_{20}FN_3O_4$
Monoisotopic protonated ion (*m/z*): 362.1511
Molecular weight: 361.37
CAS number: 100986-85-4

122: Ofloxacin

Chemical name: 7-Fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.$0^{5,13}$]trideca-5(13),6,8,11-tetraene-11-carboxylic acid
Brand name: Ocuflox
Formula: $C_{18}H_{20}FN_3O_4$
Monoisotopic protonated ion (*m/z*): 362.1511
Molecular weight: 361.37
CAS number: 82419-36-1

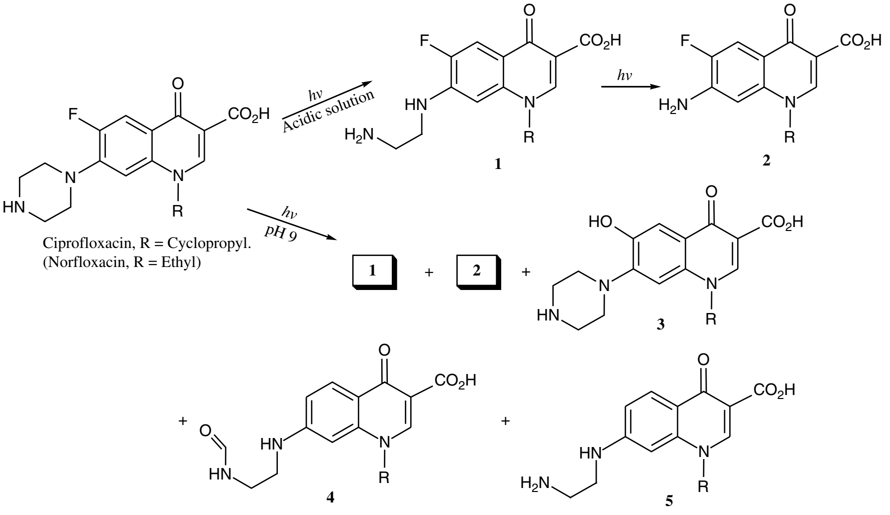
123: Norfloxacin

Chemical name: 1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid
Brand name: Noroxin
Formula: $C_{16}H_{18}FN_3O_3$

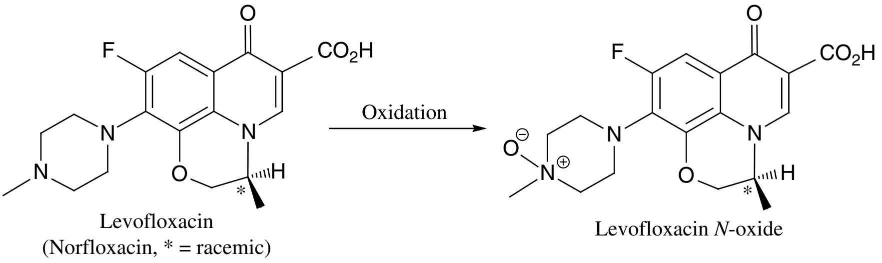
Monoisotopic protonated ion (*m/z*): 320.1405
Molecular weight: 319.33
CAS number: 70458-96-7

Ciprofloxacin and analogous fluoroquinolones are widely used antibiotics. The fluoroquinolone core is usually quite stable thermally. On the other hand, the substituted amino group on the fluoroquinolone core can undergo elimination under photochemical stress conditions; e.g., the piperazine ring of ciprofloxacin was destroyed under both artificial and sunlight exposure to yield the ethylenediamine and amine degradants, sequentially, in acidic solutions (Scheme 120–123-1) [1, 2]. With the increase of pH, the rate of photo degradation was found to be accelerated [2]. Another photo degradation study conducted at pH 9 indicated that ciprofloxacin produced three additional degradants [3]. Norfloxacin, which is structurally very similar to ciprofloxacin, also underwent this photo elimination process [4].

For ofloxacin and its *S*-enantiomer, levofloxacin, they have a methyl substituent on the piperazine ring, and the resulting tertiary amine is susceptible to nucleophilic oxidation, yielding the *N*-oxide degradant (Scheme 120–123-2) [5]. On the



Scheme 120–123-1 Photo degradation pathways of ciprofloxacin. The structures of **3–5** were proposed based on LC-MS data.



Scheme 120–123-2 Oxidation of levofloxacin.

contrary, the second amine functional group of ciprofloxacin and ofloxacin would be reluctant to undergo this kind of nucleophilic oxidation.

References

1 Torniainen, K., Mattinen, J., Askolin, C.-P., and Tammilehto, S. (1997). Structure elucidation of a photodegradation product of ciprofloxacin. *J. Pharm. Biomed. Anal.* 15: 887–894.

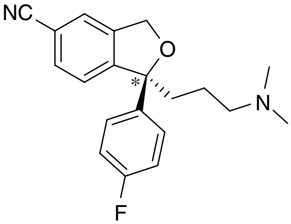
2 Torniainen, K., Askolin, C.-P., and Mattinen, J. (1997). Isolation and structure elucidation of an intermediate in the photodegradation of ciprofloxacin. *J. Pharm. Biomed. Anal.* 16: 439–445.

3 Vasconcelos, T.G., Henriques, D.M., König, A. et al. (2009). Photo-degradation of the antimicrobial ciprofloxacin at high pH: identification and biodegradability assessment of the primary by-products. *Chemosphere* 76: 487–493.

4 Córdoba-Borrego, M. and M. Córdoba-Díaz, D. Córdoba-Díaz. (1999). Validation of a high-performance liquid chromatographic method for the determination of norfloxacin and its application to stability studies (photo-stability study of norfloxacin). *J. Pharm. Biomed. Anal.* 18: 919–926.

5 Devi, M.L. and Chandrasekhar, K.B. (2009). A validated stability-indicating RP-HPLC method for levofloxacin in the presence of degradation products, its process related impurities and identification of oxidative degradant. *J. Pharm. Biomed. Anal.* 50: 710–717.

124–125: Citalopram, Escitalopram



Escitalopram
(Citalopram, * = racemic)

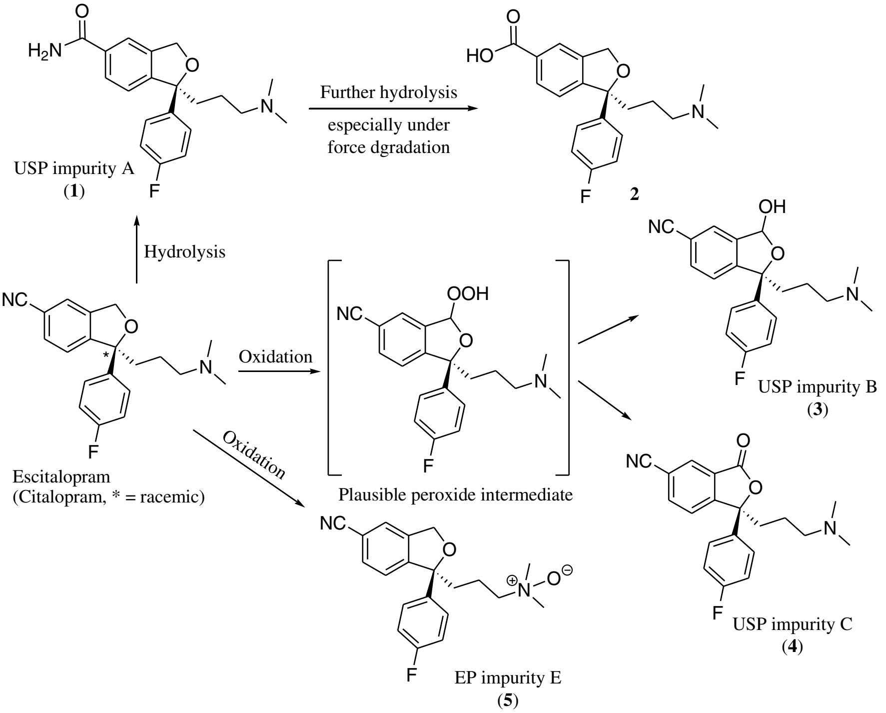
124: Citalopram

Chemical name: 1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-3*H*-2-benzofuran-5-carbonitrile
Brand name: Celexa
Formula: $C_{20}H_{21}FN_2O$
Monoisotopic protonated ion (*m/z*): 325.1711
Molecular weight: 324.39
CAS number: 59729-33-8
Common salt form: HBr, Oxalate

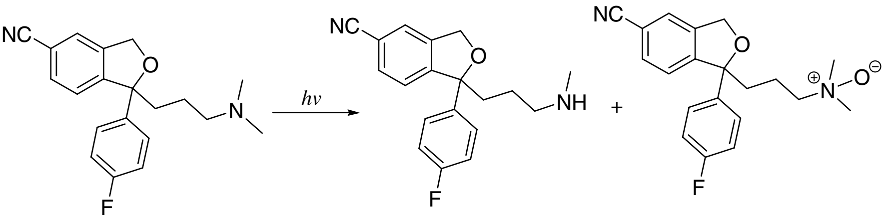
125: Escitalopram

Chemical name: (1*S*)-1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-3*H*-2-benzofuran-5 -carbonitrile
Brand name: Cipralex, Lexapro
Formula: $C_{20}H_{21}FN_2O$
Monoisotopic protonated ion (*m/z*): 325.1711
Molecular weight: 324.39
CAS number: 128196-01-0
Common salt form: Oxalate

Citalopram and its *S*-enantiomer escitalopram are selective serotonin reuptake inhibitors (SSRI), which are used clinically for the treatment of depression and anxiety. The drug molecule contains three degradable functional groups: the cyano, benzylic position, and tertiary amine groups. Hydrolysis of the cyano group should first yield the carboxamide degradant (**1**) [1, 2], which upon further hydrolysis should give the carboxylic acid degradant (**2**), especially under forced degradation conditions (Scheme 124–125-1) [2]. The benzylic position is susceptible to oxidation, particularly free radical-mediated oxidation, in which process the peroxide would be a plausible intermediate. Decomposition of the latter intermediate should give rise to the hydroxyl and keto degradants (**3** and **4**), respectively. Under a nucleophilic oxidative condition, the tertiary amine group can be oxidized to produce the *N*-oxide real degradant (**5**) [2].



Scheme 124–125-1 Degradation pathways of escitalopram.



Scheme 124–125-2 Photochemical degradation of citalopram.

In a photo degradation study of citalopram HBr in water with simulated sunlight over a period of 30 days, *N*-desmethylcitalopram (**6**) was observed as the major degradant, while citalopram *N*-oxide (**5**) was formed as the minor degradant (Scheme 124–125-2) [3].

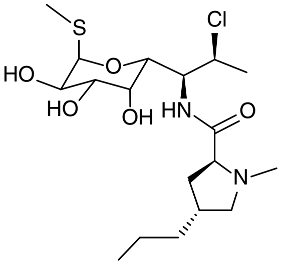
References

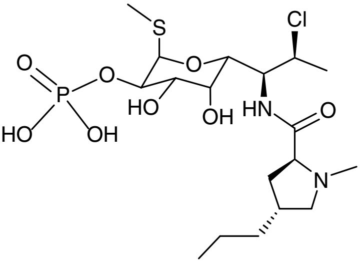
1 Sun, C., Xu, H., Pan, Y. et al. (2007). Characterization of the trace-level impurities in the bulk drug citalopram by high-performance liquid chromatography/tandem multistage mass spectrometry. *Rapid Commun. Mass Spectrom.* 21: 2889–2894.

2 Rao, R.N., Raju, A.N., and Narsimha, R. (2008). Isolation and characterization of degradation products of citalopram and process-related impurities using RP-HPLC. *J. Sep. Sci.* 31: 1729–1738.

3 Kwon, J.-W. and Armbrust, K.L. (2005). Degradation of citalopram by simulated sunlight. *Environ. Toxicol. Chem.* 24: 1618–1623.

126–127: Clindamycin, Clindamycin Phosphate


Clindamycin


Clindamycin-2-phosphate

126: Clindamycin

Chemical name: (2*S*,4*R*)-*N*-[(1*S*,2*S*)-2-Chloro-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide

Brand name: Acanya, Benzaclin, Cleocin

Formula: $C_{18}H_{33}ClN_2O_5S$

Common salt form: HCl

Monoisotopic protonated ion (*m/z*): 425.1871

Molecular weight: 424.98

CAS number: 18323-44-9

127: Clindamycin Phosphate (Clindamycin-2-Phosphate; Injectable Version of Clindamycin)

Chemical name: [(2*R*,3*R*,4*S*,5*R*,6*R*)-6-[(1*S*,2*S*)-2-Chloro-1-[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carbonyl]amino]propyl]-4,5-dihydroxy-2-methylsulfanyloxan-3-yl] dihydrogen phosphate

Brand name: Cleocin Phosphate

Formula: $C_{18}H_{34}ClN_2O_8PS$

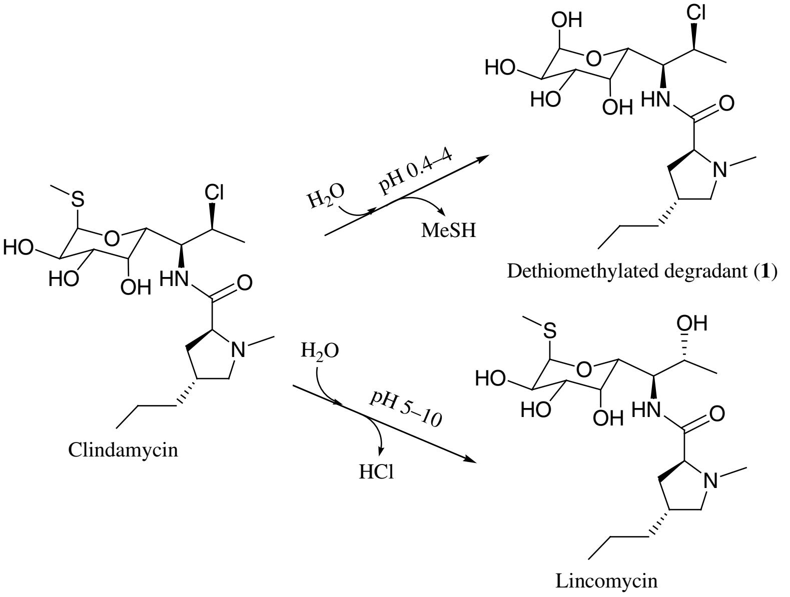
Monoisotopic protonated ion (*m/z*): 505.1535

Molecular weight: 504.96

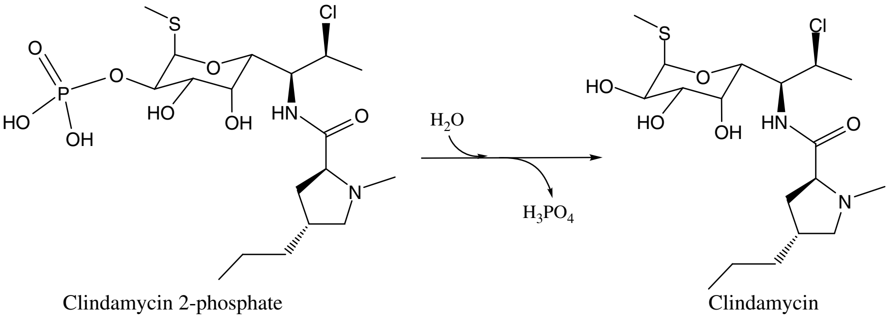
CAS number: 24729-96-2

Clindamycin is a lincosamide family antibiotics, which was developed based on lincomycin, a natural product and first member of the lincosamide family antibiotics.

Oesterling performed a comprehensive stability study of clindamycin solution in the pH range of 0.4–12 [1]; it showed that clindamycin is most stable at pH 3–5. In the acidic pH range of 0.4–4, the degradation of clindamycin is mainly the hydrolysis at the thioglycosidic linkage to produce 1-dethiomethyl-1-hydroxy clindamycin (**1**) and mercaptomethane (Scheme 126–127-1). The activation energy for the hydrolysis was found to be 38 kcal/mol. At pH 5–10, the displacement of the chloro substituent via hydrolysis became the main degradation pathway, producing



Scheme 126–127-1 Hydrolytic degradation of clindamycin.



Scheme 126–127-2 Hydrolysis of clindamycin 2-phosphate.

lincomycin as the degradant. The activation energy for this process was determined to be 29 kcal/mol. The relatively high values of both activation energies for the two hydrolytic degradation pathways suggest that both processes are not likely to take place under long-term or accelerated stability conditions.

Since the drug molecule contains both thiol ether and tertiary amine moieties, it should be susceptible to nucleophilic oxidation on both functional groups; nevertheless, no report has been found to suggest the presence of the sulfoxide and *N*-oxide degradants, even though the sulfoxide is a metabolite of clindamycin [2, 3].

Oesterling and Rowe conducted a kinetic study for the hydrolysis of clindamycin-2-phosphate, the injectable version of clindamycin, and found the activation energy was 32.9 kcal/mol [4], which is typical for the hydrolysis of an alkyl phosphate ester (Scheme 126–127-2) [5].

References

1 Oesterling, T.O. (1970). Aqueous stability of clindamycin. *J. Pharm. Sci.* 59: 63–67.

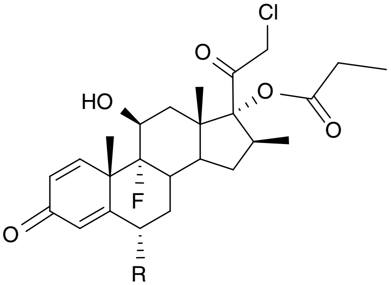
2 Sun, F.F. (1973). Metabolism of clindamycin II: urinary excretion products of clindamycin in rat and dog. *J. Pharm. Sci.* 62: 1657–1662.

3 Wynalda, M.A., Hutzler, J.M., Koets, M.D. et al. (2003). In vitro metabolism of clindamycin in human liver and intestinal microsomes. *Drug Metab. Dispos.* 31: 878–887.

4 Oesterling, T.O. and Rowe, E.L. (1970). Hydrolysis of lincomycin-2-phosphate and clindamycin-2-phosphate. *J. Pharm. Sci.* 59: 175–179.

5 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 16–47. Cambridge: Royal Society of Chemistry.

128–129: Clobetasol Propionate, Halobetasol Propionate



Clobetasol 17-propionate, R = H;

Halobetasol 17-propionate, R = F.

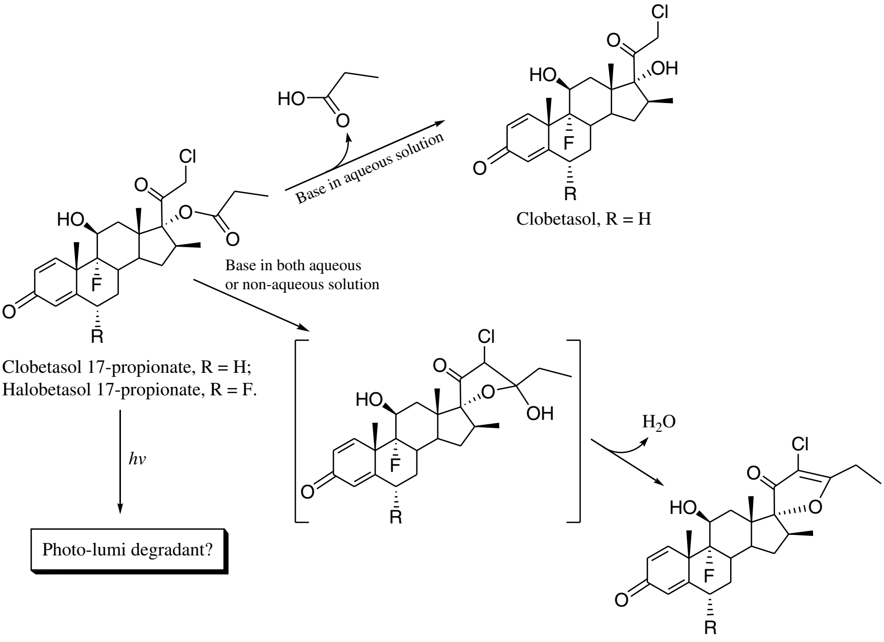
128: Clobetasol Propionate (Clobetasol 17-Propionate)

Chemical name: [(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-17-(2-Chloroacetyl)-9-fluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta [a]phenanthren-17-yl] propanoate
Brand name: Clobex, Clodan, Dermovate
Formula: $C_{25}H_{32}ClFO_5$
Monoisotopic protonated ion (*m/z*): 467.1995
Molecular weight: 466.97
CAS number: 25122-46-7

129: Halobetasol Propionate

Chemical name: [(6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-17-(2-Chloroacetyl)-6,9-difluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] propanoate
Brand name: Bryhali, Duobrii, Lexette, Ultravate
Formula: $C_{25}H_{31}ClF_2O_5$
Monoisotopic protonated ion (*m/z*): 485.1901
Molecular weight: 484.96
CAS number: 66852-54-8

Clobetasol propionate, or more specifically clobetasol 17-propionate, is a synthetic corticosteroid used for the topic treatment of various dermatologic disorders such as eczema and psoriasis. Despite being a drug product that has been on the market since the 1970s, there have been very few studies published on the degradation chemistry of clobetasol propionate. For the two forced degradation studies reported in the literature [1, 2], either no characterization of the degradants was performed [1] or only partial characterization was done [2]. The latter study, performed by Damle and Polawar, reported two base-catalyzed degradants, which appeared to be

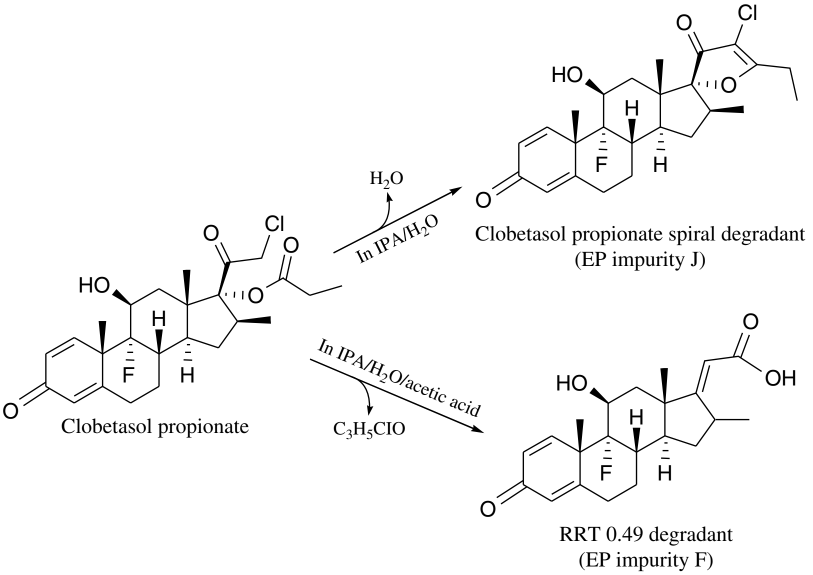


Scheme 128–129-1 Degradation pathways of clobetasol 17-propionate and halobetasol 17-propionate.

clobetasol (via cleavage of the ester linkage) and an unknown dehydrated product, respectively, based on LC-MS evidence. On the other hand, Cravotto et al. reported complete structure characterization of a dehydrated impurity (**1b**, Scheme 128–129-1) of halobetasol propionate (a structural analog of clobetasol propionate) that was also formed under base catalysis [3]. This forced degradation product (**1b**) resulted from condensation (via dehydration) between the two 17-substituents on the D-ring of the halobetasol steroid core (Scheme 128–129-1). Since clobetasol propionate and halobetasol propionate have identical substituents on the 17-position of the steroid cores, it would be reasonable to assume that the dehydrated degradant (**1a**), as observed by Damle and Polawar [2], was formed via the same Claisen-type condensation (Scheme 128–129-1). This type of dehydrative condensation was also observed with mometasone furoate, which has similar 17-position substituents [4].

It is noted that the base-catalyzed degradation of clobetasol propionate yielded two degradants [2], while that of halobetasol propionate produced only one degradant [3]. This difference could probably be attributed to the fact that different solvent systems were used in the base-catalyzed degradation studies: the study of clobetasol propionate used a mixture of methanol/0.1 N aqueous NaOH (10/1, v/v), while that of halobetasol propionate employed ethanol solution of K_2CO_3 in the absence of water.

In addition to the two 17-position substituents, the A-ring of the clobetasol propionate could also be susceptible to photochemical rearrangement, which is not



Scheme 128–129-2 Degradation pathways of clobetasol propionate leading to the formation of EP Impurity J and RRT 0.49 impurity (EP Impurity F) as the major degradant under each respective condition [7]. IPA, isopropanol. *Source:* Adapted from Tian et al. [7].

uncommon for corticosteroids containing the same A-ring such as betamethasone and its derivatives [5, 6].

In a stability study of clobetasol 17-propionate topical solution by Tian et al. [7], a major degradant was found, which was determined to be EP Impurity F through the use of LC-MS and NMR. The formation of this degradant was proposed to proceed through a Favorskii-like rearrangement mechanism under weakly acidic condition (Scheme 128–129-2). Even though this impurity is listed in the EP, there has been no clear indication that this impurity is a degradant of clobetasol 17-propionate in the literature.

References

1 Fauzee, A.F.B. and Walker, R.B. (2013). Forced degradation studies of clobetasol 17-propionate in methanol, propylene glycol, as bulk drug and cream formulations by RP-HPLC. *J. Sep. Sci.* 36: 849–856.

2 Damle, M.C. and Polawar, A.R. (2014). Stability indicating Hptlc method for the estimation of clobetasol propionate in presence of alkali induced degradation product. *Int. J. PharmTech Res.* 6: 1914–1925.

3 Cravotto, G., Giovenzana, G.B., Masciocchi, N. et al. (2007). A degradation product of halobetasol propionate – characterization and structure. *Steroids* 72: 787–791.

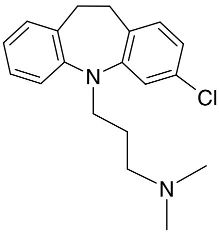
4 Sahasranaman, S., Issar, M., Toth, G. et al. (2004). Characterization of degradation products of mometasone furoate. *Pharmazie* 59: 367–373.

5 Hidaka, T., Huruumi, S., Tamaki, S. et al. (1980). *Yakugaku Zasshi* 100: 72–80.

6 Lin, M., Li, M., Buevich, A.V. et al. (2009). Rapid structure elucidation of drug degradation products using mechanism-based stress studies in conjunction with LC–MSn and NMR spectroscopy: identification of a photodegradation product of betamethasone dipropionate. *J. Pharm. Biomed. Anal.* 50: 275–280.

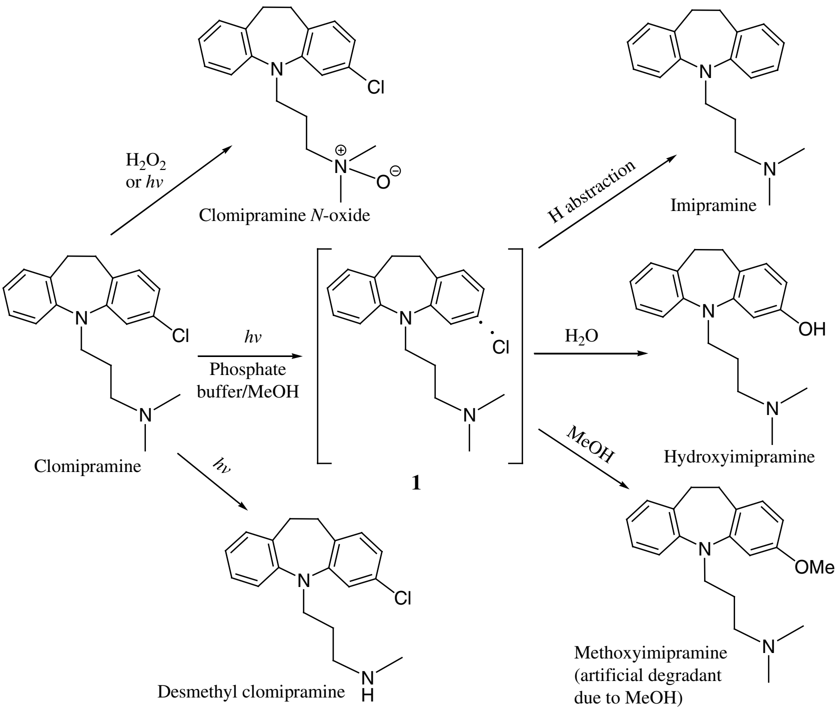
7 Tian, Y., Zhang, H., Zhu, Z. et al. A variation of Favorskii rearrangement mechanism under weakly acidic conditions: the case of clobetasol propionate degradation in solution. *Chemrxiv* https://doi.org/10.26434/chemrxiv.11809077.v1.

130: Clomipramine



Chemical name: 3-(2-Chloro-5,6-dihydrobenzo[b][1]benzazepin-11-yl)-*N*,*N*-dimethylpropan-1-amine
Brand name: Anafranil
Formula: $C_{19}H_{23}ClN_2$
Monoisotopic protonated ion (*m/z*): 315.1623
Molecular weight: 314.85
CAS number: 303-49-1
Common salt form: HCl salt

Clomipramine is a tricyclic antidepressant. It contains two likely degradable moieties: the aryl chloride and the tertiary amine. In a photo degradation study carried



Scheme 130 Degradation pathways of clomipramine.

out in a mixture of phosphate buffer and methanol, several degradants were isolated and identified [1]. It was proposed that the aryl chloride underwent homolytic cleavage to give the carbon-based radical intermediate (**1**), from which deschloro, hydroxyl, and methoxyl degradants were formed (Scheme 130). Clomipramine *N*-oxide was also found among the photo degradants, although this degradant can also be formed under non-photo degradation conditions. Due to the difference in irradiation sources, solvents, and length of the irradiation, the formation and distribution of photo degradants may be different. In a photo degradation study of clomipramine performed in pure water as the solvent, only the hydroxyl degradant was observed [2], while in another photo degradation study, desmethyl clomipramine was detected [3].

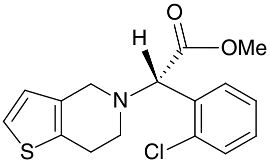
References

1 Canudas, N. and Contreras, C. (2002). Isolation and identification of the photodegradation products of the photosensitizing antidepressant drug clomipramine. Phototoxicity studies on erythrocytes. *Pharmazie* 57: 405–408.

2 Nassar, R., Trivella, A., Mokh, S. et al. (2017). Photodegradation of sulfamethazine, sulfamethoxypiridazine, amitriptyline, and clomipramine drugs in aqueous media. *J. Photochem. Photobiol. A Chem.* 336: 176–182.

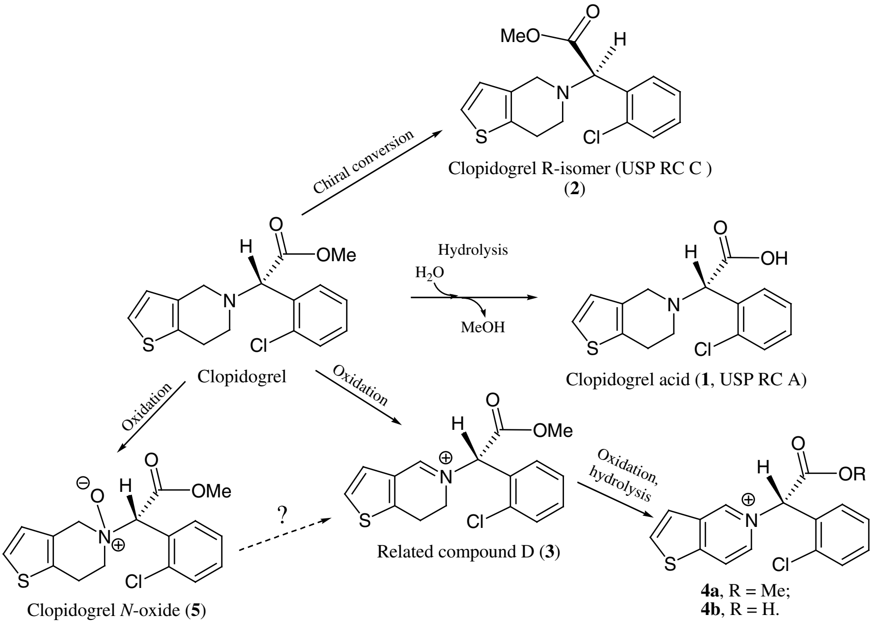
3 Székely, P., Gyéresi, A., Hancu, G. et al. (2011). Study regarding the phototoxicity of some tricyclic antidepressants. *Acta Med. Marisiensis* 57: 745–747.

131: Clopidogrel



Chemical name: methyl (2*S*)-2-(2-Chlorophenyl)-2-(6,7-dihydro-4*H*-thieno[3,2-c] pyridin-5-yl)acetate
Brand name: Plavix
Formula: $C_{16}H_{16}ClNO_2S$
Monoisotopic protonated ion (*m/z*): 322.0663
Molecular weight: 321.82
CAS number: 113665-84-2
Common salt form: Bisulfate

Clopidogrel is an antiplatelet drug, which was marketed by Sanofi–Synthelabo and Bristol-Myers Squibb worldwide under the brand names PLAVIX® and ISCOVER® and was the second best-selling drug prior to its patent expiration. Structurally, clopidogrel is a chiral drug containing a single chiral center and a methyl ester functionality. As such, clopidogrel would be expected to undergo hydrolysis and may be susceptible to racemization, leading to the formation of clopidogrel acid (**1**) and the R-isomeric degradant (**2**, Scheme 131); indeed, in an accelerated stability study of the original brand name drug product versus several generic products on the market, **1** and **2** were observed as the main degradants [1]. A degradation kinetic study of clopidogrel in methanolic phosphate buffers within the pH range of 3.0–9.0 indicated that the chiral conversion was slowest at pH 3.0, while the hydrolytic degradation was slowest at pH 5.6 [2]. In an investigation performed by Mohan et al., an oxidative degradant present in clopidogrel drug substance and product was characterized (**3**) [3, 4]. This degradant (**3**) could undergo further oxidation to form degradant **4a**; the hydrolyzed version of **4a**, namely **4b**, appeared to be present in an accelerated stability study of amorphous clopidogrel [5]. In a forced degradation study of clopidogrel with H_2O_2, the presence of clopidogrel *N*-oxide was reported, but its structure was not fully characterized due to its instability [6].



Scheme 131 Degradation pathways of clopidogrel.

References

1 Gomez, Y., Adams, E., and Hoogmartens, J. (2004). Analysis of purity in 19 drug product tablets containing clopidogrel: 18 copies versus the original brand. *J. Pharm. Biomed. Anal.* 34: 341–348.

2 Reist, M., Roy-de Vos, M., Montseny, J.-P. et al. (2000). Very slow chiral inversion of clopidogrel in rats: a pharmacokinetic and mechanistic investigation. *Drug Metab. Dispos.* 28: 1405–1410.

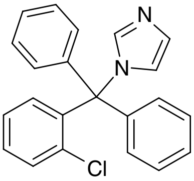
3 Mohan, A., Hariharan, M., Vikraman, E. et al. (2008). Identification and characterization of a principal oxidation impurity in clopidogrel drug substance and drug product. *J. Pharm. Biomed. Anal.* 47: 183–189.

4 Li, M. (2012). *Organic Chemistry of Drug Degradation*, 64–65. Cambridge: Royal Society of Chemistry.

5 Raijada, D.K., Prasad, B., Paudel, A. et al. (2010). Characterization of degradation products of amorphous and polymorphic forms of clopidogrel bisulphate under solid state stress conditions. *J. Pharm. Biomed. Anal.* 52: 332–344.

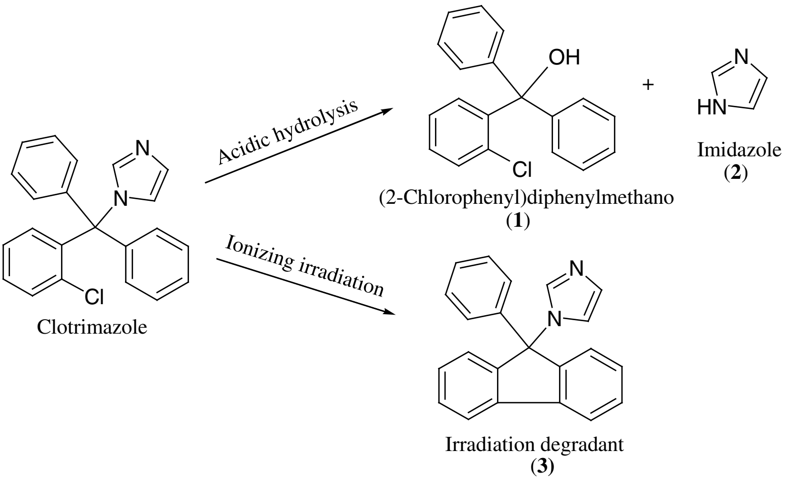
6 Mashelkar, U.C. and Renapurkar, S.D. (2010). A LCMS compatible stability-indicating HPLC assay method for clopidogrel bisulphate. *Int. J. ChemTech Res.* 2: 822–829.

132: Clotrimazole



Chemical name: 1-[(2-Chlorophenyl)diphenylmethyl]imidazole
Brand name: Lotrimin
Formula: $C_{22}H_{17}ClN_2$
Monoisotopic protonated ion (*m/z*): 345.1153
Molecular weight: 344.84
CAS number: 23593-75-1

Clotrimazole is an antifungal drug. Its main degradation pathway is of hydrolytic nature to give (2-chlorophenyl)diphenylmethanol (**1**) and imidazole (**2**, Scheme 132); this degradation is more significant at low pH [1, 2], at which the solubility of the drug is greatly enhanced as the pK of the drug is 4.7. On the other hand, clotrimazole appeared to be stable under basic conditions; in a clotrimazole solution in 0.5 N NaOH, no degradation was observed after the solution was heated at 80°C for 8 hours [3]. In an ionizing irradiation study, an intramolecular crosslinked impurity (**3**) was found as the only degradant [4].



Scheme 132 Degradation pathways of clotrimazole.

References

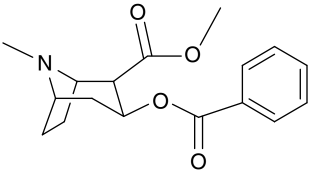
1 Solich, P., Hajkova, R., Pospisilov, M., and Sicha, J. (2002). Determination of methylparaben, propylparaben, clotrimazole and its degradation products in topical cream by RP-HPLC. *Chromatographia* 56 (Suppl): S181–S184.

2 Hajkova, R., Sklenarova, H., Matysova, L. et al. (2007). Development and validation of HPLC method for determination of clotrimazole and its two degradation products in spray formulation. *Talanta* 73: 483–489.

3 Borhade, V., Pathak, S., Sharma, S., and Patravale, V. (2012). Clotrimazole nanoemulsion for malaria chemotherapy. Part II: stability assessment, in vivo pharmacodynamic evaluations and toxicological studies. *Int. J. Pharm.* 431: 149–160.

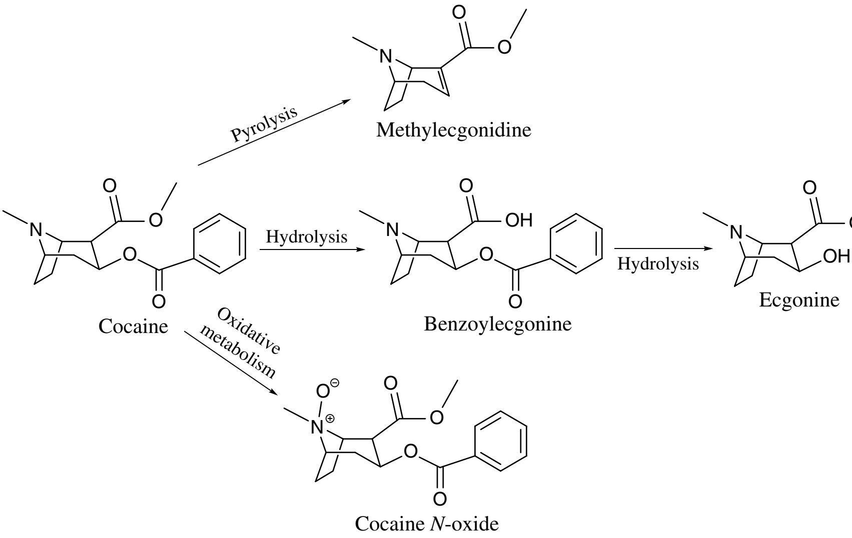
4 Marciniec, B., Dettlaff, K., and Naskrent, M. (2009). Influence of ionising irradiation on clotrimazole in the solid state. *J. Pharm. Biomed. Anal.* 50: 675–678.

133: Cocaine



Chemical name: Methyl (1*R*,2*R*,3*S*,5*S*)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1] octane-2-carboxylate
Brand name: Goprelto, Numbrino
Formula: $C_{17}H_{21}NO_4$
Monoisotopic protonated ion (*m/z*): 304.1543
Molecular weight: 303.35
CAS number: 50-36-2
Common salt form: HCl

Despite being the second most abused controlled substance on a global scale, only next to cannabis, cocaine does have a few legitimate medical applications. It contains two ester groups and one tertiary amine group, which should be susceptible to hydrolytic and oxidative degradation, respectively. In a study assessing hydrolytic stability of cocaine in aqueous solutions of pH 2.2, 5.0, and 6.8, the activation energies for the hydrolysis were found to be 97.9, 107.3, and 95.9 kJ/mol (or 23.4, 25.6, and 22.9 kcal/mol), respectively, at each pH [1]. Hence, in the pH range studied, cocaine was most hydrolytically stable at pH 5.0. From a structural perspective, one may expect that the methyl ester functionality would be much more susceptible to



Scheme 133 Degradation pathways of cocaine.

hydrolysis than the benzoyl ester functionality [2]. Indeed, a study of cocaine stability in blood samples demonstrated that only the methyl ester was hydrolyzed to produce benzoylecgonine under non-enzymatic conditions [3]. Interestingly, under pyrolysis conditions where the solid cocaine was subjected to temperatures in the order of several hundred °C, elimination became a main route of degradation, in which methylecgonidine was formed (Scheme 133) [4]. Obviously, such a high temperature employed in pyrolysis is usually not encountered in drug degradation studies. Technically, the tertiary amine moiety of cocaine could be oxidized to give cocaine *N*-oxide, a metabolite of the drug [5]; nevertheless, it has not been reported as a degradant in a pharmaceutical preparation.

References

1 Murray, J.B. and Al-Shora, H.I. (1978). Stability of cocaine in aqueous solution. *J. Clin. Pharm. Therap.* 3: 1–6.

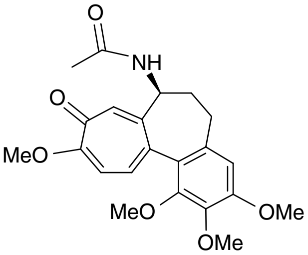
2 Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 16–47. Cambridge: Royal Society of Chemistry.

3 Isenschmid, D.S., Levine, B.S., and Caplan, Y.H. (1989). A Comprehensive study of the stability of cocaine and its metabolites. *J. Anal. Toxicol.* 13: 250–256.

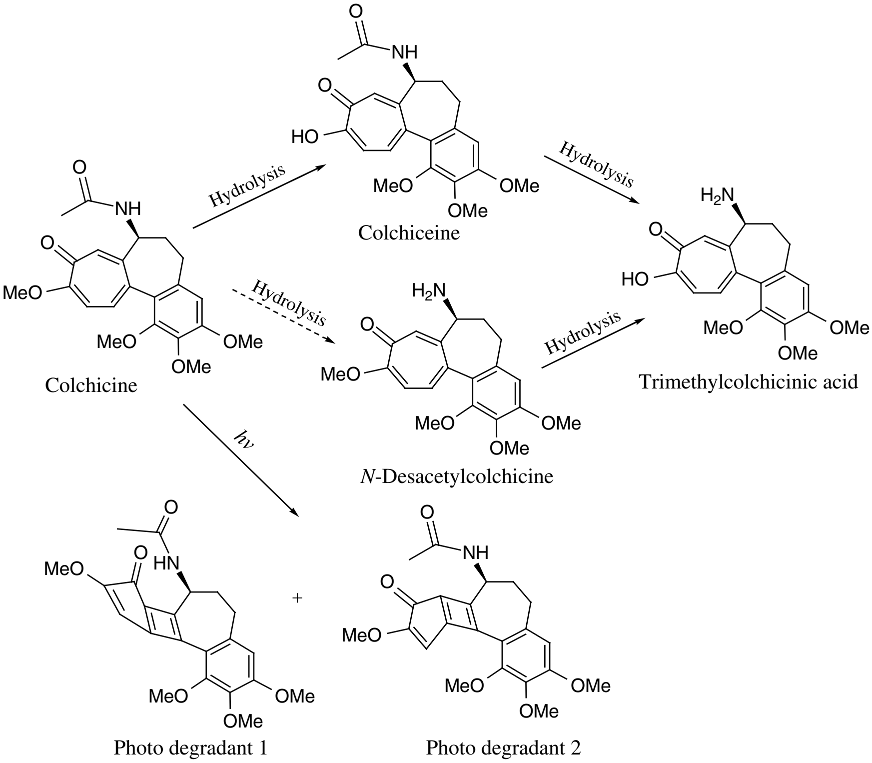
4 Cook, C.E. and Jeffcoat, A.R. National institute on drug abuse. In: *Research Monograph*, vol. 99, 97–120.

5 Wang, P.P. and Bartlett, M.G. (1999). Identification and quantitation of cocaine *N*-oxide: a thermally labile metabolite of cocaine. *J. Anal. Toxicol.* 23: 62–66.

134: Colchicine



Chemical name: *N*-[(7*S*)-1,2,3,10-Tetramethoxy-9-oxo-6,7-dihydro-5*H*-benzo[a]heptalen-7-yl]acetamide
Brand name: Colcrys, Gloperba, Mitigare
Formula: $C_{22}H_{25}NO_6$
Monoisotopic protonated ion (*m/z*): 400.1755
Molecular weight: 399.44
CAS number: 64-86-8



Scheme 134 Degradation pathways of colchicine.

Colchicine, a natural product originally isolated from plants of the genus *Colchicum*, is usually used for treating gout; the description of *Colchicum* extract for medicinal usage dates back as early as 1500 BC [1]. The first effort to elucidate the structure of colchicine appears to have occurred in 1924 [2]. Its degradation chemistry consists of hydrolytic and photochemical degradation pathways (Scheme 134), which were well summarized by Lacey and Brady [3]. When heated in 0.1 N HCl at 100°C for 1 hour, the tropolone methyl ether linkage can be mostly cleaved to produce colchiceine [4] and possibly other degradants, such as trimethylcolchicinic acid. Under photo irradiation, colchicine undergoes [2 + 2] photo cyclization to yield two isomeric degradants.

References

1 Colchicine. https://en.wikipedia.org/wiki/Colchicine (accessed 26 February 2017).

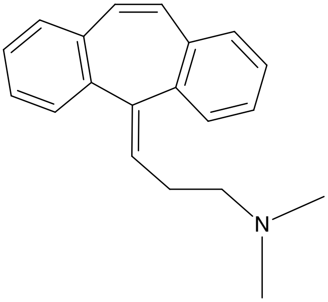
2 Dewar, M.J.S. (1945). The structure of colchicine. *Nature* 155: 141–142.

3 Lacey, E. and Brady, R.L. (1984). Separation of colchicine and related hydrolysis and photodecomposition products by high-performance liquid chromatography, using copper ion complexation. *J. Chrom. A.* 315: 233–241.

4 Borisy, G.G. and Taylor, E.W. (1967). The mechanism of action of colchicine. *J. Cell Biol.* 34: 525–533.

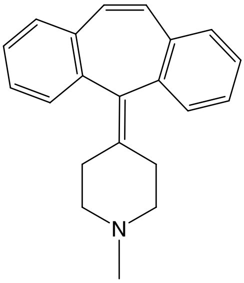
135–136: Cyclobenzaprine, Cyproheptadine

135: Cyclobenzaprine



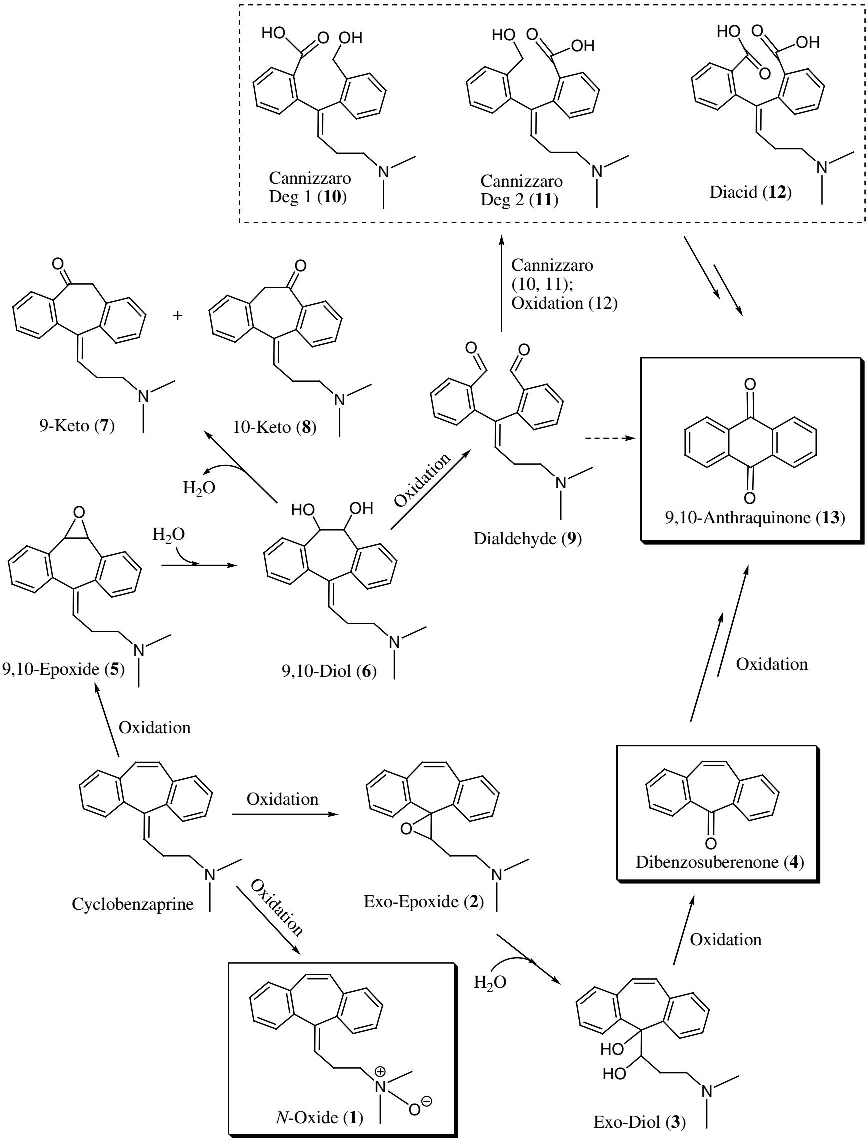
Chemical name: *N,N*-Dimethyl-3-(2-tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,9,11,13-heptaenylidene)-propan-1-amine
Brand name: Amrix, Fexmid, Flexeril
Formula: $C_{20}H_{21}N$
Common salt form: HCl
Monoisotopic protonated ion (*m/z*): 276.1747
Molecular weight: 275.39
CAS number: 303-53-7

136: Cyproheptadine

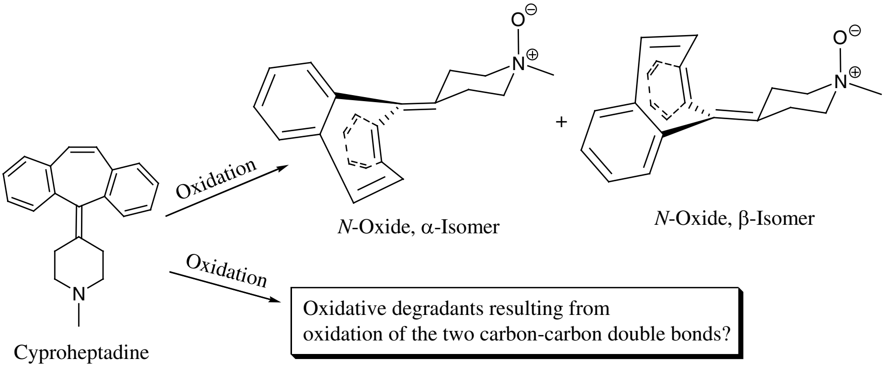


Chemical name: 1-Methyl-4-(2-tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,9,11,13-heptaenylidene)piperidine
Brand name: Periactin
Formula: $C_{21}H_{21}N$
Common salt form: HCl
Monoisotopic protonated ion (*m/z*): 288.1747
Molecular weight: 287.40
CAS number: 129-03-3

Cyclobenzaprine is used to relieve skeletal muscle spasm of local origin [1]. At the core of the molecule is a highly conjugated tricyclic ring with two phenyl groups fused together by an endocyclic and exocyclic carbon–carbon double bond. Being an electron-rich molecule, the two carbon–carbon double bonds, as well as the tertiary amine moiety of cyclobenzaprine are susceptible to oxidative degradation. Liu et al. performed a comprehensive forced degradation study of cyprobenzaprine [2]; more than a dozen oxidative degradants were observed (Scheme 135–136-1). Many of these degradants may not be very stable; nevertheless, cyclobenzaprine *N*-oxide, dibenzosubrenone, and 9,10-anthraquinone seem to be more stable than others and their presence in a topic formulation of cyclobenzaprine HCl, piroxicam, and



Scheme 135–136-1 Degradation pathways of cyclobenzaprine.



Scheme 135–136-2 Degradation pathways of cyproheptadine.

lidocaine was reported [3]. In an experimental tablet formulation, anthraquinone was formed as the primary degradant, although trace amounts of other oxidative degradants were also seen [4]. The oxidation could be suppressed by adding antioxidant, BHA, into the formulation.

Cyproheptadine, a first-generation antihistamine, has a structure very similar to that of cyclobenzaprine. Consequently, it would be reasonable to assume that it has very similar degradation pathways. Nevertheless, there have been very few reports regarding its degradation chemistry. In a study by Christy et al., it was reported that cyproheptadine can be oxidized by hydrogen peroxide, and the resulting *N*-oxide has two stereoisomers, the α and β-isomers (Scheme 135–136-2) [5].

On the other hand, amitriptyline, a tricyclic antidepressant, is structurally identical to cyprobenzaprine, except that it has a saturated 9,10-double bond. For the discussion of its degradation chemistry, refer to the monograph for amitriptyline and nortriptyline.

References

1 FDA Approval package for Flexeril. 2003. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/017821_S045_FLEXERIL.pdf (accessed 3 March 2017).

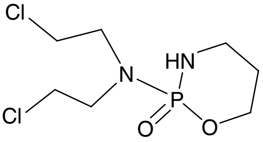
2 Liu, Y., Zhao, D., and Zhao, Z.Z. (2014). Study of degradation mechanisms of cyclobenzaprine by LC-MS/MS. *Anal. Methods* 6: 2320–2330.

3 Cioroiu, B.I., Grigoriu, I.C., Cioroiu, M.E. et al. (2016). Determination of degradation products of cyclobenzaprine hydrochloride, lidocaine and piroxicam in a semi-topical formulation: MS–MS confirmation of unknown impurities. *J. Chromatogr. Sci.* 54: 902–911.

4 Cotton, M.L. and Down, G.R.B. Cyclobenzaprine. In: *Anal. Prof. Drug Subs.*, vol. 17 (ed. K. Florey), 41–72. Elsevier.

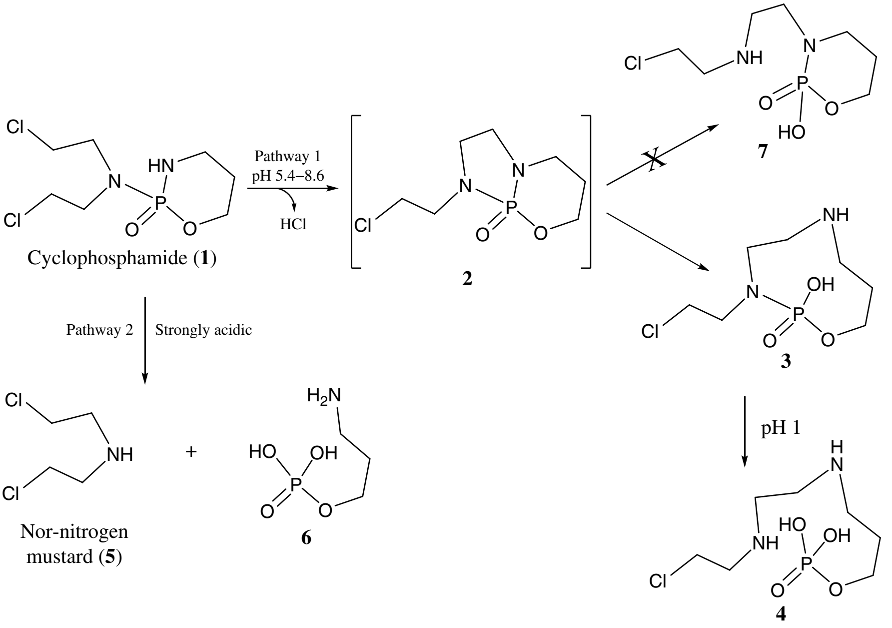
5 Christy, M.E., Anderson, P.S., Arison, B.H. et al. (1977). Stereoisomerism of cyproheptadine *N*-oxide. *J. Organomet. Chem.* 42: 378–379.

137: Cyclophosphamide [WHO List of Essential Medicines]

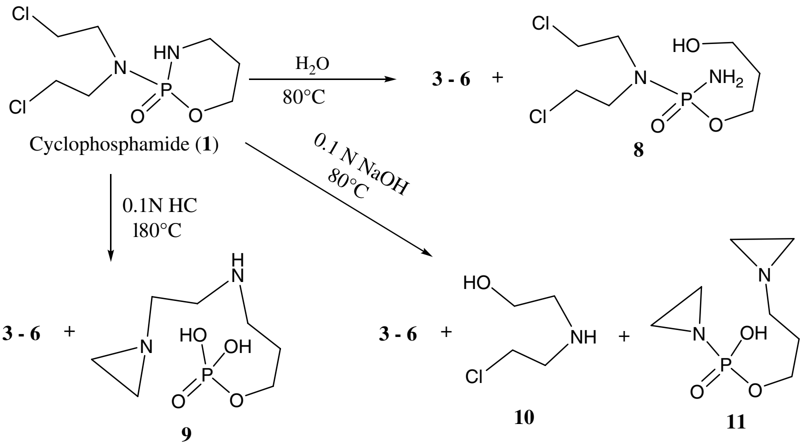


Chemical name: (*RS*)-*N*,*N*-Bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide
Brand name: Procytox
Formula: $C_7H_{15}Cl_2N_2O_2P$
Monoisotopic protonated ion (*m/z*): 261.0321
Molecular weight: 261.09
CAS number: 50-18-0

Cyclophosphamide, a pro-drug of nitrogen mustard, is the prototype of the drug family of oxazaphosphorine cytostatics. It was developed by Brock and his research team at ASTA Pharma in the 1950s [1]. Its clinical use includes the treatment of cancers and autoimmune diseases. The degradation chemistry of cyclophosphamide in aqueous solutions has been studied a few times, and those performed by ASTA Pharma are illustrated in Scheme 137-1 [2, 3]. In general, the degradation of cyclophosphamide is of hydrolytic nature, and the formation and distribution of the degradants depends upon the nature of solvents, pH, temperature, and duration of



Scheme 137-1 Degradation pathways of cyclophosphamide based on the results from references 2 and 3.



Scheme 137-2 Degradation pathways of cyclophosphamide in solution of water, 0.1 N HCl and 0.1 N NaOH at 80°C for 24 hours.

the degradation studies. In solutions with pH between 5.4 and 8.6, cyclophosphamide underwent intramolecular alkylation to form a transient intermediate (**2**), which immediately decomposed to produce the degradant with a 9-membered ring (**3**, Pathway 1). Upon further treatment in pH 1 solution, the latter degradant decomposed to yield phosphoric acid monoester **4**. In strongly acidic solutions, cyclophosphamide degraded to give *nor*-nitrogen mustard (**5**) and phosphoric acid monoester **6** (Pathway 2); at pH 1.2 and 37°C, the half-life of the degradation was 1.4 days. In the pH range of 2.2–3.4, both Pathways 1 and 2 coexist; in solid-state degradation, where the samples were heated to the melting temperature, degradation proceeded predominantly via Pathway 1 over Pathway 2 [3].

In a more recent forced degradation study [4], cyclophosphamide was subjected to solutions of 0.1 N HCl, 0.1 N NaOH, and water, respectively, at 80°C for 24 hours. While Degradant **8** was observed under all forced degradation conditions (Scheme 137-2), Degradant **9** was in 0.1 N HCl solution, and Degradants **10** and **11** were observed in 0.1 N NaOH solution. The structural elucidation was based on accurate MS and MS/MS.

References

1 Brock, N. (1989). Oxazaphosphorine cytostatics: past-present-future. Seventh Cain memorial award lecture. *Cancer Res.* 49: 1–7.

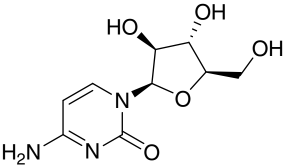
2 Gilard, V., Martino, R., Malet-Martino, M.-C. et al. (1994). Chemical and biological evaluation of hydrolysis products of cyclophosphamide. *J. Med. Chem.* 37: 3986–3993.

3 Niemeyer, U., Kutscher, B., Engel, J. et al. Degradation products of cyclophosphamide synthesis and structural studies. *Phosphorus Sulfur Silicon Relat. Elem.* 109: 473–476.

4 Shivakumar, G. and Dwivedi, J. (2015). Identification of degradation products in cyclophosphamide API by LC-QTOF mass spectrometry. *J. Liq. Chromatogr. Relat. Technol.* 38: 190–195.

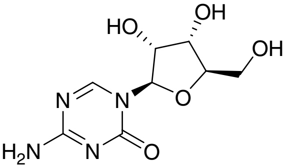
138–140: Cytarabine, Azacitadine, Decitabine

138: Cytarabine [WHO List of Essential Medicines]



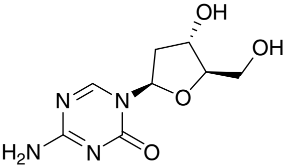
Chemical name: 1β-Arabinofuranosylcytosine; 4-Amino-1-[(2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one;
Brand name: Cytosar, Vyxeos
Formula: $C_9H_{13}N_3O_5$
Monoisotopic protonated ion (*m/z*): 244.0928
Molecular weight: 243.22
CAS number: 147-94-4
Common salt form: HCl salt

139: Azacitidine (Azacytidine)



Chemical name: 4-Amino-1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-one
Brand name: Onureg, Vidaza
Formula: $C_8H_{12}N_4O_5$
Monoisotopic protonated ion (*m/z*): 245.0880
Molecular weight: 244.21
CAS number: 320-67-2

140: Decitabine



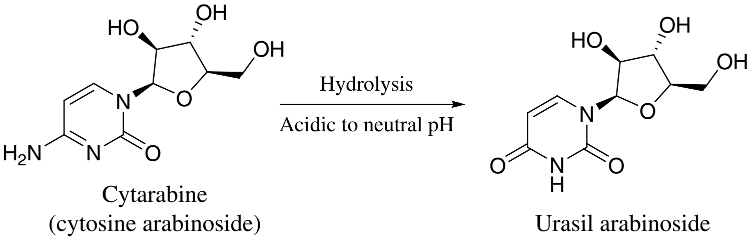
Chemical name: 4-Amino-1-[(2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2- one

Brand name: Dacogen
Formula: $C_8H_{12}N_4O_4$
Monoisotopic protonated ion (*m/z*): 229.0931
Molecular weight: 228.21
CAS number: 2353-33-5

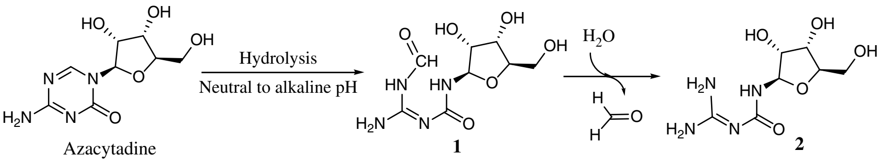
Cytarabine, azacytadine, and decitabine are analogs of the nucleoside cytidine and are clinically used for the treatment of certain types of cancers, particularly leukemia. Cytarabine was first synthesized in 1959, as a result of the research following the discovery that the structurally similar spongonucleosides, isolated from the sponge, *Cryptotethia crypta*, possess anticancer activity [1]; it was approved for clinical use in 1969 [2]. Azacitidine and its 2′-deoxy derivative, decitabine, which were discovered in the mid-1970s [3], were approved by the FDA in 2004 and 2006, respectively, for the treatment of myelodysplastic syndromes (MDS) [4, 5], conditions that can progress to acute myeloid leukemia (AML).

The main degradation pathways of cytarabine, azacytadine, and decitabine are of hydrolytic nature. In aqueous buffered solutions with acidic to neutral pH, cytarabine primarily underwent hydrolytic deamination to produce uracil arabinoside (Scheme 138–140-1) [6]. The degradant, uracil arabinoside, was present in a sterile powder of cyrarabine in low level (0.05%) [7], which was lower than the USP specification of 0.30% for this impurity [8]. Cytarabine was found to be most stable at pH 6.9 [9].

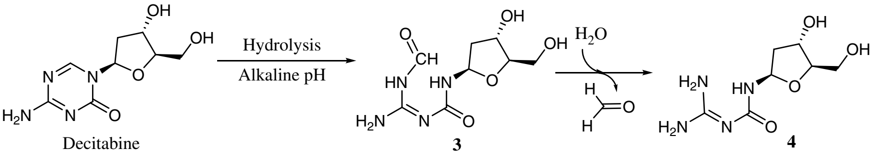
In neutral to alkaline solutions, the triazine of azacytadine would also be susceptible to hydrolytic degradation, resulting in the formation of *N*-(formylaminine)-*N*′-β-D-ribofuranosylurea (**1**) and its further des-formyl degradant (**2**, Scheme 138–140-2) [7]. This degradation pathway was also observed in a stability study of reconstituted azacitidine suspensions for injection [10].



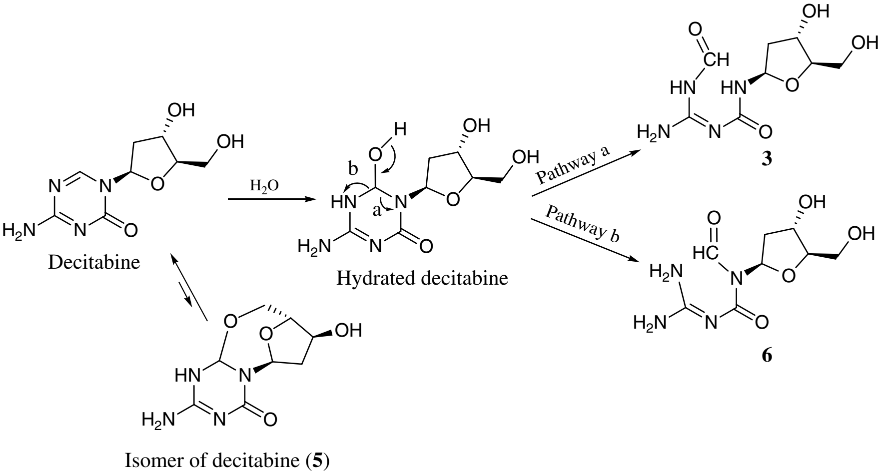
Scheme 138–140-1 Hydrolysis of cytarabine in acidic to neutral solutions.



Scheme 138–140-2 Hydrolysis of azacytadine in neutral to alkaline solutions.



Scheme 138–140-3 Hydrolysis of decitabine in alkaline solutions.



Scheme 138–140-4 Stability of decitabine in water solution. Illustrated above are the more reasonable structures (**5** and **6**) among those proposed by Liu et al. [13] for the respective isomers of decitabine and **3**. *Source:* Liu et al. [13]/John Wiley & Sons.

Decitabine, the 2′-deoxyl derivative of azacytadine, would be expected to behave similarly in terms of hydrolytic stability with regard to the triazine moiety as compared to azacytadine. Indeed, in a study performed by Lin et al. [11], decitabine was found to undergo the same sequential decomposition of the triazine moiety in alkaline solution (Scheme 138–140-3).

Nevertheless, in a pH 7.4 phosphate buffer or water, the degradation profile of decitabine was quite different from that of azacytadine: in addition to the two degradants due to the triazine ring-opening, additional unidentified degradant peaks were observed [11]. During a stability study of reconstituted decitabine solutions [12], a degradation profile similar to that observed in the pH 7.4 solution [11] was seen. In a 2006 study of decitabine stability in water by Liu et al. [13], two of the previously unidentified major degradants were found to be isomers of decitabine and **3**, respectively. Among the structures proposed by Liu et al., it appears that the following two structures (**5** and **6**) are more reasonable for the respective isomers of decitabine and **3** (Scheme 138–140-4) than the others.

References

1 Lichtman, M.A. (2013). A historical perspective on the development of the cytarabine (7 days) and daunorubicin (3 days) treatment regimen for acute myelogenous leukemia: 2013 the 40th anniversary of 7+3. *Blood Cells Mol. Dis.* 50: 119–130.

2 Cytarabine. https://en.wikipedia.org/wiki/Cytarabine (accessed 30 August 2022).

3 Cihák, A. (1974). Biological effects of 5-azacytidine in eukaryotes. *Oncology* 30: 405–422.

4 Azacitidine. https://en.wikipedia.org/wiki/Azacitidine (accessed 30 August 2022).

5 Decitabine. https://go.drugbank.com/drugs/DB01262 (accessed 30 August 2022).

6 Notari, R.E. (1967). A mechanism for the hydrolytic deamination of cytosine arabinoside in aqueous buffer. *J. Pharm. Sci.* 56: 804–809.

7 Kissinger, L.D. and Stemm, N.L. (1986). Determination of the antileukemia agents cytarabine and azacitidine and their respective degradation products by high-performance liquid chromatography. *J. Chromatogr.* 353: 309–318.

8 Cytarabine (2020). USP Monograph, official as of 01 May 2020. https://doi.org/10.31003/USPNF_M21810_05_01 (accessed 30 August 2022).

9 Notari, R.E., Chin, M.L., and Wittebort, R. (1972). Arabinosylcytosine stability in aqueous solutions: pH profile and shelflife predictions. *J. Pharm. Sci.* 61: 1189–1196.

10 Legeron, R., Xuereb, F., Djabarouti, S. et al. (2013). Chemical stability of azacitidine suspensions for injection after cold-chain reconstitution of powder and storage. *Am. J. Health-Sys. Pharm.* 70: 2137–3142.

11 Lin, K.-T., Momparler, R.L., and Rivard, G.E. (1981). High-performance liquid chromatographic analysis of chemical stability of 5-aza-2′-deoxycytidine. *J. Pharm. Sci.* 70: 1228–1232.

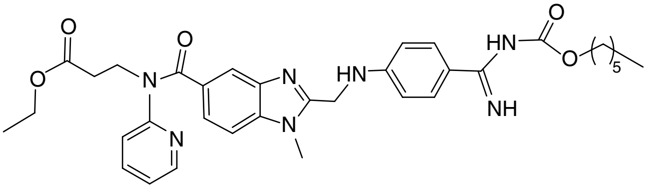
12 Kim, S.H., Heeb, R.M., and Krämer, I. (2017). Physicochemical stability of reconstituted Decitabine (Dacogen®) solutions and ready-to-administer infusion bags when stored refrigerated or frozen. *Pharm. Tech. Hosp. Pharm.* 2: 145–157. https://doi.org/10.1515/pthp-2017-0025.

13 Liu, Z., Marcucci, G., Byrd, J.C. et al. (2006). Characterization of decomposition products and preclinical and low dose clinical pharmacokinetics of decitabine (5-aza-2′-deoxycytidine) by a new liquid chromatography/tandem mass spectrometry quantification method. *Rapid Commun. Mass Spectrom.* 20: 1117–1126.

5

Dabigatran Etexilate to Duloxetine

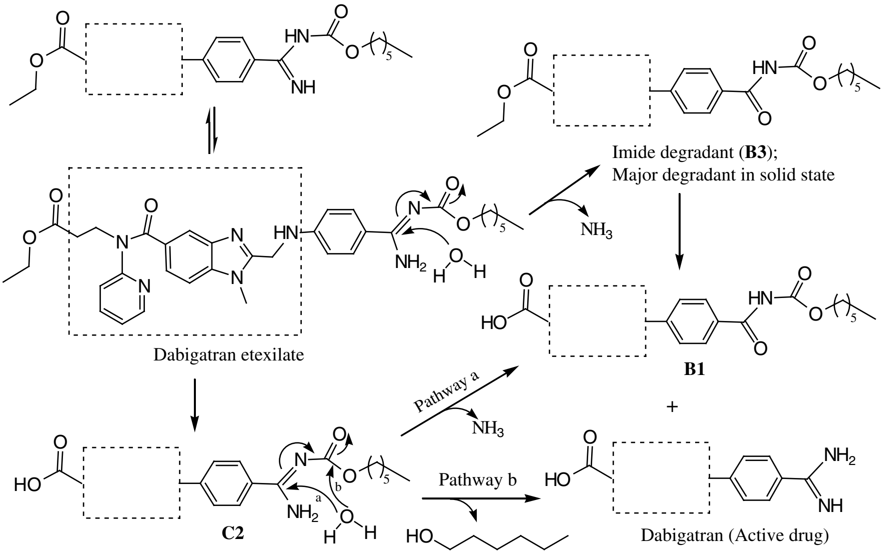
141: Dabigatran Etexilate



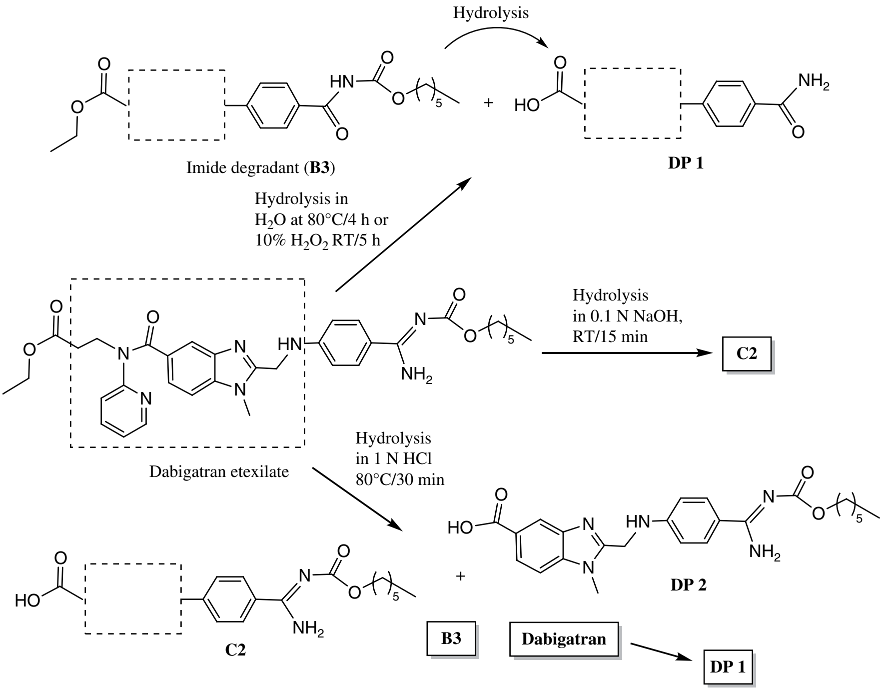
Chemical name: Ethyl 3-[[2-[[4-(*N*-hexoxycarbonylcarbamimidoyl)anilino] methyl]-1-methylbenzimidazole-5-carbonyl]-pyridin-2-ylamino]propanoate
Brand name: Pradaxa
Formula: $C_{34}H_{41}N_7O_5$
Monoisotopic protonated ion (*m/z*): 628.3242
Molecular weight: 627.73
CAS number: 1610666-09-5
Common salt form: Mesylate

Dabigatran etexilate is the prodrug of dabigatran, which itself is a direct thrombin inhibitor, used as an oral anticoagulant. The function of the prodrug is achieved via the two functionalities: the ethyl ester and carbamate, both of which would be susceptible to hydrolytic degradation. As the other parts of the drug molecule seem to be reasonably stable, the main degradation pathways of dabigatran etexilate would be hydrolytic. In a patent application [1], Miralles et al. described a few hydrolytic degradation products observed from stability studies of several tablet formulations of dabigatran etexilate (Scheme 141-1). The major one, the imide degradant (**B3**), was not reported before. Such a degradation profile in the solid state is apparently quite different from the in vivo study, where the active drug, dabigatran, is the main product from the hydrolysis of dabigatran etexilate [2]. It seems that the imide degradant **B3** tended to form rather easily, apparently more easily than **C2**, which results from hydrolysis of the ethyl ester moiety, in the solid formulations as reported

Compendium of Drug Degradation Pathways, First Edition. Min Li.



Scheme 141-1 Hydrolytic degradation pathways of formulated dabigatran etexilate in solid dosage. The nomenclature of the degradants is that used by Miralles et al. [1].



Scheme 141-2 Forced degradation of dabigatran etexilate solutions under different conditions.

in the patent application, particularly if the formulated product lacked proper protection from moisture by its packaging.

On the other hand, in certain solution forced degradation studies [3, 4], the degradation profiles (Scheme 141-2) were different from that obtained in the solid state [1]. Under the harsh conditions of these forced degradation studies, another two hydrolytic degradants, i.e., DP 1 and DP 2, were also observed in addition to B3 and C2. DP 1 could be a direct degradant from dabigatran etexilate or a secondary degradant from the imide degradant (B3), while DP 2 results from the hydrolysis of the amide linkage of the drug molecule.

References

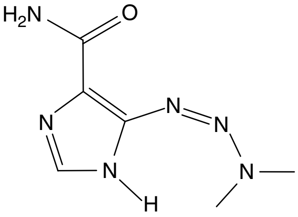
1 Miralles, J.S., Lépez, E.D., and Rao, L. US Patent Application, US 2014/0076036 A1.

2 Laizure, S.C., Parker, R.B., Herring, V.L., and Hu, Z.-Y. (2014). Identification of carboxylesterase-dependent dabigatran etexilate hydrolysis. *Drug Metab. Dispos.* 42: 201–206.

3 Swain, D., Patel, P.N., Nagaraj, G. et al. Liquid chromatographic method development for forced degradation products of dabigatran etexilate: characterisation and in silico toxicity evaluation. *Chromatographia* https://doi.org/10.1007/s10337-015-3009-5.

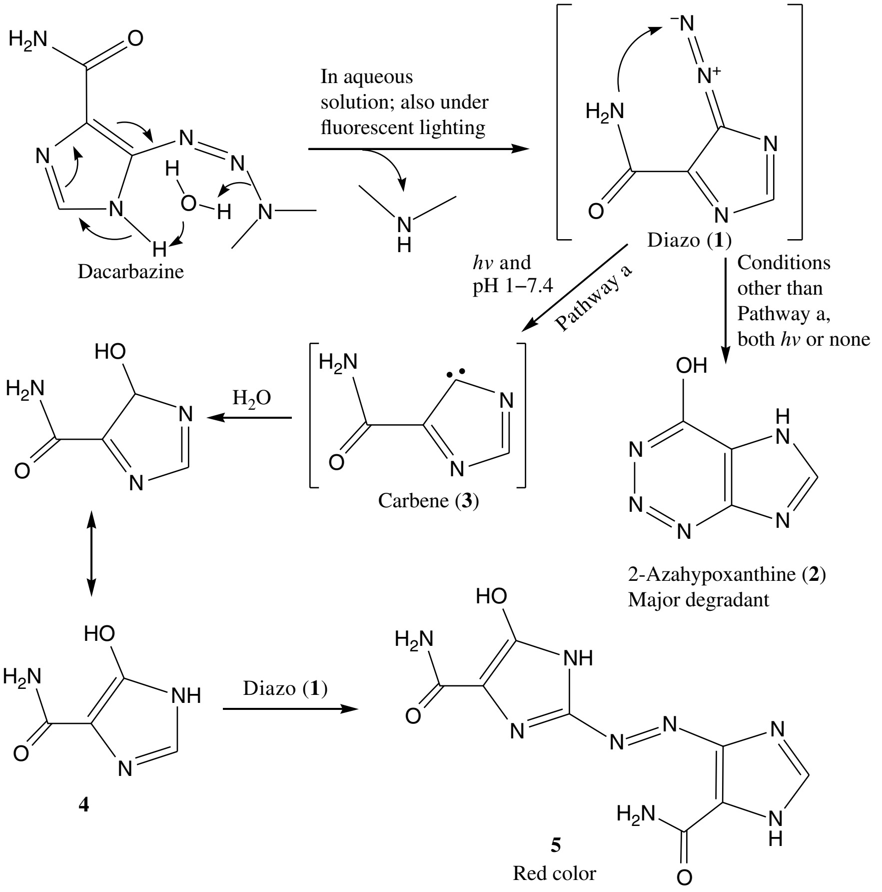
4 Amrani, F., Secrétan, P.-H., Sadou-Yayé, H. et al. (2015). Identification of dabigatran etexilate major degradation pathways by liquid chromatography coupled to multi stage higher resolution mass spectrometry. *RSC Adv.* 5: 45068–45081. https://doi.org/10.1039/C5RA04251H.

142: Dacarbazine [WHO List of Essential Medicines]



Chemical name: 4-[(*E*)-Dimethylaminodiazenyl]-1*H*-imidazole-5-carboxamide
Brand name: DTIC, DTIC-Dome
Formula: $C_6H_{10}N_6O$
Monoisotopic protonated ion (*m/z*): 183.0989
Molecular weight: 182.18
CAS number: 4342-03-4

Dacarbazine is a triazene anticancer drug with its cytotoxicity deriving from its DNA alkylating capability. Its main degradant is 2-azahypoxanthine (**2**, Scheme 142) [1],



Scheme 142 Degradation of dacarbazine.

which is a photodegradant; the overall degradation was enhanced between ~15 and 100 times at different pH under fluorescent lighting [2]. A stability study of dacarbazine in commercial amber glass vials and polyvinyl chloride bags was conducted, and the results indicated that “dacarbazine must be carefully protected from light, administered using opaque infusion tubing” [3]. When the photolysis of dacarbazine solutions was performed in pH 1–7.4, either by UV or by fluorescent lighting, the diazo intermediate (**1**) can produce a red-colored degradant (**5**) via the intermediacy of degradant **4** [4].

References

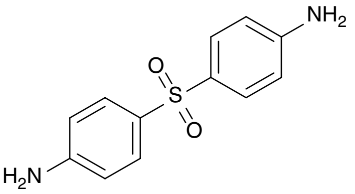
1 Fiore, D., Jackson, A.J., Didolkar, M.S., and Dandu, V.R. (1985). Simultaneous determination of dacarbazine, its photolytic degradation product, 2-Azahypoxanthine, and the metabolite 5-Aminoimidazole-4-Carboxamide in plasma and urine by high-pressure liquid chromatography. *Antimicrob. Agents Chemother.* 27: 977–979.

2 Shetty, B.V., Schowen, R.L., Slavik, M., and Riley, C.M. (1992). Degradation of dacarbazine in aqueous solution. *J. Pharm. Biomed. Anal.* 10: 675–683.

3 El Aatmani, M., Poujol, S., Astre, C. et al. (2002). Stability of dacarbazine in amber glass vials and polyvinyl chloride bags. *Am. J. Health-Sys. Pharm.* 59: 1351–1356.

4 Asahi, M., Matsushita, R., Kawahara, M. et al. (2002). Causative agent of vascular pain among photodegradation products of dacarbazine. *J. Pharm. Pharmacol.* 54: 1117–1122.

143: Dapsone [WHO List of Essential Medicines]

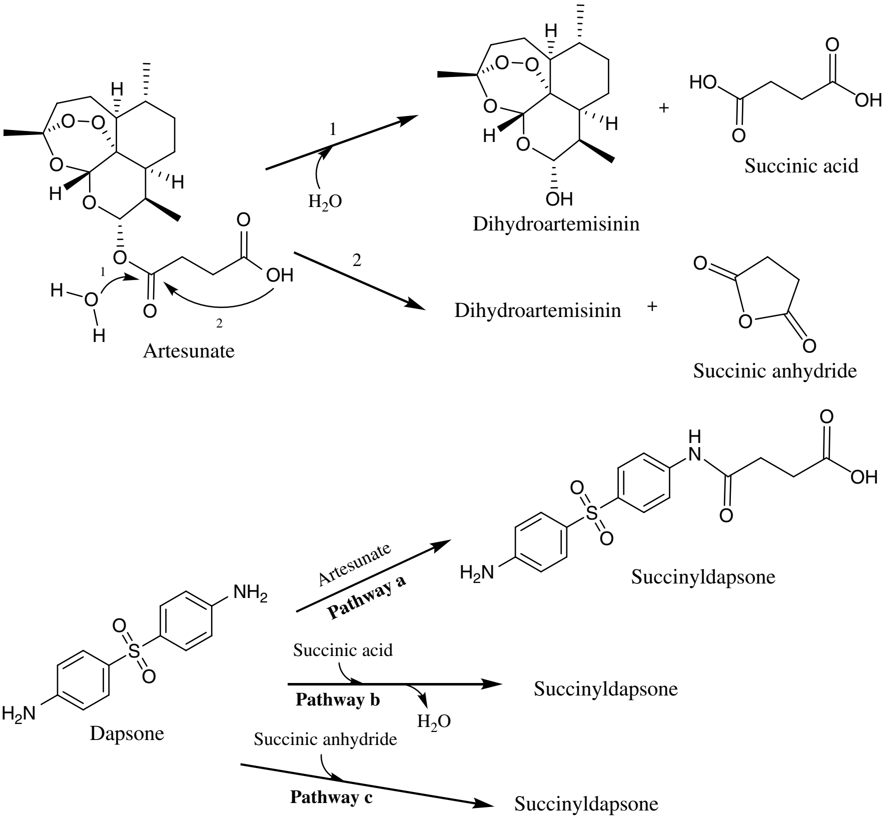


Chemical name: 4-(4-Aminophenyl)sulfonylaniline
Brand name: Aczone
Formula: $C_{12}H_{12}N_2O_2S$
Monoisotopic protonated ion (*m/z*): 249.0692
Molecular weight: 248.30
CAS number: 80-08-0

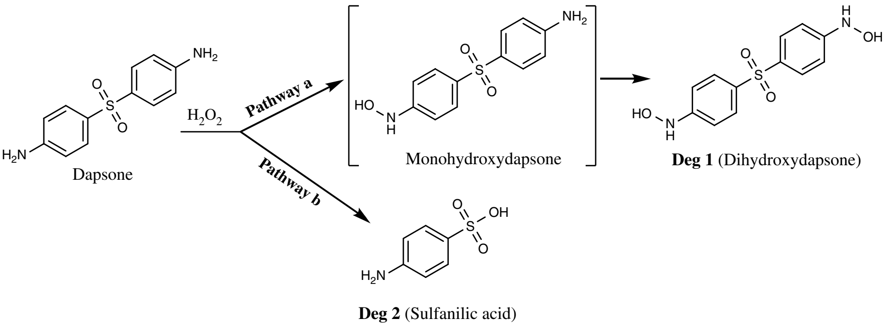
Dapsone, also known as diaminodiphenylsulfone (or DDS), is an old antibiotic for the treatment of leprosy as well as other bacterial or parasitic infections, which dates back to the 1930s [1]. Its potentially degradable functional group is the aromatic amino group, which is nucleophilic as well as oxidizable as an aniline analog. In the study of a triple combination drug for malaria, it was found that dapsone reacted with the succinic moiety of artesunate, one of the components in the triple combination drug, to form succinyl dapsone [2].

The formation of the degradant, succinyldapsone, could proceed through a direct interaction between dapsone and artesunate (Pathway a, Scheme 143-1). Nevertheless, the original authors preferred a step-wise mechanism (Pathway b, Scheme 143-1), in which dapsone is coupled with the succinic acid that is formed from the hydrolytic degradation of artesunate. Such a step-wise mechanism would not only require a quite significant hydrolytic degradation of artesunate but also a quite efficient coupling reaction between dapsone and the succinic acid formed in the hydrolysis. Unfortunately, the latter coupling reaction between a weak organic base and nonactivated acid would not be expected to be efficient, suggesting that the indirect mechanism lacks a reasonable mechanistic basis. Although the direct mechanism would be more reasonable than the step-wise one, it may suffer from a potential drawback that the point of the direct attacking may have too much steric hindrance. Another possible and perhaps more reasonable mechanism would be for artesunate to undergo an intramolecular decomposition in which succinic anhydride is formed. The anhydride is quite reactive and can react with dapsone much more efficiently (Pathway c, Scheme 143-1).

The aniline-type amino groups of dapsone would certainly be susceptible to oxidative degradation. In a forced degradation study with hydrogen peroxide, two degradants were formed (Scheme 143-2): dihydroxydapsone (*N,N′*-[sulfonyl(bis(4,1-phenylene))]-bis(hydroxylamine); Deg 1) and sulfanilic acid (Deg 2) [3]. The formation of Deg 1, dihydroxydapsone, is apparently via a nucleophilic oxidation pathway. The monohydroxydapsone, an intermediate of the nucleophilic oxidation, is also a metabolite of dapsone [4]; however, it was not reported in this forced degradation study.



Scheme 143-1 Degradation pathways of dapsone and artesunate in their combination formulation.



Scheme 143-2 Oxidative degradation of dapsone under forced degradation by hydrogen peroxide.

References

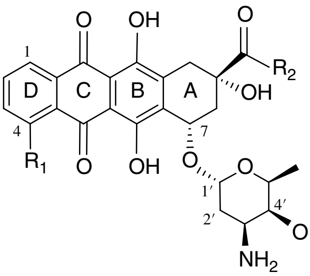
1 Dapsone. https://en.wikipedia.org/wiki/Dapsone (accessed 17 February 2018).

2 Bardsley, B., Barry, S.J., Drozdz, M.A. et al. (2011). Formation and identification of a degradant in chlorproguanil–dapsone– artesunate (Dacart™) tablets. *J. Pharm. Biomed. Anal.* 54 (3): 610–613.

3 Salama, N.N.E.-D.A., El Ries, M.A., Toubar, S. et al. (2012). Validated TLC and HPLC stability-indicating methods for the quantitative determination of dapsone. *J. Planar Chromatogr.* 25: 65–71.

4 Aberg, A.K.G., Zolotoy, A., Bain, A.I. (2000). Galenical preparations of dapsone and related sulphones, and method of therapeutic and preventative treatment of disease. WO2001041772 A1.

144–146: Daunorubicin, Doxorubicin, Idarubicin



Daunorubicin: R_1 = –OMe; R_2 = –Me.
Doxorubicin: R_1 = –OMe; R_2 = –CH_2OH.
Idarubicin: R_1 = –H; R_2 = –Me.

144: Daunorubicin

Chemical name: (7*S*,9*S*)-9-Acetyl-7-[(2*R*,4*S*,5*S*,6*S*)-4-amino-5-hydroxy-6-methyl oxan-2-yl]oxy-6,9,11-trihydroxy-4-methoxy-8,10-dihydro-7*H*-tetracene-5, 12-dione

Brand name: Cerubidine, Vyxeos

Formula: $C_{27}H_{29}NO_{10}$

Monoisotopic protonated ion (*m/z*): 528.1864

Molecular weight: 527.52

Common salt form: HCl salt

CAS number: 20830-81-3

145: Doxorubicin [WHO List of Essential Medicines]

Chemical name: (7*S*,9*S*)-7-[(2*R*,4*S*,5*S*,6*S*)-4-Amino-5-hydroxy-6-methyloxan-2-yl] oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7*H*-tetracene-5,12-dione

Brand name: Adriamycin, Caelyx, Myocet

Formula: $C_{27}H_{29}NO_{11}$

Monoisotopic protonated ion (*m/z*): 544.1813

Molecular weight: 543.52

Common salt form: HCl salt

CAS number: 23214-92-8

146: Idarubicin

Chemicalname:(7*S*,9*S*)-9-Acetyl-7-[(2*R*,4*S*,5*S*,6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-8,10-dihydro-7*H*-tetracene-5,12-dione

Brand name: Idamycin

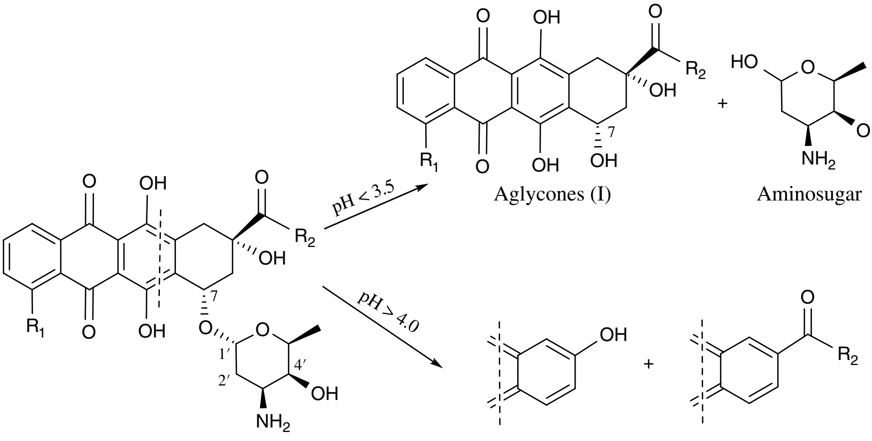
Formula: $C_{26}H_{27}NO_9$

Monoisotopic protonated ion (*m/z*): 498.1759

Molecular weight: 497.49
Common salt form: HCl salt
CAS number: 58957-92-9

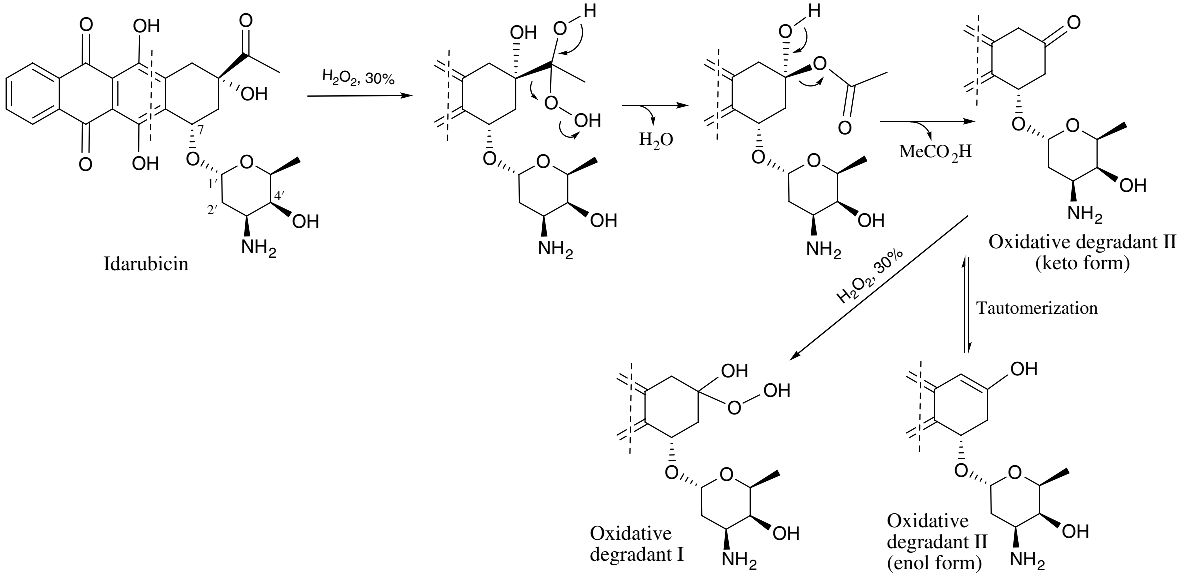
In this monograph, the degradation chemistry of daunorubicin (also known as daunomycin) and its closely related analogs, doxorubicin and idarubicin, is examined. All three drugs are anthracycline antibiotics used as chemotherapeutic agents for treating cancers. The degradation chemistry of these drugs seems to occur mainly on the glycosidic linkage, which could be cleaved under both acidic and alkaline conditions, while it was most stable in the pH range of 4.5–6.0 [1]. The degradation profile is dependent on the pH of the solution: at $pH < 3.5$, daunorubicin seemed to be exclusively hydrolyzed to give daunorubicinone (I, Scheme 144–146-1) and the amino sugar, if the degradation conditions were somewhat moderate [1, 2]. Notably, the configuration of the chiral center in I remained unchanged, indicating that the nucleophilic attack by water would occur on the glycosidic carbon (C1′), rather than on the benzilic-like position (C7) in the anthracycline D ring [3]. At $pH > 4.0$, daunorubicin primarily decomposed to yield the other two aglycone degradants (II and III, Scheme 144–146-1) [1]. Under harsher forced degradation conditions, daunorubicin produced a few more degradants [3]; nevertheless, they may not be relevant under pharmaceutical storage conditions.

In a forced degradation study of idarubicin, which is 4-desmethoxyldaunorubicin and reported to have less cardio toxicity and better efficacy, it was found to yield two oxidative degradants through the Baeyer-Villiger oxidation of the keto group (Scheme 144–146-2) [4]. However, the oxidation condition used was quite harsh (30% H_2O_2), which resulted in excessive degradation. Hence, the validity of this forced degradation study in terms of predicting the degradation chemistry of idarubicin (and its analogs) may need to be further explored.



Daunorubicin: R_1 = –OMe; R_2 = –Me.
Doxorubicin: R_1 = –OMe; R_2= $-CH_2OH$.
Idarubicin: R_1 = –H; R_2 = –Me.

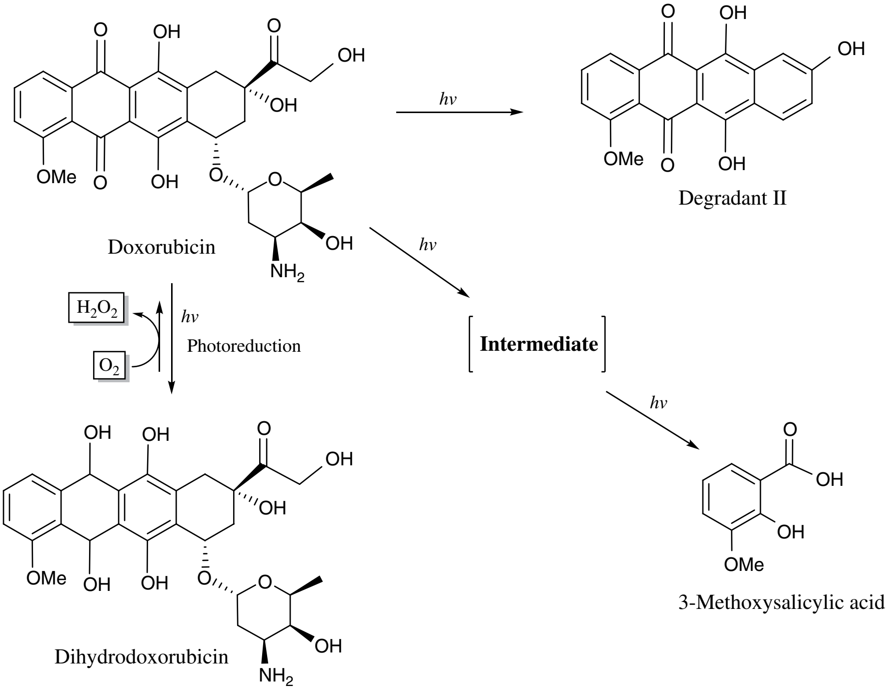
Scheme 144–146-1 Degradation of anthracycline antibiotics in solutions.



Scheme 144–146-2 Oxidative degradation of idarubicin in 30% H_2O_2. The mechanism presented is modified from that proposed by the original authors. *Source:* Kaushik and Bansal [4]/with permission of Elsevier.

The degradation kinetic parameters, activation energies, and frequency factors of daunorubicin were determined to be 114 kJ/mol and 5.5×10^{13}/s at pH 1.5 and 79 kJ/mol and 5.5×10^{6}/s at pH 8, respectively, over the temperature range of 40–60°C [1]. For a degradation kinetic study of doxorubicin, the activation energy for degradation in a 0.5 M HCl solution was found to be 92.0 kJ/mol over the temperature range 22–50°C [5].

Photochemical degradation of daunorubicin, doxorubicin, and epirubicin (4′-epimer of doxorubicin) conducted by Wood et al. [6] indicated that under intense sunlight and fluorescent lighting, these drugs quickly decomposed, particularly in solutions of low concentrations. However, in high concentration solutions, e.g., those used in typical anticancer treatment (≥500 mg/l), it was concluded "no special precautions are necessary to protect freshly prepared solutions of these agents from light during intravenous administration." The major photodegradant was reported to be degradant II (Scheme 144–146-1) [7]. In a more recent photochemical study, doxorubicin exhibits unusual photoreactivity in aqueous solutions via two pathways [8]. The first pathway is a two-step process that leads to the formation of 3-methoxysalicylic acid through an unstable intermediate (Scheme 144–146-3). The second pathway is a photocatalyzed process in which doxorubicin is recycled back and forth with its reduced form, dihydrodoxorubicin, via photoreduction of doxorubicin and subsequent spontaneous oxidation of dihydrodoxorubicin. The latter process is mediated by dissolved oxygen, which is reduced to H_2O_2 (Pathway b, Scheme 144–146-3). During this redox cycling, the amount of H_2O_2 may accumulate



Scheme 144–146-3 Photochemical degradation of doxorubicin.

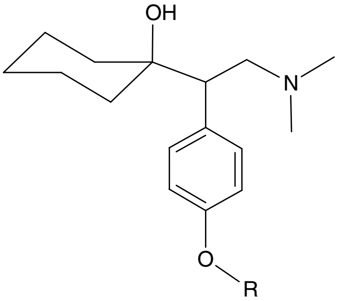
to toxic levels in pharmaceutical preparations, according to the evidence presented by the same researchers [9].

These anthracycline antitumor antibiotics can form stable complexes with metal ions such as Fe(III) and Cu(II), and under slightly acidic conditions, these metal ions can induce an appreciable amount of degradant II [10, 11]. All the identified degradants of daunorubicin do not have the aminosugar ring, commonly referred to as aglycones, and they are devoid of anticancer activity for which the aminosugar moiety is required [12].

References

1. Beijnen, J.H., van der Houwen, O.A.G.J., Voskuilen, M.C.H., and Underberg, W.J.M. (1986). Aspects of the degradation kinetics of daunorubicin in aqueous solution. *Int. J. Pharm.* 31: 75–82.
2. Arcamone, F., Franceschi, G., Orezzi, P. et al. (1964). The structure of daunomycinone. *J. Am. Chem. Soc.* 86: 5334–5335.
3. Beijnen, J.H., Potman, R.P., van Ooijen, R.D. et al. (1987). Structure elucidation and characterization of daunorubicin degradation products. *Int. J. Pharm.* 34: 247–251.
4. Kaushik, D. and Bansal, G. (2013). Characterization of degradation products of idarubicin through LC-UV, MS^n and LC–MS-TOF studies. *J. Pharm. Biomed. Anal.* 85: 123–131.
5. Wassermann, K. and Bundgaard, H. (1983). Kinetics of the acid-catalyzed doxorubicin. *Int. J. Pharm.* 14: 73–78.
6. Wood, M.J., Irwin, W.J., and Scott, D.K. (1990). Photodegradation of doxorubicin, daunorubicin and epirubicin measured by high-performance liquid chromatography. *J. Clin. Pharm. Therap.* 15: 291–300.
7. Gray, P.J. and Phillips, D.R. (1981). Ultraviolet photoirradiation of daunomycin and DNA-daunomycin complexes. *Photochem. Photobiol.* 33: 297–303.
8. Nawara, K., Krysinski, P., and Blanchard, G.J. (2012). Photoinduced reactivity of doxorubicin: catalysis and degradation. *J. Phys. Chem. A* 116: 4330–4337.
9. Nawara, K., Krysinski, P., and Blanchard, G.J. (2012). Doxorubicin is a photocatalyst for the generation of H_2O_2. *RSC Adv.* 2: 4059–4061.
10. Fiallo, M.M.L., Garnier-Suillerot, A., Matzanke, B., and Kozlowski, H. (1999). How Fe^{3+} binds anthracycline antitumour compounds: the myth and the reality of a chemical sphinx. *J. Inorg. Biochem.* 75: 105–115.
11. Fiallo, M.M.L., Haj, H.T.-B., and Garnier-Suillerot, A. (1994). Degradation of anthracycline antitumor compounds catalysed by metal ions. *Metal-Based Drugs* 1: 183–193.
12. Henry, D.W. (1979). Structure-activity relationships among daunorubicin and adriamycin analogs. *Cancer Treat. Rep.* 63: 845–854.

147–148: Desvenlafaxine, Venlafaxine



Desvenlafaxine (active metabolite of venlafaxine), R = H;
Venlafaxine, R = Me.

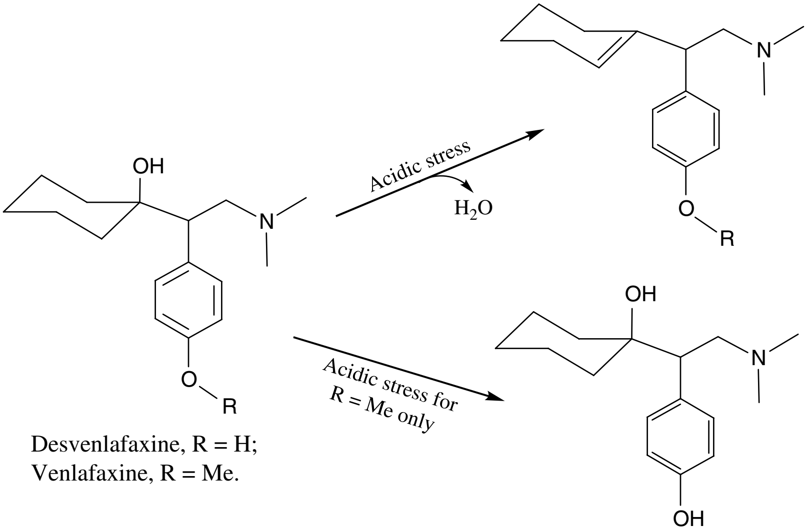
147: Desvenlafaxine

Chemical name: 4-[2-(Dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol
Brand name: Pristiq
Formula: $C_{16}H_{25}NO_2$
Monoisotopic protonated ion (*m/z*): 264.1958
Molecular weight: 263.38
CAS number: 93413-62-8
Common salt form: HCl salt, fumarate, succinate

148: Venlafaxine

Chemical name: 1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol
Brand name: Effexor
Formula: $C_{17}H_{27}NO_2$
Monoisotopic protonated ion (*m/z*): 278.2115
Molecular weight: 277.40
CAS number: 93413-69-5
Common salt form: HCl salt

The two functional groups in both desvenlafaxine and venlafaxine, which may be susceptible to degradation, are the hydroxyl and tertiary amino groups. Indeed, under acidic-forced degradation conditions, both desvenlafaxine and venlafaxine were found to undergo dehydration. Interestingly, the double bond formed as a result of the dehydration is in the cyclohexane ring (Scheme 147–148), which is supported by the presence of a single proton signal in the olefinic region [1, 2]. It needs to be pointed out that in a 2012 paper published by Pawar et al. [3], the structure proposed for dehydrated desvenlafaxine was incorrect with regard to the position of the double bond formed.



Scheme 147–148 Degradation of desvenlafaxine and venlafaxine under acidic forced degradation conditions.

A later forced degradation study of venlafaxine by Carneiro et al. found that venlafaxine not only yielded the dehydrated venlafaxine but also gave desvenlafaxine as a minor degradant under the acidic stress condition in 1 N HCl at 60°C for 24 hours [4]. Nevertheless, the minor degradant peak of desvenlafaxine disappeared upon further stress by the time point of 50 hours. The results obtained by Carneiro et al. were somewhat different from those of Asafu-Adjaye et al. [5] and Makhija and Vavia [6]: in the case of Asafu-Adjaye et al., only dehydrated venlafaxine was observed, while in the case of Makhija and Vavia, only desvenlafaxine was seen. Such differences could be attributed to the different forced degradation conditions or different chromatographic conditions.

In all the forced degradation studies cited above, both desvenlafaxine and venlafaxine were found to be stable under conditions of basic (e.g., 1 M NaOH) and hydrogen peroxide. As both desvenlafaxine and venlafaxine are aliphatic tertiary amines and, as such, they would be expected to form the *N*-oxide degradants, the observation that they were stable under the stress by hydrogen peroxide would appear to be contradictory to the prediction. Nevertheless, the observation may be explained by the fact that in the stress studies, both APIs were utilized in their salt forms, and thus, the nucleophilicity of the tertiary amine functionality was suppressed by protonation. On the other hand, with regard to the degradation behaviors of the formulated products of desvenlafaxine and venlafaxine, there seems to be no evidence to indicate that the dehydrated products are the real degradants for both products, respectively. Furthermore, the degradation of venlafaxine to desvenlafaxine would also seem to be a remote possibility for a formulated product of venlafaxine, particularly in solid dosage form.

References

1 Moussa, B.A., El-Bagary, R.I., and Al-Eryani, Y.A. (2011). Development and validation of a stability-indicating HPLC method for the analysis of desvenlafaxine succinate in the presence of its acidic induced degradation product in bulk and pharmaceutical preparation. *J. Chem. Pharm. Res.* 3: 425–437.

2 Rao, R.N. and Raju, A.N. (2006). Simultaneous separation and determination of process-related substances and degradation products of venlafaxine by reversed-phase HPLC. *J. Sep. Sci.* 29: 2733–2744.

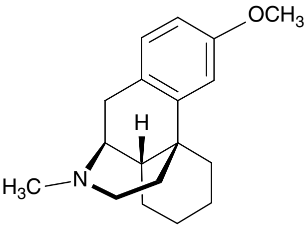
3 Pawar, S.M., Khatal, L.D., Gabhe, S.Y., and Dhaneshwar, S.R. (2012). LC–UV and LC–MS evaluation of stress degradation behavior of desvenlafaxine. *J. Pharm. Anal.* 2: 264–271.

4 Carneiro, W.J., Andrade, C.H., Braga, R.C., and de Oliveira, V. (2010). Identification of desvenlafaxine, the major active metabolite of venlafaxine, in extended-release capsules. *Rev. Eletr. Farm.* 7: 39–53.

5 Asafu-Adjaye, E.B., Faustino, P.J., Tawakkul, M.A. et al. (2007). Validation and application of a stability-indicating HPLC method for the in vitro determination of gastric and intestinal stability of venlafaxine. *J. Pharm. Biomed. Anal.* 43: 1854–1859.

6 Makhija, S.N. and Vavia, P.R. (2002). Stability indicating LC method for the estimation of venlafaxine in pharmaceutical formulations. *J. Pharm. Biomed. Anal.* 28: 1055–1059.

149: Dextromethorphan



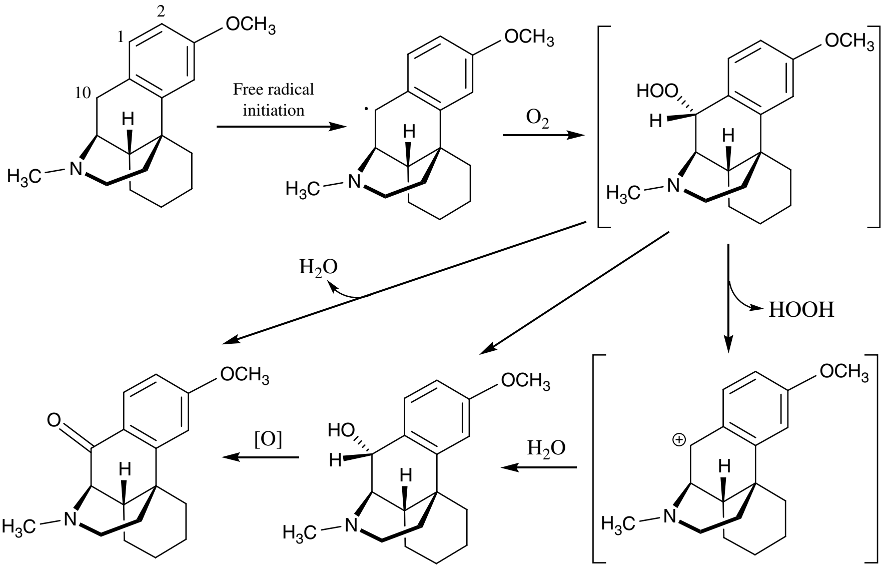
Chemical name: (1*S*,9*S*,10*S*)-4-Methoxy-17-methyl-17-azatetracyclo[7.5.3.$0^{1,10}$.$0^{2,7}$]heptadeca-2(7),3,5-triene
Brand name: Robitussin, Delsym, Others
Formula: $C_{18}H_{25}NO$
Monoisotopic protonated ion (*m/z*): 272.2009
Molecular weight: 271.40
CAS number: 125-71-3
Common salt form: HBr salt

Dextromethorphan (DM) is a widely used antitussive (cough suppressant) drug, which is typically coformulated with other active pharmaceutical ingredients in a great number of over-the-counter cold and cough medicines. The stereoisomer of dextromethorphan, levomethorphan, on the other hand, is a potent opioid analgesic but has never been developed into a clinically approved drug.

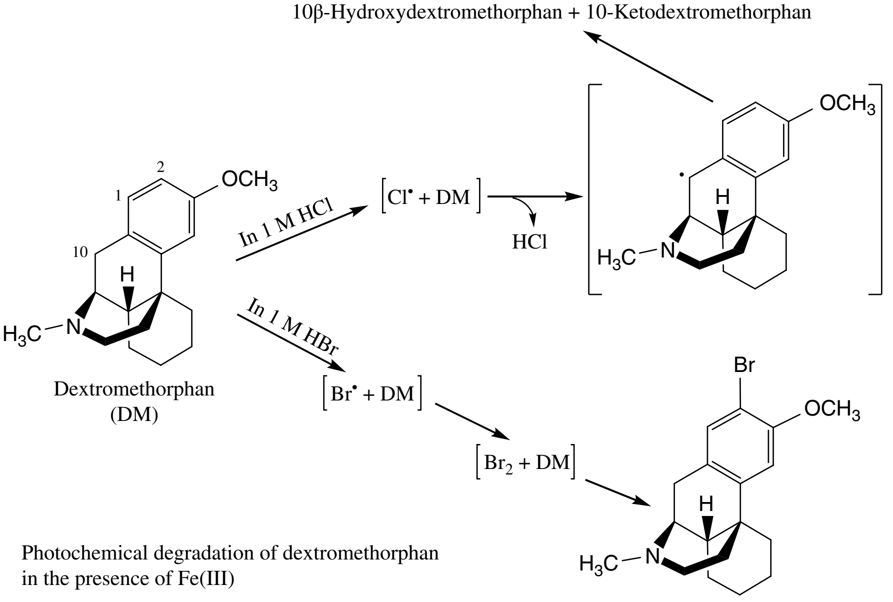
Due to its structural similarity to opioid analgesics, such as morphine, it can be reasonably expected that the benzylic position (10-position) is susceptible to free-radical mediated oxidation; this oxidation process can be initiated both photochemically [1] and nonphotochemically [2], which nonetheless should both produce the hydroperoxyl intermediate (Scheme 149-1).

The formation of the free radical should be catalyzed by redox-active transition metal ions, most likely iron ions. In a photochemical degradation study of dextromethorphan in 1 M HCl solution, Boccardi et al. found that Fe(III) was required for the photochemical oxidation to occur, in which both the 10β-hydroxyl and 10-keto degradants were formed [3]. From the hydroperoxyl intermediate, the dehydration process would lead to the formation of the 10-keto degradant, while a reductive process of the hydroperoxyl intermediate should give the 10β-hydroxyl degradant. Nevertheless, according to Boccardi et al. [3], this reductive process would be a minor process. The major process should be the one illustrated in Pathway b, Scheme 149-1: the formation of a carbocation via the elimination of H_2O_2 from the hydroperoxyl intermediate, followed by quenching of the carbocation by a water molecule. This elimination/quenching mechanism was supported by an experiment in which the corresponding 10β-methoxyl degradant was observed in good yield when the oxidation was carried out in a solution of 2 M HCl/methanol (1/1, v/v) [3].

Boccardi et al. also found that the photochemical degradation of dextromethorphan performed in a HCl solution gave different degradation profile than that

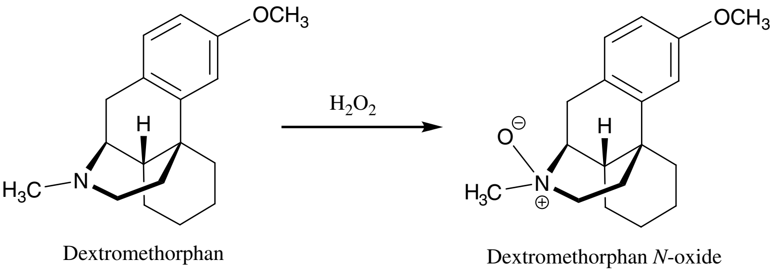


Scheme 149-1 Plausible mechanism for the oxidation on the 10-position of dextromethorphan.



Scheme 149-2 Photochemical degradation of dextromethorphan in solution in the presence of Fe(III).

performed in a HBr solution: while the former produced the 10β-hydroxyl and 10-keto degradants, the latter yielded only the 2-bromo degradant (Scheme 149-2) [3]. The difference was explained based on the reactivity of the chlorine and bromine radicals ($Cl^{\cdot}$ and $Br^{\cdot}$). The $Cl^{\cdot}$ would be capable of initiating the free radical process



Scheme 149-3 Nucleophilic oxidation of dextromethorphan.

by abstracting the 10β-H. On the contrary, Br˙ would not be capable of the H abstraction due to its lower energy as compared to that of Cl˙. Thus, Br˙ could only react with each other to form Br_2, and the latter would then brominate dextromethorphan at the 2-position via electrophilic substitution.

Under the oxidative stress condition using 3.0% hydrogen peroxide at 60°C for 3 hours, dextromethorphan *N*-oxide seemed to be the only significant degradant with 8% yield (Scheme 149-3) [4]. Another similar oxidative stress study of a formulated combination product containing guaifenesin and dextromethorphan also indicated that dextromethorphan underwent significant degradation [5].

References

1 Haefiger, Q., Brossi, A., Chopard-dit-Jean, L.H. et al. (1956). Mitteilung. Über ein photooxydationsprodukt von (+)-3-Methoxy-N-methyl-morphinan. *Helv. Chim. Acta* 39: 2053–2062.

2 Proska, B., Voticky, Z., Molanar, L. et al. (1978). 10-Oxomorphine, a decomposition product of morphine. *Chem. Zvestii* 32: 710–714.

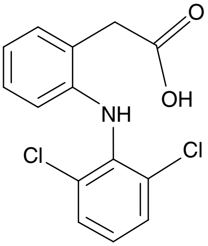
3 Boccardi, G., Mezzanzanica, P., Guzzi, U. et al. (1989). Photochemical Iron(III)-meidated autoxidation of dextromethorphan. *Chem. Pharm. Bull.* 37: 308–310.

4 Puppala, U., Srinivas, K.S.V., Reddy, K.V., and Jogi, B.R. (2018). Development and validation of a stability indicating and LCMS compatible UPLC method for estimation of assay, related substances and degradants in dextromethorphan hydrobromide active pharmaceutical ingredient. *Euro. J. Biomed. Pharm. Sci.* 5: 757–765.

5 Raju, T.V.R., Kumar, N.A., Kumar, S.R. et al. Development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of guaifenesin and dextromethorphan impurities in pharmaceutical formulations. *Chrom. Res. Int.* https://doi.org/10.1155/2013/315145.

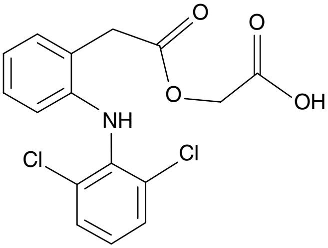
150–152: Diclofenac, Aceclofenac, Meclofenamic Acid

150: Diclofenac



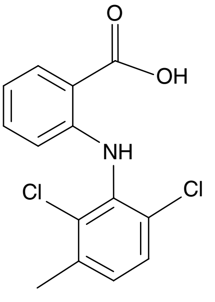
Chemical name: 2-[2-(2,6-Dichloroanilino)phenyl]acetic acid
Brand name: Arthrotec, Cataflam, Voltaren, Zipsor
Formula: $C_{14}H_{11}Cl_2NO_2$
Monoisotopic protonated ion (*m/z*): 296.0240
Molecular weight: 296.15
Common salt form: Sodium salt
CAS number: 15307-86-5

151: Aceclofenac



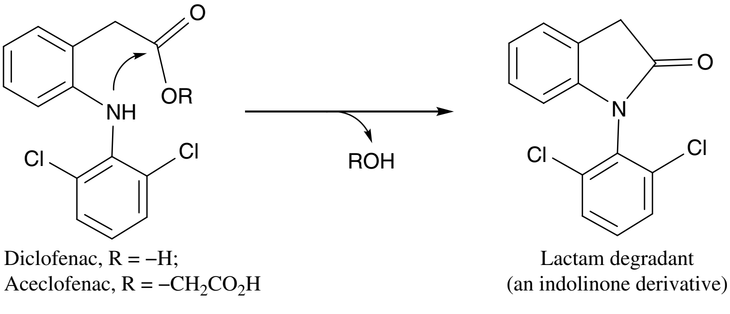
Chemical name: 2-[2-[2-(2,6-Dichloroanilino)phenyl]acetyl]oxyacetic acid
Brand name: Cincofen, Clanza, Hifenac
Formula: $C_{16}H_{13}Cl_2NO_4$
Monoisotopic protonated ion (*m/z*): 354.0294
Molecular weight: 354.18
CAS number: 89796-99-6

152: Meclofenamic Acid



Chemical name: 2-(2,6-Dichloro-3-methylanilino)benzoic acid
Brand name: Meclomen
Formula: $C_{14}H_{11}Cl_2NO_2$
Monoisotopic protonated ion (*m/z*): 296.0240
Molecular weight: 296.15
Common salt form: Sodium salt
CAS number: 644-62-2

Diclofenac is a nonsteroidal anti-inflammatory drug, which is a substituted *N*-(2,6-dichlorophenyl)aniline containing a methylenecarboxyl group ortho to the amine group. The carboxyl group could be cyclized with the amino group due to the particular spatial distance between the two groups. Indeed, during an accelerated stability study of diclofenac in liquid formulation, diclofenac underwent the cyclization between the carboxyl and amine groups, yielding the five-membered lactam degradant (Scheme 150–152-1) [1].



Diclofenac, R = –H;
Aceclofenac, R = $-CH_2CO_2H$

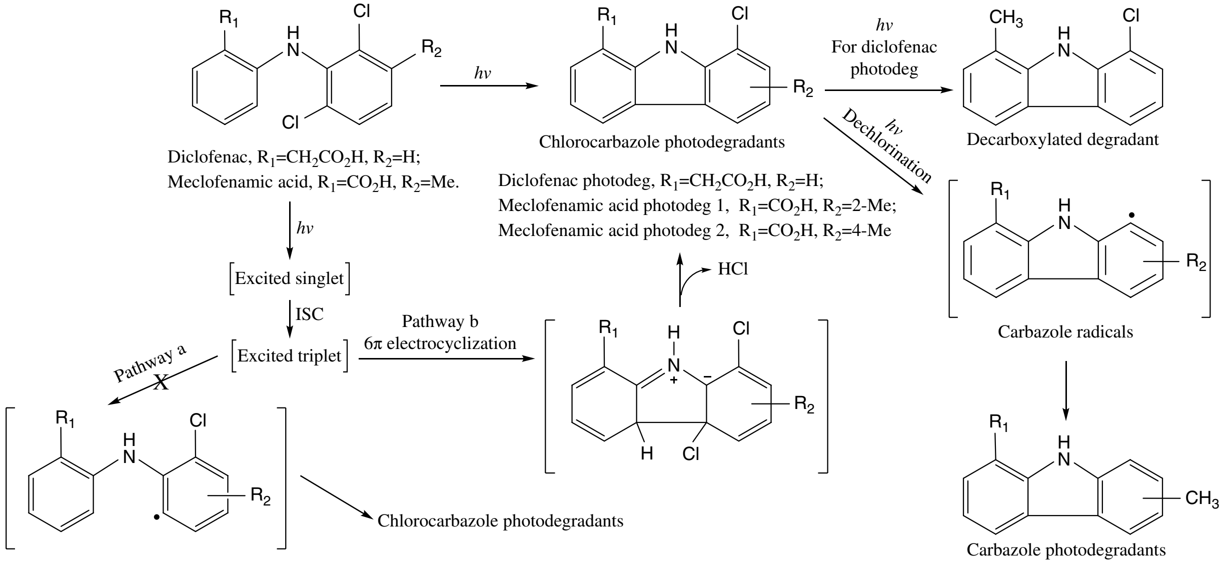
Lactam degradant
(an indolinone derivative)

Scheme 150–152-1 Degradation of diclofenac and aceclofenac due to intramolecular cyclization.

Although diclofenac was photochemically stable in the solid state, its two substituted phenyl rings were found to undergo photochemical cyclization to produce the tricyclic chlorocarbazole ring, accompanied by the elimination of one chlorine, upon UV irradiation of its solution [2]. It was believed that the formation of the carbazole photodegradant is not via a free radical pathway (Pathway a, Scheme 150–152-2) but rather a 6π-electrocyclization pathway (Pathway b, Scheme 150–152-2). A study performed by Encinas and Miranda suggested that the tricyclic chlorocarbazole degradant may be responsible for the phototoxicity of diclofenac [3]. Further photolysis of the chlorocarbazole degradant affected decarboxylation, producing the decarboxylated degradant (Scheme 150–152-2).

Aceclofenac is a prodrug of diclofenac in which the original carboxyl group is esterified by hydroxyacetic acid to form the glycolic acid ester of diclofenac. Based on its structural feature and similarity with diclofenac, aceclofenac would also be expected to undergo the same cyclization to form the same lactam degradant (Scheme 150–152-1) and the same photochemical degradation to yield the analogous tricyclic chlorocarbazole degradant (with R_1 being $-CH_2CO_2CH_2CO_2H$, Scheme 150–152-2). On the other hand, due to the blockage of the methylenecarboxyl group attached to the phenyl ring by the formation of the ester, aceclofenac would not be expected to undergo decarboxylation, unless the ester is hydrolyzed. In a compatibility study with a number of excipients, aceclofenac was found to be incompatible with magnesium stearate due to the formation of aceclofenac magnesium salt [4].

Meclofenamic acid is a desmethylene analog of diclofenac. Due to the absence of the methylene group between the carboxyl and the phenyl groups, meclofenamic acid would not be expected to undergo facile cyclization to form the analogous degradant with a four-membered ring, owing to the high tension of the four-membered ring. Nevertheless, its *N*-(2,6-dichlorophenyl)aniline core is still very similar to those of diclofenac and aceclofenac; as such, the substituted aniline core can still photocyclize to yield two analogous tricyclic chlorocarbazole degradants due to its asymmetric 2,6-dichloro-4-methylphenyl ring (Scheme 150–152-2). It also needs to point out that the chlorocarbazole degradants would not be expected to undergo the subsequent photochemical decarboxylation, because the carboxyl group in the benzoic acid moiety of meclofenamic acid is no longer "activated" for decarboxylation [5]. The chlorocarbazole degradants, nevertheless, could undergo dechlorination to give two carbazole degradants (Scheme 150–152-2).

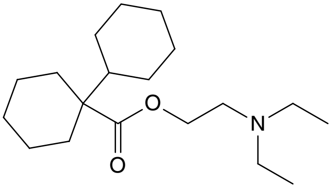


Scheme 150–152-2 Photochemical degradation of diclofenac and structurally similar meclofenamic acid. Aceclofenac is expected to undergo the same photochemical pathway.

References

1. Galmier, M.-J., Bouchona, B., Madelmont, J.-C. et al. (2005). Identification of degradation products of diclofenac by electrospray ion trap mass spectrometry. *J. Pharm. Biomed. Anal.* 38: 790–796.
2. Encinas, S., Bosca, F., and Miranda, M.A. (1998). Photochemistry of 2,6-dichlorodiphenylamine and 1-chlorocarbazole, the photoactive chromophores of diclofenac, meclofenamic acid and their major photoproducts. *Photochem. Photobiol.* 68: 640–645.
3. Encinas, S., Bosca, F., and Miranda, M.A. (1998). Phototoxicity associated with diclofenac: a photophysical, photochemical, and photobiological study on the drug and its photoproducts. *Chem. Res. Toxicol.* 11: 946–952.
4. Aigner, Z., Heinrich, R., Sipos, E. et al. (2011). Compatibility studies of aceclofenac with retard tablet excipients by means of thermal and FT-IR spectroscopic methods. *J. Therm. Anal. Calorim.* 104: 265–271.
5. Li, M. (2012). Chapter 4, section 4.2 decarboxylation. In: *Organic Chemistry of Drug Degradation*, 118–121. Cambridge: RSC Publishing.

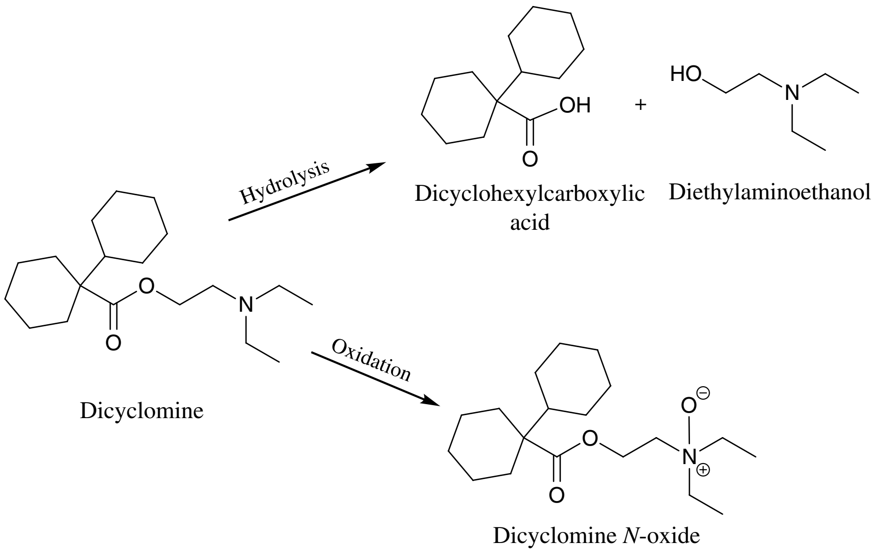
153: Dicyclomine (Dicycloverine)



Chemical name: 2-(Diethylamino)ethyl 1-cyclohexylcyclohexane-1-carboxylate
Brand name: Bentyl
Formula: $C_{19}H_{35}NO_2$
Monoisotopic protonated ion (*m/z*): 310.2741
Molecular weight: 309.49
Common salt form: HCl salt
CAS number: 77-19-0

Dicyclomine is an anticholinergic agent that is clinically used as antispasmodic for patients with irritable bowel syndrome (IBS). Despite its long and widespread clinical use since 1950, it appears that no serious study has been conducted on the stability of this drug product. Based on its structure, dicyclomine has two functional groups that may be susceptible to degradation: the ester and tertiary amine groups (Scheme 153). In one study aimed at developing a stability-indicating capillary gas-liquid chromatography–FID method for a few formulated products of dicyclomine hydrochloride, the authors demonstrated that the drug substance underwent degradation when stressed under both acidic (9 M H_2SO_4) and alkaline (1 M NaOH) conditions at 80°C for up to 45 minutes [1]. The authors assumed that the degradation was hydrolysis, and it appeared to be confined in scope. This finding is consistent with the pharmacokinetic result that the in vivo half-life of dicyclomine was reported to be 5 hours. The resistance to hydrolytic degradation, either in vitro or in vivo, may be rationalized by the fact that the ester functionality of dicyclomine is very sterically hindered due to the presence of the two nearby cyclohexane rings.

As a tertiary alkyl amine, it would be quite likely that dicyclomine could be susceptible to nucleophilic oxidation to form the corresponding *N*-oxide [2], particularly in a somewhat neutral to alkaline formulation.

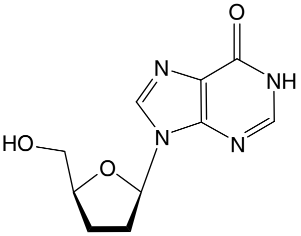


Scheme 153 Plausible degradation pathways of dicyclomine.

Reference

1 Tan, H.S.I. and Yan, Y.-P. (1989). Stability-indicating capillary gas-liquid chromatographic assay of dicyclomine hydrochloride in some pharmaceutical formulations. *J. Chromatogr.* 475: 381–389.

154: Didanosine [WHO List of Essential Medicines]

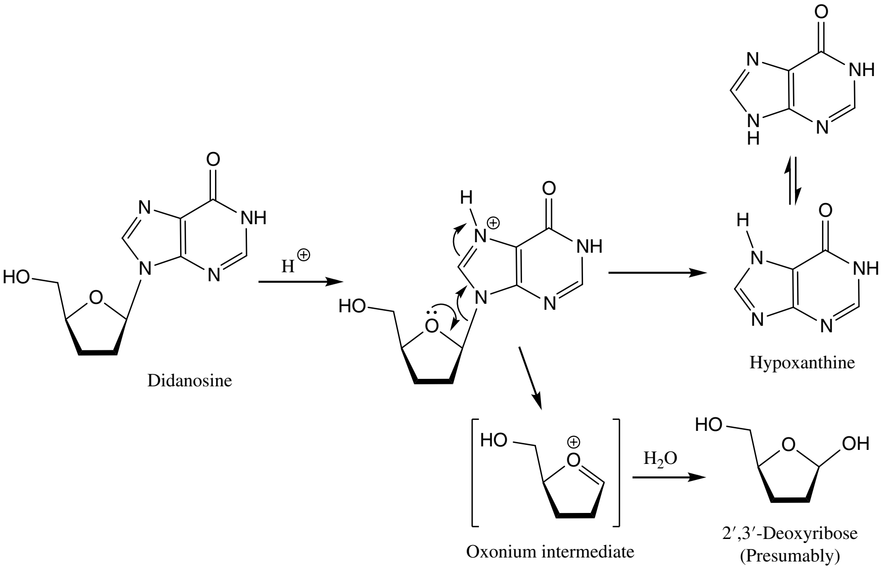


Chemical name: 9-[(2*R*,5*S*)-5-(Hydroxymethyl)oxolan-2-yl]-1*H*-purin-6-one
Brand name: Videx
Formula: $C_{10}H_{12}N_4O_3$
Monoisotopic protonated ion (*m/z*): 237.0982
Molecular weight: 236.23
CAS number: 69655-05-6

Didanosine, also known as 2′,3′-dideoxyinosine (ddI or DDI), is an HIV reverse transcriptase inhibitor. It is most commonly used in combination with HIV protease inhibitors for the treatment of AIDS. It is a dideoxypurinenucleoside analog, and its main degradation pathway is hydrolytic. Didanosine is quite unstable under acidic conditions but stable under alkaline conditions. For example, it decomposed by 30% in 6 hours in an acidic mobile phase consisting of a pH 4.0 buffer solution and methanol, giving hypoxanthine as the degradant [1]. In an earlier study, the half-life of didanosine was found to be only a few minutes in a pH 2 solution [2]. Berkers et al. studied the stability of didanosine in aqueous solutions over a pH range of 0–12 with a stability-indicating HPLC method [3]. At pH 3.2 and 3.6, the activation energies for the hydrolytic degradation were found to be 82.0 and 87.3 kJ/mol, while the frequency factors were 2.9×10^{10} and 8.8×10^{10}/s, respectively. The study also examined the stability of didanosine in the presence of several cyclodextrins. In the presence of 6×10^{-2} M hydroxypropyl-β-cyclodextrin (HP-β-CyD), the stability of didanosine was increased 5.1 times, as evidenced by the degradation rate constant of didanosine-HP-β-CyD complex (3.34×10^{-4}) versus that of uncomplexed didanosine (1.73×10^{-3}).

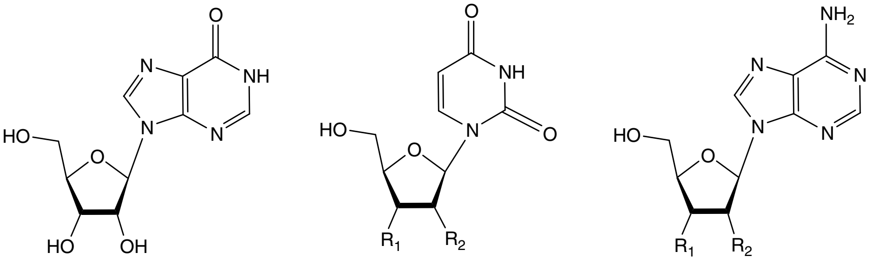
With regard to the mechanism of the acidic hydrolysis of didanosine, it appears that an A-1 mechanism would be applicable (Scheme 154), based on the study on deoxyuridine and related nucleosides by Shapiro and Kang [4].

The main acidic degradants are hypoxanthine and presumably 2′,3′-deoxyribose although it appears that the latter degradant has not been mentioned or verified in the stability studies of didanosine reported. Structurewise, didanosine is 2′,3′-deoxyinosine; nevertheless, inosine is much more stable than didanosine, i.e., 2′,3′-deoxyinosine. The activation energy (*Ea*) for acidic hydrolysis of inosine was determined to be 27.9 kcal/mol (or 117 kJ/mol) [5], which is significantly higher than that for didanosine (*Ea*: 82.0–87.3 kJ/mol) [3]. The enormous difference in the susceptibility toward acidic hydrolysis between didanosine and inosine is apparently because the 2′- and 3′-hydroxyl groups are missing in didanosine.



Scheme 154 Acidic hydrolysis of didanosine via an A-1 mechanism.

The presence of the hydroxyl groups, in particular the 2′-hydroxyl, can retard the solvolysis in neutral and acidic solution, which could explain the instability of didanosine according to Shapiro and Kang [4]. A similar example can be seen in the case of deoxyuridine and uridine, where deoxyuridine is much more hydrolyzable in acid than uridine [6]. On the other hand, in a search for acid-stable analogs of dideoxyadenosine (ddA), which is structurally similar to didanosine (ddI), introduction of a fluorine at 2′-position (2′-F-ddA) completely suppressed the acidic lability of ddA [7]. While ddA was almost immediately hydrolyzed in pH 1.0 solution at 37°C with a half-life of only 35 seconds, both epimers of 2′-F-ddA remained stable for 24 hours under the same conditions. The much-improved stability was attributed to the destabilization of the oxonium intermediate (refer to Scheme 154) by the fluorine substituent. Apparently, 2′-hydroxyl substituent can play the same role, thus explaining the instability of didanosine as compared to inosine in acidic solutions.



Inosine

Deoxyuridine: R_1 = OH, R_2 = H;
Uridine: R_1 = OH, R_2 = OH.

Dideoxyadenosine (ddA):
R_1 = H, R_2 = H;
2′-F-ara-ddA: R_1 = OH, R_2 = F.

Structures of nucleosides also discussed in this monograph.

References

1 Rebiere, H., Mazel, B., Civade, C., and Bonnet, P.-A. (2007). Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography. *J. Chromatogr. B* 850: 376–383.

2 Marquez, V.E., Tseng, C.K.H., Kelley, J.A. et al. (1987). 2′,3′-Dideoxy-2′-fluoro-ara-A. An acid-stable purine nucleoside active against human immunodeficiency virus (HIV). *Biochem. Pharmacol.* 36: 2719–2722.

3 Berkers, O., Beijnen, J.H., Tank, M.J.T.K. et al. (1993). 2′,3′-Dideoxyinosine (dd1): its chemical stability and cyclodextrin complexation in aqueous media. *J. Pharm. Biomed. Anal.* 11: 489–493.

4 Shapiro, R. and Kang, S. (1969). Uncatalyzed hydrolysis of deoxyuridine, thymidine, and 5-bromodeoxyuridine. *Biochemist* 8: 1806–1810.

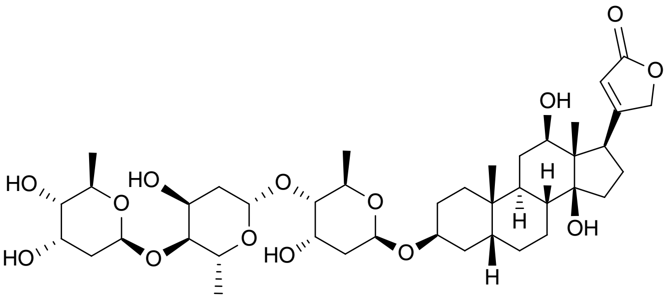
5 Suzuki, Y. (1974). The stability of inosine in acid and in alkali. *Bull. Chem. Soc. Jap.* 47: 2469–2472.

6 Pfitzner, K.E. and Moffatt, J.G. (1964). The synthesis and hydrolysis of 2′,3′-Dideoxyuridine. *J. Organomet. Chem.* 29: 1508–1511.

7 Marquez, V.E., Tseng, C.K.-H., Kelley, J.A. et al. (1987). 2′,3′-Dideoxy-2′-fluoro-ara-A. An acid-stable purine nucleoside active against human immunodeficiency virus (HIV). *Biochem. Pharmacol.* 36: 2719–2722.

155–157: Digoxin, Digitoxin, Deslanoside

155: Digoxin [WHO List of Essential Medicines]



Chemical name: 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*R*)-3-[(2*R*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-4,5-Dihydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-12,14-dihydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2*H*-furan-5-one

Brand name: Digitek, Digox, Lanoxin, Toloxin

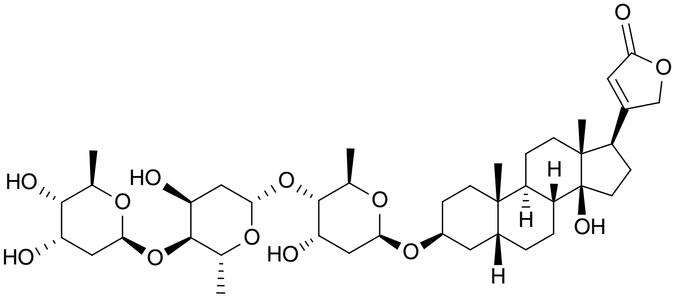
Formula: $C_{41}H_{64}O_{14}$

Monoisotopic protonated ion (*m/z*): 781.4369

Molecular weight: 780.94

CAS number: 20830-75-5

156: Digitoxin



Chemical name: 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3-[(2*R*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-4,5-Dihydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-14-hydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2*H*-furan-5-one

Brand name: Digitaline, Digitmerck

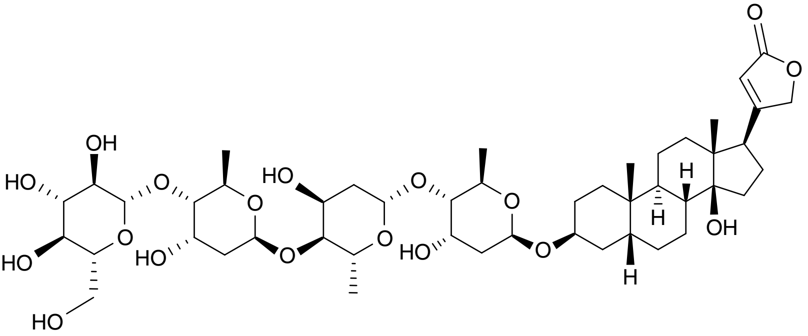
Formula: $C_{41}H_{64}O_{13}$

Monoisotopic protonated ion (*m/z*): 765.4420

Molecular weight: 764.94

CAS number: 71-63-6

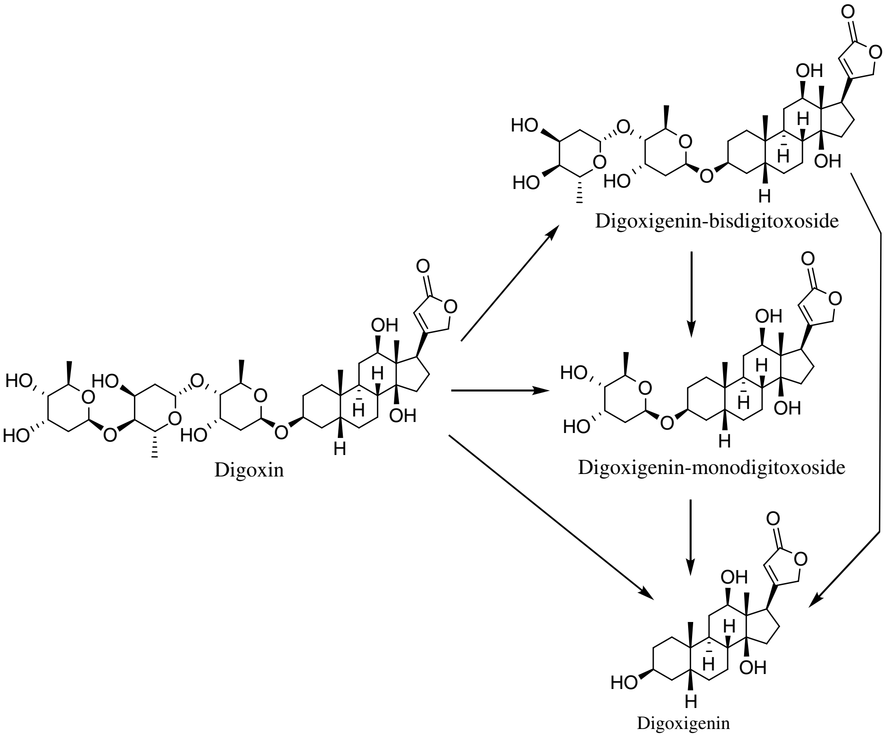
157: Deslanoside



Chemical name: 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*R*)-12,14-Dihydroxy-3-[(2*R*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-6-methyl-5-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-6-methyloxan-2-yl]oxy-6-methyloxan-2-yl]oxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2*H*-furan-5-one
Brand name: Cedilanide
Formula: $C_{47}H_{74}O_{19}$
Monoisotopic protonated ion (*m/z*): 943.4897
Molecular weight: 943.08
CAS number: 17598-65-1

Digoxin is one of the oldest medicines used for the treatment of congestive heart failure and certain types of arrhythmia such as atrial fibrillation. It was isolated from the medical herb of *Digitalis lanata*, and the medical use of the latter dates back to the 18th century. The molecule of digoxin consists of two parts: the steroid and trisaccharide parts; without the saccharide part, the steroid itself (i.e., digoxigenin) is only about 1/10 as active [1].

The trisaccharide part is made of three 2-dexoyhexose units (referred to as digitoxosides), and thus the glycosidic linkages between the digitoxosides and between the tridigitoxoside and steroid would be susceptible to hydrolytic degradation under acidic conditions [2]. Therefore, the main degradation pathway of digoxin is hydrolytic. The three glycosidic bonds can be hydrolyzed, respectively, to give digoxigenin-bisdigitoxoside and digoxigenin-monodigitoxoside and digoxigenin (Scheme 155–157) [3, 4]. Furthermore, the intermediary digoxigenin-bisdigitoxoside and digoxigenin-monodigitoxoside can be further hydrolyzed to produce digoxigenin-monodigitoxoside and digoxigenin. Sternson et al. determined the rates of hydrolysis at pH 1.0 and 37°C, and the results showed that the rates of hydrolysis were generally in the same magnitude, with the rate of digoxin to digoxigenin-bisdigitoxoside being the fastest (0.0263/min) and the rate of digoxin to digoxigenin the slowest (0.0089/min) [5]. This trend for the rates of hydrolysis can be readily understood from the perspective of the stereochemistry of digoxin as the steric hindrance of the steroid moiety should be the largest.



Scheme 155–157 Hydrolytic degradation of digoxin.

Digitoxin is a close analog of digoxin, and structurewise, it is 12-deoxydigoxin. The loss of the 12-hydroxyl group from the steroid core causes a dramatic difference in the ADME behavior of digitoxin, rendering its significant elimination via feces as opposed to digoxin's elimination via urine. Nevertheless, the chemical degradation pathway of digitoxin should be the same as that of digoxin [6]. Thus, the pathway illustrated in Scheme 155–157 would be readily applicable to digotoxin.

Deslanoside has an additional glucose unit than digoxin and digitoxin; it can be considered D-glucopyranosyldigoxin, i.e., the glucose unit is attached to the terminal digitoxose of digoxin. As the additional glucose unit has a hydroxyl group at its 2-position, the glycosidic bond between the first sugar unit (D-glucopyranosyl) and the second sugar unit is expected to be stable under acidic conditions. Hence, the acidic degradants of deslanoside would be expected to be the same as those of digoxin.

References

1 Kroneberg, G. (1959). Zuckergehalt und Wirksamkeit der Digitoxigenin- und Digoxigenindigitoxose. *Naunyn-Schmiedeberg's Areh. exp. Path. Pharmak* 287: 222–228.

2 Namchuk, M.N., McCarter, J.D., Becalski, A. et al. (2000). The role of sugar substituents in glycoside hydrolysis. *J. Am. Chem. Soc.* 122: 1270–1277.

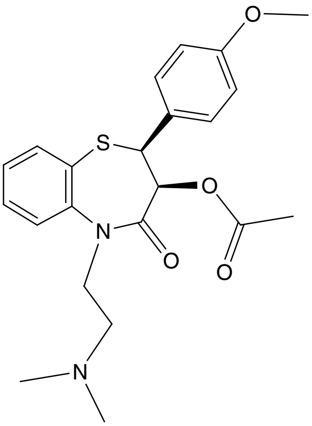
3 Kuhlmann, J., Abshagen, U., and Rietbrock, N. (1973). Cleavage of glycosidic bonds of digoxin and derivatives as function of pH and time. *Naunyn Schmiedeberg's Arch. Pharmacol.* 276: 149–156.

4 Jedlicka, A., Grafnetterova, T., and Miller, V. (2003). HPLC method with UV detection for evaluation of digoxin tablet dissolution in acidic medium after solid-phase extraction. *J. Pharm. Biomed. Anal.* 33: 109–115.

5 Sternson, L.A. and Shaffer, R.D. (1978). Kinetics of gigoxin stability in aqueous solution. *J. Pharm. Sci.* 67: 327–330.

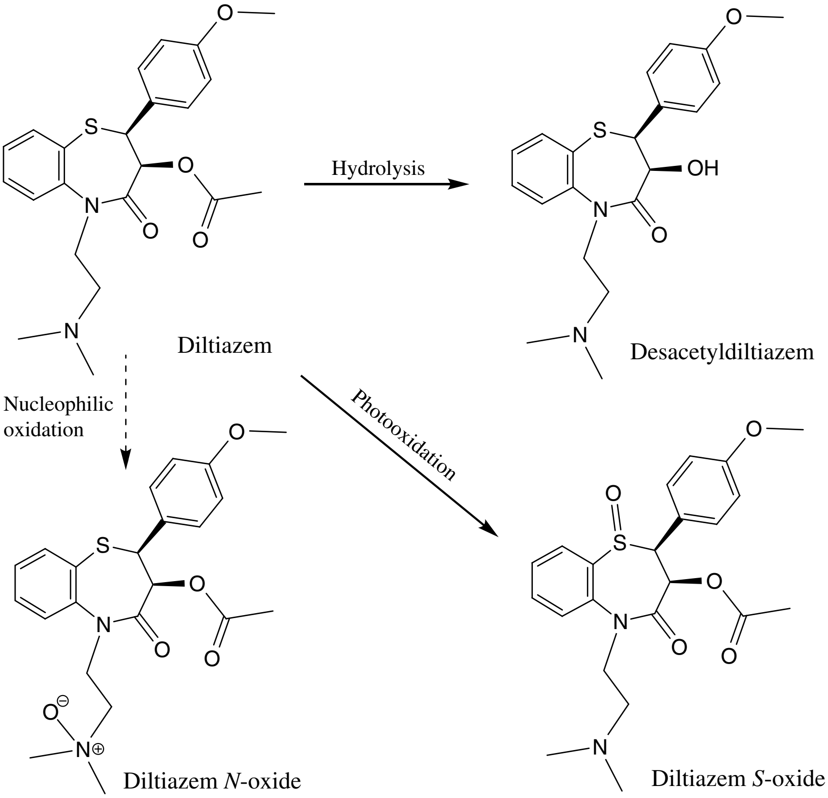
6 Desta, B., Kwong, E., and McErlane, K.M. (1982). Separation of digoxin, digitoxin and their potential metabolites, impurities or degradation products by high performance liquid chromatography. *J. Chromatogr.* 240: 137–143.

158: Diltiazem



Chemical name: [(2*S*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5- benzothiazepin-3-yl] acetate
Trade name: Cardizem, Dilacorxr
Formula: $C_{22}H_{26}N_2O_4S$
Monoisotopic protonated ion (*m/z*): 415.1686
Molecular weight: 414.52
CAS number: 42399-41-7
Common salt form: HCl salt

Diltiazem is a calcium channel blocker that is clinically used to treat hypertension and certain type of arrhythmia such as atrial fibrillation. It is a benzothiazepine derivative that contains the following functional groups or moieties that may be susceptible to various types of degradation: an acetyl ester, a tertiary amine, and a thioether. The acetyl ester functionality is particularly susceptible to hydrolytic degradation, under both acidic and alkaline conditions, leading to the formation of the deacetylated degradant, which is both a process impurity and metabolite (Scheme 158) [1, 2]. Won and Iula performed a kinetic study for the hydrolysis of diltiazem, in which the Log *k*-pH curves were determined at different temperatures; the results indicated that diltiazem is most stable at pH near 4 [3], which is consistent with a prior study [4]. Furthermore, at both ends of the pH range (1–8), the rates of the hydrolysis were similar [3]. The tertiary amine moiety of the drug molecule could be likely oxidized to produce *N*-oxide degradant, which is a known metabolite [5], although its existence in drug products has not been reported. On the other hand, desacetyldiltiazem *N*-oxide is also a metabolite [2].



Scheme 158 Degradation pathways of diltiazem. The dashed arrow indicates a likely scenario in which the *N*-oxide would be expected to form under nucleophilic oxidative conditions.

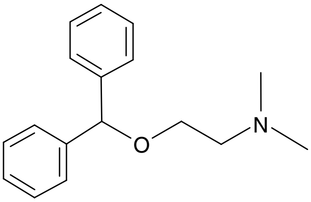
Reports of skin disorders after taking this drug prompted some researchers to study its phototoxic mechanism. According to a study by Andrisano et al., diltiazem *S*-oxide was the main photodegradant in acidic and near-neutral solutions that were exposed to UVB–UVA radiations (xenon arc lamp) [6]. In a solution of pH 9.0, photodegradation produced a few more degradants, among which the only identified degradant was the deacetylated degradant.

Muszalska and Siakowski studied the degradation of diltiazem in the solid state, and they found that under an atmosphere of moisture of 76.4%, diltiazem decomposed only to deacetylated diltiazem with the following thermodynamic parameters: *Ea* 134.5 kJ/mol, $\Delta H^{\neq}$ 132.0 kJ/mol, and $\Delta S^{\neq}$ 9.31 J/K mol [7]. The activation energy, 134.5 kJ/mol, determined under the solid state was significantly higher than the one (71.0 kJ/mol) in solution measured by Davis et al. [8].

References

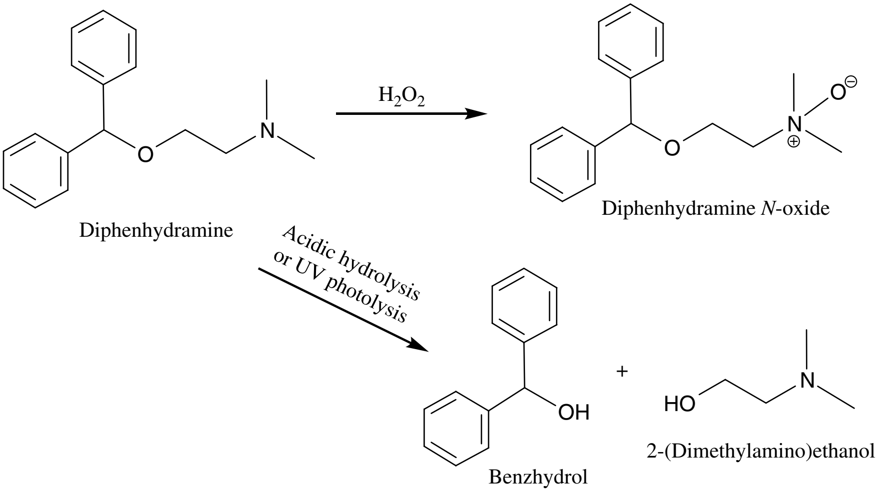
1. Mahajan, N., Deshmukh, S., and Farooqui, M. (2021). A novel stability-indicating method for known and unknown impurities profiling for diltiazem hydrochloride pharmaceutical dosage form (tablets). *Future J. Pharm. Sci.* https://doi.org/10.1186/s43094-021-00352-x.
2. Miyazaki, M., Iwakuma, T., and Tanaka, T. (1978). Synthesis of the metabolites and related compounds of diltiazem. *Chem. Pharm. Bull.* 26: 2889–2893.
3. Won, C.M. and Iula, A.K. (1992). Kinetics of hydrolysis of diltiazem. *Int. J. Pharm.* 79: 183–190.
4. Davis, S.S., Illum, L., Triccas, I.M., and Winchcomb, K.N. (1986). The simultaneous degradation and sorption of diltiazem in aqueous solution. *Int. J. Pharm.* 30: 29–33.
5. Li, R., Farmer, P.S., Xie, M. et al. (1992). Synthesis, characterization, and Ca^{2+} antagonistic activity of diltiazem metabolites. *J. Med. Chem.* 35: 3246–3253.
6. Andrisano, V., Hrelia, P., Gotti, R. et al. (2001). Photostability and phototoxicity studies on diltiazem. *J. Pharm. Biomed. Anal.* 25: 589–597.
7. Muszalska, I. and Siakowski, M. (2003). Kinetics of diltiazem hydrochloride in solid phase. *Act Polon. Pharm.–Drug Res.* 60: 27–30.
8. Davis, S.S., Illum, L., Triccas, I.M., and Winchcomb, K.N. (1986). The simultaneous degradation and sorption of diltiazem in aqueous solution. *Int. J. Pharm.* 30: 29–33.

159: Diphenhydramine



Chemical name: 2-Benzhydryloxy-*N*,*N*-dimethylethanamine
Trade name: Benadryl
Formula: $C_{17}H_{21}NO$
Monoisotopic protonated ion (*m/z*): 256.1696
Molecular weight: 255.36
CAS number: 58-73-1
Common salt form: HCl salt, Citrate

Diphenhydramine is a first-generation antihistamine medication that was discovered and commercialized in the 1940s. In addition to its indication for treatment of allergic symptoms, diphenhydramine also has sedative properties, due to which it is widely used as an over-the-counter sleeping aid, as well as a local anesthetic. There had been some studies regarding the stability of diphenhydramine between the 1950s and the 1960s, mostly by Japanese research groups, which were summarized in a monograph by Holcomb and Fusari [1]. According to the monograph, diphenhydramine was susceptible to decomposition in an acidic medium due to the hydrolytic cleavage of the ether linkage, producing benzhydrol and 2-(dimethylamino) ethanol (Scheme 159); a separate UV irradiation study of diphenhydramine also



Scheme 159 Degradation pathways of diphenhydramine.

yielded the two main degradants. In a more recent study, combination tablets of ibuprofen and diphenhydramine citrate were subjected to acidic degradation in 2 N HCl solution at 25°C for 2 hours, and no degradation product was observed, which may be due to the milder stress condition used [2]. On the other hand, diphenhydramine was fairly stable in an alkaline solution [1, 2]. In an oxidative stress study of diphenhydramine hydrochloride, it appeared that it was substantially degraded, and the degradation products included toluene, benzophenone, benzyl alcohol, benzoic acid, and several phenolic substances, in addition to 2-(dimethylamino) ethanol [1]. The results of this early oxidative stress study were quite different from those conducted in much later times, where only diphenhydramine *N*-oxide was observed as the primary degradant [2, 3]. The discrepancy is most likely due to the much harsher conditions used in the early study, as the initial degradants underwent substantial subsequent degradation. It is somewhat surprising to note that there have been very few reports for a systematic forced degradation study on diphenhydramine with emphasis on structural elucidation of degradation products [4], since those conducted in early times [1], despite its long and widespread medical use.

References

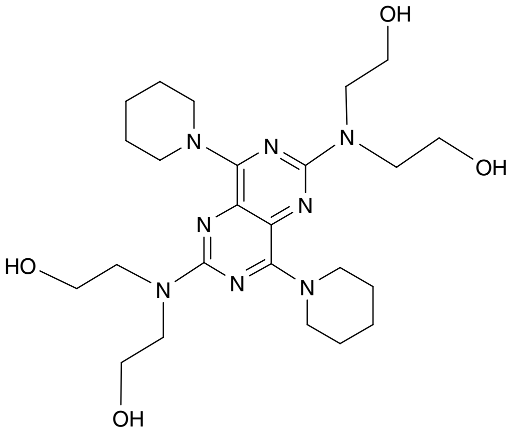
1 Holcomb, I.J. and Fusari, S.A. (1974). Diphenhydramine hydrochloride. *Ana. Prof. Drug subs.* 3: 173–232.

2 Rao, D.D., Sait, S.S., and Mukkanti, K. (2011). Development and validation of an UPLC method for rapid determination of ibuprofen and diphenhydramine citrate in the presence of impurities in combined dosage form. *J. Chromatogr. Sci.* 49: 281–286.

3 Ali, N.W., Abdelkawy, M., and Abdelhamid, N.S. (2013). Simultaneous determination of paracetamol and diphenhydramine hydrochloride mixture in the presence of their degradation products. *IOSR J. Pharm. Bio. Sci.* 6: 44–52.

4 Barbas, C., García, A., Saavedra, L., and Castro, M. (2000). Optimization and validation of a method for the determination of caffeine, 8-chlorotheophylline and diphenhydramine by isocratic high-performance liquid chromatography: stress test for stability evaluation. *J. Chrom. A* 870: 97–103.

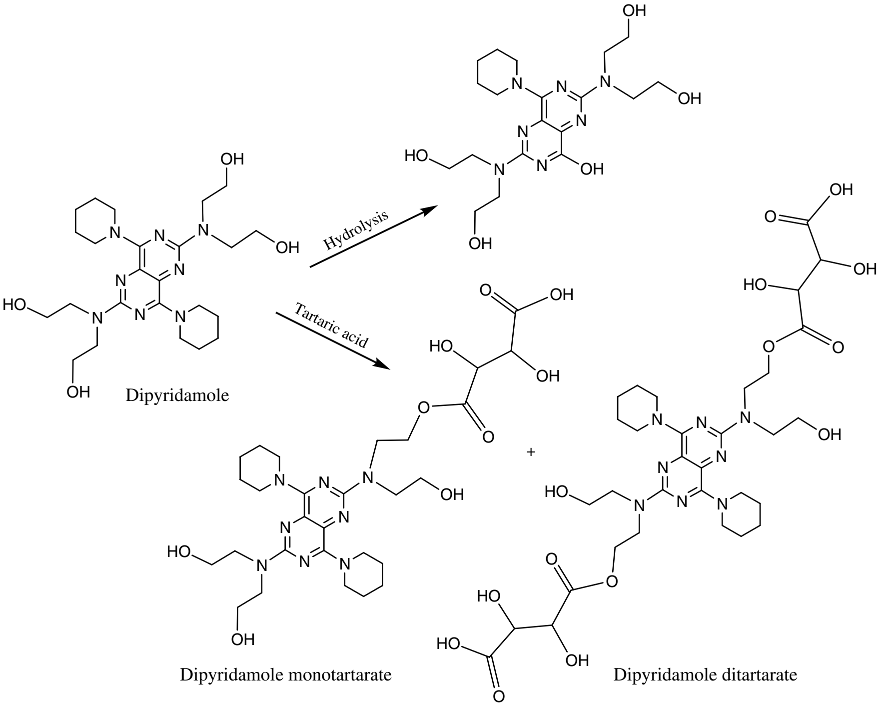
160: Dipyridamole



Chemical name: 2-[[2-[Bis(2-hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido [5,4-d]pyrimidin-6- yl]-(2-hydroxyethyl)amino]ethanol
Trade name: Persantin
Formula: $C_{24}H_{40}N_8O_4$
Monoisotopic protonated ion (*m/z*): 505.3245
Molecular weight: 504.64
CAS number: 58-32-2

Dipyridamole is a fused pyrimidine compound with certain level of structural symmetry. It was introduced as a therapeutic agent in 1959 due to its coronary vasodilator properties and later demonstrated to have antiplatelet effects by being an inhibitor of cAMP-platelet phosphodiesterase [1, 2]. Despite some controversy regarding some of its therapeutic uses, it has been widely prescribed [1, 3].

In a stability batch of a formulated dipyridamole drug product, Subbaiah et al. reported two degradation products: a hydrolytic degradant and an adduct resulting from monoesterification of the API with tartaric acid, an excipient in the formulation [4]. The latter monoester degradant was also observed in another formulated product of dipyridamole during its shelf-life stability study; furthermore, dipyridamole di-tartaric ester was also formed in the study [5]. Both the mono- and di-ester degradants, which are listed in the British Pharmacopeia [6], were greatly enriched through an accelerated stress of a 1:1 solid mixture of dipyridamole and tartaric acid at 40°C/75%RH [5]. The degradation pathway of dipyridamole as formulated in the presence of tartaric acid is summarized in Scheme 160.



Scheme 160 Degradation pathways of dipyridamole formulated with tartaric acid.

References

1 Bibbs, C.R. and Lip, G.Y.H. (1998). Do we still need dipyridamole. *Br. J. Clin. Pharmacol.* 45: 323–328.

2 Harker, L.A. and Kadatz, R.A. (1983). Mechanism of action of dipyridamole. *Thromb. Res.* 29: 39–46.

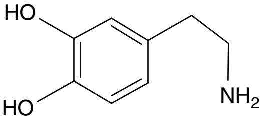
3 Spano, D., Marshall, J.-C., Marino, N. et al. (2013). Dipyridamole prevents triple-negative breast-cancer progression. *Clin. Exp. Metastasis* 30: 47–68.

4 Subbaiah, B.V., Ganesh, K.K.S., Krishna, G.V. et al. (2012). Isolation and characterisation of degradant impurities in dipyridamole formulation. *J. Pharm. Biomed. Anal.* 61: 256–264.

5 Nagadeep, J., Kamaraj, P., Arthanareeswar, M., and Vivekanand, P.A. (2021). Identification of tartaric acid adduct impurities in dipyridamole capsule formulation related substances method. *Asian J. Chem.* 33: 307–313.

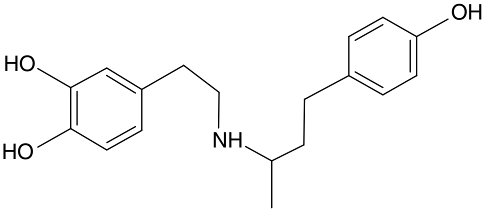
161–164: Dopamine and related catecholamines, Dobutamine, Norepinephrine, Epinephrine

161: Dopamine [WHO List of Essential Medicines]



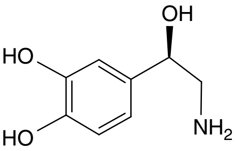
Chemical name: 4-(2-Aminoethyl)benzene-1,2-diol
Trade name: Intropin
Formula: $C_8H_{11}NO_2$
Monoisotopic protonated ion (*m/z*): 154.0863
Molecular weight: 153.18
CAS number: 51-61-6
Common salt form: HCl salt

162: Dobutamine



Chemical name: 4-[2-[4-(4-Hydroxyphenyl)butan-2-ylamino]ethyl]benzene-1,2-diol
Trade name: Dobuject
Formula: $C_{18}H_{23}NO_3$
Monoisotopic protonated ion (*m/z*): 302.1751
Molecular weight: 301.38
CAS number: 34368-04-2
Common salt form: HCl salt

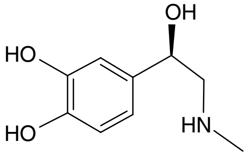
163: Norepinephrine



Chemical name: 4-[(1*R*)-2-Amino-1-hydroxyethyl]benzene-1,2-diol
Trade name: Levophed

Formula: $C_8H_{11}NO_2$
Monoisotopic protonated ion (*m/z*): 170.0812
Molecular weight: 169.18
CAS number: 51-41-2
Common salt form: Bitartrate

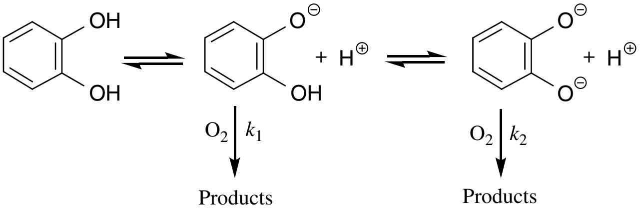
164: Epinephrine



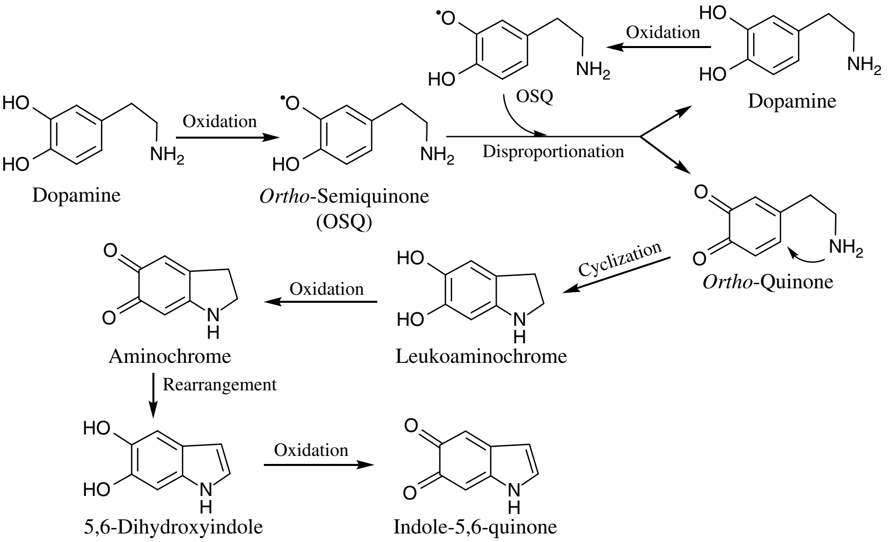
Chemical name: 4-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol
Trade name: EpiPen, Adrenaclick, and many others
Formula: $C_9H_{13}NO_3$
Monoisotopic protonated ion (*m/z*): 184.0968
Molecular weight: 183.21
CAS number: 51-43-4
Common salt form: HCl salt, bitartrate

Dopamine is a neurotransmitter when secreted by neurons in the brain, and it is biosynthesized from L-DOPA via decarboxylation. It belongs to the family of catecholamines and is the simplest form of catecholamine. When used for therapeutic purposes, dopamine is given via intravenous injection for the treatment of very low blood pressure, particularly for neonatal use; as dopamine cannot penetrate blood-brain barrier, it does not have CNS effect when administered in this way.

The catechol part of dopamine, as well as other members of the catecholamine family, is the very electron-rich 3,4-dihydroxyl-form of catechol (or 3,4-catechol; the other form of catechol being the 2,3-dihydroxyl isomer, i.e., 2,3-catechol); thus, it is very susceptible to oxidation, particularly its deprotonated forms. The pH-dependent oxidation of catechol has been well recognized, and the higher the pH, the quicker the oxidation [1, 2]. In a systematic study of catechol oxidation by molecular oxygen (O_2) in relation to pH, Maier et al. studied the oxidation rates of several catechols [3]. They found that the oxidation rates of fully deprotonated catechols (di-anions) were several orders of magnitude higher than those of mono-deprotonated anions (mono-anions). For example, for 4-ethylcatechol, which is a close analog of dopamine [4-(2′-aminoethyl)catechol], its di-anion form would be oxidized almost 4,000-folds faster than its mono-anion form (Scheme 161–164-1). This indicates that there will be an exponential increase in the oxidation rates of catechol from neutral to alkaline pH range.



Scheme 161–164-1 Mechanism for the pH-dependent oxidation of catechol by O_2 proposed by Maier et al. [3]. *Source:* Maier et al. [3]/Royal Society of Chemistry.



Scheme 161–164-2 Oxidative degradation pathway of dopamine. The pathway displayed is based on the mechanisms proposed by Maier et al. [3] as well as Bindoli et al. [4]. *Source:* Maier et al. [3] and Bindoli et al. [4].

The product from the initial oxidation of the catechol moiety of dopamine is *ortho*-quinone via the intermediacy of *ortho*-semiquinone disproportionation (Scheme 161–164-2) [4]. According to the mechanism illustrated in Scheme 161–164-1, the initial oxidation should occur on either of the anion forms. The *ortho*-quinone formed undergoes intramolecular cyclization to produce an unstable leucoaminochrome intermediate through a Michael addition by the amino group onto the *ortho*-quinone moiety. The leucoaminochrome intermediate is then rapidly oxidized to aminochrome. The latter can rearrange to 5,6-dihydroxyindole with an activation energy of 69 kJ/mol, which can be further oxidized to yield indole-5,6-quinone [5].

Dobutamine is a structural analog of dopamine with the amino group derivatized and the catechol moiety remained unchanged. Hence, its oxidative degradation pathway would be expected to be similar to that of dopamine (Scheme 161–164-2). The other naturally occurring members of the catecholamine family, which are also utilized therapeutically, include norepinephrine and epinephrine. The latter two molecules are biosynthesized from dopamine via hydroxylation and subsequent methylation. Due to the presence of the identical catechol moiety, norepinephrine and epinephrine would be expected to undergo the same or very similar oxidative degradation pathway as shown in Scheme 161–164-2. The hydroxyl groups on the benzylic positions of both molecules are usually very stable and resistant to oxidation as compared with the catechol moiety.

References

1 Joslyn, M.A. and Branch, G.E.K. (1935). The kinetics of absorption of oxygen by catechol. *J. Am. Chem. Soc.* 57: 1779–1785.

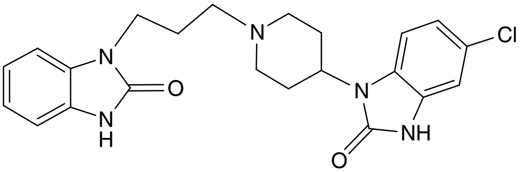
2 Roberts, N.B., Higgins, G., and Sargazi, M. (2010). A study on the stability of urinary free catecholamines and free methyl-derivatives at different pH, temperature and time of storage. *Clin. Chem. Lab. Med.* 48: 81–87.

3 Maier, G.P., Bernt, C.M., and Butler, A. (2017). Catechol oxidation: considerations in the design of wet adhesive materials. *Biomater. Sci.* https://doi.org/10.1039/c7bm00884h.

4 Bindoli, A., Rigobello, M.P., and Deeble, D.J. (1992). Biochemical and toxicological properties of the oxidation products of catecholamines. *Free Radic. Biol. Med.* 13: 391–405.

5 Bisaglia, M., Mammi, S., and Bubacco, L. (2007). Kinetic and structural analysis of the early oxidation products of dopamine. *J. Biol. Chem.* 282: 15597–15605.

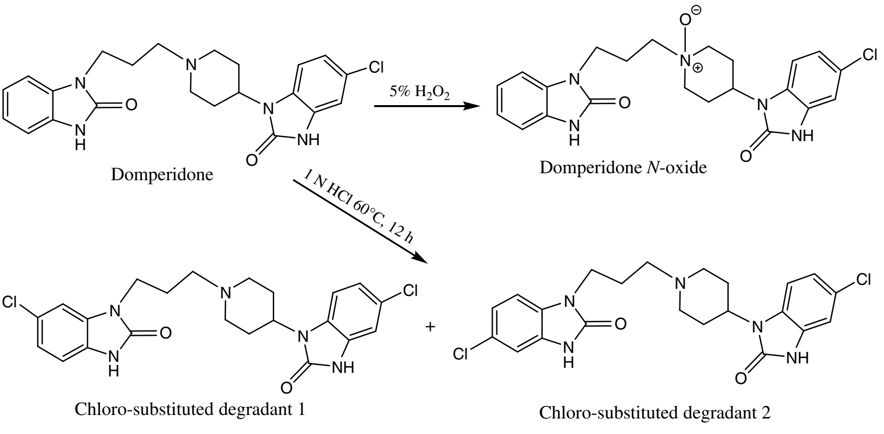
165: Domperidone



Chemical name: 6-Chloro-3-[1-[3-(2-oxo-3*H*-benzimidazol-1-yl)propyl] piperidin-4-yl]-1*H*-benzimidazol-2-one
Trade name: Motilium, Euciton, Moperidona, Nauzelin
Formula: $C_{22}H_{24}ClN_5O_2$
Monoisotopic protonated ion (*m/z*): 426.1691
Molecular weight: 425.91
CAS number: 57808-66-9
Common salt form: Maleate

Domperidone is a peripheral dopamine receptor antagonist, which has been marketed outside of the United States since 1978 [1]. Its clinical use includes the treatment of vomiting and nausea, as well as gastrointestinal motility disorders.

There are a few studies for the development of stability-indicating methods in which forced degradation was performed [2, 3]. Nevertheless, there seems to be only one study performed by Mutha et al., who conducted a full structural elucidation of the degradation products generated [4]. In the study by Mutha et al., three degradants were observed, and their structures elucidated by mass spectrometry and 1D and 2D NMR. The first degradant is the *N*-oxide of domperidone, which was generated by stress with 5% hydrogen peroxide, while the second and third degradants are a pair of isomeric chloro-substituted domperidone, which were produced by stress in 1 N HCl at 60°C for 12 hours (Scheme 165). While domperidone *N*-oxide could be



Scheme 165 Degradation pathway of domperidone under oxidative and acidic stress conditions.

a real degradant, the two chloro-substituted isomers are most likely solution degradation products due to the use of 1 N HCl in the acidic stress condition. The formation of these two artifact degradants appears to be rather unusual; nonetheless, no further study was performed with regard to their formation mechanism.

References

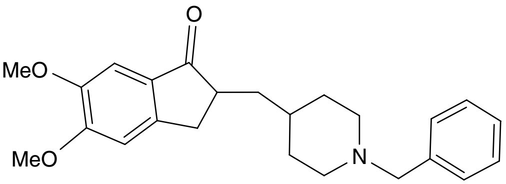
1 Barone, J.A. (1999). Domperidone: a peripherally acting dopamine2-receptor antagonist. *Ann. Pharmacother.* 33: 429–440.

2 Thanikachalam, S., Rajappan, M., and Kannappan, V. (2008). Stability-indicating HPLC method for simultaneous determination of pantoprazole and domperidone from their combination drug product. *Chromatographia* 67: 41–47.

3 Seshadri, R.K., Raghavaraju, T.V., and Chakravarthy, I.E. (2013). A single gradient stability-indicating reversed-phase LC method for the estimation of impurities in omeprazole and domperidone capsules. *Sci. Pharm.* 81: 437–458.

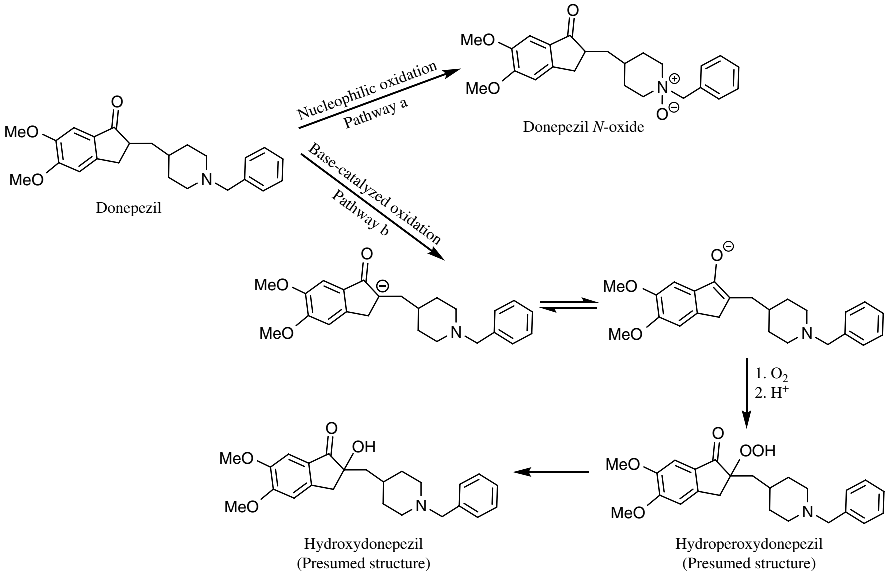
4 Mutha, A.K., Chandra, C., Vijayabhaskar, B. et al. (2019). Separation of unprecedented degradants of domperidone by ultra-performance convergence chromatography and their structure elucidation. *J. Chromatogr. Sci.* 57: 806–814.

166: Donepezil



Chemical name: 2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one
Trade name: Aricept
Formula: $C_{24}H_{29}NO_3$
Monoisotopic protonated ion (*m/z*): 380.2220.
Molecular weight: 379.50
CAS number: 120014-06-4
Common salt form: HCl salt

Donepezil is an acetylcholinesterase inhibitor and clinically used for the treatment of Alzheimer's disease. The structural moiety in this drug molecule that is most obviously susceptible to degradation is the benzyl-substituted piperidinyl tertiary amine group, which could be degraded into the corresponding *N*-oxide under nucleophilic oxidative conditions. There have been a number of papers reporting stress studies of donepezil HCl and its formulated products under different stress conditions, including oxidative stress condition. The latter stress used hydrogen peroxide in different concentrations (from 3% to 30%) and temperatures (from room temperature to 80°C). Most of these studies reported oxidative degradation products [1–6], except few that did not observe oxidative degradation [3]. The main reason for not observing the oxidative degradant is most likely due to one or more of the following factors: (i) donepezil HCl was not neutralized with base prior to the oxidation, (ii) the concentration of hydrogen peroxide used was relatively low, (iii) the stress temperature was low, e.g., at room temperature, and (iv) the method used could not elute the degradants. For those studies that reported oxidative degradation products of donepezil under hydrogen peroxide stress, no efforts were demonstrated to elucidate the structures of the degradants, except the one by Mladenovic et al., who performed high-resolution LC-MS analysis of the degradants [4], and the one by Sreelatha et al. [5], who performed both LC-MS and ^{1}H NMR analysis. In the oxidative stress study performed by Mladenovic et al. using 3.0% hydrogen peroxide at 80°C for 30 minutes, a major oxidative degradant was observed at a relative retention time of 1.79 [4]. This degradant has an additional oxygen than donepezil and thus would be most likely the *N*-oxide of donepezil, which is consistent with the results of a later study performed by Sreelatha et al., where the structure of the *N*-oxide was confirmed by ^{1}H NMR measurement [5]. The plausible mechanism for the formation of the *N*-oxide is illustrated in Scheme 166 (Pathway a).



Scheme 166 Oxidative degradation pathways of donepezil under forced degradation conditions.

In the stress studies mentioned above, nearly all of them also reported degradation products under alkaline stress conditions. Nevertheless, no structural elucidation was performed in these studies, except the ones performed by Ruela et al. [3] and Mladenovic et al. [4] in which unit-resolution LC-MS and high-resolution LC-MS were utilized, respectively, to analyze the degradants. Three major degradants were observed eluting before donepezil by both research groups [3, 4], and their formulas were found to be [donepezil + O_2–H_2], [donepezil + O], and [donepezil + O_2], respectively [4]. It was found that the [donepezil + O_2] degradant was increasing along with the time of alkaline stress and became the major degradant in 6 hours [3]. Based on these results obtained by the two groups, one can reasonably speculate that donepezil underwent base-catalyzed oxidation [7], and the [donepezil + O] and [donepezil + O_2] degradants would likely be the hydroxydonepezil and hydroperoxydonepezil. The plausible mechanism for the formation of the two base-catalyzed oxidative degradants is displayed in Scheme 166 (Pathway b).

References

1 Pappa, H., Farru, R., Vilanova, P.O. et al. (2002). A new HPLC method to determine donepezil hydrochloride in tablets. *J. Pharm. Biomed. Anal.* 27: 177–182.

2 Kafkala, S., Matthaiou, S., Alexaki, P. et al. (2008). New gradient high-performance liquid chromatography method for determination of donepezil hydrochloride assay and impurities content in oral pharmaceutical formulation. *J. Chrom. A* 1189: 392–397.

3 Ruela, A.L.M., Santos, M.G., Figueiredo, E.C., and Pereira, G.R. (2014). LC-PDA and LC-MS studies of donepezil hydrochloride degradation behaviour in forced stress conditions. *J. Braz. Chem. Soc.* 25: 2094–2101.

4 Mladenovic, A.R., Jadranin, M.B., Pavlovic, A.D. et al. (2015). Liquid chromatography and liquid chromatography-mass spectrometry analysis of donepezil degradation products. *Chem. Ind. Chem. Eng. Q.* 21: 447–455.

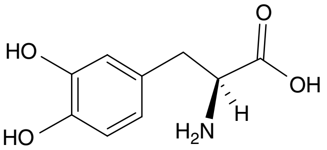
5 Sreelatha, P., Reddy, D.V., Palavai, S.R., and Devi, B.R. (2016). Impurity profiling for donepezil hydrochloride tablet formulations and characterisation of potential degradant. *Am. J. PharmTech Res.* 6: 213–229.

6 Korany, M.A., Mahgoub, H., Haggag, R.S. et al. (2017). Development of a green stability-indicating HPLC–DAD method for the determination of donepezil hydrochloride in the presence of its related substance and degradation products. *J. Liq. Chromatogr. Relat. Technol.* 40: 930–942.

7 Li, M. (2012). Section 3.4, Carbanion/enolate-mediated autooxidation (base-catalyzed autooxidation). In: *Organic Chemistry of Drug Degradation*, 61–62. Cambridge: RSC.

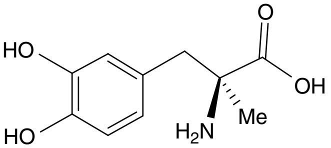
167–168: L-Dopa (Levodopa), Methyldopa

167: L-Dopa (Levodopa)



Chemical name: (2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)propanoic acid
Trade name: Sinemet, Larodopa, Dopar, Inbrija
Formula: $C_9H_{11}NO_4$
Monoisotopic protonated ion (*m/z*): 198.0761
Molecular weight: 197.19
CAS number: 59-92-7

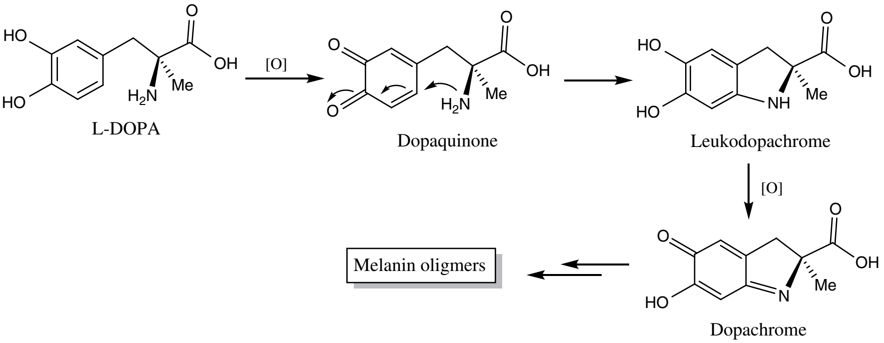
168: Methyldopa



Chemical name: (2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid
Trade name: Aldomet, Aldoril, Dopamet
Formula: $C_{10}H_{13}NO_4$
Monoisotopic protonated ion (*m/z*): 212.0917
Molecular weight: 211.21
CAS number: 555-30-6
Common salt form: HCl salt

Levodopa, also known as L-DOPA, is L-3,4-dihydroxyphenylalanine. Biosynthetically, it is the precursor of dopamine, and thus, it is used as a treatment for Parkinson's disease, which is characterized by a decrease in the production of dopamine in the brain. Conversion of levodopa into dopamine is catalyzed by the enzyme levodopa decarboxylase, and as the enzyme is not only present in the brain but also in the peripheral tissues, levodopa is typically coadministered with an inhibitor of the decarboxylase, such as carbidopa, to prevent levodopa decarboxylation before it reaches the brain.

Compared to dopamine, the structure of levodopa has an extra carboxyl group, and the remaining parts are the same. Thus, the catecholamine moiety of levodopa undergoes a similar degradation pathway as that of dopamine (Scheme 167–168; refer to Scheme 161–164-2 for the oxidative degradation pathway of dopamine) [1].



Scheme 167–168 Oxidative degradation pathway of L-DOPA. The intermediates are unstable, which were not detected by Zhou et al. in their stress study [1]. *Source:* Zhou et al. [1]/Oxford University Press.

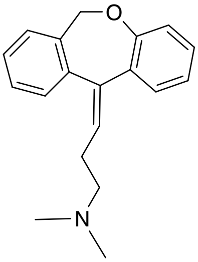
Methyldopa is a medication for treating hypertension, and it is a methyl derivative of levodopa, with its methyl group replacing the alpha-H of levodopa. Due to the high similarity of its structure with that of L-DOPA, the degradation chemistry of methyldopa should be very similar to that of levodopa.

Reference

1 Zhou, Y.Z., Alany, R.G., Chuang, V., and Wen, J. (2012). Studies of the rate constant of L-DOPA oxidation and decarboxylation by HPLC. *Chromatographia* 75: 597–606.

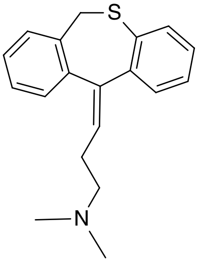
169–170: Doxepin, Dothiepin

169: Doxepin



Chemical name: (3*E*)-3-(6*H*-Benzo[c][1]benzoxepin-11-ylidene)-*N,N*-dimethylpropan-1-amine
Trade name: Sinequan
Formula: $C_{19}H_{21}NO$
Monoisotopic protonated ion (*m/z*): 280.1696
Molecular weight: 279.38
Common salt form: HCl salt
CAS number: 1668-19-5

170: Dothiepin (Dosulepin)

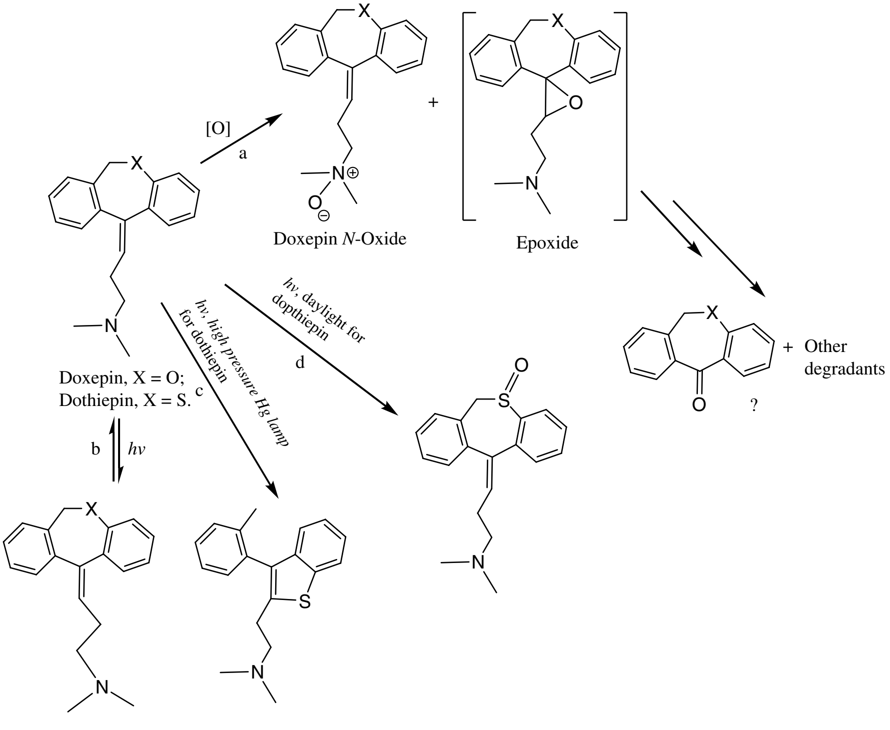


Chemical name: (3*Z*)-3-(6*H*-Benzo[c][1]benzothiepin-11-ylidene)-*N,N*-dimethylpropan-1-amine
Trade name: Prothiaden, Dopress
Formula: $C_{19}H_{21}NS$
Monoisotopic protonated ion (*m/z*): 296.1467
Molecular weight: 295.44
CAS number: 113-53-1
Common salt form: HCl salt

Doxepin is used clinically for the treatment of depression and anxiety disorders. Structurally, it is similar to the drugs in the tricyclic antidepressant family, such as amitriptyline, particularly in the part of the tertiary amine moiety that contains a

C═C double bond linked to the tricyclic ring. Hence, the degradation chemistry of the tertiary amine moiety would be expected to be similar to that of amitriptyline and related drugs. Specifically, the tertiary amine group could undergo nucleophilic oxidation to yield the corresponding *N*-oxide, while the C═C double bond might be susceptible to epoxidation (particularly under photo-oxidative conditions) and photoisomerization, respectively (Pathway a, Scheme 169–170). In a study by Po and Irwin [1], irradiation of a doxepin aqueous solution with a mercury lamp led to the increase of the cis-isomer (Pathway b, Scheme 169–170). In the presence of nitrogen, there was another photo-rearranged degradant, while in the presence of air, the degradation products were much more complex. There was a study in which doxepin was found to slightly degrade to give one degradant after being subjected to hydrolysis in 2 M HCl at 90°C for 24 hours [2]. Nevertheless, the structure of the acidic degradant proposed does not seem to be reasonable.

Dothiepin, also known as dosulepin, is the sulfur analog of doxepin in that the oxygen atom of doxepin is replaced by a sulfur atom. It is also employed as a treatment for depression. Due to its structure being highly similar to that of doxepin, the degradation chemistry of dothiepin would be expected to follow that of doxepin, except for the moiety containing the sulfur atom. In a photostability study of dothiepin in aqueous solutions, when irradiation was carried out under high pressure mercury lamp, dothiepin was found to undergo another isomerization in which the



Scheme 169–170 Probable degradation pathways of doxepine and dothiepin; the presumed oxidative degradation is based on the degradation chemistry of amitriptyline.

7-membered ring was opened and the sulfur attached to the carbon-carbon double bond (Pathway c, Scheme 169–170) [3]. When the irradiation of dothiepin was performed under daylight, the main degradation was the oxidation of the sulfur moiety, leading to the formation of *S*-oxide (Pathway d, Scheme 169–170).

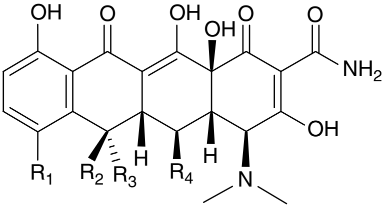
References

1 Po, A.L.W. and Irwin, W.J. (1980). The photochemical stability of cis- and trans-isomers of tricyclic neuroleptic drugs. *J. Pharm. Pharmacol.* 32: 25–29.

2 Maślanka, A., Krzek, J., and Stolarczyk, M. (2011). Stability studies of clonazepam, diazepam, haloperidol, and doxepin with diverse polarities in an acidic environment. *J. AOAC Inter.* 94: 1791–1799.

3 Tammilehto, S. and Torniainen, K. (1989). Photochemical stability of dothiepin in aqueous solutions. *Int. J. Pharm.* 52: 123–128.

171–175: Doxycycline, Chlortetracycline, Oxytetracycline, Tetracycline, Minocycline



Doxycycline: R_1=H, R_2=Me, R_3=H, R_4=OH;
Chlortetracycline: R_1=Cl, R_2=Me, R_3=OH, R_4=H;
Oxytetracycline: R_1=H, R_2=Me, R_3=OH, R_4=OH;
Tetracycline: R_1=H, R_2=Me, R_3=H, R_4=H;
Minocycline: R_1=N(Me)$_2$, R_2=H, R_3=H, R_4=H.

171: Doxycycline [WHO List of Essential Medicines]

Chemical name: (4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aR*)-4-(Dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-2-carboxamide
Trade name: Doxy, Doryx, Vibramycin
Formula: $C_{22}H_{24}N_2O_8$
Monoisotopic protonated ion (*m/z*): 445.1605
Molecular weight: 444.44
CAS number: 564-25-0
Common salt form: HCl salt

172: Chlortetracycline [WHO List of Essential Medicines]

Chemical name: (4*S*,4*aS*,5*aS*,6*S*,12*aR*)-7-Chloro-4-(dimethylamino)-1,6, 10,11,12*a*-pentahydroxy-6- methyl-3,12-dioxo-4,4*a*,5,5*a*-tetrahydrotetracene-2-carboxamide
Trade name: Aueromycin
Formula: $C_{22}H_{23}ClN_2O_8$
Monoisotopic protonated ion (*m/z*): 479.1216
Molecular weight: 478.88
CAS number: 57-62-5
Common salt form: HCl salt

173: Oxytetracycline [WHO List of Essential Medicines]

Chemical name: (4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aR*)-4-(Dimethylamino)-1,5,6,10,11,12a-hexahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide
Trade name: Terramycin, Urobiotic
Formula: $C_{22}H_{24}N_2O_9$

Monoisotopic protonated ion (*m/z*): 461.1555
Molecular weight: 460.43
CAS number: 79-57-2
Common salt form: HCl salt

174: Tetracycline [WHO List of Essential Medicines]

Chemical name: (4*S*,4*aS*,5*aS*,6*S*,12*aR*)-4-(Dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide
Trade name: Achromycin, Pylera, Sumycin
Formula: $C_{22}H_{24}N_2O_8$
Monoisotopic protonated ion (*m/z*): 445.1605
Molecular weight: 444.44
CAS number: 60-54-8
Common salt form: HCl salt

175: Minocycline

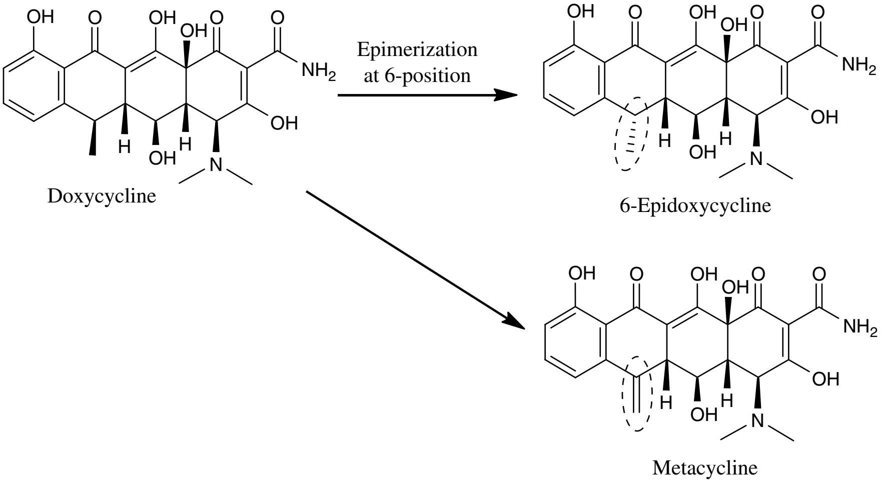
Chemical name: (4*S*,4*aS*,5*aR*,12*aR*)-4,7-Bis(dimethylamino)-1,10,11,12*a*-tetrahydroxy-3,12-dioxo-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-2-carboxamide
Trade name: Minocin, Minomycin, Akamin, Amzeeq
Formula: $C_{23}H_{27}N_3O_7$
Monoisotopic protonated ion (*m/z*): 458.1922
Molecular weight: 457.48
CAS number: 10118-90-8
Common salt form: HCl salt

Doxycycline is the most prescribed member in the tetracycline family of antibiotics that have broad antibacterial activity. The other members of the family include chlortetracycline, oxytetracycline, tetracycline, and minocycline. They all have the same core structure of a linear fused tetracyclic nucleus, with each of the fused rings named as A, B, C, and D rings, respectively.

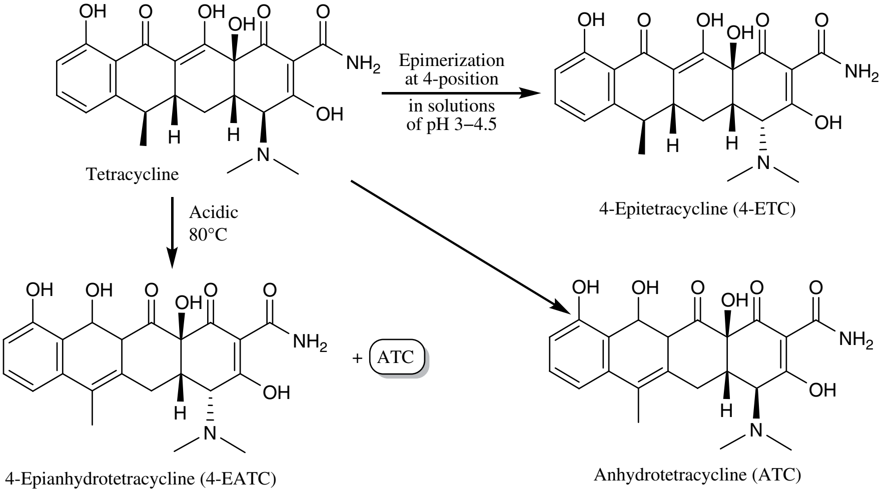
In general, the family of tetracycline drugs displays poor stability, and their main degradation pathway is the epimerization at either the 4- or the 6-position of the core structure [1, 2]. Other degradation types may also occur depending on the specific structure of a tetracycline, pH of the environment, and other factors such as excipients used in their formulated products.

Injac et al. reported two degradation products, 6-epidoxycycline and metacycline, after subjecting doxycycline capsules to 70°C for 90 days (Scheme 171–175-1) [1]. Metacycline, which is also a synthetic intermediate of doxycycline, and 6-epidoxycycline are both controlled at no more than 2% by USP and EP.

Pena et al. performed acidic stress of tetracycline (TC) and found it underwent epimerization at the 4-position to form 4-epitetracycline (4-ETC) in a weak pH range of 3–4.5 (Scheme 171–175-2) [3]. In solution of $pH < 2$, anhydrotetracycline was produced, while at the elevated temperature of 80°C, both anhydrotetracycline (ATC) and 4-epianhydrotetracycline (4-



Scheme 171–175-1 Degradation of doxycycline capsules under stress at 70°C for 90 days [1].



Scheme 171–175-2 Degradation of tetracycline under conditions of acidic stress and elevated temperature of 80°C.

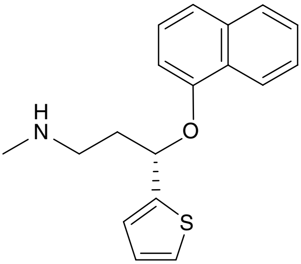
References

1 Injac, R., Djordjevic-Milic, V., and Srdjenovic, B. (2007). Thermostability testing and degradation profiles of doxycycline in bulk, tablets, and capsules by HPLC. *J. Chromatogr. Sci.* 45: 623–628.

2 Skulason, S., Ingolfsson, E., and Kristmundsdottir, T. (2003). Development of a simple HPLC method for separation of doxycycline and its degradation products. *J. Pharm. Biomed. Anal.* 33: 667–672.

3 Pena, A., Palilis, L.P., Lino, C.M. et al. (2000). Determination of tetracycline and its major degradation products by chemiluminescence.

176: Duloxetine



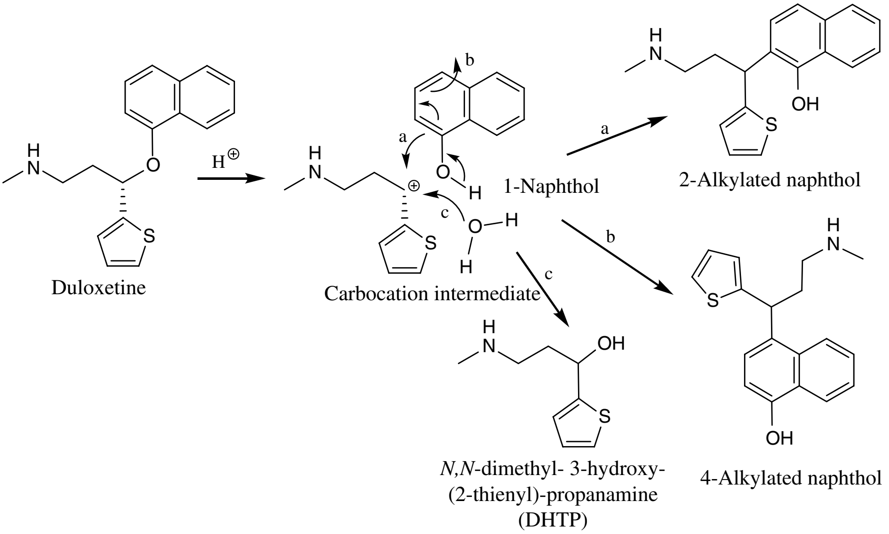
Chemical name: (3*S*)-*N*-Methyl-3-naphthalen-1-yloxy-3-thiophen-2-ylpropan-1-amine
Trade name: Cymbalta
Formula: $C_{18}H_{19}NOS$
Monoisotopic protonated ion (*m/z*): 298.1260
Molecular weight: 297.42
CAS number: 116539-59-4
Common salt form: HCl salt

Duloxetine is an inhibitor for the reuptake of both serotonin and norepinephrine. It is clinically used for the treatment of major depression, as well as neuropathic pain and other indications. The 1-naphthyl ether linkage is the primary weak point of the drug molecule that is readily degradable under acidic conditions; various forced degradation studies have demonstrated its acidic lability [1–4]. In these studies, the structures of certain degradation products were identified or specified based on solid evidence such as NMR and X-ray crystallography [1] or authentic reference compounds [2]. Based on the results of these studies, the degradation pathway of duloxetine is summarized in Scheme 176.

Under acidic conditions, the 1-naphthyl ether moiety is first protonated, which is followed by the cleavage of the ether linkage to generate 1-naphthol and the carbocation intermediate. The latter intermediate, which can be stabilized by conjugation with the thiophene ring, is then intercepted by either 1-naphthol or water to yield 2- and 4-alkylated derivatives of 1-naphthol, as well as *N,N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine (DHTP).

Yet, in another forced degradation study, the apparent overstress generated 17 degradation products under various stress conditions, and the structures of 15 of the 17 degradants were proposed solely based on LC-MS and MS-MS analyses [5]. The structures proposed in this study do not seem to be relevant for the degradation of duloxetine under real-life scenarios.

In summary, duloxetine is reasonably stable in conditions other than acidic ones. Under photolytic stress conditions, duloxetine HCl API was found to be stable in solid but unstable in solution. On the other hand, the secondary amine moiety of duloxetine may be incompatible with aldehyde-containing excipients due to potential Maillard reaction between the secondary amine and aldehyde functionalities [6].



Scheme 176 Degradation pathway of duloxetine under acidic conditions. The chiral centers in the three degradants, 2-alkyldated naphthol, 4-alkylated naphthol, and DHTP, are most likely racemized.

References

1 Arava, V.R., Siripalli, U.B.R., and Bandatmakuru, S.R. (2007). Novel acid catalysed rearrangement of duloxetine. *Indian J. Chem.* 46B: 1695–1698.

2 Sinha, V.R., Anamika, R., and Kumria, J.R.B. (2009). Stress degradation studies on duloxetine hydrochloride and development of an RP-HPLC method for its determination in capsule formulation. *J. Chromatogr. Sci.* 47: 589–593.

3 Raman, N.V.V.S.S., Harikrishna, K.A., Prasad, A.V.S.S. et al. (2010). Determination of duloxetine hydrochloride in the presence of process and degradation impurities by a validated stability-indicating RP-LC method. *J. Pharm. Biomed. Anal.* 51: 994–997.

4 Reddy, P.R.M., Sreeramulu, J., Naidu, P.Y., and Reddy, A.R. (2010). Stability indicating fast LC for the simultaneous estimation of intermediates and degradants of duloxetine hydrochloride. *Chromatographia* 71: 95–100.

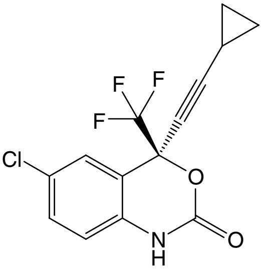
5 Chadha, R., Bali, A., and Bansal, G. (2016). Characterization of stress degradation products of duloxetine hydrochloride employing LC–UV/PDA and LC–MS/TOF studies. *J. Pharm. Biomed. Anal.* 121: 39–55.

6 Wirth, D.D., Baertschi, S.W., Johnson, R.A. et al. (1998). Maillard reaction of lactose and fluoxetine hydrochloride, a secondary amine. *J. Pharm. Sci.* 87: 31–39.

6

Efavirenz to Hydroxychloroquine

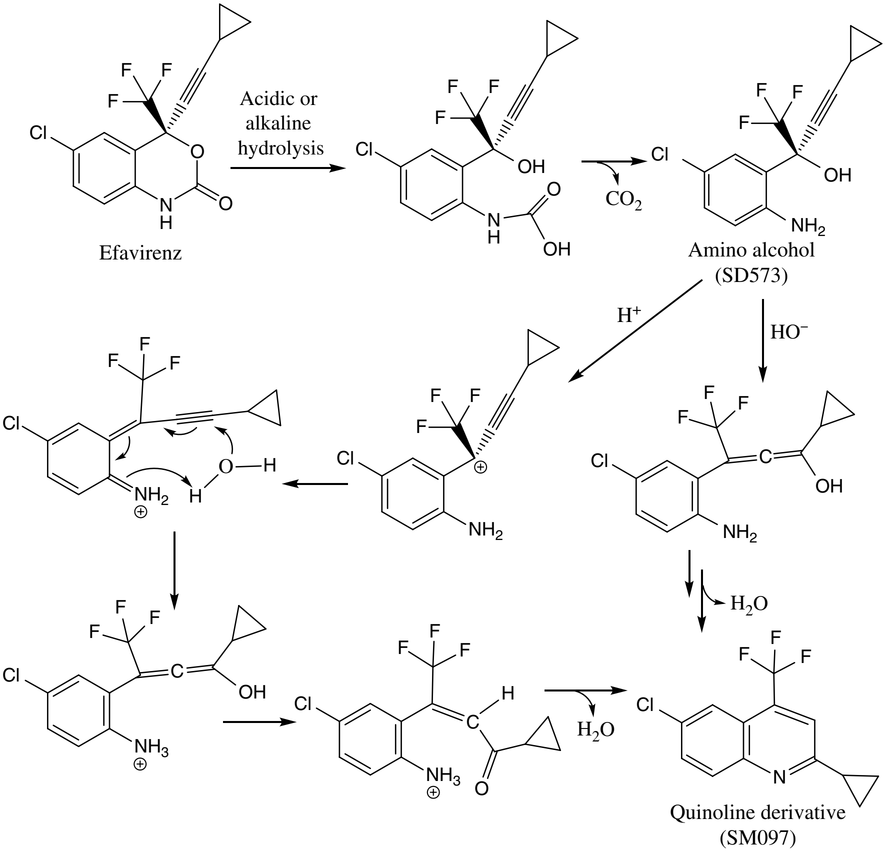
177: Efavirenz



Chemical name: (4*S*)-6-Chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1*H*-3,1-benzoxazin-2-one
Trade name: Stoctin, Sustiva
Formula: $C_{14}H_9ClF_3NO_2$
Monoisotopic protonated ion (*m/z*): 316.0347
Molecular weight: 315.67
CAS number: 154598-52-4

Efavirenz is a non-nucleoside inhibitor of the reverse transcriptase of human immunodeficiency virus type-1 (HIV-1), the virus that causes AIDS (acquired immune deficiency syndrome). It contains a fused cyclic carbamate moiety, which is susceptible to hydrolytic degradation under either acidic or alkaline condition [1, 2]. The initial degradation product is an amino alcohol (code name SD573) [3], which can further degrade to a quinoline derivative (code name SM097) [3] via Rupe or Meyer–Shuster rearrangement depending on the catalysis by either acidic or alkaline conditions, respectively (Scheme 177) [1]. Maurin et al. studied the kinetics of efavirenz across a wide range of pH and found that it displayed a V-shape pH-rate profile with the most stable pH range at ~3–5 [1].

Compendium of Drug Degradation Pathways, First Edition. Min Li.



Scheme 177 Degradation of efavirenz under acidic or alkaline condition.

References

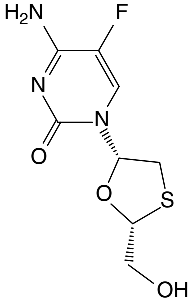
1 Maurin, M.B., Rowe, S.M., Blom, K., and Pierce, M.E. (2002). Kinetics and mechanism of hydrolysis of efavirenz. *Pharm. Res.* 19: 517–521.

2 de Aquino Ribeiro, J.A., Moreira de Campos, L.M., Alves, R.J. et al. (2007). Efavirenz related compounds preparation by hydrolysis procedure: setting reference standards for chromatographic purity analysis. *J. Pharm. Biomed. Anal.* 43: 298–303.

3 Alexander, A.J., Zhang, L., Hooker, T.F., and Tomasella, F.P. (2013). Comparison of supercritical fluid chromatography and reverse phase liquid chromatography for the impurity profiling of the antiretroviral drugs lamivudine/BMS-986001/efavirenz in a combination tablet. *J. Pharm. Biomed. Anal.* 78–79: 243–251.

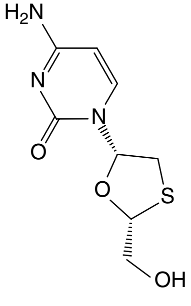
178–179: Emtricitabine (FTC), Lamivudine (3TC)

178: Emtricitabine (FTC)



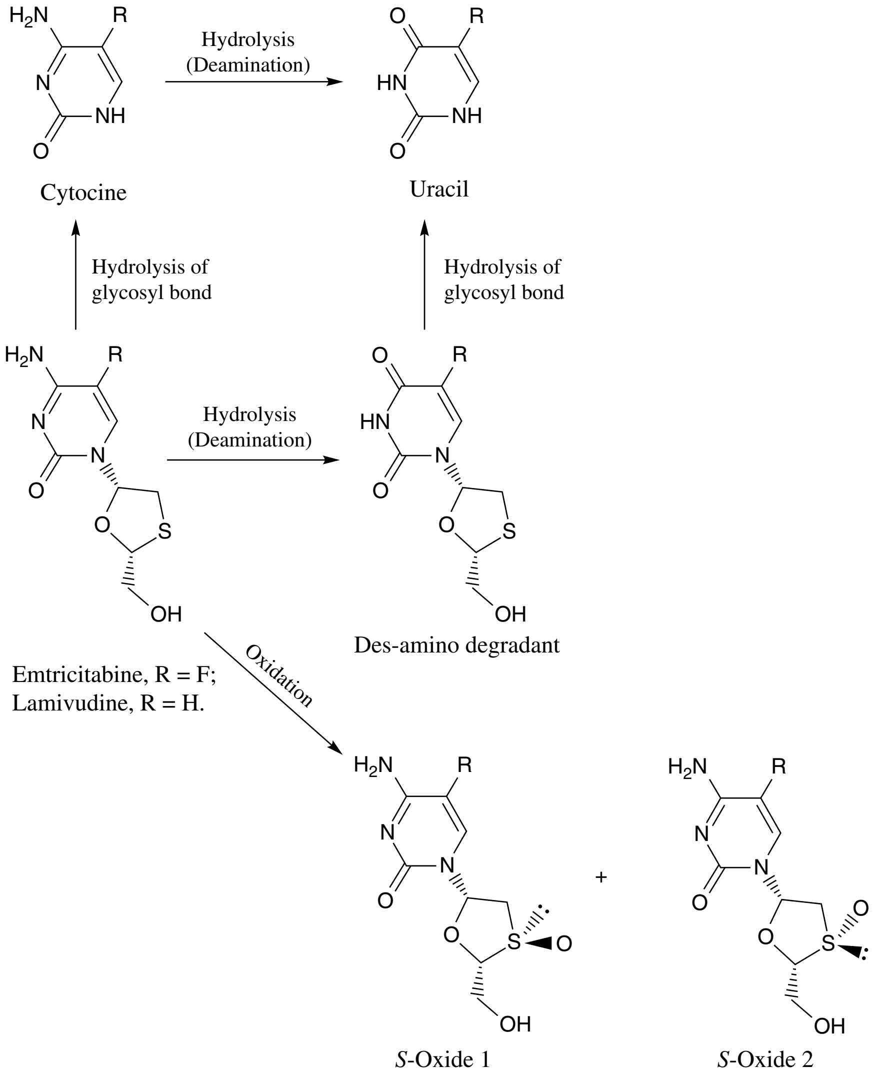
Chemical name: 4-Amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one
Trade name: Emtriva
Formula: $C_8H_{10}FN_3O_3S$
Monoisotopic protonated ion (*m/z*): 248.0500
Molecular weight: 247.24
CAS number: 143491-57-0

179: Lamivudine



Chemical name: 4-Amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2-one
Trade name: Epivir, Zeffix, Heptovir
Formula: $C_8H_{11}N_3O_3S$
Monoisotopic protonated ion (*m/z*): 230.0594
Molecular weight: 229.25
CAS number: 134678-17-4

Emtricitabine, also referred to as FTC, is a nucleoside analog that inhibits the HIV-1 reverse transcriptase, and it is typically utilized in combination with other anti-AIDS medications. FTC consists of three functional groups or structural moieties



Scheme 178–179 Degradation pathways of emtricitabine and lamivudine.

that are susceptible to degradation: the amino group can be hydrolyzed to produce the des-amino degradant, the sulfur in the modified sugar ring can be subjected to nucleophilic oxidation to give the sulfoxide degradant (*S*-oxide), and the glycosyl linkage could be cleaved to yield 5-fluorocytocine [1]. In an oral liquid formulation, the des-amino and sulfoxide degradants were observed, while in solid dosage forms such as capsules and tablets, only the sulfoxide degradant was detected [2]. Both the des-amino and sulfoxide degradants can be generated under the conditions of acidic hydrolysis and hydrogen peroxide oxidation, respectively, as reported by Seshachalam et al. and Ashenafi et al. [3, 4]. It needs to be noted that the structure of the des-amino degradant presented in the papers of Seshachalam et al. and Ashenafi et al. was incorrect.

Lamivudine, also known as 3TC, is the des-fluoro analog of emtricitabine, which is also an inhibitor of the HIV-1 reverse transcriptase. Due to the very high similarity between the two drug molecules, their degradation pathways are expected to be the same or very similar, which turned out to be true according to a forced degradation study performed by Bedse et al. [5]. The degradation pathways for Emtricitabine and lamivudine are shown in Scheme 178–179. It is interesting to note that the two oxidative degradants of lamivudine, i.e., the two diastereomeric lamivudine *S*-oxides, were separated in the study by Bedse et al., while their counterparts of emtricitabine were not resolved by the HPLC analyses in the previous studies [3, 4]. Further looking into the HPLC methods of emtricitabine shows that the peak of *S*-oxides eluted quite early, suggesting the methods lack proper resolving power to separate the two structurally very similar diastereomers.

References

1 Hamarapurkar, P.D. and Parate, A.N. (2013). HPLC method for the determination of emtricitabine and related degradation substances. *J. Chromatogr. Sci.* 51: 419–424.

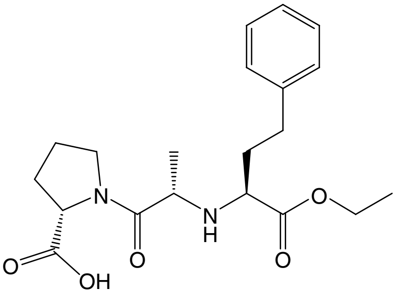
2 Ashenafi, D., Ungerbock, M., Hoogmartens, J., and Adams, E. (2013). Liquid chromatographic analysis of various formulations containing emtricitabine. *Chromatographia* 76: 1495–1503.

3 Seshachalam, U., Haribabu, B., and Chandrasekhar, K.B. (2007). Development and validation of a stability-indicating liquid chromatographic method for determination of emtricitabine and related impurities in drug substance. *J. Sep. Sci.* 30: 999–1004.

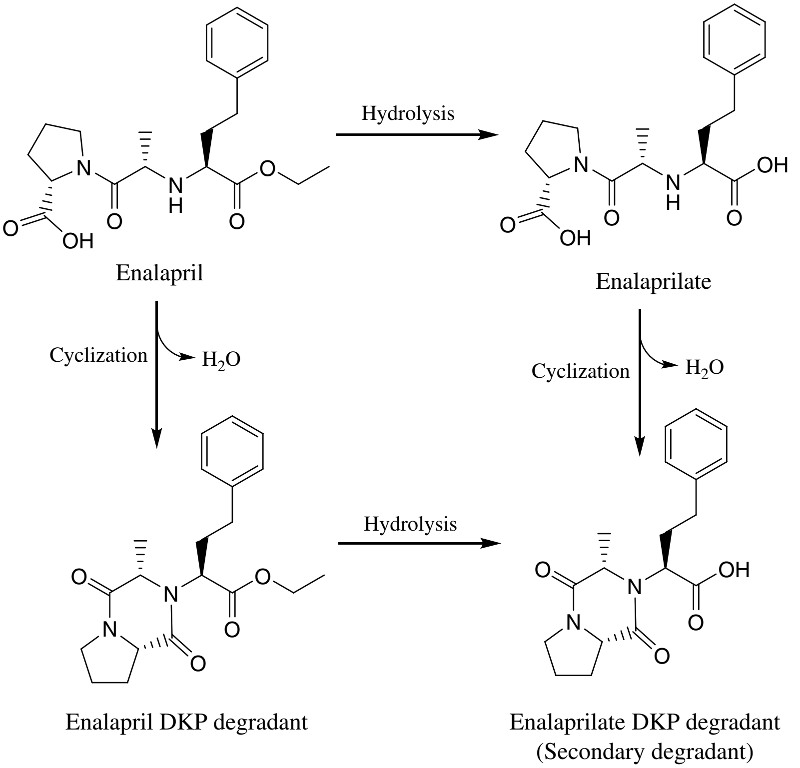
4 Ashenafi, D., Verbeek, A., Hoogmartens, J., and Adams, E. (2009). Development and validation of an LC method for the determination of emtricitabine and related compounds in the drug substance. *J. Sep. Sci.* 32: 1823–1830.

5 Bedse, G., Kumar, V., and Singh, S. (2009). Study of forced decomposition behavior of lamivudine using LC, LC–MS/TOF and MS^n. *J. Pharm. Biomed. Anal.* 49: 55–63.

180: Enalapril



Chemical name: (2*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]pyrrolidine-2-carboxylic acid
Trade name: Vasotec
Formula: $C_{20}H_{28}N_2O_5$
Monoisotopic protonated ion (*m/z*): 377.2071
Molecular weight: 376.45
CAS number: 75847-73-3
Common salt form: Maleate



Scheme 180 Degradation pathways of enalapril.

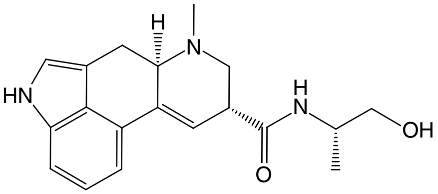
Enalapril is clinically used for the treatment of hypertension. It is a prodrug that is hydrolyzed in vivo by esterases into its active metabolite, enalaprilate, which is an angiotensin-converting enzyme (ACE) inhibitor. The ethyl ester functionality of enalapril can also be hydrolyzed to form enalaprilate, particularly under alkaline conditions [1, 2]. Under acidic conditions, the main degradation pathway seems to be the formation of the so-called diketopiperazine (DKP) degradant, which is a common degradation pathway for many ACE inhibitors that possess a free carboxyl and a secondary amine group [3]. In formulated enalapril products, both enalaprilate and the DKP degradants were observed in meaningful amounts [4, 5]. In forced degradation studies in acidic and alkaline solutions, secondary degradation products could be formed in significant amounts [6]. Like some of the other ACE inhibitors, enalapril could be present in two rotamers due to the hindered rotation around the amide bond; the two rotamers would usually appear as two split peaks in HPLC analysis, which could be converted into a single peak with the control of several factors of the HPLC methods such as column temperature, pH of the mobile phase, and choice of the organic modifier [7]. In a study of enalapril maleate in solid, the formation of the DKP degradant was found to be independent of the moisture [8]. The degradation pathways of enalapril are shown in Scheme 180.

References

1. Shiromani, P.K. and Bavitz, J.F. (1986). Effect of moisture on the physical and chemical stability of granulations and tablets of the angiotensin converting enzyme inhibitor, enalapril maleate. *Drug Dev. Ind. Pharm.* 12: 2467–2480.
2. Ip, D.P. and Brenner, G.S. (1987). Enalapril Maleate. In: *Analytical Profiles of Drug Substances*, vol. 16 (ed. K. Florey), 207–243. London: Academic Press.
3. Li, M. (2012). Various types and mechanisms of degradation reactions. In: *Organic Chemistry of Drug Degradation*, 110–146. Cambridge, UK: RSC Publishing.
4. Qin, X.-Z., DeMarco, J., and Ip, D.P. (1995). Simultaneous determination of enalapril, felodipine and their degradation products in the dosage formulation by reversed phase high-performance liquid chromatography using a Spherisorb C_8 column. *J. Chromatogr. A* 707: 245–254.
5. Al-Omari, M.M., Abdelah, M.K., Badwan, A.A., and Jaber, A.M.Y. (2001). Effect of the drug-matrix on the stability of enalapril maleate in tablet formulations. *J. Pharm. Biomed. Anal.* 25: 893–902.
6. Bhardwaj, S.P. and Singh, S. (2008). Study of forced degradation behavior of enalapril maleate by LC and LC–MS and development of a validated stability-indicating assay method. *J. Pharm. Biomed. Anal.* 46: 113–120.
7. Trabelsi, H., Bouabdallah, S., Sabbah, S. et al. (2000). Study of the cis–trans isomerization of enalapril by reversed-phase liquid chromatography. *J. Chromatogr. A* 871: 189–199.
8. Stanisz, B. (2003). Evaluation of stability of enalapril maleate in solid phase. *J. Pharm. Biomed. Anal.* 31: 375–380.

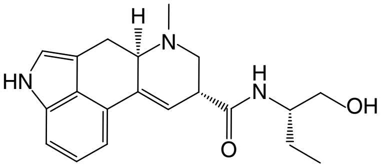
181–183: Ergometrine, Methylergometrine, Ergotamine

181: Ergometrine (Ergonovine) [WHO List of Essential Medicines]



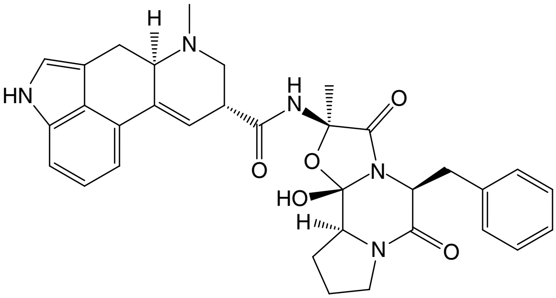
Chemicalname: (6a*R*,9*R*)-*N*-[(2*S*)-1-Hydroxypropan-2-yl]-7-methyl-6,6a,8,9-tetrahydro-4*H*-indolo[4,3-fg]quinoline-9-carboxamide
Trade name: Ergotrate, Ergostat, Syntometrine
Formula: $C_{19}H_{23}N_3O_2$
Monoisotopic protonated ion (*m/z*): 326.1863
Molecular weight: 325.41
CAS number: 60-79-7
Common salt form: Maleate

182: Methylergometrine [WHO List of Essential Medicines]



Chemical name: (6a*R*,9*R*)-*N*-[(2*S*)-1-Hydroxybutan-2-yl]-7-methyl-6,6a,8,9-tetrahydro-4*H*-indolo[4,3-fg]quinoline-9-carboxamide
Trade name: Methergine
Formula: $C_{20}H_{25}N_3O_2$
Monoisotopic protonated ion (*m/z*): 340.2020
Molecular weight: 339.44
CAS number: 113-42-8
Common salt form: Maleate

183: Ergotamine

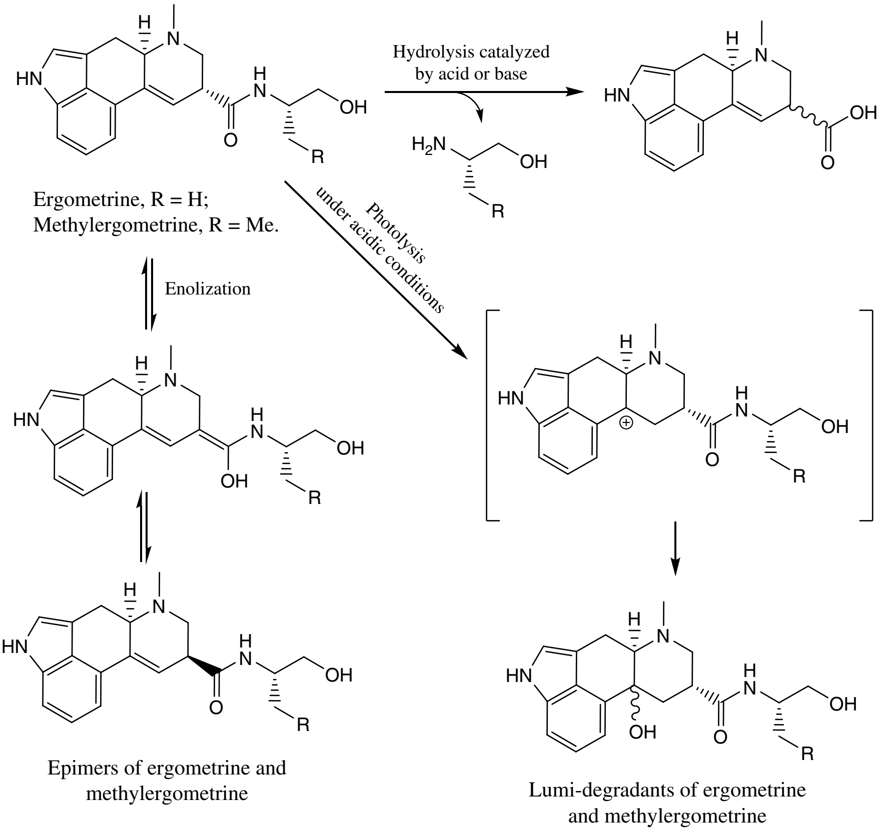


Chemical name: (6a*R*,9*R*)-*N*-[(1*S*,2*S*,4*R*,7*S*)-7-Benzyl-2-hydroxy-4-methyl-5,8-dioxo-3-oxa-6,9-diazatricyclo[7.3.0.0^{2,6}]dodecan-4-yl]-7-methyl-6,6a,8,9-tetrahydro-4*H*-indolo[4,3-fg]quinoline-9-carboxamide
Trade name: Cafergot, Ergomar, Migergot
Formula: $C_{33}H_{35}N_5O_5$
Monoisotopic protonated ion (*m/z*): 582.2711
Molecular weight: 581.67
CAS number: 379-79-3
Common salt form: Tartrate

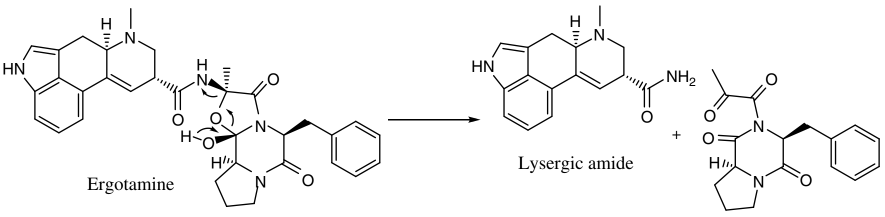
Ergometrine, also known as ergonovine, is a member of the ergot alkaloids, which are produced by fungi of the *Claviceps* species, especially *Claviceps purpurea* on rye and other cereal-producing plants. Although the medical use of ergot, as an aid in childbirth, dates back to the middle ages, it was not until 1935 when Dudley and Moir identified ergometrine as the component responsible for the traditional clinical effect of ergot [1]. Today, ergometrine is used clinically for treating heavy bleeding after childbirth through contraction of the uterus, and it is commonly used in combination with oxytocin [2]. Although methylergometrine is a methyl analog of ergometrine, it originates from being an active metabolite of methysergide, a medication for migraine, via demethylation of the latter [3]. Clinically, methylergometrine is also employed for the treatment of migraine in addition to its use in childbirth [4].

Both ergometrine and methylergometrine are amides of lysergic acid, and the latter is a precursor for LSD. Hydrolysis of ergometrine and methylergometrine can produce lysergic acid [5, 6]. For this reason, ergometrine is a controlled substance. The lysergic part, which is a common structure moiety in both ergometrine and methylergometrine, contains a fused indole ring, a tertiary amine functional group, and a carbon-carbon double bond conjugated to the indole ring. The α-proton of the conjugated double bond is activated by the amide carbonyl group and, thus, can undergo enolization, particularly under alkaline or acidic conditions, which leads to the formation of the epimeric degradants for both ergometrine and methylergometrine [7]. Furthermore, the conjugated double bond is susceptible to photochemical hydration under somewhat acidic conditions as well [8]. These two types of degradation pathways, i.e., epimerization and photochemical hydration, can also be operative in the derivatives (including their degradants) of ergometrine and methylergometrine that contain the same structural moieties. The degradation pathways of ergometrine and methylergometrine are summarized in Scheme 181–182-1.

Ergotamine is also a member of the ergot alkaloids and has been used medicinally since the 17th century with similar indications as some other members of the alkaloid family. Nowadays, it is most widely used in combination with caffeine for treating migraine (e.g., Cafergot) [9]. It shares the same lysergic moiety as those of ergometrine and methylergometrine. Nevertheless, the amine part of ergotamine is much more structurally complex than those of ergometrine and methylergometrine. Thus, on one hand, it is expected that ergotamine undergoes the same degradation pathways as those of ergometrine and methylergometrine (refer to Scheme 181–183-1) with regard to its lysergic moiety [10]. On the other hand, the amine part of ergometrine contains one cyclic amide bond and forming a hemi-acetal linkage with the nearby hydroxyl group. The hemi-acetal linkage could be susceptible to degradation under acidic or alkaline catalysis, which leads to the formation of lysergic amide (Scheme 181–183-2) [10]. This dissociative degradation pathway is different from that of ergometrine and methylergometrine, in that the latter two would produce lysergic acid rather than lysergic amide.



Scheme 181–183-1 Degradation pathways of ergometrine and methylergometrine.



Scheme 181–183-2 Dissociative degradation of ergotamine due to its amine part; the paper citing this degradation only showed the structure of lysergic amide. *Source:* Adapted from Bethke et al. [10].

References

1 Dudley, H.W. and Moir, C. (1935). The substance responsible for the traditional clinical effect of ergot. *BMJ* 1: 520–523.

2 Ergometrine – Wikipedia. https://en.wikipedia.org/wiki/Ergometrine (accessed 2 February 2023).

3 Müller-Schweinitzer, E. and Tapparelli, C. (1986). Methylergometrine, an active metabolite of methysergide. *Cephalalgia* 6: 35–41.

4 Methylergometrine – Wikipedia. https://en.wikipedia.org/wiki/Methylergometrine (accessed 2 February 2023).

5 Reif, V.D. (1982). Ergonovine maleate. In: *Analytical Profiles of Drug Substances*, vol. 11 (ed. K. Florey), 273–312. London: Academic Press.

6 Marigny, K., Lohezic-Ledevehat, F., Aubin, F., and Burgot, G. (2006). Stability of oral liquid preparations of methylergometrine. *Pharmazie* 61: 701–705.

7 Hafner, M., Sulyok, M., Schuhmacher, R. et al. (2008). Stability and epimerisation behaviour of ergot alkaloids in various solvents. *World Mycotoxin J.* 1: 67–78.

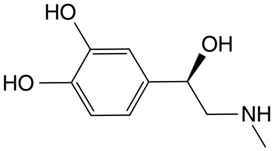
8 Merkel, S., Köppen, R., Koch, M. et al. (2012). Lumi-ergometrine – structural identification and occurrence in sclerotia. *Mycotoxin Res.* 28: 59–66.

9 Ergotamine – Wikipedia. https://en.wikipedia.org/wiki/Ergotamine (accessed 2 February 2023).

10 Bethke, H., Delz, B., and Stich, K. (1976). Determination of the content and purity of ergotamine preparations by means of high-pressure liquid chromatography. *J. Chromatogr.* 123: 193–203.

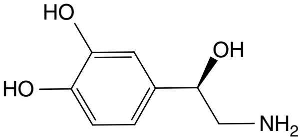
184–186: Epinephrine, Norepinephrine, Isoprenaline

184: Epinephrine [L-Adrenaline or R-Adrenaline]



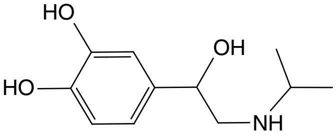
Chemical name: 4-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol
Formula: $C_9H_{13}NO_3$
Monoisotopic protonated ion (*m/z*): 184.0968
Molecular weight: 183.207
CAS number: 51-43-4
Common salt form: HCl salt, Acetate, Bitartrate

185: Norepinephrine [Noradrenaline]



Chemical name: 4-[(1*R*)-2-Amino-1-hydroxyethyl]benzene-1,2-diol
Trade name: Levophed
Formula: $C_8H_{11}NO_3$
Monoisotopic protonated ion (*m/z*): 170.0812
Molecular weight: 169.18
CAS number: 51-41-2
Common salt form: Bitartrate

186: Isoprenaline [Racemate]



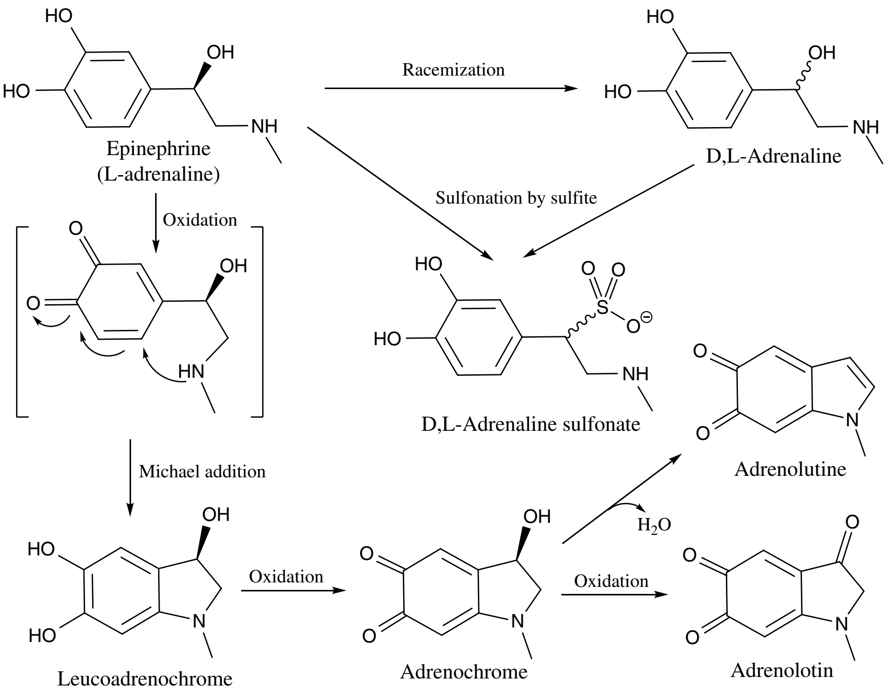
Chemical name: 4-[1-Hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,2-diol
Trade name: Isuprel
Formula: $C_{11}H_{17}NO_3$
Monoisotopic protonated ion (*m/z*): 212.1281
Molecular weight: 211.26
CAS number: 7683-59-2
Common salt form: HCl salt

Epinephrine and norepinephrine are both neurotransmitters and hormones secreted by the adrenal glands to help deal with body's acute stress. Clinically, they have been used to treat allergic reactions and restore cardiac rhythm; the latter application typically occurs in emergency rooms. Isoprenaline, the isopropyl analog of epinephrine, is a nonselective beta-adrenergic receptor agonist that is clinically used for treating bradycardia, heart block, and other symptoms such as congestive heart failure and septic shock.

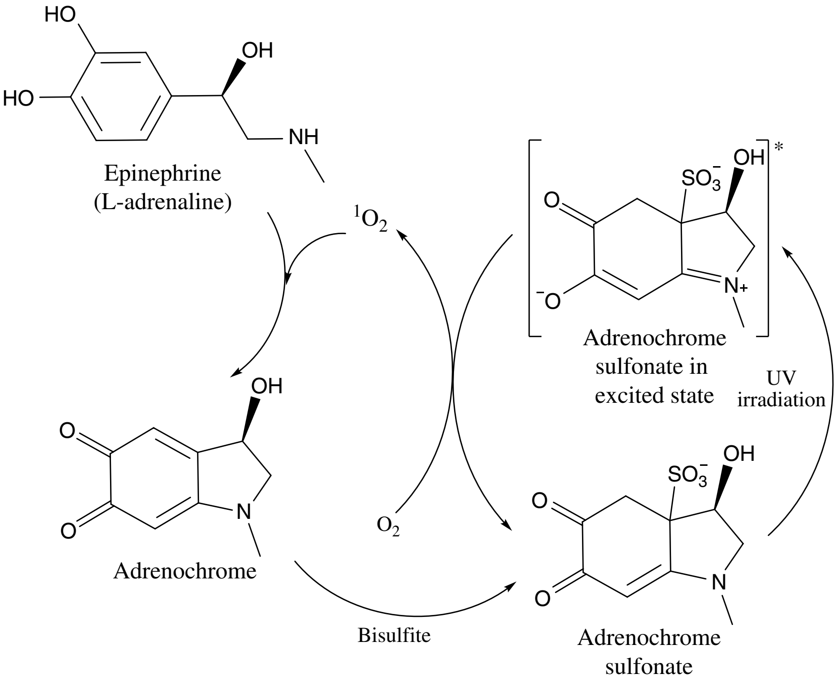
Chemically, epinephrine, norepinephrine, and isoprenaline are all catecholamines. Their catechol moieties are quite electron rich and, thus, are very susceptible to oxidative degradation, particularly under catalysis by redox reactive transition metal ions, exposure to light, and increased pH [1]. These drug substances are usually formulated in aqueous solutions as injectable formats. The most common antioxidative measure to preserve the active ingredients in solutions is the use of antioxidants, typically sulfite or bisulfite salts, in addition to measures such as reduction of oxygen content (by nitrogen), buffered pH, and protection from light. Although bisulfite can effectively suppress the oxidative degradation [2], a drawback of this protective measure is that it can cause the interaction between the drug substances and sulfites or bisulfites, resulting in the formation of the corresponding sulfonates [3–5]. Racemization of the chiral center could occur in the absence of bisulfite, and the presence of bisulfite did not seem to accelerate the racemization of epinephrine [3]. The formation of the sulfonate was found to be significant in a study where the formulated product was subjected to simulated environmental stress during storage and transportation [1]. The degradation pathways of epinephrine, as summarized by Stepensky et al. [6], are shown in Scheme 184–186-1 with some modification according to the original studies by Schroeter et al. [3] and by Grunert and Wollmann [7]. The initial oxidation of the catechol moiety of epinephrine leads to the formation of 3,4-benzoquinone, which is followed by the Michael addition of the amino group to result in leucoadrenochrome. Further oxidation would produce adrenochrome and adrenolotin, respectively. On the other hand, dehydration of adrenochrome would yield adrenolutin [7].

In a comprehensive review on epinephrine and norepinephrine stability in injectable solutions [8], it was indicated that the use of metal chelates such as diethylenetriaminepentaacetic acid (DTPA) and 8-hydroxyquinoline sulfate could significantly enhance the stability of epinephrine solutions. Interestingly, EDTA did not show any stabilization effect and even promoted degradation at pH 5.5. On the other hand, the combination of different control strategies, such as the use of antioxidants and DTPA, may yield a synergistic protective effect.

In a study aiming to identify the role of sulfites in promoting photochemical degradation of epinephrine [9], Brustugun et al. found that bisulfite could react with adrenochrome, one of the intermediate oxidative degradants of epinephrine, to form adrenochrome sulfonate [10]. Upon UV irradiation at 350 nm, the excited state of adrenochrome sulfonate collides with triplet molecular oxygen to generate singlet oxygen, the presence of which was supported by the characteristic luminescence signal detected at 1270 nm. The singlet oxygen would inflict oxidative degradation of epinephrine, resulting in the formation of more adrenochrome, which could further react with bisulfite to produce another batch of adrenochrome sulfonate, forming a catalytic cycle of degradation (Scheme 184–186-2).



Scheme 184–186-1 Degradation pathways of epinephrine (L-adrenaline) via oxidation and racemization. The degradation pathways were adapted from Stepensky et al. [6] with modifications according to the original studies by Schroeter et al. [3] and by Grunert and Wollmann [7]. *Source:* Stepensky et al. [6]; Schroeter et al. [3]; Grunert and Wollmann [7].



Scheme 184–186-2 Photochemical degradation of epinephrine in the presence of bisulfite.

References

1 Church, W.H., Hu, S.S., and Henry, A.J. (1994). Thermal degradation of injectable epinephrine. *Am. J. Emerg. Med.* 12: 306–309.

2 Wollman, H. and Raether, G. (1993). Efficacy testing of stabilizing agents in epinephrine model solutions: 19: stability of drugs and preparations. *Pharmazie* 38: 37–42.

3 Schroeter, L.C., Higuchi, T., and Schuler, E.E. (1958). Degradation of epinephrine induced by bisulfite. *J. Am. Pharm. Assoc.* 47: 723–728.

4 Higuchi, T. and Schroeter, L.C. (1959). Reactivity of bisulfite with a number of pharmaceuticals. *J. Am. Pharm. Assoc.* 48: 535–540.

5 Higuchi, T. and Schroeter, L.C. (1960). Kinetics and mechanism of formation of sulfonate from epinephrine and bisulfite. *J. Am. Chem. Soc.* 82: 1904–1907.

6 Stepensky, D., Chorny, M., Dabour, Z., and Schumacher, I. (2004). Long-term stability study of l-adrenaline injections: kinetics of sulfonation and racemization pathways of drug degradation. *J. Pharm. Sci.* 93: 969–980.

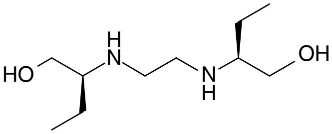
7 Grunert, R. and Wollmann, H. (1982). Effect of ultraviolet, visible light on drugs of the phenylalkylamine series with a view toward their stability in plastic containers. 17. Stability of drugs, preparations. 83. Problems of the use of plastic containers for liquid pharmaceuticals. *Pharmazie* 37: 798–799.

8 Hoellein, L. and Holzgrabe, U. (2012). Ficts and facts of epinephrine and norepinephrine stability in injectable solutions. *Int. J. Pharm.* 434: 468–480.

9 Brustugun, J., Tønnesen, H.H., Klem, W., and Kjønniksen, I. (2000). Photodestabilization of epinephrine by sodium metabisulfite. *PDA J. Pharm. Sci. Technol.* 54: 136–143.

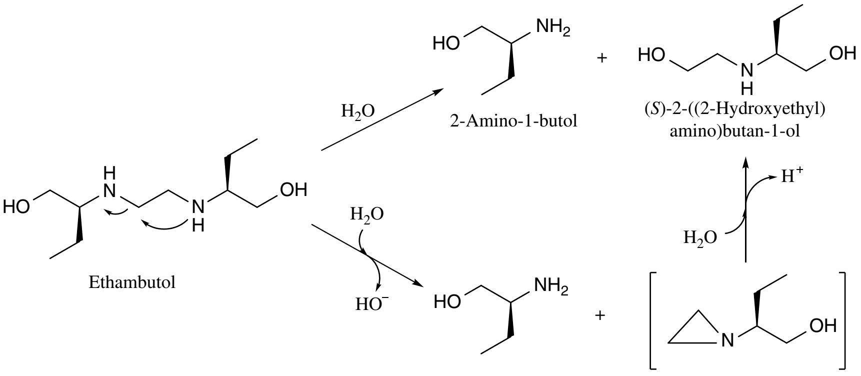
10 Brustugun, J., Kristensen, S., and Tønnesen, H.H. (2004). Photostability of epinephrine – the influence of bisulfite and degradation products. *Pharmazie* 59: 457–463.

187: Ethambutol



Chemical name: (2*S*)-2-[2-[[(2*S*)-1-Hydroxybutan-2-yl]amino]ethylamino]butan-1-ol
Trade name: Etibi, Myambutol
Formula: $C_{10}H_{24}N_2O_2$
Monoisotopic protonated ion (*m/z*): 205.1911
Molecular weight: 204.31
CAS number: 74-55-5
Common salt form: HCl salt

Ethambutol is an aminoalcohol clinically employed for treating tuberculosis (TB) since the 1960s, which remains to be a major global health threat causing 1.5 million deaths each year and 1.7 billion infections as of 2018 [1]. 2-Amino-1-butanol was reported to be a degradant of ethambutol; nevertheless, no mechanistic explanation was provided in these studies [2, 3]. It could be formed under hydrolytic degradation conditions, either via direct hydrolysis or through a tricyclic aziridine intermediate (Scheme 187). Under either mechanism, the second degradant, (*S*)-2-((2-hydroxyethyl)amino)butan-1-ol, would be expected to form; however, no such degradant has been reported. Ethambutol is usually used in combination with other anti-TB drugs, such as isoniazid, rifampicin, and pyrazinamide, and a number of studies found that the presence of ethambutol promoted the degradation occurred between isoniazid and rifampicin, which was attributed to the hygroscopicity and acidic nature of ethambutol hydrochloride [4–6].



Scheme 187 Possible degradation pathway of ethambutol.

References

1 Tuberculosis. CDC archive, last reviewed: April 6, 2020. https://archive.cdc.gov/#/details?q=tuberculosis&start=0&rows=10&url=https://www.cdc.gov/globalhealth/newsroom/topics/tb/index.html (accessed 26 June 2025).

2 Ragonese, R., Macka, M., Hughes, J., and Petocz, P. (2002). The use of the Box–Behnken experimental design in the optimisation and robustness testing of a capillary electrophoresis method for the analysis of ethambutol hydrochloride in a pharmaceutical formulation. *J. Pharm. Biomed. Anal.* 27: 995–1007.

3 da Silva, J.A.F., de Castro, N.V., de Jesus, D.P. et al. (2010). Fast determination of ethambutol in pharmaceutical formulations using capillary electrophoresis with capacitively coupled contactless conductivity detection. *Electrophoresis* 31: 570–574.

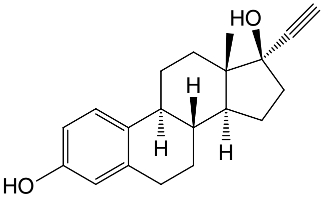
4 Bhutani, H., Singh, S., and Jindal, K.C. (2005). Drug-drug interaction studies on first-line anti-tuberculosis drugs. *Pharm. Dev. Technol.* 10: 517–524.

5 Bhutani, H., Mariappan, T.T., and Singh, S. (2004). The physical and chemical stability of anti-tuberculosis fixed-dose combination products under accelerated climatic conditions. *Int. J. Tuberc. Lung Dis.* 8: 1073–1080.

6 Bhutani, H., Singh, S., Jindal, K.C., and Chakraborti, A.K. (2005). Mechanistic explanation to the catalysis by pyrazinamide and ethambutol of reaction between rifampicin and isoniazid in anti-TB FDCs. *J. Pharm. Biomed. Anal.* 39: 892–899.

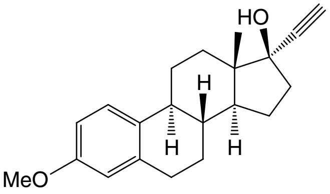
188–189: Ethinylestradiol, Mestranol

188: Ethinylestradiol



Chemical name: (8*R*,9*S*,13*S*,14*S*,17*R*)-17-Ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol
Formula: $C_{20}H_{24}O_2$
Monoisotopic protonated ion (*m/z*): 297.1849
Molecular weight: 296.41
CAS number: 57-63-6

189: Mestranol

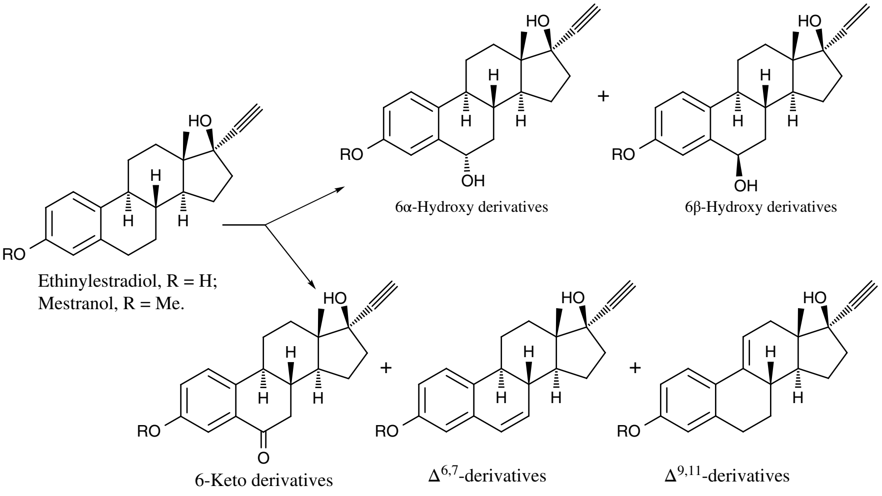


Chemical name:
Formula: $C_{21}H_{26}O_2$
Monoisotopic protonated ion (*m/z*): 311.2006
Molecular weight: 310.44
CAS number: 72-33-3

Ethinylestradiol and its methyl derivative, mestranol, have long been utilized as oral contraceptive agents.[1] Nevertheless, there seem to be only a limited number of studies looking into the degradation behaviors of both drug molecules. In a thermal degradation study of ethinylestradiol and mestranol [1], both drug molecules underwent oxidative degradation at the 6-benzylic position of the steroid core after the solid samples were subjected to heating at 178°C and 147°C, respectively, for a few hours while exposing to air, yielding the corresponding 6α-hydroxy- and 6β-hydroxy-derivatives as the major degradants and 6-keto- and $\Delta^{6,7}$-derivatives as the minor

---

[1] Ethinylestradiol has been known since 1938: H.H. Inhoffen, W. Logemann, W. Hohlweg, and A. Serini, Chem. Ber., 71: 1024 (1938), while mestranol was disclosed in a 1954 patent: F.B. Colton, U.S. Patent 2,666,769. January 19, 1954.



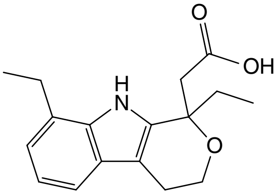
Scheme 188–189 Oxidative degradation pathways of ethinylestradiol and mestranol.

degradants. According to a paper by Ekhato et al. [2], the above four degradants were observed in formulated ethinylestradiol products stored under ambient laboratory conditions for 3 years. In addition, $\Delta^{9,11}$-degradant was also observed. The oxidative degradation pathways of ethinylestradiol and mestranol are illustrated in Scheme 188–189. Li et al. reported improved syntheses of these oxidative degradants of ethinylestradiol and mestranol [3].

References

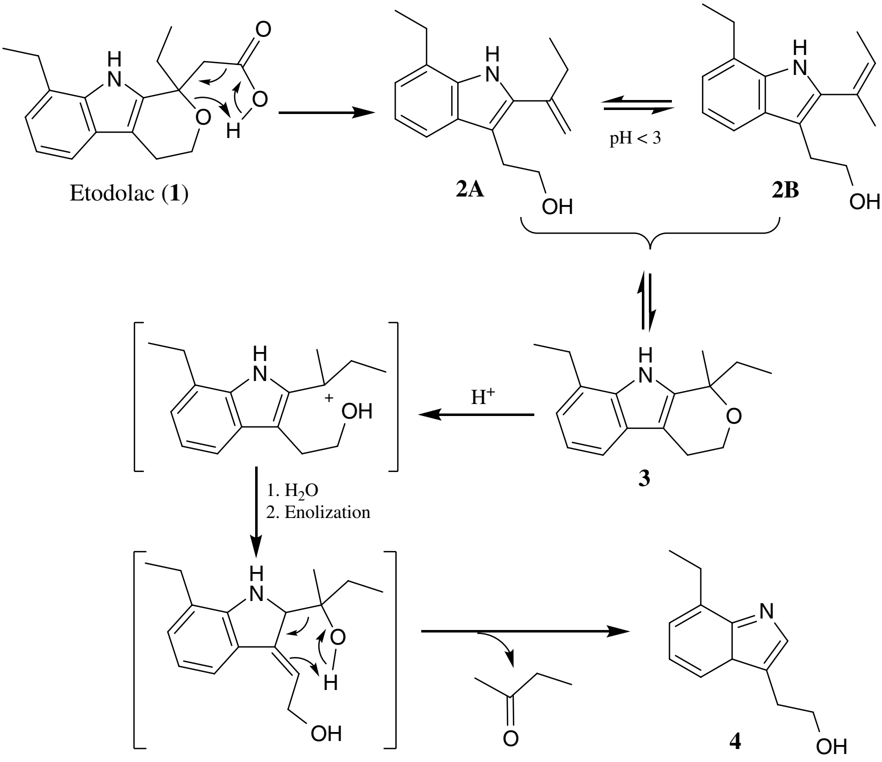
1. Cotter, M.L., Levine, S.D., Mallory, R., and Shaw, C. (1978). Thermal degradation of mestranol and ethinyl estradiol. *Tetrahedron Lett.* 22: 1939–1942.
2. Ekhato, I.V., Hurley, T., Lovdahl, M. et al. (2002). Norethindrone acetate (NA) and ethinyl estradiol (EE) related oxidative transformation products in stability samples of formulated drug product: synthesis of authentic references. *Steroids* 67: 165–174.
3. Li, H., Song, Y., and Peng, X. (2008). Improved synthesis of mestranol and ethinyl estradiol (EE) related degradation products as authentic references. *Steroids* 73: 488–494.

190: Etodolac



Chemical name: 2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b]indol-1-yl)acetic acid
Trade name: Lodine
Formula: $C_{17}H_{21}NO_3$
Monoisotopic protonated ion (*m/z*): 288.1594
Molecular weight: 287.36
CAS number: 41340-25-4

Etodolac is a selective cyclooxygenase inhibitor clinically used for treating pain, fever, etc. Lee et al. studied its degradation pathway and kinetics in acidic aqueous solutions [1]. The degradation started with the decarboxylation of the drug molecule (**1**) via a concerted mechanism through a six-membered intermediate. The concerted mechanism was consistent with the small positive entropy of the activation ($\Delta S^{\neq}$) as determined in the study. The initial degradation product from the decarboxylation was degradant **2A**, which was partially converted to degradant **2B** at pH below 3. Both **2A** and **2B** appeared to be intermediary degradants as **3** was observed as the major degradant, which further degraded into degradant **4** through a retro-aldo-like process [2] in which butanone was eliminated. The retro-aldo-like process was probably made possible via the enolization of the open ring form of **3**. The degradation pathway of etodolac under acidic conditions is summarized in Scheme 190.



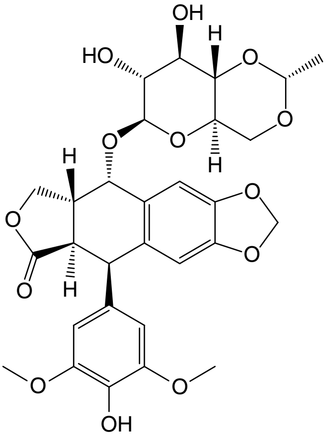
Scheme 190 Degradation pathway of etodolac under acidic conditions.

References

1 Lee, Y.J., Padula, J., and Lee, H.-K. (1988). Kinetics and mechanisms of etodolac degradation in aqueous solutions. *J. Pharm. Sci.* 77: 81–86.

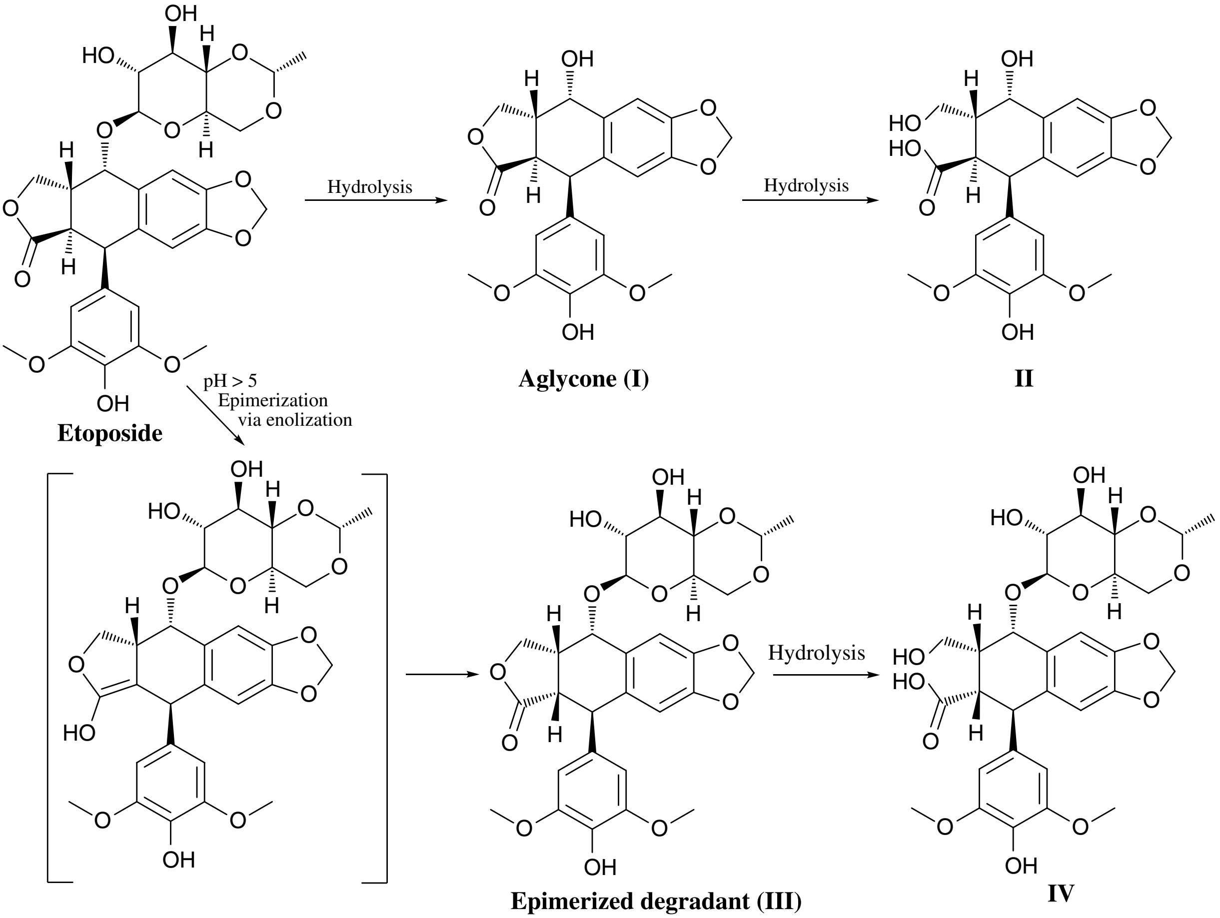
2 Li, M. (2012). Aldol condensation and retro-aldol. In: *Organic Chemistry of Drug Degradation*, 124–127. Cambridge, UK: RSC Publishing.

191: Etoposide [WHO List of Essential Medicines]



Chemical name: (5*S*,5a*R*,8a*R*,9*R*)-5-[[(2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7,8-Dihydroxy-2-methyl-4,4a,6,7,8,8a-hexahydropyrano[3,2-d][1,3]dioxin-6-yl]oxy]-9-(4-hydroxy-3,5-dimethoxyphenyl)-5a,6,8a,9-tetrahydro-5*H*-[2]benzofuro[6,5-f][1,3]benzodioxol-8-one
Trade name: Vepesid, Etopophos, Toposar
Formula: $C_{29}H_{32}O_{13}$
Monoisotopic protonated ion (*m/z*): 589.1916
Molecular weight: 588.56
CAS number: 33419-42-0

Etoposide is a chemotherapeutic agent for the treatment of a variety of cancers, including lung cancer, lymphoma, leukemia, testicular cancer, and ovarian cancer [1]. It is a semi-synthetic epipodophyllotoxin from the wild mandrake; structurally, it is a glycoside that contains a modified D-glucose unit. Both the sugar unit and the lactone moiety of etoposide are susceptible to hydrolytic degradation, and its degradation pathways mostly result from the hydrolysis of these two structural moieties (Scheme 191) [2]. In acidic solutions with pH below 5.0, the glycosidic linkage was cleaved to give the aglycone 4′-demethylepipodophyllotoxin (I), which could further degrade to form the corresponding *trans*-hydroxyl carboxylic acid via the opening of the lactone ring (II). In solutions with pH greater than 5.0, the epimerization of the *trans*-fused lactone ring became the most significant degradation pathway, in which the isomeric degradant with the *cis*-fused lactone ring (IV) was formed. The lactone moiety of IV could further decompose to give the corresponding *cis*-hydroxyl carboxylic degradant (V) [2, 3]. Overall, etoposide displayed a V-shaped pH stability profile with the most stable region near pH 5 [2]. In a study by Tian et al., etoposide in liquid emulsion and aqueous solution was found to be most stable at pH 5.0 with half-lives of 54.7 hour and 38.6 minutes, respectively, at 80°C [4]. Joel et al. studied the stability of both i.v. and oral-formulated etoposide solutions and found significant concentration-dependent degradation, with the more concentrated solutions degraded faster than less concentrated ones [5].

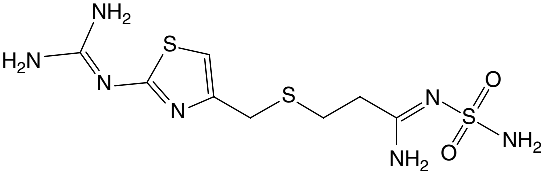


Scheme 191 Degradation pathways of etoposide under acidic and alkaline solutions.

References

1. Etoposide. https://en.wikipedia.org/wiki/Etoposide (accessed 5 April 2023).
2. Beijnen, J.H., Holthuis, J.J.M., Kerkdijk, H.G. et al. (1988). Degradation kinetics of etoposide in aqueous solution. *Int. J. Pharm.* 41: 169–178.
3. Strife, R.J., Jardine, I., and Colvin, M. (1980). Analysis of the anticancer drugs VP16-213 and VM 26 and their metabolites by high performance liquid chromatography. *J. Chromatogr.* 182: 211–220.
4. Tian, L., He, H., and Tang, X. (2007). Stability and degradation kinetics of etoposide-loaded parenteral lipid emulsion. *J. Pharm. Sci.* 96: 1719–1728.
5. Joel, S.P., Clark, P.I., and Slevin, M.L. (1995). Stability of the i.v. and oral formulations of etoposide in solution. *Cancer Chemother. Pharmacol.* 37: 117–124.

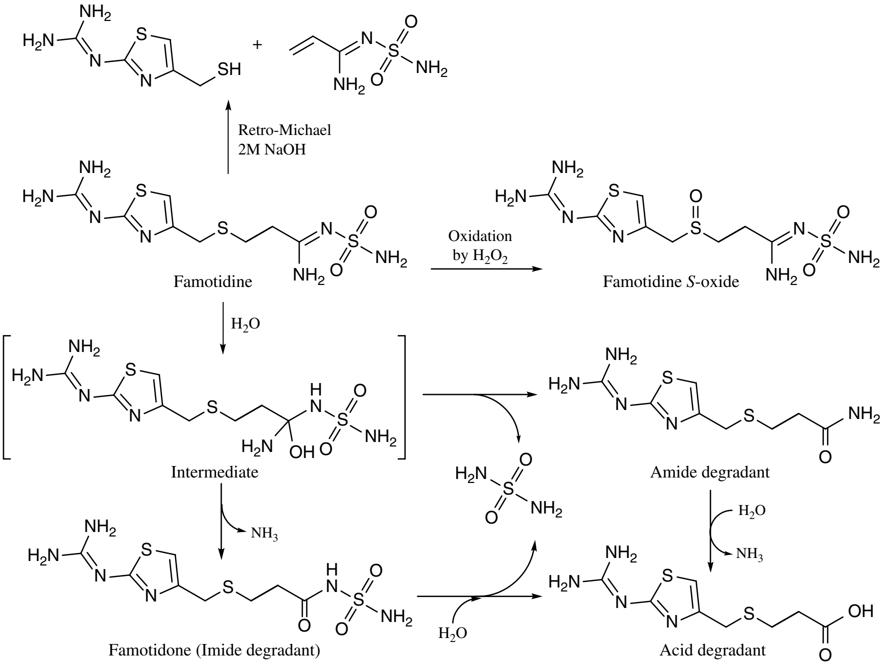
192: Famotidine



Chemical name: 3-[[2-(Diaminomethylideneamino)-1,3-thiazol-4-yl] methylsulfanyl]-*N′*-sulfamoylpropanimidamide
Trade name: Pepcid
Formula: $C_8H_{15}N_7O_2S_3$
Monoisotopic protonated ion (*m/z*): 338.0522
Molecular weight: 337.44
CAS number: 76824-35-6

Famotidine is a histidine-2 (H_2) receptor antagonist that pharmacologically suppresses gastric secretion. It is clinically used for the treatment of duodenal ulcers, gastric ulcers, gastritis, and gastroesophageal reflux. Its main degradation is hydrolytic, resulting from the hydrolysis of its *N'*-sulfamoylpropanimidamide moiety under both acidic and alkaline conditions, yielding famotidone and several other degradants [1]. According to a study by Islam and Narurkara, the maximum stability of famotidine occurred at pH 6.3 [2]. A later study reported that in a 2M NaOH solution, famotidine was also found to undergo retro-Michael addition, resulting in the breakage of the thiol ether linkage [3]. Nevertheless, the acidic hydrolysis appears to be more pharmacologically relevant, as the acidic degradation is believed to be mostly responsible for the poor bioavailability of famotidine. The pseudo-first-order reaction rate constants were found to be 0.392/hour (or 6.53×10^{-3}/minutes; $t_{1/2}$ 1.77 hour) and 8.1×10^{-3}/minutes in 0.1 N HCl and simulated gastric fluid at 37°C, respectively [4, 5]. In another kinetic study, the activation energy for famotidine degradation was reported to be 16.000–17.563 kcal/mol within the pH range of 1.71–4.00 [1].

When treated with hydrogen peroxide, famotidine can be converted into its *S*-oxide [6], which is also a metabolite of famotidine [7]. The overall degradation pathways of famotidine are illustrated in Scheme 192.



Scheme 192 Degradation pathways of famotidine.

References

1 Junnarkar, G.H. and Stavchansky, S. (1995). Isothermal and nonisothermal decomposition of famotidine in aqueous solution. *Pharm. Res.* 12: 599–604.

2 Islam, M.S. and Narurkar, M.M. (1993). Solubility, stability and ionization behaviour of famotidine. *J. Pharm. Pharmacol.* 45: 682–686.

3 Singh, S., Kumar, S., Sharda, N., and Chakrabort, A.K. (2001). New findings on degradation of famotidine under basic conditions: Identification of a hitherto unknown degradation product and the condition for obtaining the propionamide intermediate in pure form. *J. Pharm. Sci.* 91: 253–257.

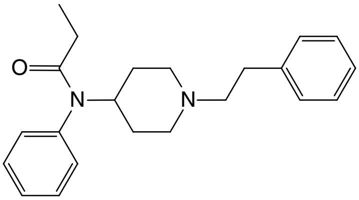
4 Suleiman, M.S., Muti, H.Y., Abdel-Hamid, M.E. et al. (1989). A stability-indicating HPLC analysis of famotidine and its application to kinetic studies. *Anal. Lett.* 22: 1499–1512.

5 El-Shaheny, R., Radwan, M.O., Belal, F., and Yamadaf, K. (2020). Pentabromobenzyl-RP versus triazole-HILIC columns for separation of the polar basic analytes famotidine and famotidone: LC method development combined with in silico tools to follow the potential consequences of famotidine gastric instability. *J. Pharm. Biomed. Anal.* 186: 113305.

6 Kelani, K.M., Aziz, A.M., Hegazy, M.A., and Fattah, L.A. (2002). UV-spectrophotometric stability indicating methods for the quantitative determination of cimetidine, famotidine, and ranitidine hydrochloride in the presence of their oxidative derivatives. *Anal. Lett.* 35: 1055–1073.

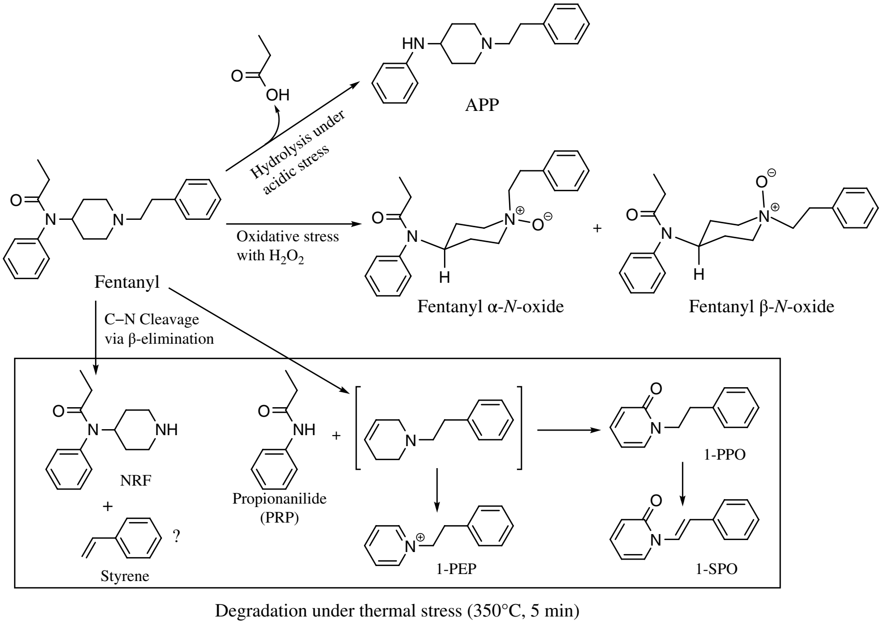
7 Al-Omar, M.A. and Al-Mohizea, A.M. (2009). Famotidine. In: *Profiles of Drug Substances, Excipients and Related Methodology*, vol. 34 (ed. H.G. Brittain), 115–151. Elsevier.

193: Fentanyl [WHO List of Essential Medicines]



Chemical name: *N*-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide
Trade name: Sublimaze, Abstral, Durogesic, Fentanest
Formula: $C_{22}H_{28}N_2O$
Monoisotopic protonated ion (*m/z*): 337.2274
Molecular weight: 336.48
CAS number: 437-38-7
Common salt form: Citrate

Fentanyl, first synthesized by Paul Janssen in 1959, is a highly potent synthetic narcotic, used primarily as a pain reliever for cancer patients and those after painful surgical operations [1]. It is also used as a sedative in combination with a benzodiazepine such as midazolam [2]. On the other hand, abuse of fentanyl has become a major issue in the United States, particularly in recent years. It is a Schedule II-controlled substance by the US DEA. Fentanyl contains an acyl group and a tertiary amine moiety, which are susceptible to hydrolytic and oxidative degradation, respectively. Garg et al. performed a comprehensive forced degradation study of fentanyl under acidic, alkaline, oxidative, thermal, and photolytic conditions [3]. Acidic stress of fentanyl in 5N HCl/acetonitrile (3/6, v/v) at 70°C for 24 hours generated ~35% yield of *N*-phenyl-1-(2-phenylethyl)-piperidin-4-amine (PPA), while it was stable in alkaline stress in 5N NaOH/acetonitrile (3/6, v/v) at the same temperature and duration. Oxidative stress of fentanyl (neutral base) in 0.3% hydrogen peroxide/acetonitrile (1/10, v/v) at ambient temperature produced two diastereomeric *N*-oxide degradants, with the major β-diastereomer formed in 6.51%. On the contrary, a previous oxidative stress of fentanyl citrate in 3% hydrogen peroxide by Lambropoulos et al. did not induce any degradation [4]. The inactivity of fentanyl citrate toward the nucleophilic oxidation under the apparently stronger oxidative stress condition employed by Lambropoulos et al. is most likely because the nucleophilicity of the tertiary amine functionality is inhibited by protonation via citric acid. Heating of fentanyl at 350°C for 5 minutes yielded five major degradants, three of which were identified in a previous thermal stress study [5]. The degradation pathways of fentanyl are shown in Scheme 193. The degradation that occurred under thermal stress (350°C, 5 minutes) seems to start from two pathways of C—N cleavage via β-elimination, followed by several steps of oxidation.



Scheme 193 Degradation pathways of fentanyl. The proposed pathways for the degradation that occurred under thermal stress are somewhat different from those described by Garg et al. [3]. *Source:* Garg et al. [3]/with permission of Elsevier.

References

1 Fentanyl. https://en.wikipedia.org/wiki/Fentanyl (accessed 19 May 2023).

2 Lobb, D., Clarke, A., and Lai, H. (2018). Administration order of midazolam/fentanyl for moderate dental sedation. *J. Dent. Anesth. Pain Med.* 18: 47–56.

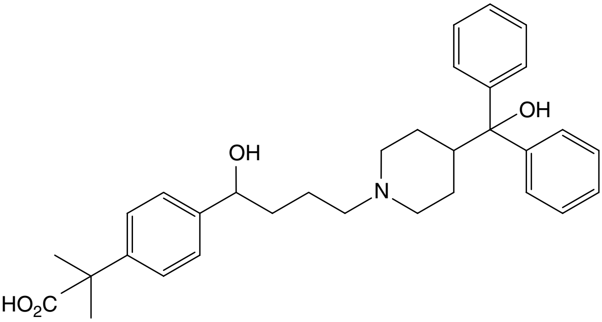
3 Garg, A., Solas, D.W., Takahashi, L.H., and Cassella, J.V. (2010). Forced degradation of fentanyl: identification and analysis of impurities and degradants. *J. Pharm. Biomed. Anal.* 53: 325–334.

4 Lambropoulos, J., Spanos, G.A., Lazaridis, N.V. et al. (1999). Development and validation of an HPLC assay for fentanyl and related substances in fentanyl citrate injection, USP. *J. Pharm. Biomed. Anal.* 20: 705–716.

5 Rabinowitz, J.D., Wensley, M., Lloyd, P. et al. (2004). Fast onset medications through thermally generated aerosols. *J. Pharmacol. Exp. Ther.* 309: 769–775.

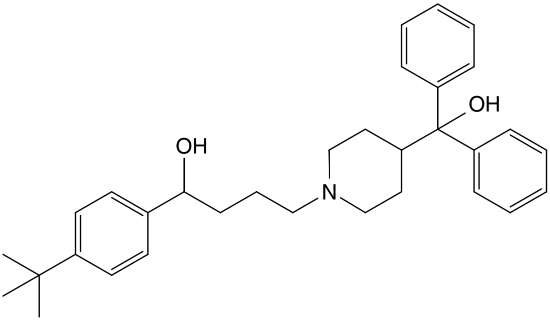
194–195: Fexofenadine, Terfenadine

194: Fexofenadine

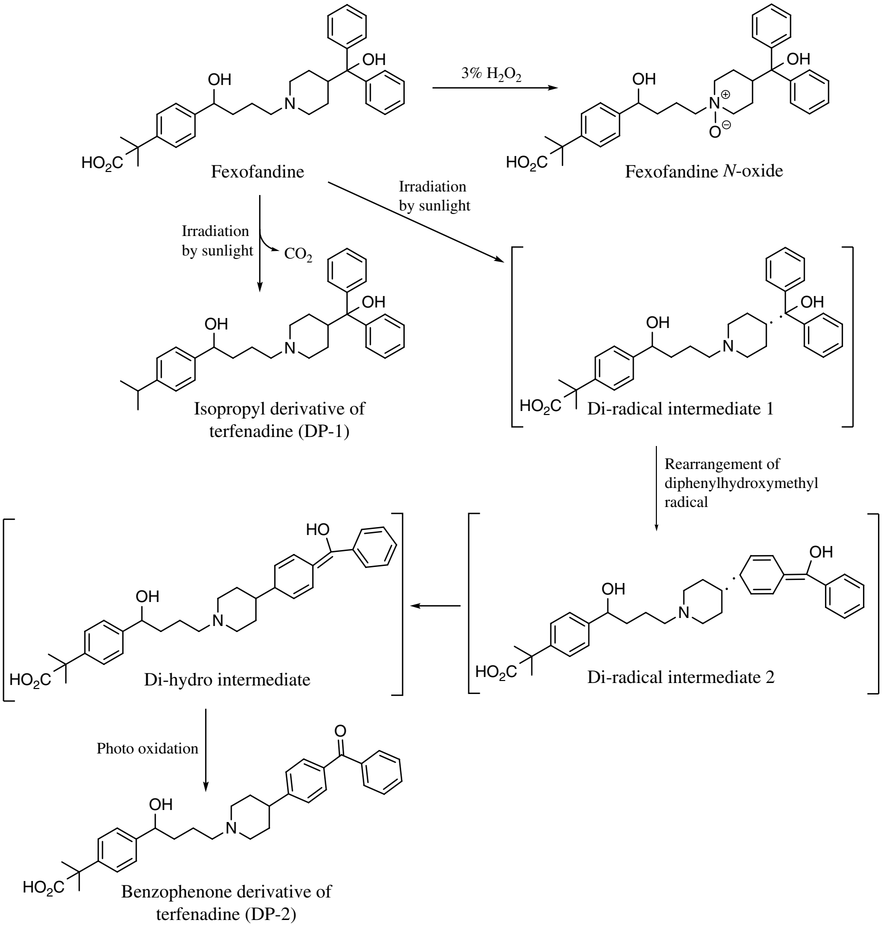


Chemical name: 2-[4-[1-Hydroxy-4-[4-[hydroxy(diphenyl)methyl]piperidin-1-yl] butyl]phenyl]-2-methylpropanoic acid
Trade name: Allegra
Formula: $C_{32}H_{39}NO_4$
Monoisotopic protonated ion (*m/z*): 502.2952
Molecular weight: 501.67
CAS number: 153439-40-8
Common salt form: HCl salt

195: Terfenadine

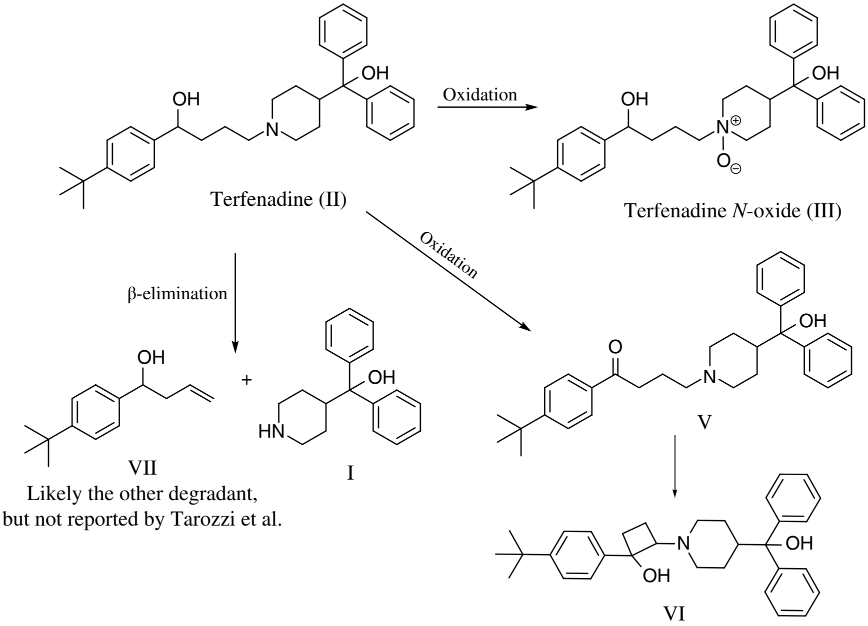


Chemical name: 1-(4-tert-Butylphenyl)-4-[4-[hydroxy(diphenyl)methyl]piperidin-1-yl]butan-1-ol
Formula: $C_{32}H_{41}NO_2$
Monoisotopic protonated ion (*m/z*): 472.3210
Molecular weight: 471.67
CAS number: 50679-08-8



Scheme 194–195-1 Degradation pathways of fexofenadine.

Terfenadine was originally intended to be developed as a potential tranquilizer, but, instead, it was brought to market in 1985 as the first of so-called second-generation antihistamines, which have minimal sedative effect due to their inability to cross the blood-brain barrier [1]. Terfenadine was withdrawn from the US market in 1998 due to the cardiac risk at high doses, and it was superseded by its carboxylic metabolite, fexofenadine. Both drugs contain a piperidine moiety, a tertiary amine functionality, and thus are susceptible to oxidation to form the corresponding *N*-oxide degradants. In a forced degradation study of fexofenadine by Vaghela et al., its *N*-oxide degradant was formed in ~2% yield after being subjected to stress in 3% hydrogen peroxide at 60°C for 5 hours [2]. In a photostability study of fexofenadine drug substance in methanolic solutions with UV irradiation at 254 nm, two major photodegradants (DP-1 and DP-2) were observed. While DP-1 apparently resulted from the photodecarboxylation, the formation of DP-2 does not appear to be straightforward. Although the original authors did not provide a probable formation mechanism for DP-2, it would likely be mediated by a free radical process, in which the initially formed diphenylhydroxymethyl radical would undergo a rearrangement



Scheme 194–195-2 Photodegradation pathways of terfenadine. The nomenclature of the degradants is that of Tarozzi et al., except for VII, which was not reported in the original paper. *Source:* Tarozzi et al. [5]/John Wiley & Sons.

subsequent photooxidation (Scheme 194–195-1) [3]. This photo-initiated free radical process would be similar to a photo-Fries rearrangement process [4]. These two photodegradants were also present in fexofenadine tablets after being subjected to the same UV irradiation at 254 nm, indicating that the formulated fexofenadine-formulated products should be protected from light.

In another photostability study of terfenadine by Tarozzi et al., terfenadine was exposed to both UV and natural sunlight, giving rise to different and complex degradation product distributions. The photodegradants formed under natural sunlight irradiation were analyzed by LC-MS, and their structures were proposed (Scheme 194–195-2) [5]. It needs to be noted that Degradant I, reported by Tarozzi et al., would likely result from a β-elimination process, and the other degradant stemming from this process would be Degradant VII or its analog, which was not reported in the original paper.

References

1 Terfenadine. https://en.wikipedia.org/wiki/Terfenadine (accessed 20 May 2023).

2 Vaghela, B., Rao, S.S., Reddy, A.M. et al. (2012). Identification and characterization of an oxidative degradation product of fexofenadine, development and validation of a stability-indicating RP-UPLC method for the estimation of process related impurities and degradation products of fexofenadine in pharmaceutical formulations. *Sci. Pharm.* 80: 295–309.

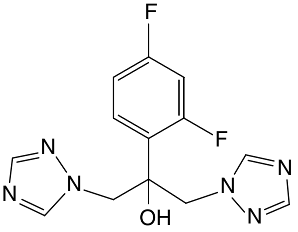
3 Breier, A.R., Nudelman, N.S., Steppe, M., and Schapoval, E.E.S. (2008). Isolation and structure elucidation of photodegradation products of fexofenadine. *J. Pharm. Biomed. Anal.* 46: 250–257.

4 Li, M. (2012). Photo-Fries rearrangement. In: *Organic Chemistry of Drug Degradation*. 173–174. Cambridge, UK: RSC Publishing.

5 Tarozzi, A., Andrisano, V., Fiori, J. et al. (2003). Photomutagenic properties of terfenadine as revealed by a stepwise photostability, phototoxicity and photomutagenicity testing approach. *Photochem. Photobiol.* 77: 356–361.

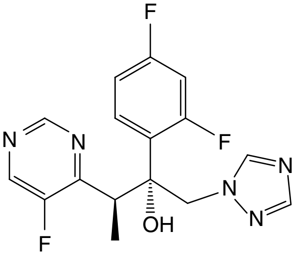
196–197: Fluconazole, Voriconazole

196: Fluconazole [WHO List of Essential Medicines]



Chemical name: 2-(2,4-Difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl)propan-2-ol
Trade name: Diflucan
Formula: $C_{13}H_{12}F_2N_6O$
Monoisotopic protonated ion (*m/z*): 307.1113
Molecular weight: 306.28
CAS number: 86386-73-4

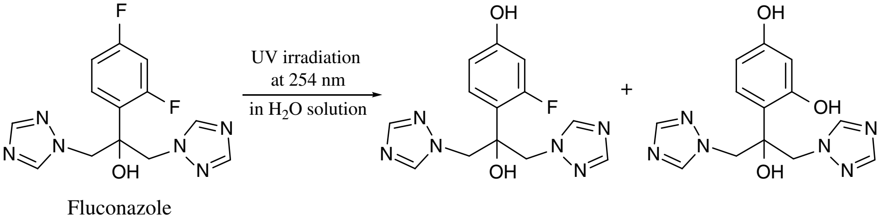
197: Voriconazole



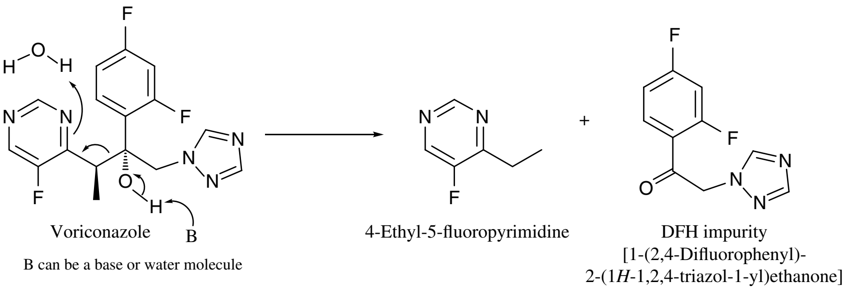
Chemical name: (2*R*,3*S*)-2-(2,4-Difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1,2,4-triazol-1-yl)butan-2-ol
Trade name: Vfend
Formula: $C_{16}H_{14}F_3N_5O$
Monoisotopic protonated ion (*m/z*): 350.1223
Molecular weight: 349.32
CAS number: 188416-29-7

Fluconazole is a first-generation antifungal drug based on a triazole structural moiety. Despite its long history since its commercialization in 1998, there have been very limited studies with regard to its stability or degradation chemistry. Among those few studies, there seems to be no reporting of structures for the potential degradants observed in the forced degradation, except for one photostability study [1]. In this study, a number of degradants were observed in the photostress of fluconazole in aqueous solutions with UV irradiation at 254 nm, two of which were determined to be monohydroxylated and dihydroxylated defluorination products, respectively. As the photostability experiments were performed in pure aqueous environment, the corresponding simple defluorinated degradation products were not seen, apparently due to a lack of a proper proton radical donor, which would have been available if an organic cosolvent is present (Scheme 196–197-1) [2]. In a forced degradation study by Corrêa et al., fluconazole was found to be mostly stable under the conditions of acidic (0.1 M HCl) and alkaline (0.1 M NaOH) stress, either at room temperature or 90°C for up to 6 hours [3]. While no degradation was observed after being stressed in 3% hydrogen peroxide at room temperature for 1 hour, heating the 3% hydrogen peroxide stress solution at 90°C produced a possible degradant peak; nevertheless, the structure of this possible degradant was not elucidated. In another oxidative degradation study, fluconazole was found to produce a degradant in ~32% yield after being stressed in 35% hydrogen peroxide solution at 150°C for 30 minutes [4]. Nevertheless, the degradant formed under such an extreme condition would most likely be artificial.

On the other hand, voriconazole produced one degradant (DFH impurity) under an alkaline condition (0.1 M NaOH, room temperature, 30 minutes) (Scheme 196–197-2) [5]. The formation of this particular degradant may be due to the introduction of 5-fluoropyrimidinyl group in voriconazole, which made the breakage of the C—C bond possible via a concerted rearrangement mechanism. The other degradant 4-ethyl-5-fluoropyrimidine, which would be expected to form from this degradation process, was not reported in the study, which may be attributed to its volatility. A later study indicated that voriconazole underwent the same degradation under thermal stress conditions at 60°C for 24 hours [6].



Scheme 196–197-1 Photodegradation pathway of fluconazole in pure aqueous solution.



Scheme 196–197-2 Degradation of voriconazole under alkaline and thermal stress conditions.

References

1 Corrêa, J.C.R., Reichman, C., Vianna-Soares, C.D., and Salgado, H.R.N. (2011). Stability study of fluconazole applying validated bioassay and stability-indicating LC methods. *J. Anal. Bioanal. Tech.* 2: 1000126.

2 Li, M. (2012). Photochemical Degradation. In: *Organic Chemistry of Drug Degradation*, 165–197. Cambridge, UK: RSC Publishing.

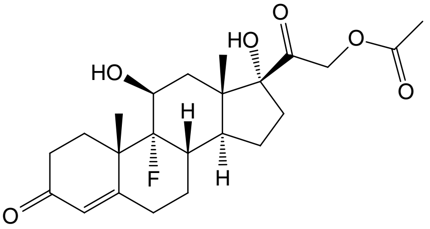
3 Chen, Z.-F., Ying, G.G., Jiang, Y.-X. et al. (2014). Photodegradation of the azole fungicide fluconazole in aqueous solution under UV-254: kinetics, mechanistic investigations and toxicity evaluation. *Water Res.* 52: 83–91.

4 Lotfy, H.M., Abdel-Aleem, A.-A.A.-B., and Monir, H.H. (2013). Stability-indicating high performance liquid chromatography determination of fluconazole in the presence of its oxidative degradation product – kinetic and stress study. *J. Liq. Chromatogr. Relat. Technol.* 36: 1013–1029.

5 Adams, A.I.H., Gosmann, G., Schneider, P.H., and Bergold, A.M. (2009). LC stability studies of voriconazole and structural elucidation of its major degradation product. *Chromatographia* 69: 115–122.

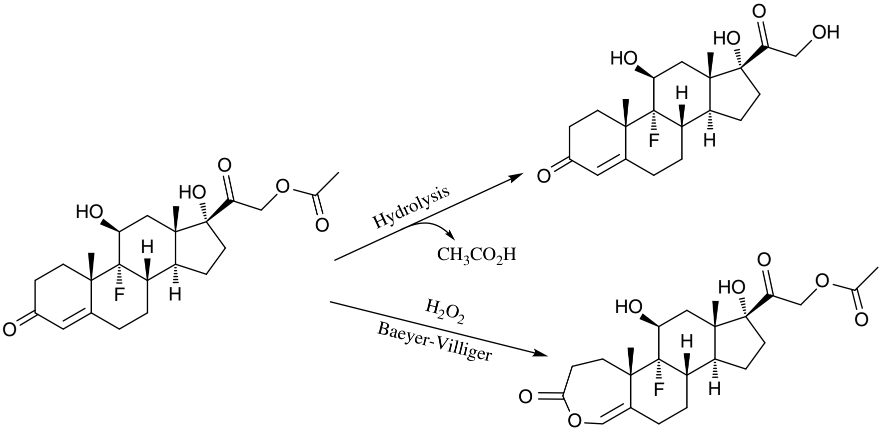
6 Shaikh, K.A. and Patil, A.T. (2012). A validated stability-indicating liquid chromatographic method for determination of degradation impurities and diastereomers in voriconazole tablets. *Sci. Pharm.* 80: 879–888.

198: Fludrocortisone Acetate [WHO List of Essential Medicines]



Chemical name: (11β)-9-Fluoro-11,17-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate
Trade name: Florinef
Formula: $C_{23}H_{31}FO_6$
Monoisotopic protonated ion (*m/z*): 423.2177
Molecular weight: 422.49
CAS number: 514-36-3

Fludrocortisone is an adrenal steroid clinically used to treat primary and secondary adrenocortical insufficiency [1]. It was the first synthetic corticosteroid drug and the first fluorine-containing drug [2], which is usually administered in its 21-acetate prodrug form. Hence, hydrolysis is its primary degradation pathway, in which fludrocortisone is generated (Scheme 198). On the other hand, oxidative insertion between 3,4-positions of the A-ring via the Baeyer–Villiger mechanism could be a potential degradation pathway, as such a pathway was reported for other corticosteroids containing the 1,2-dihydro A ring moiety [3, 4].

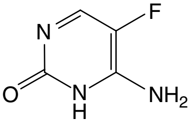


Scheme 198 Hydrolytic and potential oxidative degradation pathways of fludrocortisone.

References

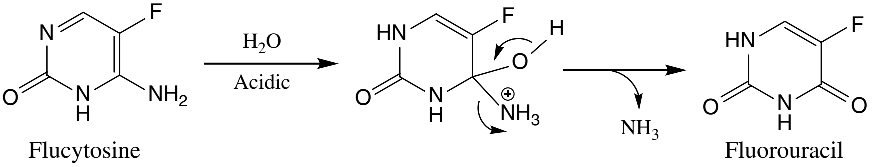
1. Rahman, M. and Anjum, F. (2023). *Fludrocortisone*. StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK564331/ (accessed 27 May 2023).
2. Fludrocortisone. https://en.wikipedia.org/wiki/Fludrocortisone (accessed 27 May 2023).
3. Caspi, E., Chang, Y.W., and Dorfman, R.I. (1962). Degradation of corticosteroids. VII. The synthesis of 7-membered ring-A enol-lactones. *J. Med. Pharm. Chem.* 5: 714–719.
4. Pinhey, J.T. and Schaffner, K. (1968). Baeyer-Villiger oxidation of Δ^4-3-oxo-steroids and 4,5-epoxy-3-oxo-steroids. A route to some novel A-nor-steroids. *Aust. J. Chem.* 21: 1873–1889.

199: Flucytosine [WHO List of Essential Medicines]



Chemical name: 6-Amino-5-fluoro-1*H*-pyrimidin-2-one
Trade name: Ancobon
Formula: $C_4H_4FN_3O$
Monoisotopic protonated ion (*m/z*): 130.0411
Molecular weight: 129.09
CAS number: 2022-85-7

Flucytosine, introduced into the market in the 1950s, is an antifungal drug specifically for treating serious *Candida* infections and cryptococcosis, and for this indication, it is used in combination with another antifungal drug amphotericin B [1]. It exerts its pharmacological and therapeutic effects by transforming in vivo into fluorouracil, which is its synthetic precursor as well as degradation product due to deamination formed under hydrolytic conditions (Scheme 199) [2]. There are a limited number of forced degradation studies, in which a major degradant was observed under both hydrolytic (acidic) and oxidative (H_2O_2) conditions. Nevertheless, the structure of the degradant was not identified in these studies [3, 4].



Scheme 199 Deamination of flucytosine under acidic conditions.

References

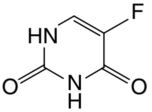
1 Flucytosine. https://en.wikipedia.org/wiki/Flucytosine (accessed 20 May 2023).

2 Cavrini, V., Bonazzi, D., and Di Pietra, A.M. (1991). Analysis of flucytosine dosage forms by derivative UV spectroscopy and liquid chromatography. *J. Pharm. Biomed. Anal.* 9: 401–407.

3 Annapurna, M.M., Baswani, R., and Pradhan, D.P. (2019). New stability indicating RP-UFLC method for the determination of flucytosine – an anti fungal agent. *Acta Sci. Pharm. Sci.* 3: 38–46.

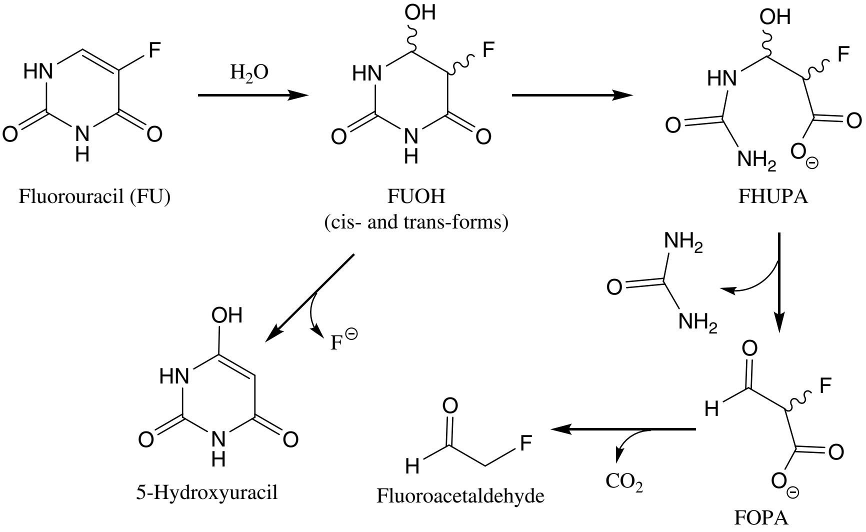
4 Venkata, N.P. and Seru, G. (2021). A new stability indicating RP-HPLC method for the estimation of flucytosine in presence of an internal standard. *Res. J. Pharm. Technol.* 14: 289–294.

200: Fluorouracil [WHO List of Essential Medicines]



Chemical name: 5-Fluoro-1*H*-pyrimidine-2,4-dione
Formula: $C_4H_3FN_2O_2$
Monoisotopic protonated ion (*m/z*): 131.0251
Molecular weight: 130.08
CAS number: 51-21-8

Fluorouracil, also known as 5-fluorouracil (5-FU or FU), is one of the widely used drugs for the treatment of various cancers. Its mode of action is believed to block thymidylate synthase and thus inhibit the production of DNA [1]. During the process of developing a stability-indicating method, fluorouracil was found to be more unstable under alkaline conditions than acidic one although the forced degradation products were not identified [2]. In a degradation study in aqueous solutions, Legay et al. investigated the hydrolytic pathway of fluorouracil in NaOH and Tris solutions, respectively [3]. In NaOH solutions, the initial degradation product was a pair of 6-hydroxyl intermediates (FUOH), formed via Michael addition of the hydroxide ion onto fluorouracil (Scheme 200). Subsequent degradation proceeded via either defluorination to afford 5-hydroxyuracil or hydrolysis of the imide linkage to give two diastereomeric, open-ring degradants (FHUPA). Cleavage of the C—N bond led to 2-fluoro-3-oxopropanoic acid (FOPA), which would be prone to decarboxylation,



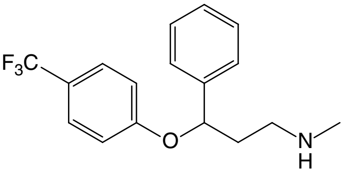
Scheme 200 Hydrolytic degradation pathway of fluorouracil.

resulting in the formation of fluoroacetaldehyde. Both 2-fluoro-3-oxopropanoic acid and fluoroacetaldehyde are cardiotoxic [4]. In Tris solutions, fluoroacetaldehyde was found to form several condensation products between the functional groups of Tris and fluoroacetaldehyde.

References

1. Fluorouracil. https://en.wikipedia.org/wiki/Fluorouracil (accessed 25 May 2023).
2. Alanazi, F.K., Yassin, A.E., El-Badry, M. et al. (2009). Validated high-performance liquid chromatographic technique for determination of 5-fluorouracil: applications to stability studies and simulated colonic media. *J. Chromatogr. Sci.* 47: 558–563.
3. Legay, R., Massou, S., Azéma, J. et al. (2014). Hydrolytic pathway of 5-fluorouracil in aqueous solutions for clinical use. *J. Pharm. Biomed. Anal.* 98: 446–462.
4. Fournet, A., Gilard, V., Malet-Martino, M. et al. (2000). Stability of commercial solutions of 5-fluorouracil for continuous infusion in an ambulatory pump. *Cancer Chemother. Pharmacol.* 46: 501–506.

201: Fluoxetine [WHO List of Essential Medicines]

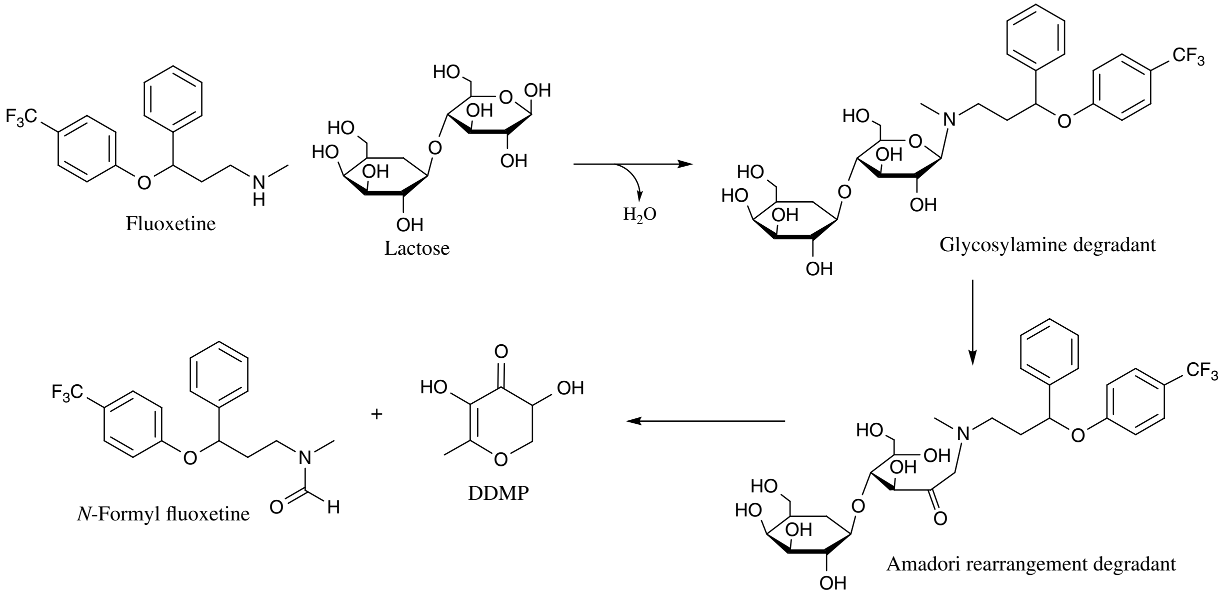


Chemical name: *N*-Methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine
Trade name: Prozac
Formula: $C_{17}H_{18}F_3NO$
Monoisotopic protonated ion (*m/z*): 310.1413
Molecular weight: 309.33
CAS number: 54910-89-3
Common salt form: HCl salt

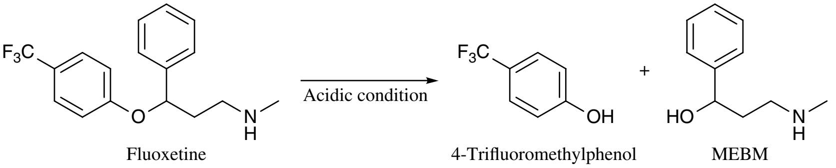
Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), clinically used for the treatment of major depressive disorder, obsessive-compulsive disorder, eating disorder, and panic disorder, among others. It was discovered in 1970 and received the US FDA's approval in 1987 [1]. Fluoxetine was the third most prescribed SSRI in the USA with approximately 27 million prescriptions in 2018 [2]. It is a secondary amine containing a 4-trifluoromethylphenyl ether moiety. The secondary amine moiety renders fluoxetine to undergo the Maillard reaction with excipients containing reducing sugars, for example, lactose. Wirth et al. reported the glycosylamine degradant and Amadori rearrangement degradant of fluoxetine, two of the degradation products formed in the early stage of the Maillard reaction (Scheme 201-1) [3]. Further degradation of the Amadori rearrangement degradant gave *N*-formylfluoxetine and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) as the major degradants.

The ether moiety of fluoxetine is susceptible to acidic hydrolysis, resulting in the formation of 4-trifluoromethylphenol and 3-phenyl-3-hydroxypropylamine (also known as MEBM) (Scheme 201-2) [4, 5].

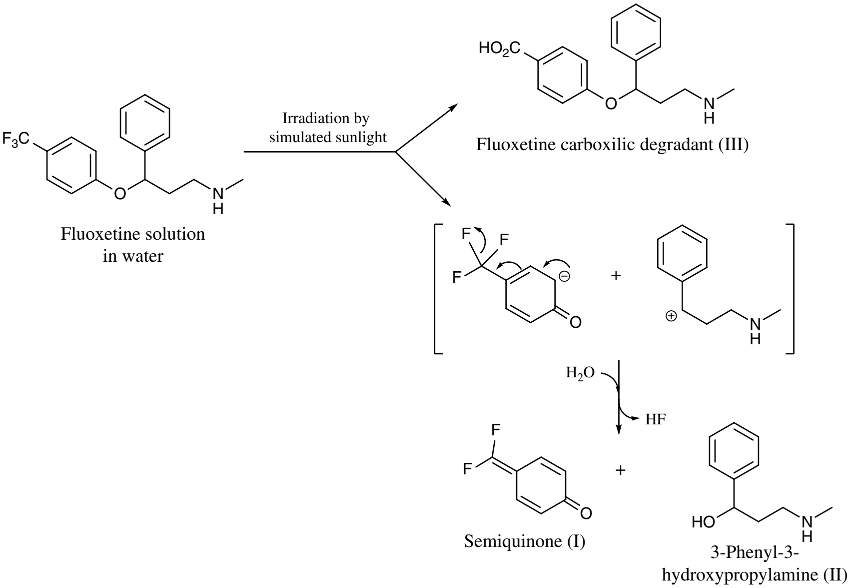
There are a number of papers studying the fate of fluoxetine discharged into the environment via wastewater by conducting photochemical stability studies in aqueous solutions under natural or simulated sunlight. Irradiation of a solution of fluoxetine in water with simulated sunlight caused the *O*-dealkylation as the major photodegradation pathway, in which 4-(difluoromethylene)-2,5-cyclohexadiene-1-one (I, a semiquinone), and 3-phenyl-3-hydroxypropylamine (II) were formed. The minor photodegradation pathway was found to be the photonucleophilic substitution of the trifluoromethyl group, in which the corresponding carboxylic degradant (III) was produced (Scheme 201-3) [6]. The same study suggested that this



Scheme 201-1 Degradation of fluoxetine in the presence of lactose via the Maillard reaction.



Scheme 201-2 Acidic degradation of fluoxetine.



Scheme 201-3 Photodegradation of fluoxetine.

photonucleophilic substitution appeared to be a common photodegradation pathway for molecules containing a trifluoromethyl group, for example, fluometuron and flutalanil [6].

In a photodegradation study of fluoxetine in solid state spiked with metal ions, fluoxetine was found to produce 4-trifluoromethylphenol and 3-phenyl-3-hydroxypropylamine [7], the degradation profile of which appeared to be similar to that under acidic stress conditions (refer to Scheme 201-2).

References

1 Fluoxetine. https://en.wikipedia.org/wiki/Fluoxetine (accessed 27 May 2023).

2 Correia, D., Domingues, I., Faria, M., and Oliveira, M. (2023). Effects of fluoxetine on fish: what do we know and where should we focus our efforts in the future? *Sci. Total Environ.* 857: 159486.

3 Wirth, D.D., Baertschi, S.W., Johnson, R.A. et al. (1998). Maillard reaction of lactose and fluoxetine hydrochloride, a secondary amine. *J. Pharm. Sci.* 87: 31–39.

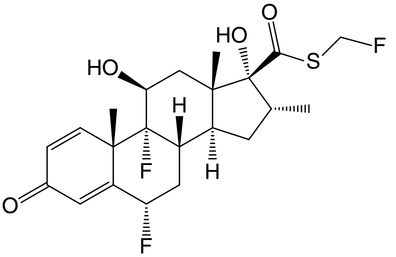
4 Risley, D.S. and Bopp, R.J. (1990). Fluoxetine. *Anal. Prof. Drug Sub.* 19: 193–219.

5 Pathak, A. and Rajput, S.J. (2009). Development of a stability-indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms. *J. Chromatogr. Sci.* 47: 605–611.

6 Lam, M.W., Young, C.J., and Mabury, S.A. (2005). Aqueous photochemical reaction kinetics and transformations of fluoxetine. *Environ. Sci. Technol.* 39: 513–522.

7 Maslanka, A., Hubicka, U., Krzek, J. et al. (2013). Determination of fluoxetine in the presence of photodegradation products appearing during UVA irradiation in a solid phase by chromatographic-densitometric method, kinetics and identification of photoproducts. *Acta Chromatogr.* 25: 465–481.

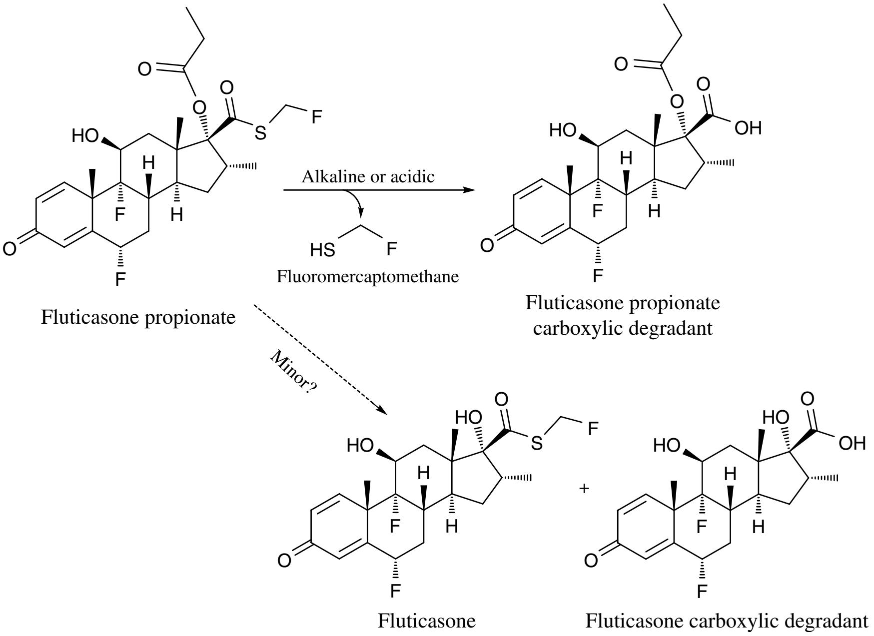
202: Fluticasone [Propionate; Furoate] [WHO List of Essential Medicines]



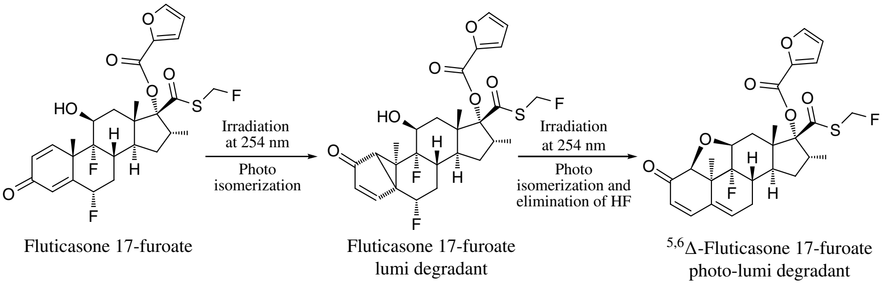
Chemical name: *S*-(Fluoromethyl) (6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-6, 9-difluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthrene-17-carbothioate
Trade name: Flovent, others
Formula: $C_{22}H_{27}F_3O_4S$
Monoisotopic protonated ion (*m/z*): 445.1655
Molecular weight: 444.51
CAS number: 54910-89-3

Fluticasone is a potent anti-inflammatory corticosteroid with low systemic activity [1]. It is much more commonly available in its 17-ester forms, 17-propionate and 17-furoate, for dermal, nasal, and inhalation delivery. When used for the treatment of asthma and chronic obstructive pulmonary disease (COPD), fluticasone 17-esters are typically used in combination with salmeterol, a beta-2 agonist bronchodilator. Both the 17-ester functionality and 21-thiol ester should be susceptible to hydrolytic degradation; nevertheless, it appeared that the 21-thiol ester is much more vulnerable than the 17-ester functionality. In both the forced degradation studies by da Silva Sangoi et al. and Ludvigsson et al. [2, 3], fluticasone 17-propionate produced one major degradant under alkaline and acidic stress conditions, with the alkaline one causing much more degradation. Mass spectrometric analysis of this major degradant indicated that it was the carboxylic degradant of fluticasone 17-propionate (Scheme 202-1) [3]. It should be pointed out that during the hydrolytic degradation, another degradant, fluoromethanethiol, should be formed concurrently; this degradant would not be detected by a typical UV detector.

In its steroid core, fluticasone contains a 2,5-cyclodienone A-ring, which is photochemically reactive and would yield so-called lumi and photo-lumi degradants for this class of corticosteroids [4]. Indeed, in a photodegradation study of fluticasone furoate, the corresponding lumi and photo-lumi degradants were detected (Scheme 202-2) [5], while in a photodegradation study of fluticasone propionate, only the lumi degradant was observed (Scheme 202-3) [6], which could be attributed to insufficient photolysis, as the photo-lumi degradant is a downstream

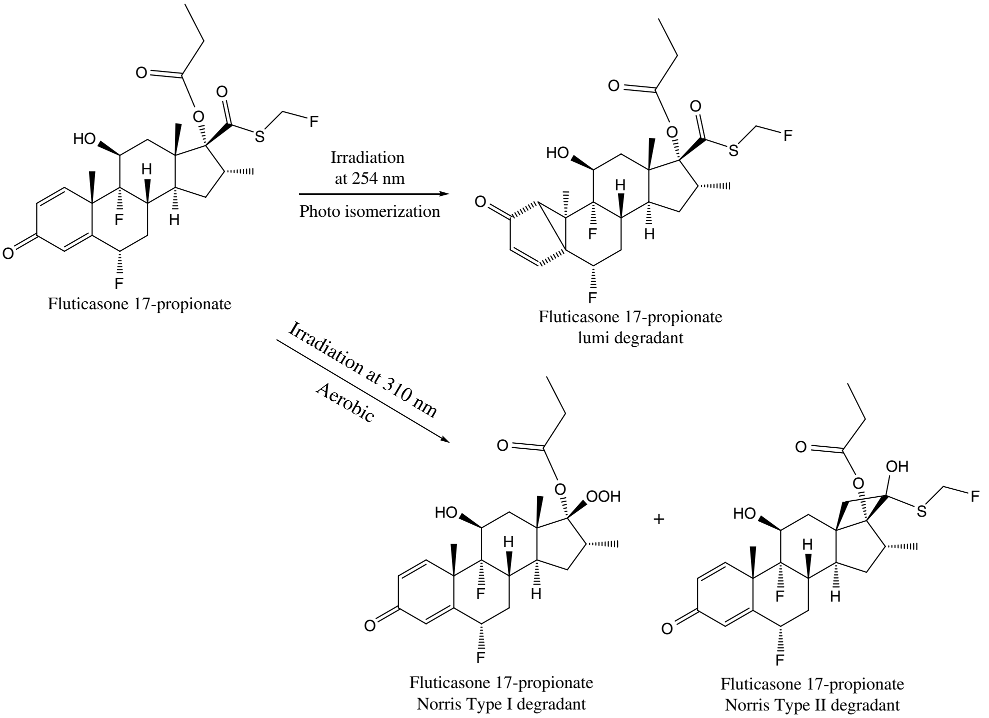


Scheme 202-1 Hydrolytic degradation of fluticasone propionate.



Scheme 202-2 Photodegradation of fluticasone 17-furoate.

degradant upon further photolysis [4]. In the latter study of fluticasone propionate, irradiation at 310 nm caused the formation of other two degradants [5], resulting from Norris type I and II photodegradation mechanisms [4], respectively.



Scheme 202-3 Photodegradation of fluticasone via photoisomerization and Norris Type I and II photoreactions. It is noted that in the original paper [6], the structures of fluticasone and its degradants are erroneous with regard to certain stereo configurations of these molecules. *Source:* Adapted from Ahmad [6].

References

1 Bryson, H.M. and Faulds, D. (1992). Intranasal fluticasone propionate. *Drugs* 43: 760–775.

2 da Silva Sangoi, M., da Silva, L.M., D'Avila, F.B., and Dalmora, S.L. (2010). Determination of fluticasone propionate in nasal sprays by a validated stability-indicating MEKC method. *J. Chromatogr. Sci.* 48: 641–646.

3 Ludvigsson, J.W., Andersson, T., and Kjellberg, V. (2016). A new method to identify hydrolytic degradants in drug substances with UPLC-MS using ^{18}O-labelled water. *J. Pharm. Biomed. Anal.* 122: 9–15.

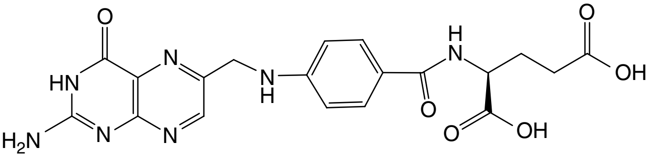
4 Li, M. (2012). Photochemical Degradation. In: *Organic Chemistry of Drug Degradation*, 165–197. Cambridge, UK: RSC Publishing.

5 Bardsley, B., Smith, M.S., and Gibbon, B.H. (2010). Structure elucidation and spectroscopic analysis of photodegradants of the anti-rhinitis drug fluticasone furoate. *Org. Biomol. Chem.* 8: 1876–1880.

6 Ahmad, W. (2016). Photodegradation behaviour of fluticasone propionate under different irradiation conditions. *Int. J. Pharm. Sci. Res.* 7: 390–395.

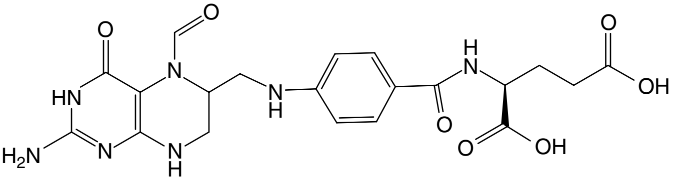
203–204: Folic Acid, Folinic Acid

203: Folic Acid [WHO List of Essential Medicines]



Chemical name: (4-(((2-Amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino) benzoyl)-L-glutamic acid
Formula: $C_{19}H_{19}N_7O_6$
Monoisotopic protonated ion (*m/z*): 442.1470
Molecular weight: 441.40
CAS number: 59-30-3
Common salt form: Sodium salt

204: Folinic Acid

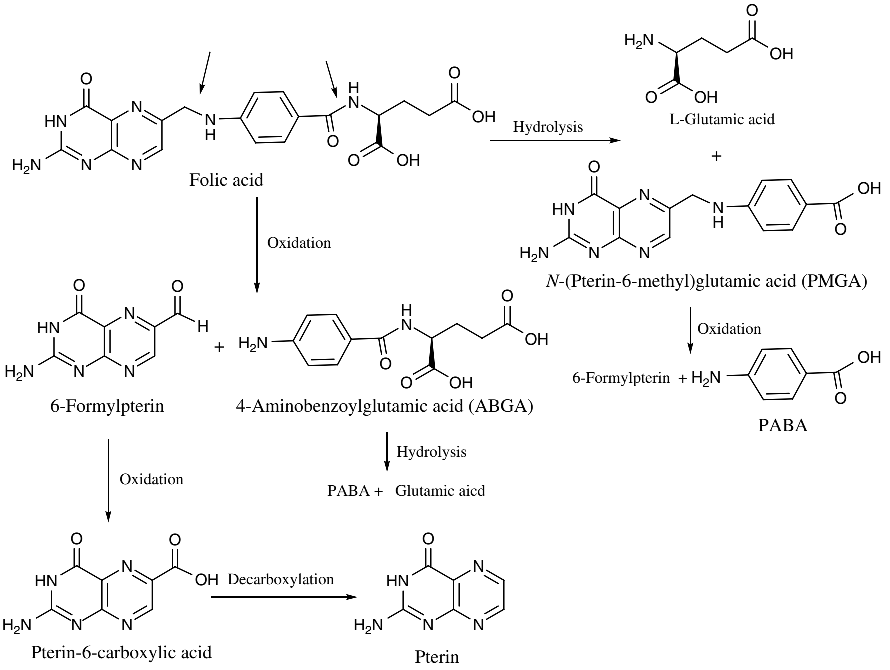


Chemical name: (4-(((2-Amino-5-formyl-4-oxo-3,4,5,6,7,8-hexahydropteridin-6-yl)methyl)amino)benzoyl)-L-glutamic acid
Trade name: Leucovorin, others
Formula: $C_{20}H_{23}N_7O_7$
Monoisotopic protonated ion (*m/z*): 474.1732
Molecular weight: 473.45
CAS number: 58-05-9
Common salt form: Calcium salt; sodium salt

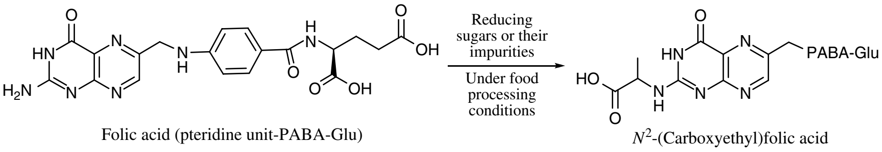
Folic acid, along with its congeners in the folate family, is also known as vitamin B_9.

Usually available in its synthetic form, folic acid is used as a dietary supplement and a therapeutic agent. It is an essential nutrient required for many critical cellular pathways as a one-carbon source, including DNA methylation, synthesis, and maintenance [1].

Also known as pteroylglutamic acid, folic acid is the simplest member of a complex family of closely related compounds that are made of a 4-[(pteridine-6-ylmethyl) amino]benzoic acid unit conjugated with one or more L-glutamate units [2]. As the 4-[(pteridine-6-ylmethyl)amino]benzoic acid unit can be further divided into 2-amino-4-hydroxypteridine and 4-aminobenzoic acid (PABA) subunits, folic acid



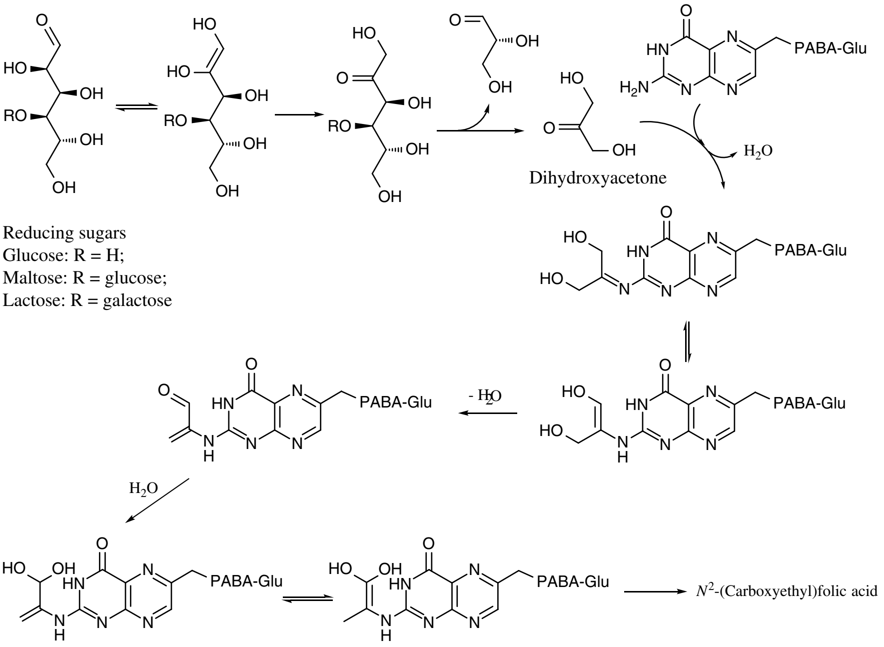
Scheme 203–204-1 Degradation pathways of folic acid under various forced degradation conditions.



Scheme 203–204-2 Isolated degradation product of folic acid in the presence of reducing sugars under food processing conditions.

can be viewed as consisting of three linearly connected structural components: 2-amino-4-hydroxypteridine (AHP), 4-aminobenzoic acid, and glutamic acid. Its degradation chemistry is largely related to these structural features described above. In forced degradation studies under stress conditions of acidic, alkaline, thermal, oxidative, and photochemical, folic acid was found to degrade into its components, subcomponents, and further degradants such as pterin-6-carboxylic acid and pterin (Scheme 203–204-1) [3–6].

The two arrows associated with folic acid (Scheme 203-204-1) indicate the two bonds vulnerable to the respective oxidative and hydrolytic dissociation, and as such, four component degradants (L-glutamic acid, PMGA, 6-formylpterin, and ABGA) could occur as primary degradants. Further degradation of the primary degradants could lead to the formation of secondary and possibly tertiary



Scheme 203–204-3 Plausible degradation mechanism of folic acid in the presence of reducing sugars under food processing conditions.

degradants, e.g., pterin-6-carboxylic acid and pterin. It needs to be pointed out that not all degradants, primary, secondary, or tertiary, would occur at the same time under each forced degradation condition. Furthermore, oxidative degradation could take place under acidic or alkaline forced degradation conditions as long as oxygen is present in the stress system.

The two degradants, 6-formylpterin and pterin-6-carboxylic acid, could undergo type II photosensitized oxidation in which reactive oxygen species (ROS) can be produced, causing cellular oxidative stress [7].

As folic acid is also used in food fortification, Schneider et al. studied the stability of folic acid under conditions that can occur during food processing and preparation. It was found that folic acid reacted with reducing sugars and sugar impurities, such as maltose, lactose, and dihydroxyacetone (a sugar degradation product), to yield a major glycation degradant, *N*-[1-(carboxyethyl)]folic acid, in addition to PABA (Scheme 203–204-2) [8]. Although the degradation reactions between primary and secondary amines with reducing sugars are generally categorized into the broad scope of the Maillard reaction, which typically proceeds through the glycosylamine and Amadori intermediates [9], the formation of *N*-[1-(carboxyethyl)]folic acid was proposed as a direct reaction between folic acid and the sugar degradant, dihydroxyacetone (Scheme 203–204-3) [8].

Folinic acid or more specifically L-folinic acid, also known as levoleucovorin, is the N^5-formyl derivative of tetrahydrofolic acid. It is used clinically as an antidote to counteract the toxic effects of folic acid antagonists, such as methotrexate, an inhibitor of the enzyme dihydrofolate reductase (DHFR). It is also used in combination with the chemotherapy agent 5-fluorouracil for treating advanced metastatic colorectal cancer [10]. Due to its high similarity to folic acid, its thermal degradation pathway would be expected to be similar to that of folic acid, i.e., it could be degraded into its primary and possibly further degradants such as formyltetrahydropterin, tetrahydropterin, 4-aminobenzoic acid, and glutamic acid.

References

1 Crider, K.S., Yang, T.P., Berry, R.J., and Bailey, L.B. (2012). Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. *Adv. Nutr.* 3: 21–38.

2 Vora, A., Riga, A., Dollimore, D., and Alexander, K. (2004). Thermal stability of folic acid in the solid-state. *J. Therm. Anal. Calorim.* 75: 709–717.

3 Gazzali, A.M., Lobry, M., Colombeau, L. et al. (2016). Stability of folic acid under several parameters. *Eur. J. Pharm. Sci.* 93: 419–430.

4 Akhtar, M.J., Khan, M.A., and Ahmad, I. (2003). Identification of photoproducts of folic acid and its degradation pathways in aqueous solution. *J. Pharm. Biomed. Anal.* 31: 579–588.

5 Akhtar, M.J., Khan, M.A., and Ahmad, I. (1999). Photodegradation of folic acid in aqueous solution. *J. Pharm. Biomed. Anal.* 19: 269–275.

6 Grncharoska, K., Todevska, E.L., Spirevska, I.S. et al. (2022). A stability-indicating HPLC method for determination of folic acid and its related substances in tablets. *Maced. Pharm. Bull.* 68: 27–36. http://bulletin.mfd.org.mk/volumes/Volume%2068_1/68_1_002.pdf (accessed 14 August 2023).

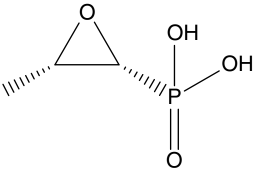
7 Goossens, J.-F., Thuru, X., and Bailly, C. (2021). Properties and reactivity of the folic acid and folate photoproduct 6-formylpterin. *Free Radic. Biol. Med.* 171: 1–10.

8 Schneider, M., Klotzsche, M., Werzinger, C. et al. (2002). Reaction of folic acid with reducing sugars and sugar degradation products. *J. Agric. Food Chem.* 50: 1647–1651.

9 Li, M. (2012). Degradation via the Maillard Reaction. In: *Organic Chemistry of Drug Degradation*, 150–153. Cambridge, UK: RSC Publishing.

10 Levoleucovorin. https://pubchem.ncbi.nlm.nih.gov/compound/135398559 (accessed 29 May 2023).

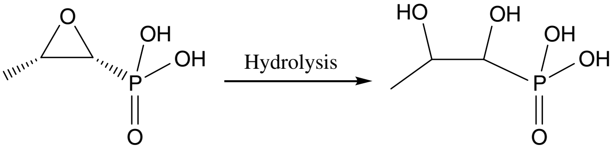
205: Fosfomycin [WHO List of Essential Medicines]



Chemical name: [(2*R*,3*S*)-3-Methyloxiran-2-yl]phosphonic acid
Trade name: Monurol
Formula: $C_3H_7O_4P$
Monoisotopic protonated ion (*m/z*): 139.0155
Molecular weight: 138.06
CAS number: 23155-02-4
Common salt form: Sodium salt

Fosfomycin, originally known as phosphomycin, is an antibiotic used primarily for the treatment of lower urinary tract infections. Discovered in 1969 and approved by the US FDA in 1996, it is a relatively old drug but experiencing a comeback in recent years [1, 2].

Fosfomycin is a phosphinic acid derivative containing an epoxide functional group, which is critical to its antibacterial activity but also susceptible to hydrolytic degradation. Bundgaard studied the degradation kinetics of fosfomycin in acidic solutions with pH ranging between 0.25 and 4.0 [3]. The half-lives of fosfomycin were found to be 27, 37, and 133 minutes at pH 1.0, 1.2, and 2.0, respectively. The hydrolytic degradation product is assumed to be the opening ring dihydroxyl degradant (Scheme 205). The stereochemistry of the degradant could be altered during the hydrolytic degradation, dependent on the hydrolysis conditions.

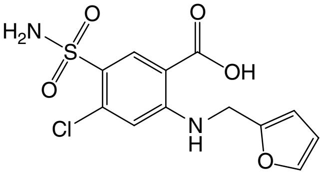


Scheme 205 Hydrolytic degradation of fosfomycin.

References

1 Fosfomycin. https://en.wikipedia.org/wiki/Fosfomycin (accessed 29 May 2023).
2 Fajfr, M., Balik, M., Cermakova, E., and Bostik, P. (2020). Effective treatment for uncomplicated urinary tract infections with oral fosfomycin, single center four year retrospective study. *Antibiotics (Basel).* 9: 511.
3 Bundgaard, H. (1980). Acid-catalyzed hydrolysis of fosfomycin and its implication in oral absorption of the drug. *Int. J. Pharm.* 6: 1–9.

206: Furosemide [WHO List of Essential Medicines]

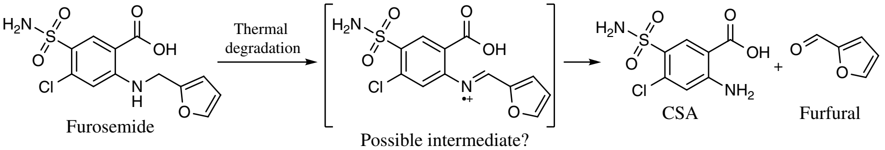


Chemical name: 4-Chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid
Trade name: Furoscix, Lasix
Formula: $C_{12}H_{11}ClN_2O_5S$
Monoisotopic protonated ion (*m/z*): 331.0150
Molecular weight: 330.74
CAS number: 54-31-9
Common salt form: Sodium salt

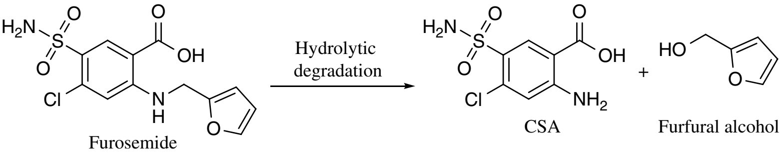
Furosemide is a diuretic used clinically for treating edema caused by heart failure, liver scarring, or kidney disease. It is also used for the treatment of hypertension in patients with kidney or heart impairment [1]. It was first approved for medical use in the 1960s and continues to be a widely prescribed medication.

Furosemide is a highly substituted aniline derivative, and its C—N linkage of the *N*-substituted furan-2-ylmethyl group is susceptible to dissociation under both thermal and photodegradation conditions [2–4]. In a thermal degradation kinetic study of furosemide in solid state, the main degradant observed was 4-chloro-5-sulfamoylanthranilic acid (CSA; also known as saluamine) [2]. The activation energy for the solid-state thermal degradation of furosemide was 47.7 kcal/mol as measured by TGA, which is meaningfully lower than a typical activation energy for C—N breakage at 59 kcal/mol [3]. This solid-state degradation would be likely of oxidative nature, and thus, the other degradant would be 2-furaldehyde (furfural), although it was not reported in the original paper [2], probably due to the volatile nature of 2-furaldehyde (Scheme 206-1).

In acidic solutions, furosemide was found to decompose to CSA and furfural alcohol (Scheme 206-2), and the degradation was accelerated dramatically as the pH was decreased: the apparent first-order rate constant of hydrolysis was determined to be 67.02×10^{-3}/minute at pH 1.5, which is greater than 500 times versus that at pH 7.17.

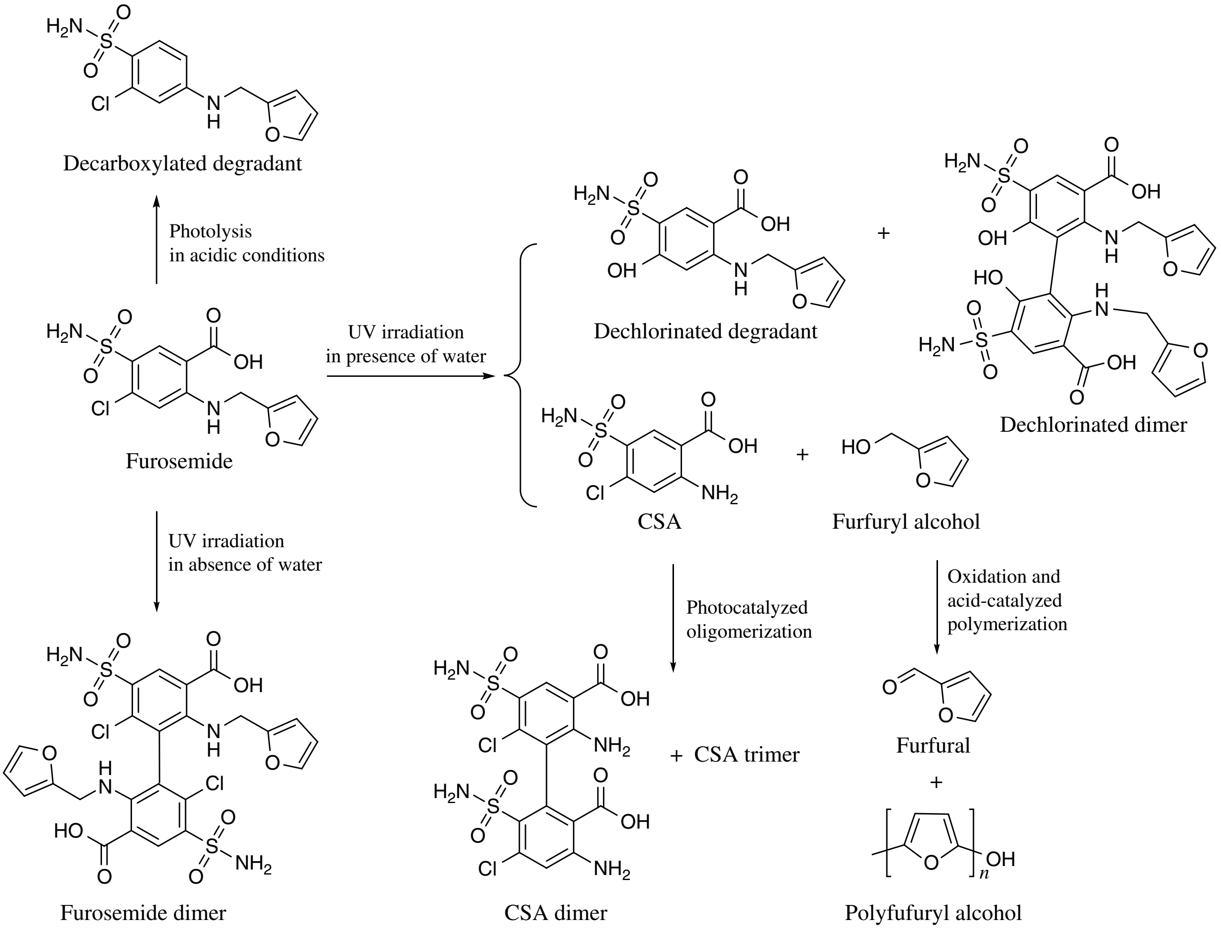


Scheme 206-1 Thermal degradation of furosemide.



Scheme 206-2 Hydrolytic degradation of furosemide.

With regard to the photodegradation pathways of furosemide, Giannetti et al. provided a good summary in their research article (Scheme 206-3) [5]. Under photolytic conditions in the presence of water, the cleavage products were CSA and furfural alcohol. Furfural alcohol would undergo further degradation: aerial oxidation to form furfural aldehyde and acid-catalyzed oligomerization/polymerization, giving rise to colored degradants [6, 7]. On the other hand, CSA could also undergo photocatalyzed dimerization, trimerization, and oligomerization, in addition to the photo-dechlorinated product and its dimer. In the absence of water, photolysis would lead to the formation of dechlorinated dimer. Photodecarboxylation of furosemide was also observed in a study in aqueous solutions by Bundgaard et al. [8].



Scheme 206-3 Photodegradation pathways of furosemide.

References

1 Furosemide. https://en.wikipedia.org/wiki/Furosemide (accessed 30 May 2023).

2 Adrjanowicz, K., Kaminski, K., Grzybowska, K. et al. (2011). Effect of cryogrinding on chemical stability of the sparingly water-soluble drug furosemide. *Pharm. Res.* 28: 3220–3236.

3 Beyers, H., Malan, S.F., van der Watt, J.G., and de Villiers, M.M. (2000). Structure-solubility relationship and thermal decomposition of furosemide. *Drug Dev. Ind. Pharm.* 26: 1077–1083.

4 Cruz, J.E., Maness, D.D., and Yakatan, G.J. (1979). Kinetics and mechanism of hydrolysis of furosemide. *Int. J. Pharm.* 2: 275–281.

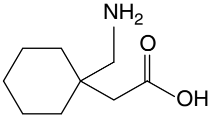
5 Giannetti, M., Canale, V.C., Micheli, L. et al. (2023). An insight into the degradation processes of the anti-hypertensive drug furosemide. *Molecules* 28: 381.

6 Kim, T., Assary, R.S., Marshall, C.L. et al. (2011). Acid-catalyzed furfuryl alcohol polymerization: characterizations of molecular structure and thermodynamic properties. *Chem. Cat. Chem.* 3: 1451–1458.

7 Shibutani, K., Nakai, J., Aphichartsuphapkhajorn, K. et al. (2021). The activation of furfuryl alcohol polymerization by oxygen and its enhanced mechanical properties. *J. Appl. Polym. Sci.* 138: 50311.

8 Bundgaard, H., Nørgaard, T., and Nielsen, N.M. (1988). Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions. *Int. J. Pharm.* 42: 217–224.

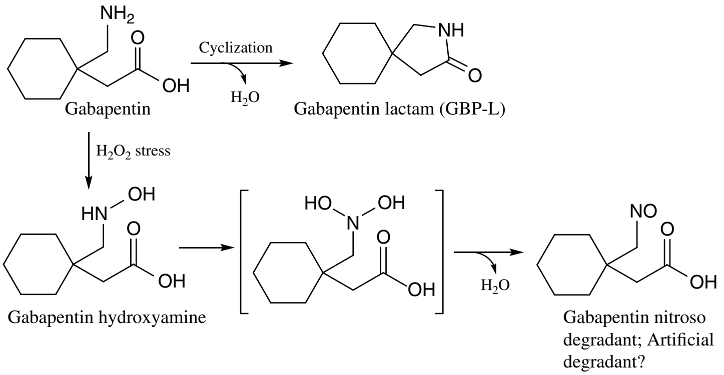
207: Gabapentin



Chemical name: 2-[1-(Aminomethyl)cyclohexyl]acetic acid
Trade name: Neurontin
Formula: $C_9H_{17}NO_2$
Monoisotopic protonated ion (*m/z*): 172.1332
Molecular weight: 171.24
CAS number: 60142-96-3

Gabapentin, originally developed for the treatment of epilepsy, is a structural analogue of the neurotransmitter γ-aminobutyric acid (GABA) [1]. Its main clinical use is to relieve neuropathic pain caused by diabetic neuropathy, postherpetic neuralgia, and central pain [2]. In 2020, it was the 10th most prescribed medication in the US.

Gabapentin is a γ-amino acid, and its main degradation product is the lactam (3,3-pentamethylene-4-butyrolactam, or GBP-L) that is formed via cyclization between the carboxyl and amino groups (Scheme 207) [3]; the lactam degradant was shown to cause seizures in an animal model [4]. It was reported to be most stable at pH around 6, where the zwitterion form dominates [5]. Zong et al. studied the factors impacting lactamization during gabapentin tablets manufacturing process and found that the lactamization rate in the milled gabapentin samples was correlated to increased surface area, milling time, and lower moisture level [6]. The stabilization effect by higher moisture (> 31% relative humidity) was attributed to a moisture-driven competitive annealing process, which would decrease the milling-induced crystal defects. In a forced degradation study, the amino group of gabapentin was found to be oxidized into a nitroso group [7]; this stress-generated degradant is most likely to be an artificial degradant, as the oxidative stress with hydrogen peroxide seemed to be quite harsh.



Scheme 207 Degradation pathways of gabapentin.

References

1 Gabapentin. https://en.wikipedia.org/wiki/Gabapentin (accessed 4 June 2023).

2 Goa, K.L. and Sorkin, E.M. (1993). A review of its pharmacological properties and clinical potential in epilepsy. *Drugs* 46: 409–427.

3 Ciavarella, A.B., Gupta, A., Sayeed, V.A. et al. (2007). Development and application of a validated HPLC method for the determination of gabapentin and its major degradation impurity in drug products. *J. Pharm. Biomed. Anal.* 43: 1647–1653.

4 Potschka, H., Feuerstein, T.J., and Löscher, W. (2000). Gabapentin-lactam, a close analogue of the anticonvulsant gabapentin, exerts convulsant activity in amygdala kindled rats. *Naunyn Schmiedeberg's Arch. Pharmacol.* 361: 200–205.

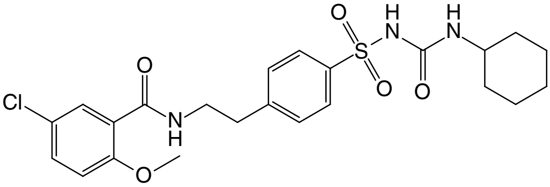
5 Zour, E., Lodhi, S.A., Nesbitt, R.U. et al. (1992). Stability studies of gabapentin in aqueous solutions. *Pharm. Res.* 9: 595–600.

6 Zong, Z., Desai, S.D., Kaushal, A.M. et al. (2011). The stabilizing effect of moisture on the solid-state degradation of gabapentin. *AAPS PharmSciTech* 12: 924–931.

7 Ragham, P.K. and Chandrasekhar, K.B. (2016). Development and validation of a stability-indicating RP-HPL C-CAD method for gabapentin and its related impurities in presence of degradation products. *J. Pharm. Biomed. Anal.* 125: 122–129.

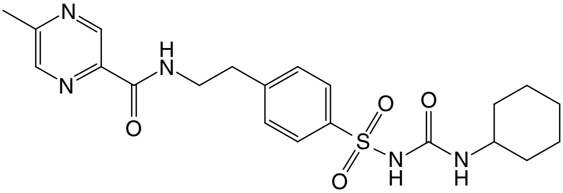
208–209: Glibenclamide, Glipizide

208: Glibenclamide (Glyburide)



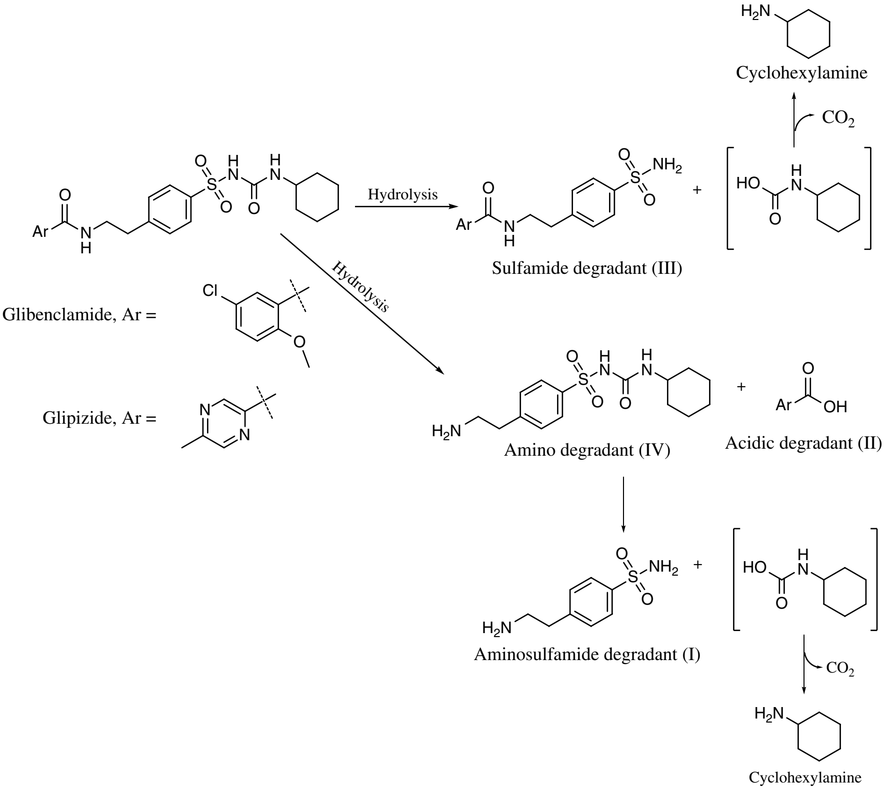
Chemical name: 5-Chloro-*N*-[2-[4-(cyclohexylcarbamoylsulfamoyl)phenyl] ethyl]-2-methoxybenzamide
Trade name: Diabeta, Glucovance, Glynase
Formula: $C_{23}H_{28}ClN_3O_5S$
Monoisotopic protonated ion (*m/z*): 494.1511
Molecular weight: 494.00
CAS number: 10238-21-8

209: Glipizide



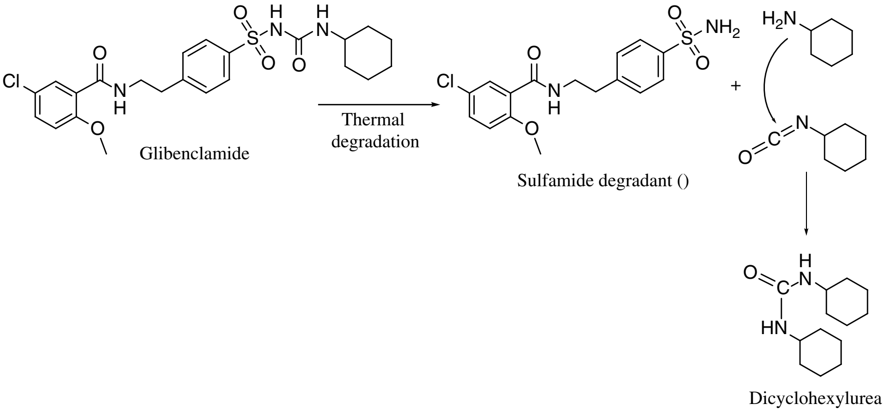
Chemical name: *N*-[2-[4-(Cyclohexylcarbamoylsulfamoyl)phenyl]ethyl]-5-methylpyrazine-2-carboxamide
Trade name: Glucotrol
Formula: $C_{21}H_{27}N_5O_4S$
Monoisotopic protonated ion (*m/z*): 446.1857
Molecular weight: 445.54
CAS number: 29094-61-9

Both glibenclamide and glipizide are antidiabetic drugs of the sulfonylurea family for the treatment of type II diabetes. Glibenclamide was discovered in the 1960s and approved by the US FDA in 1984 [1]. It works by inhibiting the ATP-sensitive K^+ channels, causing depolarization of the cells and subsequent insulin release [2].

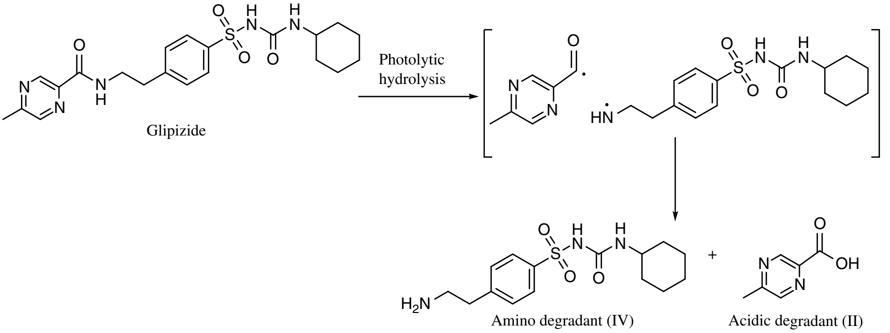


Scheme 208–209-1 Hydrolytic degradation pathways of glibenclamide and glipizide.

The sulfonylurea and benzamide moieties of both drug molecules are susceptible to hydrolytic degradation. The most vulnerable linkage is the sulfonyl-urea bond, and its breakage results in the formation of the sulfamide (III) and cyclohexylamine degradants (Scheme 208–209-1) [3–5]. For glipizide, the rates of hydrolysis under acidic and alkaline conditions were found to be comparable [5, 6], which would be expected to be the same for glibenclamide. Hydrolysis of the benzamide linkage produced degradant IV. In a thermal degradation study of amorphous glibenclamide, it decomposed to 1,3-dicyclohexylurea, probably via the intermediate of cyclohexylisocyanate (Scheme 208–209-2) [7]. Under photochemical stress of glipizide in solution, degradant IV was also formed, and its accelerated formation was proposed through a diradical intermediate produced in the homolysis of the diazole-carbonyl linkage (Scheme 208–209-3) [5, 8].



Scheme 208–209-2 Thermal degradation of glibenclamide.



Scheme 208–209-3 Probable formation mechanism of glipizide degradant IV under photochemical stress conditions.

References

1 Glibenclamide. https://en.wikipedia.org/wiki/Glibenclamide (accessed 4 June 2023).

2 Luzi, L. and Pozza, G. (1997). Glibenclamide: an old drug with a novel mechanism of action? *Acta Diabetol.* 34: 239–244.

3 Bansal, G. and Singh, M. (2008). Ultraviolet-photodiode array and high-performance liquid chromatographic/mass spectrometric studies on forced degradation behavior of glibenclamide and development of a validated stability-indicating method. *J. AOAC Int.* 91: 709–719.

4 Gupta, S. and Bansal, G. (2011). Validated stability-indicating HPLC-UV method for simultaneous determination of glipizide and four impurities. *J. AOAC Int.* 94: 523–530.

5 Bansal, G., Singh, M., Jindal, K.C., and Singh, S. (2008). LC and LC-MS study on establishment of degradation pathway of glipizide under forced decomposition conditions. *J. Chromatogr. Sci.* 46: 510–517.

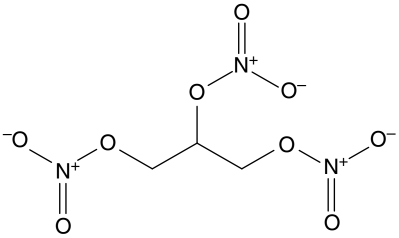
6 Gedawy, A., Al-Salami, H., and Dass, C.R. (2020). Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions. *Saudi Pharm. J.* 28: 362–368.

7 Rehder, S., Sakmann, A., Rades, T., and Leopold, C.S. (2012). Thermal degradation of amorphous glibenclamide. *Eur. J. Pharm. Biopharm.* 80: 203–208.

8 Rosenthal, I. (1970). Photochemistry of the amido group. In: *The Chemistry of Amides* (ed. I.J. Zabicky), 289–308. London: Interscience Publisher.

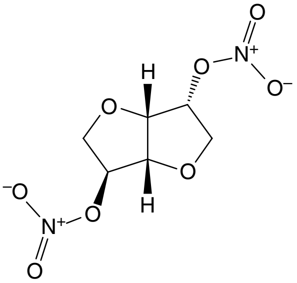
210–211: Glyceryl Trinitrate, Isosorbide Dinitrate

210: Glyceryl Trinitrate (Nitroglycerin)



Chemical name: 1,3-Dinitrooxypropan-2-yl nitrate
Formula: $C_3H_5N_3O_9$
Monoisotopic protonated ion (*m/z*): 228.0099
Molecular weight: 227.09
CAS number: 55-63-0

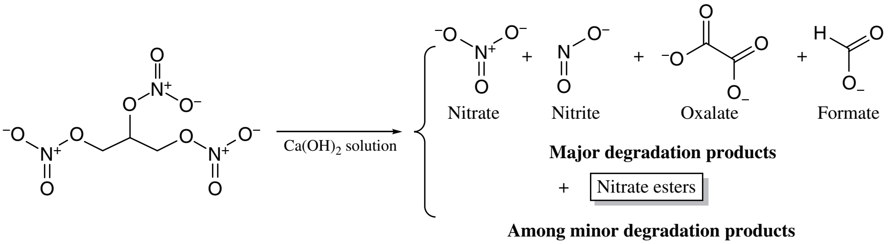
211: Isosorbide Dinitrate [WHO List of Essential Medicines]



Chemical name: [(3*S*,3a*S*,6*R*,6a*S*)-3-Nitrooxy-2,3,3a,5,6,6a-hexahydrofuro[3,2-b] furan-6-yl] nitrate
Trade name: Bidil, Dilatrate, Isordil
Formula: $C_6H_8N_2O_8$
Monoisotopic protonated ion (*m/z*): 237.0353
Molecular weight: 236.14
CAS number: 87-33-2

Glyceryl trinitrate, commonly known as nitroglycerin, was discovered by the Italian chemist Ascanio Sobrero in 1847, and its first medical use was initiated by the English physician William Murrell in 1878 [1]. It is used for the treatment of angina pectoris, or chest pain, caused by insufficient blood flow to the heart muscle. Glyceryl trinitrate relieves the symptoms of angina via its metabolic release of nitric oxide, NO, a powerful vasodilator [2].

It is a trinitrate ester, and its degradation is mainly hydrolytic. In a degradation kinetic study in calcium hydroxide solutions, degradation of glyceryl trinitrate was

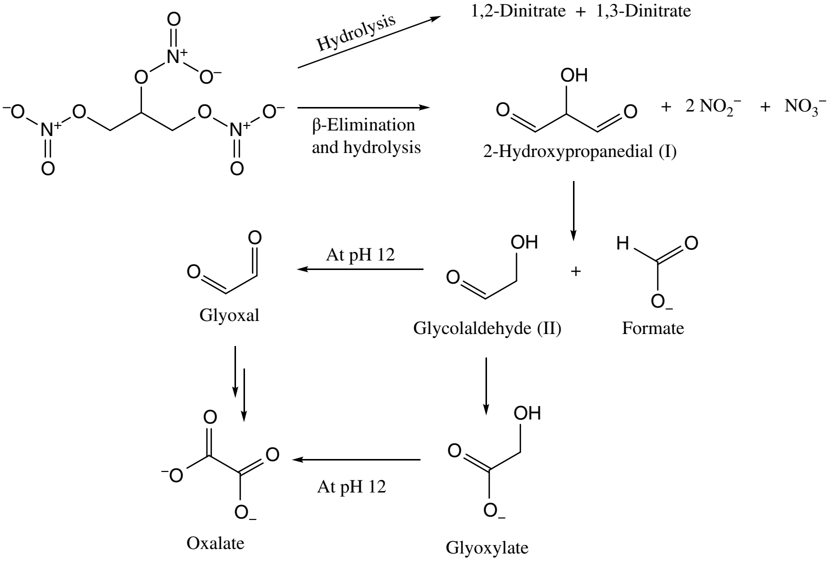


Scheme 210–211-1 Degradation of glyceryl trinitrate in alkaline solutions.

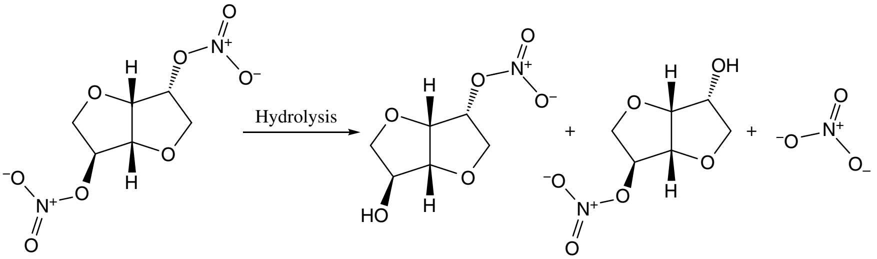
found to proceed via a second-order reaction with an activation energy of 27.53 kcal/mol [3]. The major degradation products were calcium nitrate and calcium nitrite (and the corresponding diesters), with calcium oxalate, nitrate esters, and calcium formate among the minor degradants (Scheme 210–211-1).

Halasz et al. studied the degradation product distribution of glyceryl trinitrate at pH 9 and pH 12, respectively, under heating at 50°C by either conventional way or by microwaving [4]. Although the degradation was much higher under microwave heating, the major degradation products were largely the same. Based on the degradation product distribution, a degradation mechanism was proposed, which involved two critical β-elimination steps to generate two equivalent nitrites (Scheme 210–211-2) [4]. It needs to be pointed out that none of the dinitrate degradants, mononitrate degradants, and glycerin were detected in the study by Halasz et al. [4].

Isosorbide dinitrate is also a member of the nitrate esters, which was originally reported in 1939 [4]. Its mode of action for treating angina is similar to that of



Scheme 210–211-2 Proposed mechanism for degradation of glyceryl trinitrate under alkaline conditions.



Scheme 210–211-3 Hydrolytic degradation of isosorbide dinitrate.

glyceryl trinitrate. The onset of action for isosorbide dinitrate is 30 minutes [5], which makes it a long-acting nitrate ester drug, whereas glyceryl trinitrate is a short-acting nitrate ester drug [6]. Isosorbide dinitrate is also utilized for the treatment of heart failure due to systolic dysfunction, and for this indication, it is usually used in combination with hydralazine [5]. The nature of its degradation is also hydrolytic, with the two monoesters, i.e., isosorbide-5-mononitrate (5-ISMN) and isosorbide-2-mononitrate (2-ISMN) as the major degradants (Scheme 210–211-3) [7, 8], which is quite different from that of glyceryl trinitrate degradation. The difference in their chemical stability behaviors appears to be consistent with their respective short- and long-acting modes.

References

1 Nitroglycerin. https://en.wikipedia.org/wiki/Nitroglycerin (accessed 10 June 2023).

2 Hashimoto, S. and Kobayashi, A. (2003). Clinical pharmacokinetics and pharmacodynamics of glyceryl trinitrate and its metabolites. *Clin. Pharm.* 42: 205–221.

3 Capellos, C., Fisco, W.J., Ribaudo, C. et al. (1984). Basic hydrolysis of glyceryl nitrate esters. III. Trinitroglycerin. *Int. J. Chem. Kinet.* 16: 1027–1051.

4 Halasz, A., Thiboutot, S., Ampleman, G., and Hawari, J. (2010). Microwave-assisted hydrolysis of nitroglycerin (NG) under mild alkaline conditions: new insight into the degradation pathway. *Chemosphere* 79: 228–232.

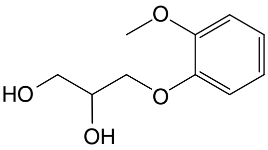
5 Isosorbide_dinitrate. https://en.wikipedia.org/wiki/Isosorbide_dinitrate (accessed 10 June 2023).

6 Schaumann, W. (1989). Pharmacokinetics of isosorbide dinitrate and isosorbide-5-mononitrate. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 27: 445–453.

7 Carlson, M., Thompson, R.D., and Snell, R.P. (1988). Determination of isosorbide dinitrate in pharmaceutical products by HPLC. *J. Chromatogr. Sci.* 26: 574–578.

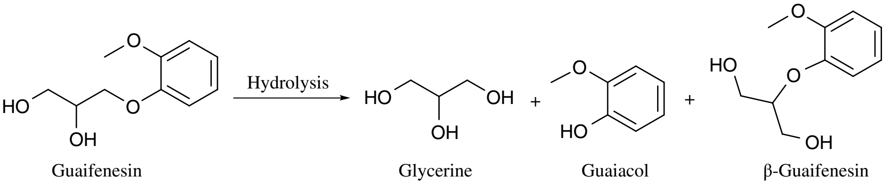
8 Liu, W.Y. (1989). Determination of isosorbide dinitrate and its degradation products in pharmaceuticals by gradient RP-HPLC. *Yao Xue Bao (Acta Pharm. Sinica)* 24: 797–800.

212: Guaifenesin



Chemical name: 3-(2-Methoxyphenoxy)propane-1,2-diol
Formula: $C_{10}H_{14}O_4$
Monoisotopic protonated ion (*m/z*): 199.0965
Molecular weight: 198.22
CAS number: 93-14-1

Guaifenesin is an expectorant drug clinically used to help the elimination of sputum from the respiratory tract. It is often used in combination with other types of medications, such as dextromethorphan (cough suppressant), pseudoephedrine (decongestant), codeine, and acetaminophen (pain relievers). Structurally, it is an aryl alkyl ether formed between guaiacol and glycerin. Its degradation pathways are primarily the hydrolytic breakage of the alkyl ether bond and isomerization, resulting in the formation of guaiacol and glycerin and isomeric beta-guaifenesin, respectively (Scheme 212) [1–4]. In a forced degradation study performed by Reddy et al. [2], under acidic condition (1N HCl at 60°C for 12 hours), it appeared that the predominant degradation was isomerization, while under alkaline condition (1N NaOH at 60°C for 12 hours), both isomerization and ether bond cleavage were observed.



Scheme 212 Hydrolytic degradation of guaifenesin.

References

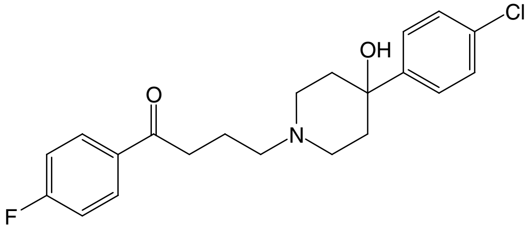
1 Grosa, G., Grosso, E.D., Russo, R., and Allegrone, G. (2006). Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup. *J. Pharm. Biomed. Anal.* 41: 798–803.

2 Reddy, S.P., Babu, K.S., Kumar, N., and Sekhar, Y.S. (2011). Development and validation of stability indicating the RP-HPLC method for the estimation of related compounds of guaifenesin in pharmaceutical dosage forms. *Pharm. Methods* 2: 229–234.

3 Raju, T.V.R., Kumar, N.A., Kumar, S.R. et al. (2013). Development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of guaifenesin and dextromethorphan impurities in pharmaceutical formulations. *Chromatogr. Res. Int.* 2013: 315145.

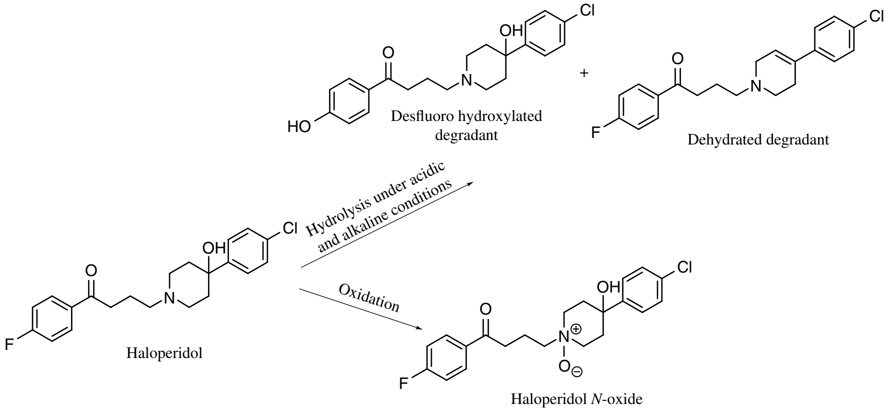
4 Rathnakar, N. and Shanker, D.G. (2020). Stability-indicating simultaneous method development and validation of guaifenesin and dextromethorphan HBr by reverse-phase high-performance liquid chromatography. *Int. J. Pharm. Qual. Assur.* 11: 262–270.

213: Haloperidol [WHO List of Essential Medicines]



Chemical name: 4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one
Trade name: Haldol
Formula: $C_{21}H_{23}ClFNO_2$
Monoisotopic protonated ion (*m/z*): 376.1474
Molecular weight: 375.87
CAS number: 52-86-8
Common salt form: Lactate

Discovered by Paul Janssen in 1958, haloperidol remains to be a typical antipsychotic medication used for the treatment of schizophrenia, bipolar disorder, acute psychosis, and agitation, among others [1]. The fluoro and hydroxyl groups are potentially susceptible to hydrolytic degradation, particularly under strong forced alkaline and acidic degradation conditions, to produce the hydroxylated desfluoro and dehydrated degradants [2, 3]. On the other hand, its tertiary amine moiety is susceptible to nucleophilic oxidation to form two diastereomeric *N*-oxide degradants (Scheme 213).



Scheme 213 Hydrolytic and oxidative degradation pathways of haloperidol.

References

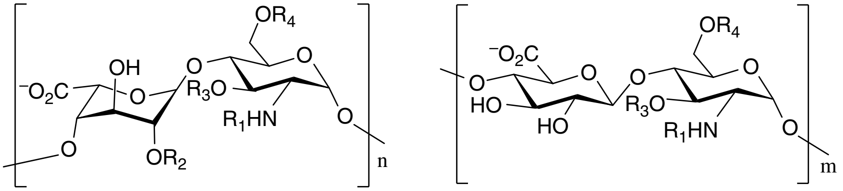
1 Haloperidol. https://en.wikipedia.org/wiki/Haloperidol (accessed 14 June 2023).

2 Trabelsi, H., Bouabdallah, S., Bouzouita, K., and Safta, F. (2002). Determination and degradation study of haloperidol by high performance liquid chromatography. *J. Pharm. Biomed. Anal.* 29: 649–657.

3 Monser, L. and Trabels, H. (2003). A rapid LC method for the determination of haloperidol and its degradation products in pharmaceuticals using a porous graphitic carbon column. *J. Liq. Chromatogr. Relat. Technol.* 26: 257–266.

214–215: Heparin, Enoxaparin

214: Heparin [WHO List of Essential Medicines]

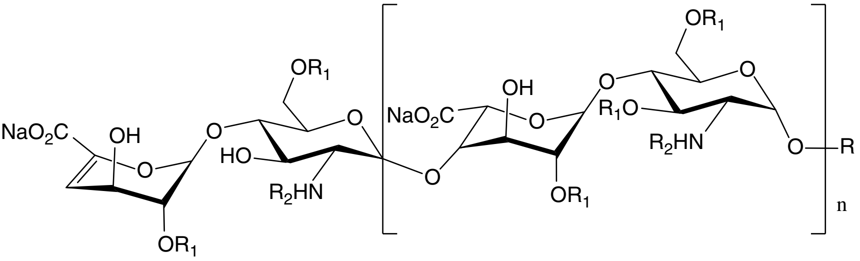


R_1 = H, SO_3^-, or Acetyl; R_2, R_3, R_4 = H or SO_3^-

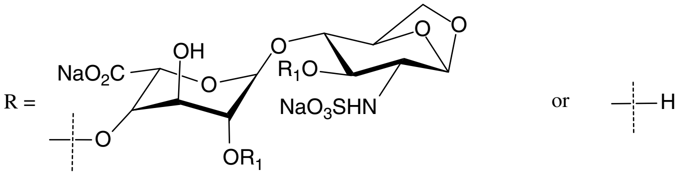
Major and minor heparin disaccharide repeating units. Left: →4)-L-iduronic acid-(1→4)-αD-glucosamine (1→, which accounts for ~85%. Right: →4)-D-glucuronic acid-(1→4)-D-glucosamine (1→, which accounts for the majority of the remaining ~15%. Each of the monosaccharide units has various degree of *N*- and *O*-sulfation, with occasional *N*-acetylation (~5% in porcine mucosal heparin).

Chemical name: IUPAC name not applicable
Formula: N/A
Monoisotopic protonated ion (*m/z*): N/A
Molecular weight: ~12,000–~15,000 (average); 3,000–30,000 (range)
CAS number: 9005-49-6

215: Enoxaparin



R_1 = H, or SO_3Na; R_2 = SO_3Na or Acetyl.



15 – 25%, n = 0 – 20

Remaining of 15 – 25%, n = 1 – 21

(low-molecular-weight heparin)

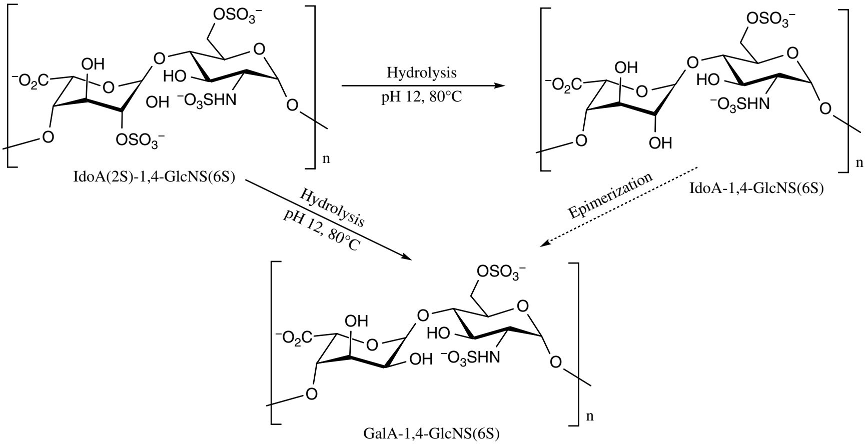
Chemical name: IUPAC name not applicable
Trade name: Lovenox
Formula: N/A
Monoisotopic protonated ion (*m/z*): N/A
Molecular weight: ~4500 (average)
CAS number: 679809-58-6 (Sodium salt)
Common salt form: Sodium salt

Heparin, a highly sulfated glycosaminoglycan, is a naturally occurring anticoagulant present in mast cells of mammals. Heparin exerts its anticoagulant activity primarily via inducing or enhancing the inhibition of thrombin (Factor IIa) and Factor Xa by antithrombin III. Clinically, it is used for preventing and treating conditions that involve undesirable formation of blood clots, including deep vein thrombosis, pulmonary embolism, atrial fibrillation, and acute coronary syndrome [1].

The early work conducted by Jay McLean in the laboratory of Prof. William H. Howell at Johns Hopkins University in the 1910s led to the eventual discovery of heparin by Howell's group in 1922 [2]. Although Howell named both substances "heparin," i.e., the first one prepared by McLean in 1918 and the second one by Howell in 1922, due to their originating from dog liver, the one prepared by McLean was not the heparin as we know it today. McLean's "heparin" was likely a lipophilic mixture containing phospholipids and inositol phosphatides, among other components; all possess anticoagulant activity [3]. In 1929, Charles H. Best, the co-discoverer of insulin (along with fellow Canadian Frederick Banting), and his graduate student, Arthur Charles, decided to optimize the purification of heparin. Their effort resulted in a few papers published in 1933, which outlined the purification protocols for isolating heparin from bovine liver [4]. In 1935, Erik Jorpes of Karolinska Institute, who had visited Best in 1929 in Canada [4], purified heparin and determined its structure [5]. The heparin prepared by Best's group and Jorpes' was pure enough to rid the toxic side effects of the previous heparin preparations and thus enabled commercial production of heparin for clinical use in the mid-1930s. Nowadays, heparin is prepared via extraction and subsequent purification from the intestinal mucosa of pigs or cattle.

Heparin is a water-soluble, heterogeneous mixture of congeners consisting of highly sulfated glycosaminoglycan units. The molecular weight distribution of heparin ranges from ~3,000 to ~30,000 Da, with an average at ~12,000–15,000 Da [1]. The level of heterogeneity in heparin varies depending on its sources – either species-wise or different organs of the same species. The sulfated substituents account for ~40% of its average molecular weight. The simplest presentation of heparin in terms of molecular composition may be defined by its predominant (~85%) repeating disaccharide units of 1,4-linked L-iduronate (IdoA)-D-glucosamine (GlcN). The majority of the remaining ~15% repeating disaccharide units are 1,4-linked D-glucuronate (GlcA)-D-glucosamine (GlcN). Each of the monosaccharide units has various degree of *N*- and *O*-sulfation, and overall, for every tetrasaccharide unit, it contains close to five sulfate groups [6]. For porcine mucosal heparin, it contains approximately 20 hexosamine residues per chain, the vast majority of which are *N*-sulfated, with a single *N*-acetylation per chain on average [7].

The degradation pathways of heparin are hydrolytic as several of its structural features are susceptible to hydrolytic degradation with increasing levels of difficulty: *N*-sulfamate, *O*-sulfonate, and glycosidic bonds. In general, *N*-sulfamate linkage is somewhat less stable hydrolytically than *O*-sulfonate linkage; nevertheless, the individual stability of a particular sulfate linkage is also influenced by its surrounding environment as well as pH of the media. On the other hand, the glycosidic linkage is significantly more stable. Nagasawa et al. were able to achieve complete desulfation, with no depolymerization (i.e., no glycosidic cleavage), by heating heparin pyridinium salt solutions in DMSO containing up to 10% water or methanol at 80–100°C [8]. This study also found that the loss of *N*-sulfate preceded that of *O*-sulfate, and that both the *N*-sulfamate and *O*-sulfonate bonds are less stable than the *N*-acetamide bond as no de-*N*-acetylation was observed under the solvolytic conditions. Kozlowski et al. examined heparin hydrolysis in an acidic environment and found that desulfation was responsible for most of the heparin molecular weight reduction (due to hydrolytic degradation at pH 1, 40°C) [9]. Jandik et al. studied the stability of heparin sodium derived from porcine intestinal mucosa, which is probably most pharmaceutically relevant, in 0.1 N HCl, 0.1 NaOH, and deoxygenated neutral phosphate buffer solutions, respectively, at different temperatures [10]. While the degradation mechanisms under acidic and alkaline conditions were quite different, heparin in neutral phosphate buffer was relatively stable upon heating at 100°C for up to 500 hours. Kozlowski et al. also studied the stability of heparin solutions in pH 7–12 and under temperatures of 40°C, 60°C, and 80°C [11]. No significant *N*-desulfation or 6-*O*-desulfation, nor glycosidic bond dissociation, was observed. Nevertheless, stereochemical transformation of α-L-iduronate 2-*O*-sulfate to α-L-galacturonate occurred in 0.1 M phosphate buffer at pH 12 and 80°C (Scheme 214–215).



Scheme 214–215 Stereochemical transformation of α-L-iduronate 2-*O*-sulfate to α-L-galacturonate in pH 12 solution at 80°C.

Enoxaparin, available as its sodium salt, is one of the low-molecular-weight heparin preparations or LMWH, which was approved by the US FDA in 1993 for indications similar to those of regular heparin [12], commonly referred to as unfractionated heparin (UFH) when compared to LMWH. The average molecular weight of enoxaparin is ~4,000–5,000, as compared to ~12,000–15,000 for UFH, and it displayed improved pharmacokinetic and pharmacodynamic profiles than UFH [13]. Enoxaparin is prepared from UFH via controlled depolymerization, i.e., controlled hydrolysis of the glycosidic bonds, by using alkaline hydrolysis of the benzylic ester of UFH. Thus, enoxaparin retains the essential major and minor disaccharide units as mentioned above. Due to the high structural similarity between enoxaparin and UFH, the degradation chemistry of enoxaparin would be expected to be similar to that of UFH.

References

1 Heparin. https://en.wikipedia.org/wiki/Heparin (accessed 27 August 2023).

2 Mueller, R.L. and Scheidt, S. (1994). History of drugs for thrombotic disease. Discovery, development, and directions for the future. *Circulation* 89: 432–449.

3 Silver, J.M., Turner, D.L., and Tocantins, L.M. (1959). Lipid anticoagulants. *Prog. Hematol.* 2: 264–281.

4 Lim, G.B. (2017). Discovery and purification of heparin. *Nat. Rev. Cardiol.* https://doi.org/10.1038/nrcardio.2017.171.

5 Jorpes, E. (1936). On heparin, its chemical nature and properties. *Acta Med. Scand.* 88: 427–433.

6 Wolfrom, M.L., Weisblat, D.I., Karabinos, J.V. et al. (1943). Chemical studies on crystalline barium acid heparinate. *J. Am. Chem. Soc.* 65: 2077–2085.

7 Bae, J.H., Desai, U.R., Pervin, A. et al. (1994). Interaction of heparin with synthetic antithrombin III peptide analogues. *Biochem. J.* 301: 121–129.

8 Nagasawa, K., Inoue, Y., and Kamata, T. (1977). Solvolytic desulfation of glycosaminoglycuronan sulfates with dimethyl sulfoxide containing water or methanol. *Carbohydr. Res.* 58: 47–55.

9 Kozlowski, A.M., Yates, E.A., Roubroeks, J.P. et al. (2021). Hydrolytic degradation of heparin in acidic environments: nuclear magnetic resonance reveals details of selective desulfation. *ACS Appl. Mater. Interfaces* 13: 5551–5563.

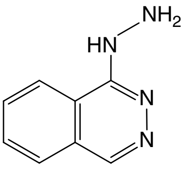
10 Jandik, K.A., Kruep, D., Cartier, M., and Linhardt, R.J. (1996). Accelerated stability studies of heparin. *J. Pharm. Sci.* 85: 45–51.

11 Kozlowski, A.M., Yates, E.A., Roubroeks, J.P. et al. (2023). Monitoring the stability of heparin: NMR evidence for the rearrangement of sulfated iduronate in phosphate buffer. *Carbohydr. Polym.* 308: 120649.

12 Enoxaparin. https://go.drugbank.com/drugs/DB01225 (accessed 2 September 2023).

13 Iqbal, Z. and Cohen, M. (2011). Enoxaparin: a pharmacologic and clinical review. *Expert. Opin. Pharmacother.* 12: 1157–1170.

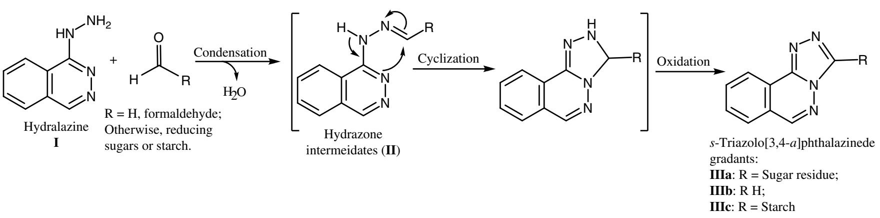
216: Hydralazine [WHO List of Essential Medicines]



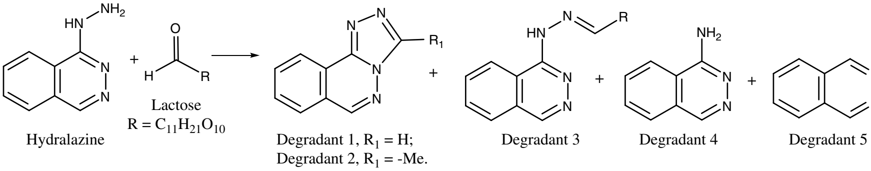
Chemical name: Phthalazin-1-ylhydrazine
Trade name: Apresoline, Bidil
Formula: $C_8H_8N_4$
Monoisotopic protonated ion (*m/z*): 161.0822
Molecular weight: 160.18
CAS number: 86-54-4
Common salt form: HCl salt

Hydralazine is a potent peripheral vasodilator for treating essential hypertension and is available in both oral and injectable dosage forms. Its injectable forms are used during hypertensive crises in emergency rooms, operating rooms, and intensive care units. Originally synthesized by J. Druey at Ciba as an antimalaria treatment in the 1940s, it received US FDA approval for treating hypertension in 1953 [1, 2].

The reactive hydrazino group of hydralazine is the main reason for the instability of the drug as well as for some of its toxicity, such as genotoxicity. The functional group is both fairly strongly nucleophilic and susceptible to oxidative and hydrolytic degradation [1, 3, 4]. The hydrazino group, in tandem with its nearby 2,3-azine moiety, is also capable of chelating metal ions [5]. Hence, hydralazine can readily condense with aldehydes and ketones, which are often present in some of the pharmaceutical excipients or as their impurities. Noda et al. reported that hydralazine rapidly reacted with formaldehyde to form the cyclized triazolophthalazine degradant under weakly acidic conditions (Scheme 216-1) and used this reaction for quantitative HPLC determination of formaldehyde [6], which is a common impurity in certain excipients and other sources. When the source of aldehydes is from reducing sugars or starch, which contains terminal glucoses, hydralazine can react with the reducing sugars to form similar cyclized triazolophthalazine degradants (Scheme 216-1), which have UV–Vis absorbance at longer wavelengths, thus causing discoloration of the drug product as reported by Lesson and Zhao [7].



Scheme 216-1 Degradation of hydralazine in the presence of aldehyde-containing excipients or their impurities.

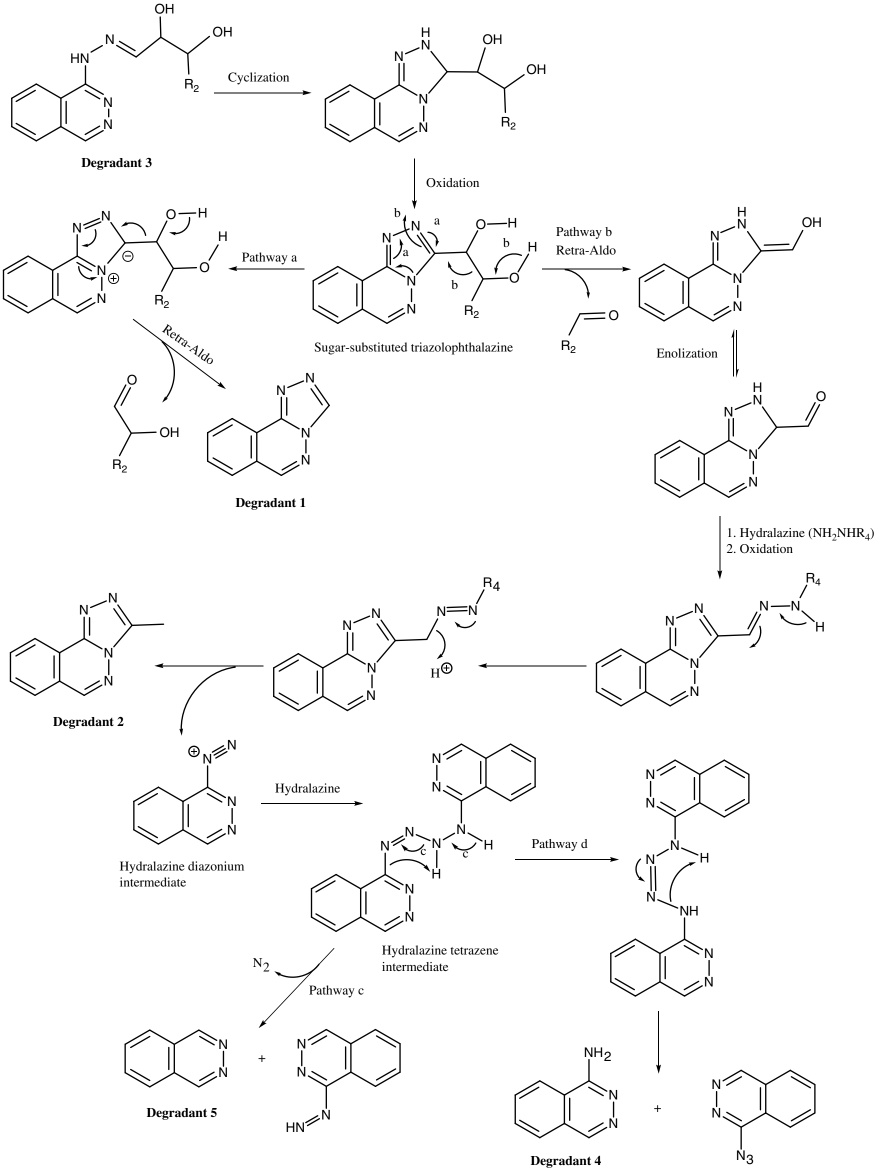


Scheme 216-2 Degradation of hydralazine due to the presence of lactose. *Source:* Adapted from Dong et al. [8].

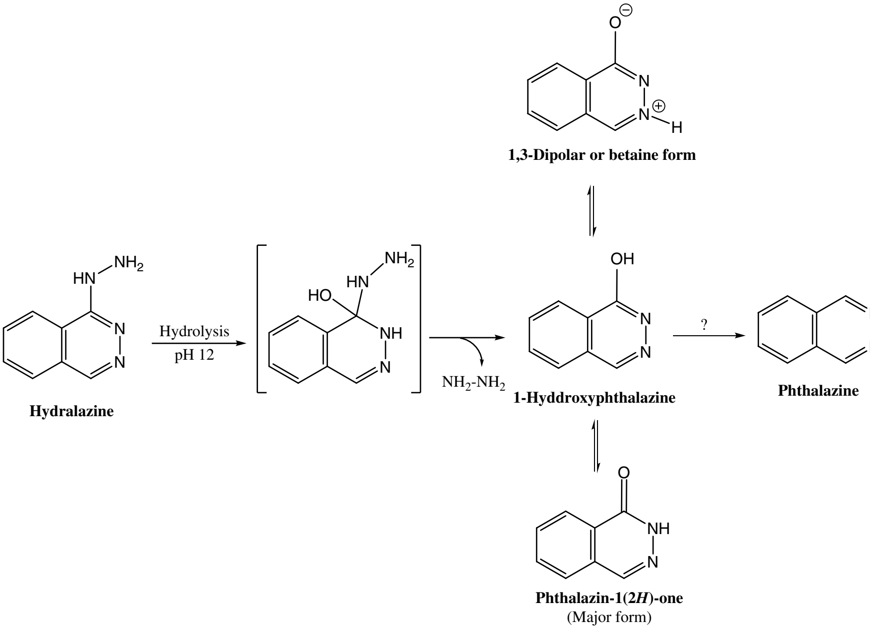
In a forced degradation study of hydralazine tablets, Dong et al. found four hydralazine degradants formed due to the presence of lactose, an excipient of the formulation [8]. The structures of the degradants were proposed based on LC-MS analysis as well as prior knowledge of hydralazine degradation (Scheme 216-2). However, Dong et al. did not propose probable formation mechanisms for these degradants, and thus, the present author attempted to fill this gap as outlined in Scheme 216-3.

For the formation of Degradant 1, Lesson and Zhao proposed a hydrolytic dissociation mechanism, which was supported by their experiment of converting the sugar-substituted degradant (**IIIa**, Scheme 215-1) into Degradant **IIIb** (same as Degradant 1 in Scheme 215-2) in acidic solution [7]. Nevertheless, in solid phase, it would also be possible for the sugar-substituted degradants, such as Degradants **IIIa**, **IIIb**, and sugar-substituted triazolophthalazine, to undergo retro-aldol reaction to produce not only Degradant 1 but also Degradant 2 (Scheme 215-3). Furthermore, for the formation of Degradant 2, it would be difficult to explain by a hydrolytic process.

Halasi and Nairn studied the hydrolysis kinetics of hydralazine in pH 1–12 at temperatures of 35°C, 50°C, and 70°C and determined that hydralazine has maximum stability near pH 3.5, and its activation energy for hydrolysis at this pH was determined to be 17.9 kcal/mol [9]. In theory, the hydrolysis of hydralazine produces hydrazine [10], which appears to be a common pathway for drugs containing the hydrazine moiety in liquid formulations. In such a hydrolytic mechanism, 1-hydroxyphthalazine would be expected to be the other degradant, rather than phthalazine itself, which is, nevertheless, contradictory to the observation that phthalazine was formed after hydralazine was stressed in pH 12 alkaline solutions (Scheme 216-4). 1-Hydroxyphthalazine can be present in three tautomeric forms, and its synthesis was reported as early as 1893 [11].



Scheme 216-3 Mechanisms for the formation of hydralazine degradants were proposed by the author of this book; the degradants were observed in the forced degradation study of hydralazine tablets by Dong et al. [8]. *Source:* Adapted from Dong et al. [8].



Scheme 216-4 Hydrolytic degradation of hydralazine in pH 12 solutions.

References

1. Israili, Z.H. and Dayton, P.G. (1977). Metabolism of hydralazine. *Drug Metab. Rev.* 6: 283–305.
2. Hydralazine. https://en.wikipedia.org/wiki/Hydralazine (accessed 2 September 2023).
3. Reidenberg, M.M., Drayer, D., deMarco, A.L., and Bello, C.T. (1973). Hydralazine elimination in man. *Clin. Pharmacol. Ther.* 14: 970–977.
4. Barbeau, D. L. (2010). U.S. Patent No. 7,728,133. Washington, DC: U.S. Patent and Trademark Office.
5. Jačić, J.K., Bajuk-Bogdanović, D., Savić, S. et al. (2023). Coordination of hydralazine with Cu^{2+} at acidic pH promotes its oxidative degradation at neutral pH. *J. Inorg. Biochem.* 243: 112181.
6. Noda, H., Minemoto, M., Noda, A., and Ushio, T. (1986). High performance liquid chromatographic determination of formaldehyde accomplished using hydralazine. *Chem. Pharm. Bull.* 34: 3499–3501.
7. Lesson, T. and Zhao, D.-C. (1996). Interactions between drug substances and excipients. 1. Fluorescence and HPLC studies of triazolophthalazine derivatives from hydralazine hydrochloride and starch. *J. Pharm. Sci.* 85: 326–329.
8. Dong, X., Tao, W., Liu, M. et al. (2016). Study on the related substances in hydralazine hydrochloride tablets by LC-MS/MS. *Chinese J. Pharm. Anal.* 36: 155–161. (in Chinese).

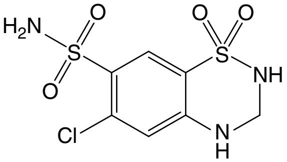
9 Halasi, S. and Nairn, J.G. (1990). Stability studies of hydralazine hydrochloride in aqueous solutions. *PDA J. Pharm. Sci. Technol.* 44: 30–34.

10 Gyllenhaal, O., Gronberg, L., and Vessman, J. (1990). Determination of hydrazine in hydralazine by capillary gas chromatography with nitrogen-selective detection after benzaldehyde derivatization. *J. Chromatogr.* 511: 303–315.

11 Dennis, N., Katritzky, A.R., and Ramaiah, M. (1974). 1.3-Dipolar character of six-membered aromatic rings. Part X. Pyridazine and benzopyridazine betaines. *J. Chem. Soc., Perkin Trans. 1* I: 1506–1514.

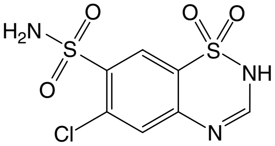
217–218: Hydrochlorothiazide, Chlorothiazide

217: Hydrochlorothiazide [WHO List of Essential Medicines]



Chemical name: 6-Chloro-3,4-dihydro-2*H*-benzo[e][1,2,4]thiadiazine-7-sulfonamide
Trade name: Hydrodiuril
Formula: $C_7H_8ClN_3O_4S_2$
Monoisotopic protonated ion (*m/z*): 297.9718
Molecular weight: 297.73
CAS number: 58-93-5

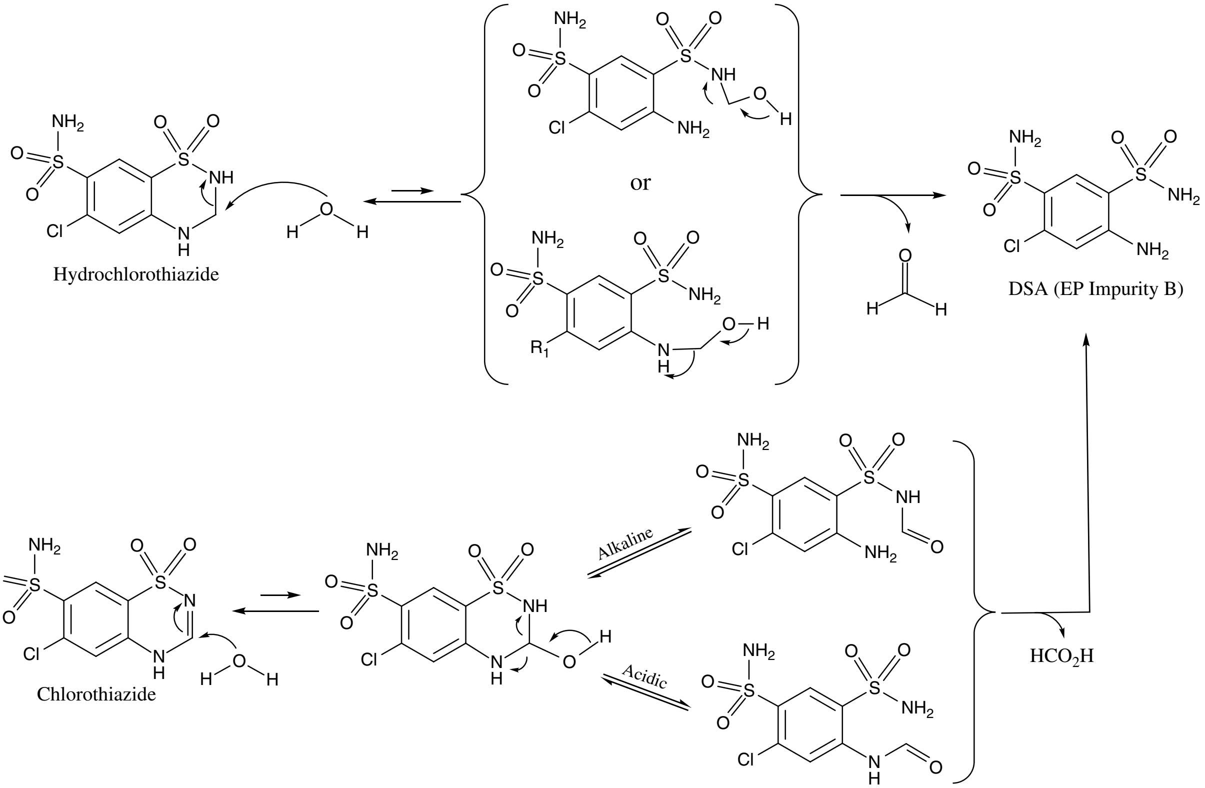
218: Chlorothiazide [WHO List of Essential Medicines]



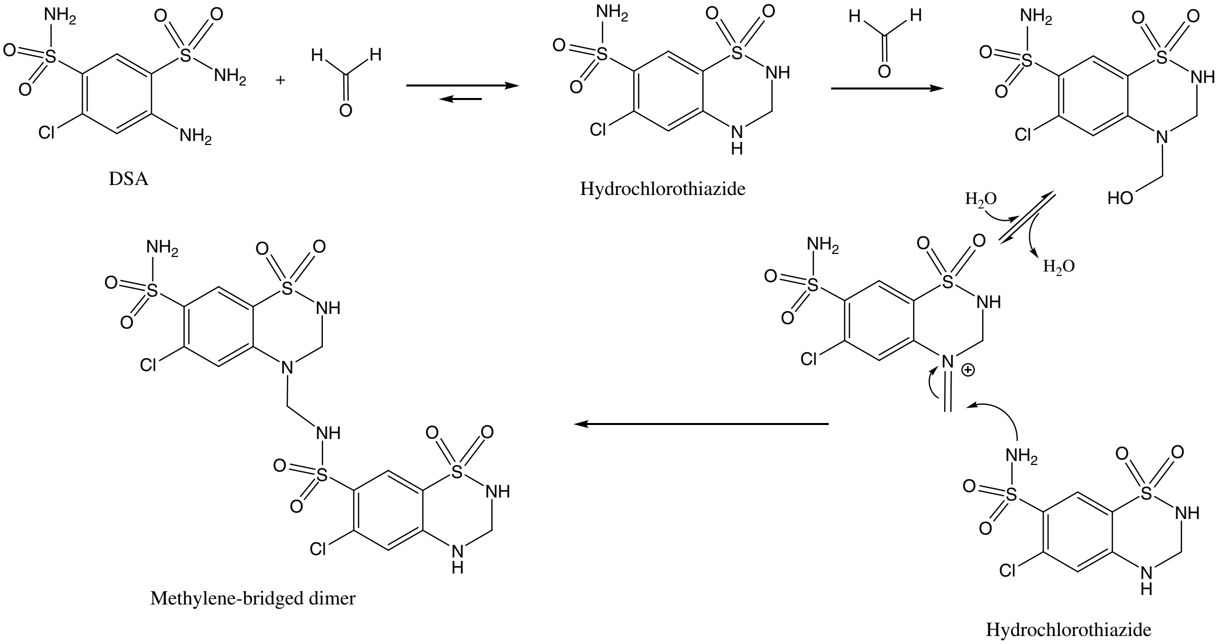
Chemical name: 6-Chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide
Trade name: Diuril
Formula: $C_7H_6ClN_3O_4S_2$
Monoisotopic protonated ion (*m/z*): 295.9561
Molecular weight: 295.71
CAS number: 58-94-6

Hydrochlorothiazide and chlorothiazide belong to the thiazide diuretic family, which are used for the treatment of hypertension, often recommended as first-line treatment, and for fluid retention conditions associated with heart failure, nephrotic syndrome, and cirrhosis [1, 2]. Both drugs were approved for clinical use in the 1950s, with chlorothiazide coming onto the market a few years earlier than hydrochlorothiazide [2, 3].

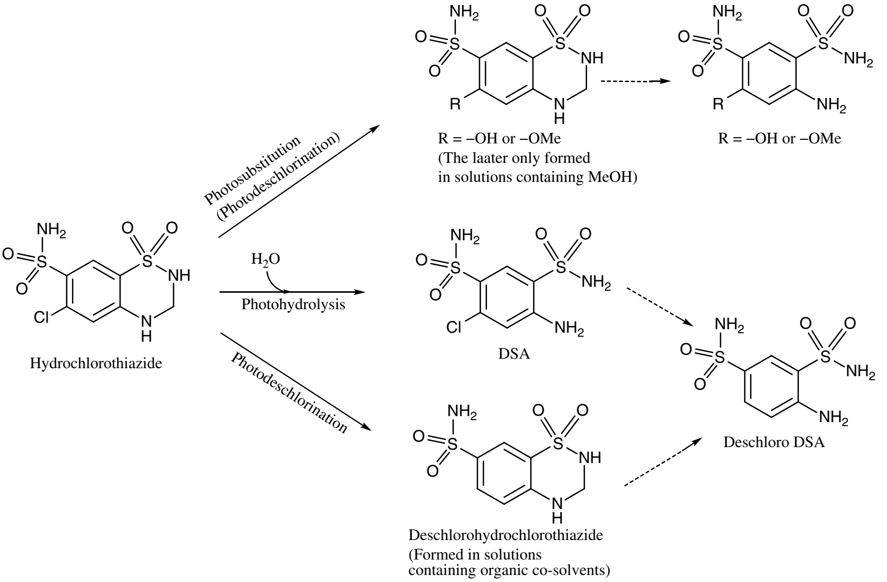
The main degradation pathways of hydrochlorothiazide and chlorothiazide are of hydrolytic and photolytic nature. The hydrolytic degradation gives rise to 4-amino-6-chloro-1,3-benzendisulfonamine (EP impurity B, also known as DSA), which is also a starting material for both hydrochlorothiazide and chlorothiazide (Scheme 217–218-1) [4]. Chlorothiazide was found to be most stable near neutral



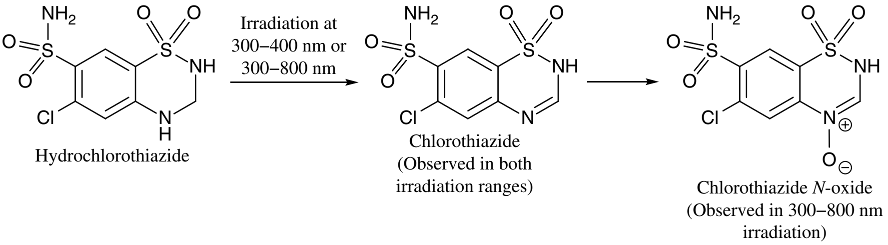
Scheme 217–218-1 Hydrolytic degradation of hydrochlorothiazide and chlorothiazide.



Scheme 217–218-2 Formation of the dimeric degradant of hydrochlorothiazide.



Scheme 217–218-3 Photosubstitution, photohydrolysis, and photodehydrogenation of hydrochlorothiazide. The dotted lines denote the secondary degradation pathways, which may occur under extended photolysis.



Scheme 217–218-4 Photooxidation of hydrochlorothiazide.

pH [5], and it seems that chlorothiazide may be hydrolytically more stable than hydrochlorothiazide [6].

Hydrochlorothiazide is synthesized by the reaction of DSA with formaldehyde, as such the excess formaldehyde can cause the formation of a methylene-bridged dimeric process impurity [7, 8]. During the hydrolytic degradation of hydrochlorothiazide, formaldehyde is also formed, which might further react with another molecule of hydrochlorothiazide to produce the same methylene-bridged dimeric impurity [7]. In such a case, the dimeric impurity would be a degradant (Scheme 217–218-2).

The photodegradation pathways of hydrochlorothiazide and chlorothiazide appear to be quite similar. The photodegradants and their distribution depend on the conditions of the photolytic degradation, which include wavelength, energy/duration, and solvents (for photodegradation in solutions). In Tamat and Moore's pioneering photodecomposition study of hydrochlorothiazide in methanolic and aqueous solutions [9], hydrochlorothiazide was found to undergo dechlorination (a photosubstitution process), hydrolysis, and reduction (Scheme 217–218-3). The same photodegradants were observed in a photodegradation study by Brigante et al., in which hydrochlorothiazide was irradiated by simulated or natural sunlight in pure water or sewage plant-treated water [10]. The most abundant photodegradant was the hydrolytic degradant, DSA, followed by the photosubstituted degradant, i.e., 6-hydroxydeschloro degradant, and the secondary photodegradant, 6-hydroxydeschloro DSA.

On the other hand, Revelle et al. reported that hydrochlorothiazide underwent photodehydrogenation (a form of photooxidation) in a methanolic solution of hydrochlorothiazide to yield chlorothiazide under irradiation of 300–400 nm [11]. Gumieniczek et al. also reported that chlorothiazide was one of the photooxidative degradants of hydrochlorothiazide, with another one being chlorothiazide *N*-oxide, after hydrochlorothiazide was subjected to UV–Vis photolysis of 300–800 nm (Scheme 217–218-4) [12].

References

1 Sica, D.A., Carter, B., Cushman, W., and Hamm, L. (2011). Thiazide and loop diuretics. *J. Clin. Hypertens.* 13: 639–643.

2 Hydrochlorothiazide. https://en.wikipedia.org/wiki/Hydrochlorothiazide (accessed 15 September 2023).

3 Chlorothiazide. https://en.wikipedia.org/wiki/Chlorothiazide (accessed 15 September 2023).

4 Mollica, J.A., Rehm, C.R., and Smith, J.B. (1969). Hydrolysis of hydrochlorothiazide. *J. Pharm. Sci.* 58: 635–636.

5 Yamana, T., Mizukami, Y., and Tsuji, A. (1968). Studies on the stability of drugs. XVII. Stability of benzothiadiazines. (8). Kinetics of acid-base catalysed hydrolysis of chlorothiazide. *Chem. Pharm. Bull.* 16: 396–404.

6 Huang, G., Smith, R.M., Albishri, H.M., and Lin, J.-M. (2010). Thermal stability of thiazide and related diuretics during superheated water chromatography. *Chromatographia* 72: 1177–1181.

7 Franolic, J.D., Lehr, G.J., Barry, T.L., and Petzinger, G. (2001). Isolation of a 2:1 hydrochlorothiazide–formaldehyde adduct impurity in hydrochlorothiazide drug substance by preparative chromatography and characterization by electrospray ionization LC–MS. *J. Pharm. Biomed. Anal.* 26: 651–663.

8 Fang, X., Bibart, R.T., Mayr, S. et al. (2001). Purification and identification of an impurity in bulk hydrochlorothiazide. *J. Pharm. Sci.* 90: 1800–1809.

9 Tamat, S.R. and Moore, D.E. (1983). Photolytic decomposition of hydrochlorothiazide. *J. Pharm. Sci.* 72: 180–183.

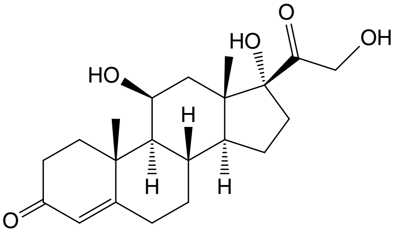
10 Brigante, M., DellaGreca, M., Previtera, L. et al. (2005). Degradation of hydrochlorothiazide in water. *Environ. Chem. Lett.* 2: 195–198.

11 Revelle, L.K., Musser, S.M., Rowe, B.J., and Feldman, I.C. (1997). Identification of chlorothiazide and hydrochlorothiazide UV-A photolytic decomposition products. *J. Pharm. Sci.* 631–634.

12 Gumieniczek, A., Galeza, J., Mroczek, T. et al. (2018). Kinetics and characterization of degradation products of dihydralazine and hydrochlorothiazide in binary mixture by HPLC-UV, LC-DAD and LC-MS methods. *Chromatographia* 81: 1147–1162.

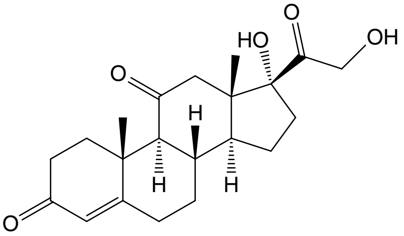
219–220: Hydrocortisone, Cortisone

219: Hydrocortisone [WHO List of Essential Medicines]



Chemical name: (8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-Dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthren-3-one
Formula: $C_{21}H_{30}O_5$
Monoisotopic protonated ion (*m/z*): 363.2166
Molecular weight: 362.47
CAS number: 50-23-7

220: Cortisone



Chemical name: (8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Hydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-1,2,6,7,8,9,12,14,15,16-decahydrocyclopenta[a]phenanthrene-3,11-dione
Formula: $C_{21}H_{28}O_5$
Monoisotopic protonated ion (*m/z*): 361.2010
Molecular weight: 360.45
CAS number: 53-06-5

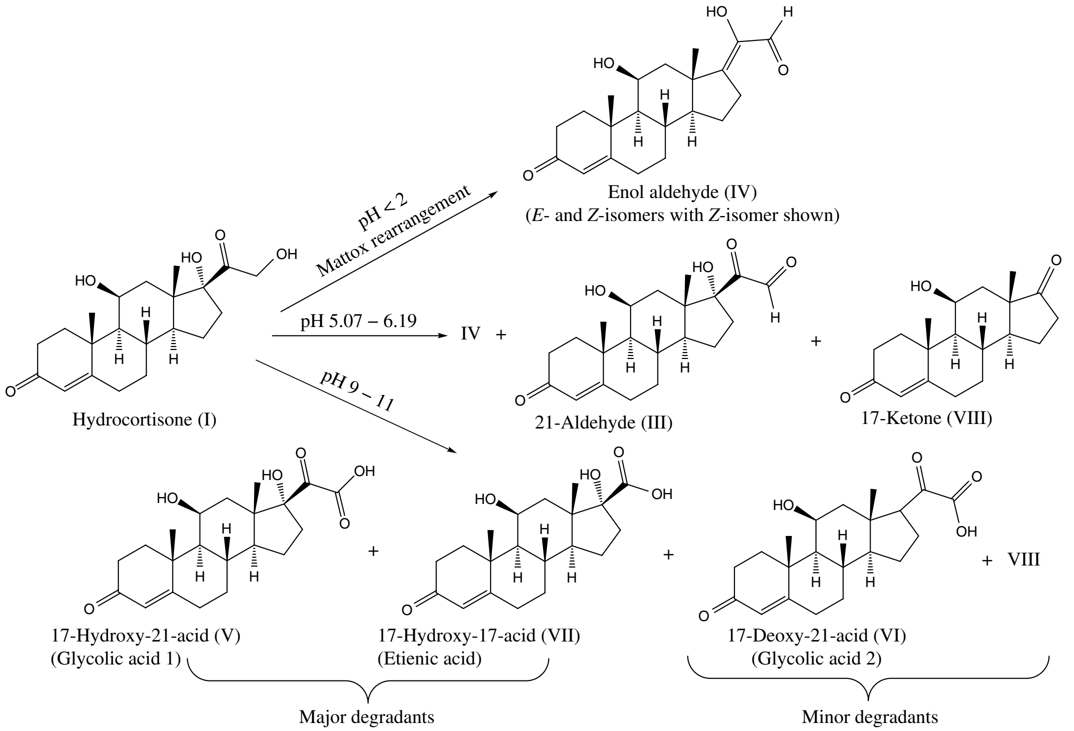
Hydrocortisone is the medicinal name for the endogenous hormone cortisol, which was patented for medicinal use in 1936 and approved in 1941. It is a glucocorticoid steroid, and its pharmacological effects are mainly anti-inflammation and immune suppression [1].

On the other hand, cortisone is an oxidative metabolite of hydrocortisone, and clinically, it is used as a prodrug for hydrocortisone, because cortisone can be converted into hydrocortisone by the enzyme 11β-hydroxysteroid dehydrogenase type 1 in the liver [2].

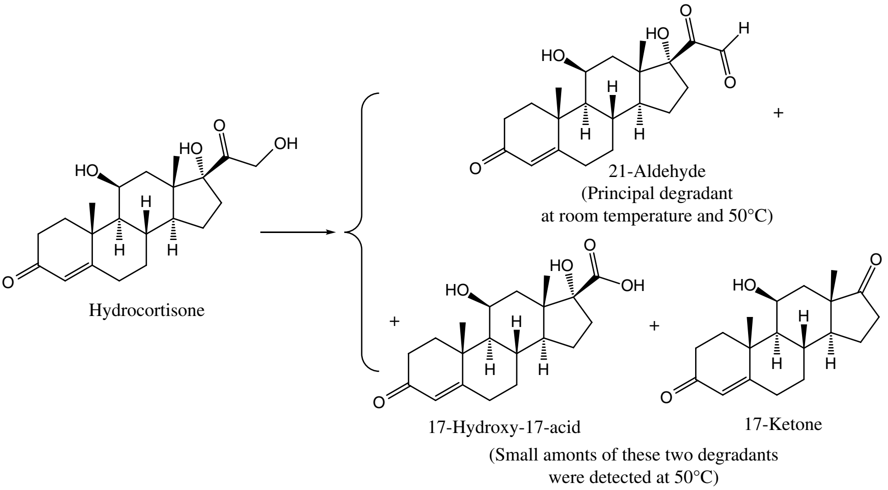
Like other structurally similar steroids, the degradation of both drugs occurs mostly on their D rings, and the types of degradation include dehydration (referred to as the Mattox rearrangement) [3], oxidation, and subsequent degradation such as intramolecular Cannizzaro rearrangement [4]. Hansen and Bundgaard studied hydrocortisone stability in aqueous solutions of different pHs [5]. In strongly acidic solutions, hydrocortisone underwent dehydration to form two enol aldehyde degradants, a process referred to as the Mattox rearrangement (Scheme 219–220-1). In weakly acidic to near-neutral solutions, hydrocortisone also degraded to oxidative degradants, 21-aldehyde (also called 21-dehydrohydrocortisone) and 17-keto degradants, in addition to the enol aldehyde degradants. In alkaline solutions, the degradation appeared more complicated: the major degradants were 17-hydroxy-21-acid (glycolic acid 1) and 17-hydroxy-17-acid (etienic acid), both of which seemed to stem from further oxidative degradation of 21-aldehyde. The minor degradants observed in the alkaline solutions were 17-deoxy-21-acid and 17-ketone, both of which would appear to result from further degradation of the enol aldehyde degradants according to more recent studies [4, 6].

Timmins and Gary studied hydrocortisone degradation behavior in zinc oxide lotion and found that the degradation was oxidative: the principal degradant was 21-aldehyde, and the minor degradants were 17-hydroxy-17-acid and 17-ketone (Scheme 219–220-2) [7]. The results of this study are consistent with those of Hansen and Bundgaard [5]; the nominal pH of the zinc oxide lotion would be somewhat alkaline.

The 11-hydroxyl group of hydrocortisone is usually quite stable and resistant toward oxidation. Nevertheless, it was reported that in certain crystal forms of hydrocortisone 17-esters, photooxidation of 11-hydroxyl into the corresponding 11-keto was observed (Scheme 219–220-3) [7].



Scheme 219–220-1 Degradation pathways of hydrocortisone in aqueous solutions of different pH [5]. The oxidative degradant, 21-aldehyde (III), is also referred to as 21-dehydrohydrocortisone. The numbering of the compounds follows that of the authors [5]. *Source:* Hansen and Bundgaard [5]/with permission of Elsevier.



Hydrocortisone

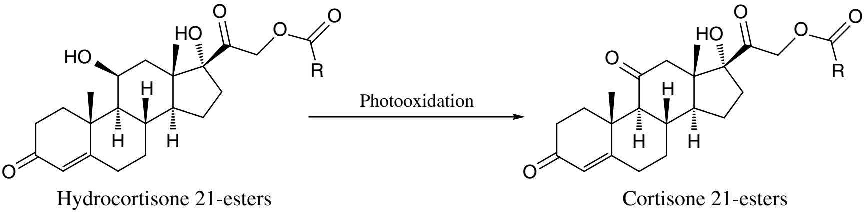
21-Aldehyde
(Principal degradant
at room temperature and 50°C)

17-Hydroxy-17-acid

17-Ketone

(Small amonts of these two degradants
were detected at 50°C)

Scheme 219–220-2 Degradation pathways of hydrocortisone in a zinc oxide lotion [7]. *Source:* Timmins and Gary [7]/John Wiley & Sons.



Hydrocortisone 21-esters

Cortisone 21-esters

Scheme 219–220-3 Photooxidation of the 11-hydroxyl group of certain crystal forms of hydrocortisone 17-esters in solid state. *Source:* Byrn and Kessler [8]/with permission of Elsevier.

References

1 Hydrocortisone. https://en.wikipedia.org/wiki/Hydrocortisone (accessed 22 November 2023).

2 Cortisone. https://en.wikipedia.org/wiki/Cortisone (accessed 22 November 2023).

3 Mattox, V.R. (1952). Steroids derived from bile acids. XV. The formation of a glyoxal side chain at C-17 from steroids with dihydroxyacetone and Δ16-ketol side chains. *J. Am. Chem. Soc.* 74: 4340–4347.

4 Li, M., Chen, B., Monteiro, S., and Rustum, A.M. (2009). Mechanism of base-catalyzed autooxidation of corticosteroids containing 20-keto-21-hydroxyl side chain. *Tetrahedron Lett.* 50: 4575–4581.

5 Hansen, J. and Bundgaard, H. (1980). Studies on the stability of corticosteroids V. The degradation pattern of hydrocortisone in aqueous solution. *Int. J. Pharm.* 6: 307–319.

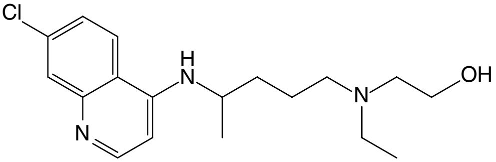
6 Li, M., Wang, X., Chen, B. et al. (2009). Forced degradation of betamethasone sodium phosphate under solid state: formation, characterization, and mechanistic study of all four 17,20-diastereomers of betamethasone 17-deoxy-20-hydroxy-21-oic acid. *J. Pharm. Sci.* 98: 894–904.

7 Timmins, P. and Gary, E.A. (1983). Degradation of hydrocortisone in zinc oxide lotion. *J. Clin. Hosp. Pharm.* 8: 79–85.

8 Byrn, S.R. and Kessler, D.W. (1987). The solid state reactivity of the crystal forms of hydrocortisone esters. *Tetrahedron* 43: 1335–1343.

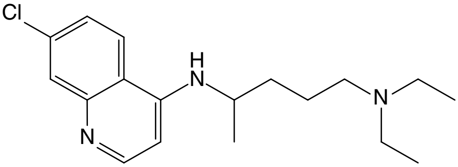
221–222: Hydroxychloroquine, Chloroquine

221: Hydroxychloroquine [WHO List of Essential Medicines]



Chemical name: 2-[4-[(7-Chloroquinolin-4-yl)amino]pentyl-ethylamino]ethanol
Trade name: Plaquenil, Sovuna
Formula: $C_{18}H_{26}ClN_3O$
Monoisotopic protonated ion (*m/z*): 336.1837
Molecular weight: 335.88
CAS number: 118-42-3
Common salt form: Sulfate

222: Chloroquine [WHO List of Essential Medicines]



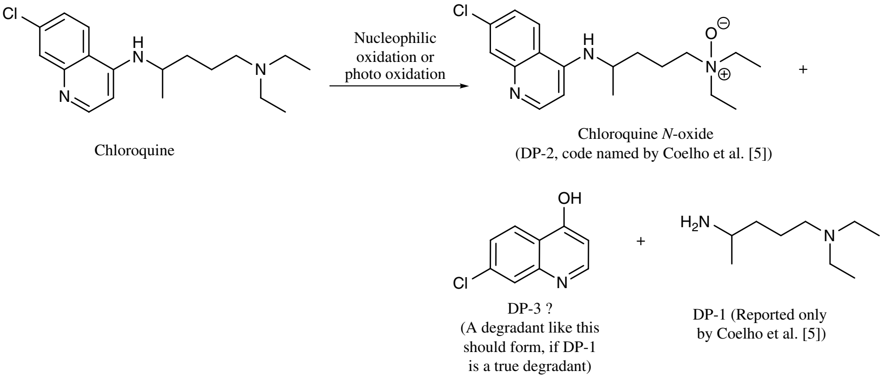
Chemical name: N^4-(7-Chloroquinolin-4-yl)-N^1,N^1-diethylpentane-1,4-diamine
Formula: $C_{18}H_{26}ClN_3$
Monoisotopic protonated ion (*m/z*): 320.1888
Molecular weight: 319.88
CAS number: 54-05-7
Common salt form: HCl salt, Sulfate, Phosphate

Hydroxychloroquine and chloroquine are primarily used for preventing and treating malaria in cases where the particular malaria strains have not developed resistance toward these two drugs. Chloroquine was discovered by Hans Andersag and coworkers at Bayer Laboratories in 1934, followed up on the lead by quinine, the natural product isolated from the bark of a cinchona tree [1]. Hydroxychloroquine was subsequently discovered among the chloroquine analogs [2]. In addition to their use as antimalarial agents, both drugs are utilized for autoimmune diseases such as rheumatoid arthritis and lupus [3].

Both drug molecules have the same moieties that are susceptible to degradation, which are the tertiary amine and chloroquinoline functional groups. The former moiety can be oxidized into the corresponding *N*-oxide under either nucleophilic oxidative or photooxidative conditions [4–6]. For example, Doddaga and Peddakonda reported chloroquine *N*-oxide as the major oxidative degradant of chloroquine [4], while Coelho et al. also identified chloroquine *N*-oxide (code name DP-2) as one of the major oxidative degradants of chloroquine (Scheme 221–222-1) [5]. In addition, Coelho et al. also reported another degradant, code name DP-1, in their oxidative stress of hydroxychloroquine in 3% hydrogen peroxide solution. Likewise, hydroxychloroquine can undergo the same oxidative degradation to give hydroxychloroquine *N*-oxide [6].

It needs to be pointed out that the structure of DP-1 was proposed solely based on mass spectrometric data. If DP-1 is a true degradant, it should be formed via cleavage off the chloroquinoline core, thus causing the simultaneous formation of another degradant that should bear the remaining chloroquinoline core, e.g., a degradant such as DP-3. Nevertheless, no additional degradant was observed in the LC-MS analysis presented by the authors. On the other hand, in the HPLC-UV chromatogram of the forced oxidative degradation solution, DP-1 showed up as a strong peak at the UV detection wavelength of 250 nm, which is contradictory to the fact that DP-1 should have essentially no UV absorption due to its lack of UV chromophore. These data presented by the authors cast considerable doubt on the validity of DP-1.

On the other hand, the chloroquinoline moiety can be susceptible to photochemical degradation. In 1982, Moore and Hemmens investigated photosensitization of a number of antimalarial drugs, including chloroquine and hydroxychloroquine. Under UV irradiation at 365 nm, chloroquine was found to undergo significant dechlorination, particularly with increasing pH; the degradation products resulting from the dechlorination were not studied. Nevertheless, it can be reasonably assumed that the hydroxylated deschloro degradant should be among the major photodegradants (Scheme 221–222-2) [7]. In the photodegradation study of chloroquine in solutions of pH 5.0, 7.0, and 8.0 performed by Karim et al. [8], it was found



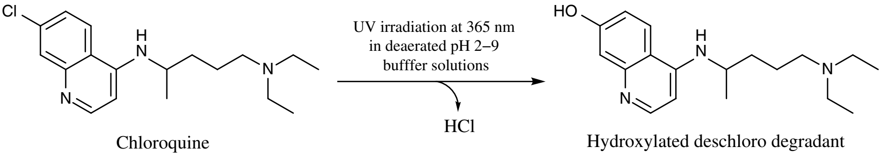
Scheme 221–222-1 Oxidative degradation of chloroquine. The validity of DP-1 appears to be questionable based on the data presented by Coelho et al. [5], who also stated "... it is necessary [to perform] complimentary studies to confirm the proposed structure." in their paper. *Source:* Coelho et al. [5]/with permission of Elsevier.

that chloroquine degraded the fastest at pH 8.0, and desethylchloroquine (II, Scheme 221–222-3) was identified as one of the degradants.

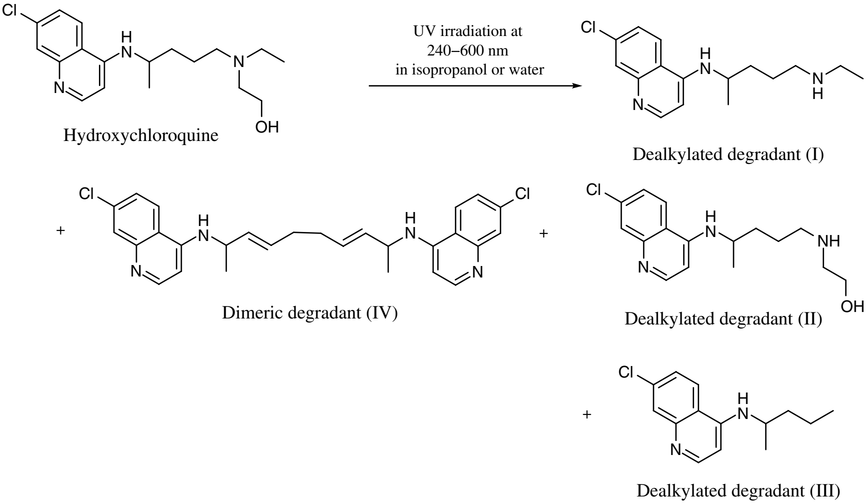
In 1988, Tonnesen et al. studied photolysis of hydroxychloroquine in neutral base form in either isopropanol or water solution with irradiation at 240–600 nm [9]. Three dealkylated degradants (I–III) and one dimeric degradant (IV) were identified based on mass spectrometric data and NMR measurement (Scheme 221–222-3).

In 2013, Saini and Bansal found that in alkaline solutions (acetonitrile/0.1 M NaOH; 2/3, v/v), hydroxychloroquine degraded into six degradants, five of which were structurally elucidated on the basis of mass spectrometric analysis (Scheme 221–222-4) [10]. One of the five degradants, desethyl hydroxychloroquine (3), was previously reported [11], while the other four degradants had not been reported according to the authors.

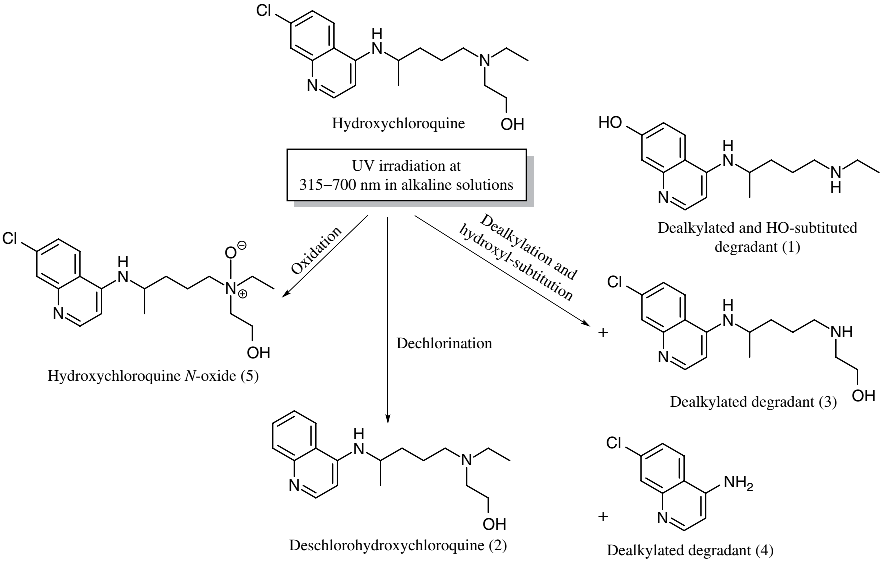
By combining the above photochemical degradation results, it is clearly that photochemical dealkylation can occur at both the tertiary amine and secondary amine sites.



Scheme 221–222-2 Photodechlorination of chloroquine in pH 2–9 buffer solutions.



Scheme 221–222-3 Photolysis of hydroxychloroquine in either isopropanol or water solution with irradiation of 240–600 nm.



Scheme 221–222-4 Photolysis of hydroxychloroquine in alkaline solutions (acetonitrile/0.1 M NaOH, v/v) with irradiation of 315–700 nm.

References

1 Hydroxychloroquine. https://en.wikipedia.org/wiki/Hydroxychloroquine (accessed 11 November 2023).

2 Chloroquine. https://en.wikipedia.org/wiki/Chloroquine (accessed 11 November 2023).

3 Ben-Zvi, I., Kivity, S., Langevitz, P., and Shoenfeld, Y. (2012). Hydroxychloroquine: from malaria to autoimmunity. *Clin. Rev. Allergy Immunol.* 42: 145–153.

4 Doddaga, S. and Peddakonda, R. (2011). Chloroquine-N-oxide, a major oxidative degradation product of chloroquine: identification, synthesis and characterization. *J. Pharm. Biomed. Anal.* 81–82: 118–125.

5 Coelho, A.S., Chagas, C.E.P., de Padua, R.M. et al. (2017). A comprehensive stability-indicating HPLC method for determination of chloroquine in active pharmaceutical ingredient and tablets: identification of oxidation impurities. *J. Pharm. Biomed. Anal.* 145: 248–254.

6 Dongala, T., Katari, N.K., Palakurthi, A.K. et al. (2020). Stability indicating LC method development for hydroxychloroquine sulfate impurities as available for treatment of COVID-19 and evaluation of risk assessment prior to method validation by quality by design approach. *Chromatographia* 83: 1269–1281.

7 Moore, D.E. and Hemmens, V. (1982). Photosensitization by antimalarial drugs. *Photochem. Photobiol.* 36: 71–77.

8 Karim, E.I.A., Ibrahim, K.E.E., Abdelrahman, A.N., and Fell, A.F. (1994). Photodegradation studies on chloroquine phosphate by high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* 12: 667–674.

9 Tonnesen, H.H., Grislingaas, A.-L., Woo, S.O., and Karlsen, J. (1988). Photochemical stability of antimalarials. I. Hydroxychloroquine. *Int. J. Pharm.* 43: 215–219.

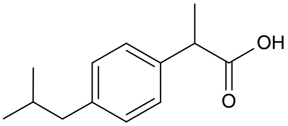
10 Saini, B. and Bansal, G. (2013). Characterization of four new photodegradation products of hydroxychloroquine through LC-PDA, ESI-MS^n and LC-MS-TOF studies. *J. Pharm. Biomed. Anal.* 84: 224–231.

11 Dongre, V.G., Ghugare, P.D., Karmuse, P. et al. (2009). Identification and characterization of process related impurities in chloroquine and hydroxychloroquine by LC/IT/MS, LC/TOF/MS and NMR. *J. Pharm. Biomed. Anal.* 49: 873–879.

7

Ibuprofen to Nitrofurantoin

223: Ibuprofen



Chemical name: (*R*,*S*)-2-(4-(2-Methylpropyl)phenyl)propanoic acid
Trade name: Motrin, others
Formula: $C_{13}H_{18}O_2$
Monoisotopic protonated ion (*m/z*): 207.1380
Molecular weight: 206.29
CAS number: 154598-52-4

Ibuprofen is one of the most widely used nonsteroidal anti-inflammatory drugs (NSAIDs) and its production is nearly 15,000 tons per year [1]. It is clinically employed as an anti-inflammatory, analgesic, and antipyretic agent. It was discovered by Stewart Adams and John Nicholson at Boots Pure Drug Co., Ltd., in the United Kingdom during the 1950s [2]. Ibuprofen received the first market approval in the United Kingdom in 1969 as a prescription drug and became an over-the-counter (OTC) drug in the 1980s. It inhibits the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [3]. Ibuprofen is available in many formulation types, including tablet, hard shell capsule, and soft gel capsule.

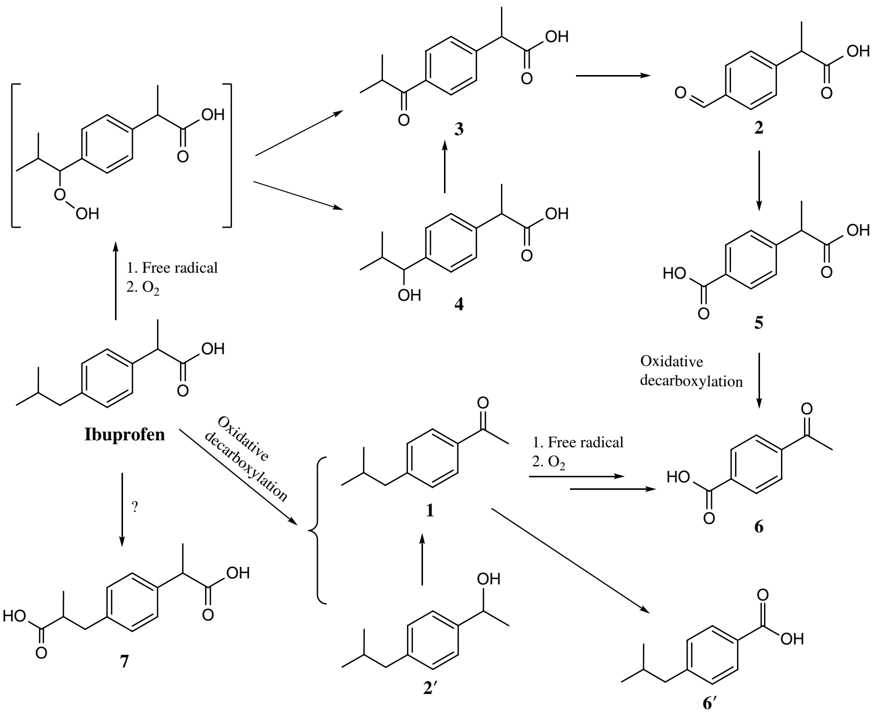
Ibuprofen is a 2-aryl-substituted propionic acid, which has two benzylic positions that could be susceptible to free-radical-mediated oxidative degradation. Thus, its main degradation pathway should be of oxidative nature [4]. Indeed, its major oxidative degradants were reported to be 4-isobutylacetophenone (**1**), 2-(4-formylphenyl)propionic acid (**2**), 2-(4-isobutyrylphenyl)propionic acid (**3**), and 2-(4-(1-hydroxy-2-methylpropyl)phenyl)propanoic acid (**4**) [5, 6]. In an accelerated stress study of ibuprofen tablet batches from different manufacturers under 70°C/75%RH, additional oxidative degradants (**5**–**7**) were also observed in some batches. For those batches that experienced significant degradation, they all contained polyethylene glycols (PEGs), an excipient family known to have varying

Compendium of Drug Degradation Pathways, First Edition. Min Li.

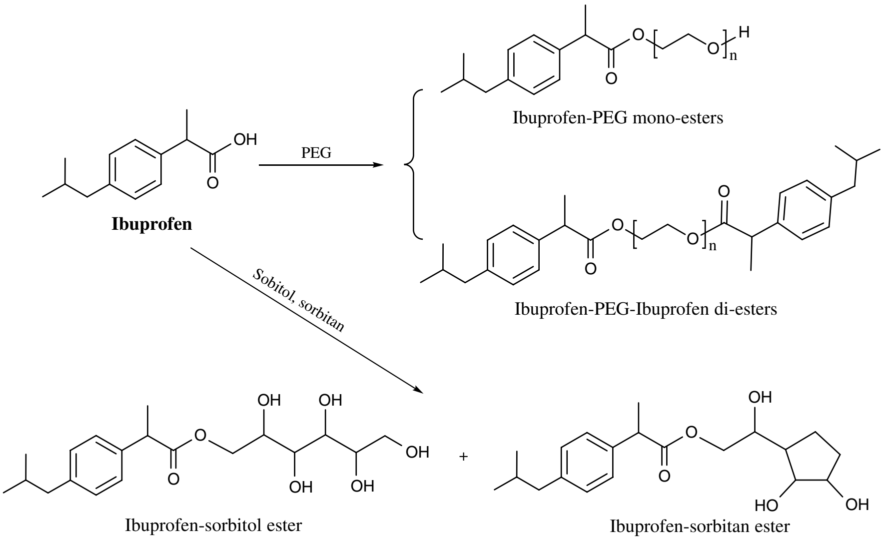
degrees of hydroperoxide impurities resulting from its rather facile autooxidation [7]. In the batch that showed the most degradation also contains polysorbate 80, another excipient that undergoes autooxidation easily. These results are consistent with a most likely scenario that ibuprofen underwent free-radical-mediated oxidative degradation within these formulated formats; a probable degradation mechanism is shown in Scheme 223-1, with some inputs based on the study of Caviglioli et al. [8]

Degradants **2**–**5** were formed due to the oxidation on the benzylic position of the 2-methylpropyl substituent, degradants **1**, **2′**, and **6′** were produced due to the oxidation on the benzylic position of the propionic moiety, while degradant **6** was generated due to the oxidation on both benzylic positions. The formation of 7 appears rather unusual; on the other hand, its structure was merely inferred from mass spectrometric data [8].

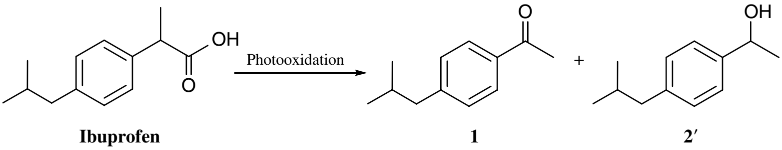
In a long-term stability study of ibuprofen soft gel capsules, four degradants were formed via esterification of the API with the excipients PEG, sorbitol, and sorbitan (Scheme 223-2) [9]. PEG was used as a filler of the soft gel, while sorbitol and sorbitan served as plasticizers for the shell. The same or similar esterification degradants were observed in a forced degradation study of ibuprofen soft gel capsules [10].



Scheme 223-1 Probable oxidative degradation mechanism of ibuprofen, with some inputs based on the study of Caviglioli et al. [8]. *Source:* Caviglioli et al. [8] / with permission of Elsevier.



Scheme 223-2 Probable formation mechanism of ibuprofen esters in soft gel capsules containing PEG as the capsule fill and sorbitol and sorbitan as plasticizers for the shell.



Scheme 223-3 Photooxidation of ibuprofen. Degradant 1 and 2′ were the main degradants in solutions, while 1 was observed as the degradant in the solid state under irradiation by natural sunlight.

Although ibuprofen appeared stable photochemically in the solid state, it is photolabile in solutions. The main photodegradants were reported to be **1** and **2′** (Scheme 223-3) [11, 12]. On the other hand, Farmer et al. showed ibuprofen underwent significant degradation in the solid state under natural sunlight, and the main degradant was **1** [13].

References

1 Myers, R.L. (2007). *The 100 Most Important Chemical Compounds: A Reference Guide*. New York: Bloomsbury Publishing.

2 Varrassi, G., Pergolizzi, J.V., Dowling, P., and Paladini, A. (2020). Ibuprofen safety at the golden anniversary: are all NSAIDs the same? A narrative review. *Adv. Ther.* 37: 61–82. https://doi.org/10.1007/s12325-019-01144-9.

3 Rainsford, K.D. (2009). Ibuprofen: pharmacology, efficacy and safety. *Inflammopharm.* 17: 275–342.

4 Dondoni, A., Dall'Occo, T., Fantin, G. et al. (1986). Studies on the actual and potential impurities in ibuprofen. *Farmaco. Prat.* 41: 237–244.

5 Asmus, P.A. (1985). Determination of 2-(4-isobutylphenyl)propionic acid in bulk and compressed tablets by reversed-phase high performance liquid chromatography. *J. Chromat.* 331: 169–176.

6 Cory, W.C., Harris, C., and Martinez, S. (2010). Accelerated degradation of ibuprofen in tablets. *Pharm. Dev. Tech.* 15: 636–643.

7 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*. Cambridge, UK: RSC Publishing.

8 Caviglioli, G., Valeria, P., Brunella, P. et al. (2002). Identification of degradation products of ibuprofen arising from oxidative and thermal treatments. *J. Pharm. Biomed. Anal.* 30: 499–509.

9 Dousa, M., Meca, L., Gibala, P. et al. (2017). Esterification of ibuprofen in soft gelatin capsules formulations: identification, synthesis and liquid chromatography separation of the degradation products. *J. Chromat. Sci.* 1–8: https://doi.org/10.1093/chromsci/bmx036.

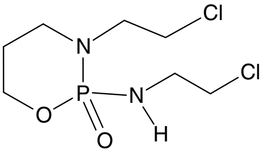
10 Farias, F.F., Martins, V.A.P., Dorr, F.A. et al. (2021). Forced degradation study and characterization of main impurities of ibuprofen soft gelatin capsules by LC–MS-QTOF. *Pharmazie* 76: 138–144.

11 Castell, J.V., Gomez, M.J., Miranda, M.A., and Morera, I.M. (1987). Photolytic degradation of ibuprofen. Toxicity of the isolated photoproducts on fibroblasts and erythrocytes. *Photochem. Photobiol.* 46: 991–996.

12 Iovino, P., Chianese, S., Canzano, S. et al. (2016). Ibuprofen photodegradation in aqueous solutions. *Environ. Sci. Pollut. Res.* https://doi.org/10.1007/s11356-016-7339-0.

13 Farmer, S., Anderson, P., Burns, P., and Velagaleti, R. (2002). Forced degradation of ibuprofen in bulk drug and tablets and determination of specificity, selectivity, and the stability-indicating nature of the USP ibuprofen assay method. *Pharm. Tech.* 28–42. https://www.pharmtech.com.

224: Ifosfamide [WHO List of Essential Medicines]

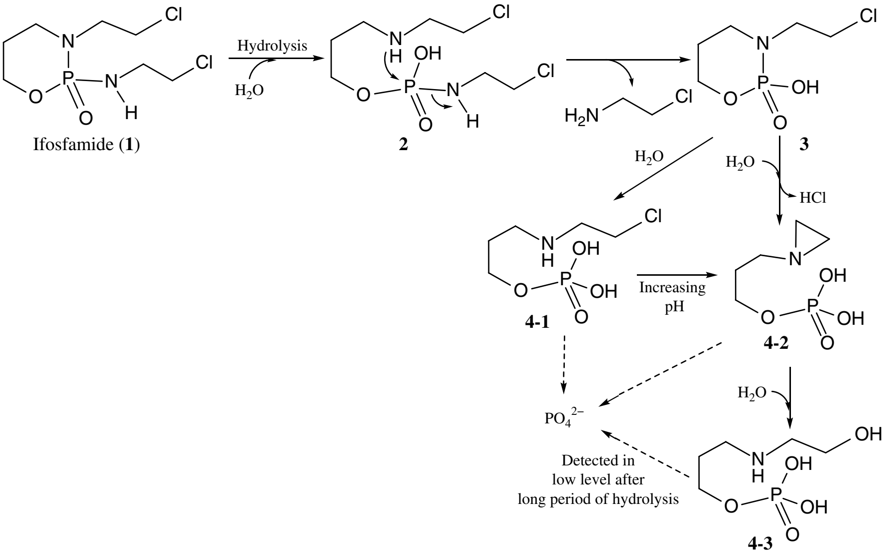


Chemical name: *N*,3-Bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amide 2-oxide
Trade name: Ifex, others
Formula: $C_7H_{15}Cl_2N_2O_2P$
Monoisotopic protonated ion (*m/z*): 261.0321
Molecular weight: 261.08
CAS number: 3778-3773-2

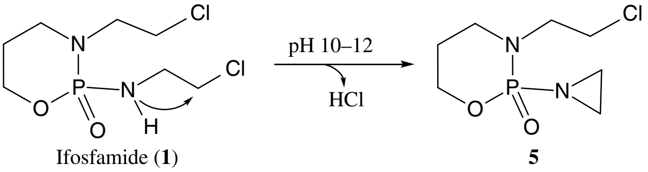
Ifosfamide is an oxazaphosphorine anticancer alkylating agent, which is isomeric to cyclophosphamide. It came onto the US market in 1987 [1], which is much later than cyclophosphamide. Like cyclophosphamide, ifosfamide is also a prodrug and differs from cyclophosphamide in that its two chloroethyl groups are attached to the endocyclic and exocyclic nitrogen atoms, respectively, as opposed to both attaching to the same exocyclic nitrogen atom as in the case of cyclophosphamide. Its degradation pathway is essentially hydrolysis, which is also the case for cyclophosphamide, although their degradation products and distributions may be different. The two P–N bonds are more labile toward hydrolysis than the P–O bond. According to the study by Gilard et al., the first step of the hydrolysis started at the endocyclic P–N bond, giving rise to an open-chain phosphoramidic acid monoester (**2**, Scheme 224-1)[2]. Degradant **2** then underwent an intramolecular cyclization to produce the 2-hydroxyoxazaphosphorine degradant (**3**). Last, the endocyclic P–N bond of **3** was hydrolyzed to yield the three phosphoric acid monoesters (**4**). The phosphoric ester linkage is usually more stable than the corresponding phosphoric amide linkage, as evidenced by the fact that the hydrolytic activation energies of phosphoric esters are usually higher than those of phosphoric amides [3]. Thus, it would not be surprising to find that the free-phosphate anion, PO_4^{2-}, was only present at low levels, even after a long period of hydrolysis [2].

Before the aforementioned study of Gilard et al., some studies on the stability of ifosfamide were performed, with the first one reported by Zon et al. [4]. Nevertheless, these studies appeared fragmented, and some of the results may be erroneous [5] because the derivatization method used in the analysis might cause sample degradation, as demonstrated by Gilard et al. [6]. In strongly alkaline conditions, the main degradant was reported to be the aziridine degradant (**5**, Scheme 224-2) [7, 8].

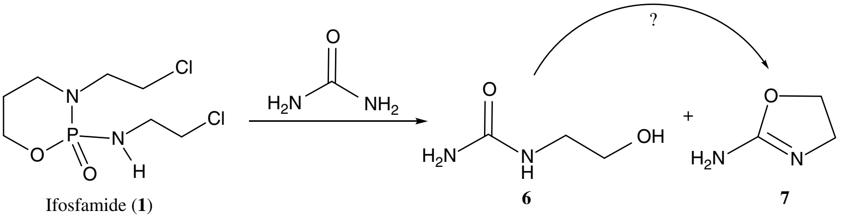
Mufioz et al. studied the degradation kinetics of ifosfamide in aqueous solutions of pH 3.6–9.8 and found that the degradation followed first-order kinetics and the activation energy for the degradation was 118 kJ/mol at pH 5.0 [9]. Furthermore,



Scheme 224-1 Hydrolytic degradation pathway of ifosfamide in acidic to neutral solutions.



Scheme 224-2 Degradation of ifosfamide in solutions of pH 10–12.



Scheme 224-3 Degradation of ifosfamide in the presence of urea.

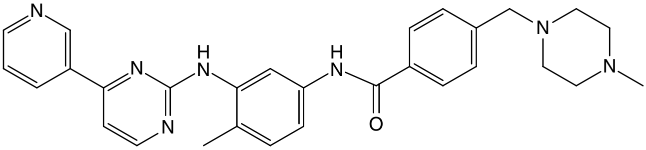
ifosfamide was found to be most stable between pH 4 and 7.9; this result was largely consistent with a previous study in which ifosfamide was found to be most stable in the pH region of 4–9 [10].

In a urea-containing formulation of ifosfamide, two degradants were found to result from the interaction between the API and urea (Scheme 224-3) [11].

References

1. Ifosfamide. https://en.wikipedia.org/wiki/Ifosfamide (accessed September 18 2023).
2. Gilard, V., Martino, R., Malet-Martino, M. et al. (1999). Chemical stability and fate of the cytostatic drug ifosfamide and its *N*-dechloroethylated metabolites in acidic aqueous solutions. *J. Med. Chem.* 42: 2542–2560.
3. Li, M. (2012). Hydrolytic degradation. In: *Organic Chemistry of Drug Degradation*, 16–47. Cambridge: Royal Society Chemistry Publishing.
4. Zon, G., Ludeman, S.M., and Egan, W. (1977). High-resolution nuclear magnetic resonance investigations of the chemical stability of cyclophosphamide and related phosphoramidic compounds. *J. Am. Chem. Soc.* 99: 5785–5795.
5. Highley, M.S., Momerency, G., Van Cauwenberghe, K. et al. (1995). Formation of chloroethylamine and 1,3-oxazolidine-2-one following ifosfamide administration in humans. *Drug Metab. Dispos.* 23: 433–437.
6. Gilard, V., Martino, R., Malet-Martino, M., and Niemeyer, U. (1997). Stability of commercial formulations and aqueous solutions of ifosfamide. *Drug Metab. Dispo.* 25: 927–931.
7. Kaijser, G.P., Beijnen, J.H., Bult, A. et al. (1991). A systematic study on the chemical stability of ifosfamide. *J. Pharm. Biomed. Anal.* 9: 1061–1067.
8. Kaijser, G.P., Aalbers, T., Beijnen, J.H. et al. (1996). Chemical stability of cyclophosphamide, trofosfamide, and 2- and 3-dechloroethylifosfamide in aqueous solutions. *J. Oncol. Pharm. Pract.* 2: 15–21.
9. Mufioz, M., Bonjoch, J., Prat, J. et al. (1996). Degradation kinetics of ifosfamide in aqueous solution. *Inter. J. Pharm.* 139: 249–253.
10. Kaijser, G.P., Beijnen, J.H., Bult, A. et al. (1991). A systematic study on the chemical stability of Ifosfamide. *J. Pharm. Biomed. Anal.* 9: 1061–1067.
11. Yüce, I. and Morlock, G.E. (2018). All on one high-performance thin-layer chromatographyplate: solvent-free nanomole-scaled on-surface synthesis, workup and online high-resolution mass spectrometry for elucidation of two new degradation products in anifosfamide formulation. *J. Chromat. A* 1 (572): 145–151.

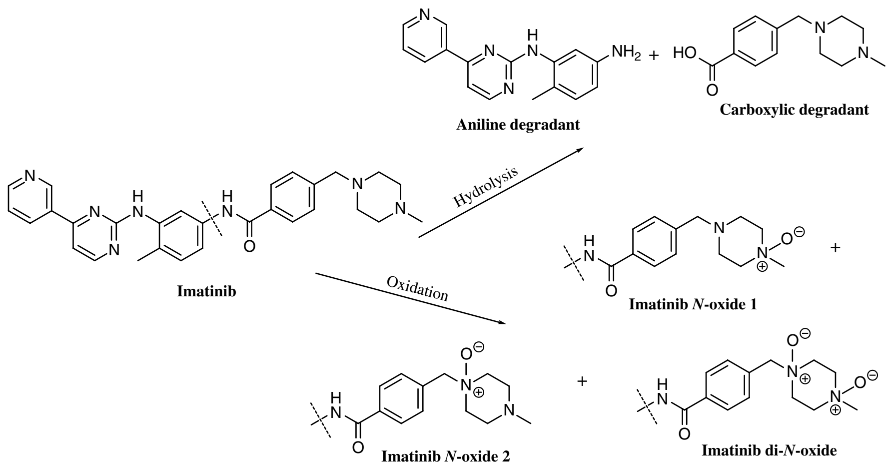
225: Imatini [WHO List of Essential Medicines]



Chemical name: 4-[(4-Methylpiperazin-1-yl)methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide
Trade name: Gleevac, Glivac
Formula: $C_{29}H_{31}N_7O$
Monoisotopic protonated ion (*m/z*): 494.2663
Molecular weight: 493.62
CAS number: 152459-95-5
Salt form: Mesylate

Imatinib is an inhibitor of several nonreceptor tyrosine kinases, including ABL, BCR-ABL, and KIT tyrosine kinases. It is used for the treatment of chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL) in addition to other tumors [1, 2]. CML is a rare clonal hematopoietic stem cell disorder, manifested by the presence of the so-called Philadelphia chromosome (Ph) [3]. The formation of Ph is due to a reciprocal translocation between the long arms of chromosomes 9 and 22, which causes the generation of a 210 k fusion protein that possesses kinase activity (BCR-ABL tyrosine kinase). The fusion protein is present in 95% of CML patients and 15–30% ALL patients. Imatinib was discovered in the late 1990s by scientists at Ciba-Geigy, which was later merged with Sandoz to become Novartis [2, 4]. The clinical use of imatinib for treating CML was spearheaded by Brian Druker of Oregon Health & Science University and it was approved by the US FDA in 2001 as the first selective tyrosine kinase inhibitor for the treatment of a cancer [4], marking a new paradigm for cancer-targeted therapies with various tyrosine kinases as drug targets [5–7].

Imatinib has a central amide linkage, which would be susceptible to hydrolytic degradation to yield the aniline and carboxyl degradants (Scheme 225) [8, 9]. It also possesses a piperazine moiety, which would be susceptible to oxidative degradation. Under harsh oxidative stress conditions, e.g., 30% hydrogen peroxide or 1% hydrogen peroxide at 60°C, the two nitrogen atoms of the piperazine moiety could all undergo nucleophilic oxidation, leading to the formation of two mono-*N*-oxide and one di-*N*-oxide [9]. Under a less harsh oxidative condition, only *N*-oxide 1 was observed [10]. It needs to be pointed out that under oxidative conditions, such as those using hydrogen peroxide as the oxidizing reagent, hydrolytic degradants like the aniline degradant could also be formed, which does not make it an oxidative degradant (as incorrectly noted in Reference [9]). Furthermore, the structural characterization of the imatinib degradants, formed under various stress conditions, was primarily based on mass spectrometric analysis. Thus, some of the degradant structures proposed do not appear to be reasonable [10]; further structural



Scheme 225 Hydrolytic and oxidative degradation pathways of Imatinib.

confirmation by complementary techniques such as NMR spectroscopy seems to be necessary.

Although imatinib absorbs light in the visible range, it was shown to be stable under simulated light, and no triplet excited state could be detected, which was attributed to a substructure of imatinib that may act as a self-quencher for the excited state [11].

References

1 Wong, S. and Witte, O.N. (2004). The BCR-ABL Story: bench to bedside and back. *Annu. Rev. Immunol.* 22: 247–306.

2 Imatinib. https://en.wikipedia.org/wiki/Imatinib (accessed December 21 2023).

3 Druker, B.J. (2002). STI571 (GleevecTM) as a paradigm for cancer therapy. *Trends Mol. Med.* 8 (4 Suppl): S14–S18.

4 Capdeville, R., Buchdunger, E., Zimmermann, J., and Matte, A. (2002). Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat. Rev. Drug Discov.* 1: 493–502.

5 Druker, B.J. (2004). Imatinib as a paradigm of targeted therapies. *Adv. Cancer Res.* 91: 1–30.

6 Arora, A. and Scholar, E.M. (2005). Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharmacol. Exper. Ther.* 315: 971–979.

7 Fischer, O.M., Streit, S., Hart, S., and Ullrich, A. (2003). Beyond Herceptin and Gleevec. *Curr. Opin. Chem. Bio.* 7: 490–495.

8 Al-Hadiya, B.M.H., Bakheit, A.H.H., and Abd-Elgalil, A.A. (2014). Chapter six - imatinib mesylate. *Prof. Drug Subs. Excip. Rel. Methodol.* 39: 265–297.

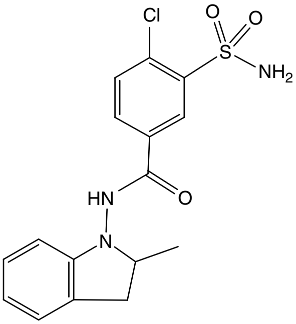
9 Ren, Z., Zhang, X., Wang, H., and Jin, X. (2016). Using an innovative quality-by-design approach for the development of a stability-indicating UPLC/

QTOF-ESI-MS/MS method for stressed degradation products of imatinib mesylate. *RSC Adv.* 6: 13050.

10 Nageswaria, A., Reddya, K.V.S.R.K., and Mukkanti, K. (2012). Stability-indicating UPLC method for determination of imatinib mesylate and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms. *J. Pharm. Biomed. Anal.* 66: 109–115.

11 Secrétan, P.H., Karoui, M., Sadou-Yaye, H. et al. (2019). Imatinib: major photocatalytic degradation pathways in aqueous media and the relative toxicity of its transformation products. *Sci. Total Environ.* 655: 547–556.

226: Indapamide [WHO List of Essential Medicines]

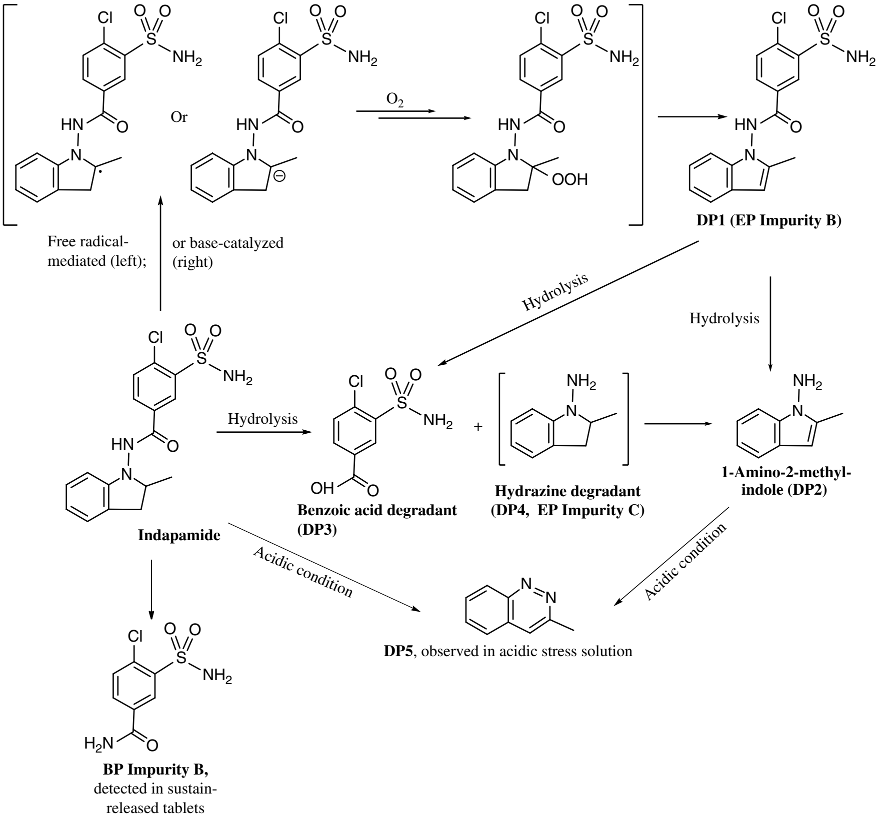


Chemical name: 4-Chloro-*N*-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoylbenzamide
Trade name: Arifon, Lozol, Natrilix, others
Formula: $C_{16}H_{16}ClN_3O_3S$
Monoisotopic protonated ion (*m/z*): 366.0674
Molecular weight: 365.83
CAS number: 26807-65-8

Indapamide is a thiazide-like diuretic indicated for the treatment of hypertension and heart failure [1]. It is also used in combination with perindopril, an ACE inhibitor.

Indapamide has an amide-like linkage, which would be labile toward hydrolytic stress and expected to yield the hydrazine degradant (DP4, Impurity C of European Pharmacopeia), and the benzoic acid degradant (DP3) (Scheme 226), which are also the starting materials for the synthesis of the drug [2]. Nevertheless, the hydrazine degradant (DP4) was not observed in the forced degradation studies performed by two research groups [3, 4]. Instead, the oxidized form of the hydrazine degradant (DP2) was produced under either acidic [3] or alkaline [4] conditions. The 2,3-dihydroindole moiety of indapamide is susceptible to oxidative degradation. It seems that the hydrazine degradant (DP4) might be oxidized during the hydrolytic stress to form DP2, or DP2 might be generated from DP1, an oxidative degradant of indapamide that can be formed under either oxidative stress conditions (such as by hydrogen peroxide or $CuCl_2$) or alkaline stress conditions. Under the latter condition, indapamide may undergo a base-catalyzed autooxidation process (Scheme 226) [5]. Palaric et al. also reported a novel degradant (DP5) that apparently stemmed from the expansion of the 1-aminoindole ring into the cinnoline ring [4, 6].

In a test of generic indapamide sustained-release tablets, Yao et al. detected 4-chloro-*N*-(2-methyl-1*H*-indol-1-yl)-3-sulfamoylbenzamide (BP Impurity B) [7], which could be a likely degradant. Such types of amide degradants, due to the cleavage of the hydrazide *N*–*N* linkage, were reported for other hydrazide drugs such as isoniazid [8]. Nevertheless, the exact mechanism for the cleavage remains unclear.



Scheme 226 Degradation pathways of indapamide under forced degradation conditions (all degradants except BP Impurity B) and in sustained-release tablets (BP Impurity B only).

References

1 Indapamide. https://en.wikipedia.org/wiki/Indapamide (accessed September 18 2023).

2 Heghes, S.C., Rus, L.M., Rus, L.-L. et al. (2017). HPLC-UV determination of indapamide in the presence of its main synthesis and degradation impurities. Method validation. *Farmacia* 65: 755–760.

3 Attia, K.A.M., Nassar, M.W.I., El-Din, M.M.K.S. et al. (2016). A stability-indicating QTRAP LC–MS/MS method for identification and structural characterization of degradation products of indapamide. *Anal. Methods* 8: 1836–1851.

4 Palaric, C., Molinié, R., Cailleu, D. et al. (2019). A deeper investigation of drug degradation mixtures using a combination of MS and NMR data: application to indapamide. *Molecules* 24: 1764.

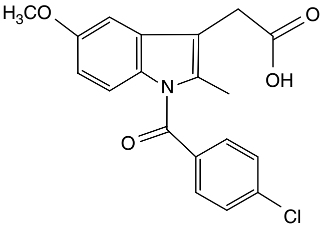
5 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*, 48–109. Cambridge: Royal Society Chemistry Publishing.

6 Somei, M. and Ura, K. (1978). Ring enlargement reaction of 1-aminoindoles to cinnoline derivatives. *Chem. Lett.* 707–708.

7 Yao, W., Zhou, S., and Cheng, Q. (2023). Simultaneous analysis of indapamide and related impurities in sustained-release tablets by a single-run HPLC-PDA method. *Wuhan Univ. J. Nat. Sci.* 28: 333–340.

8 Prajapati, P. and Agrawal, Y.K. (2015). Development of a green method for separation and identification of the degradation impurity of isoniazid by SFC-MS/MS. *Anal. Methods* 7: 7776–7783.

227: Indomethacin (Indometacin) [WHO List of Essential Medicines]



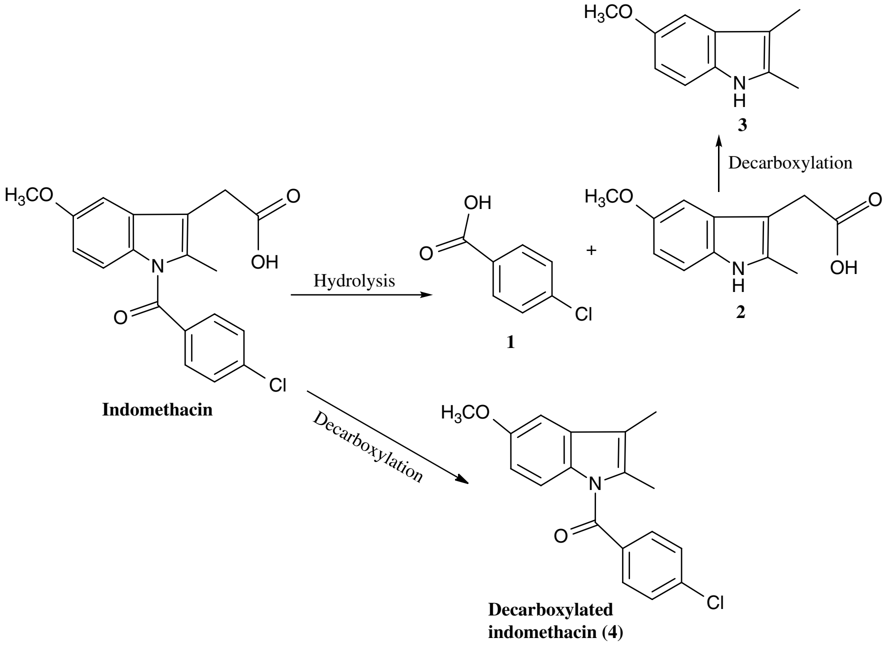
Chemical name: 2-{1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl}acetic acid
Trade name: Coversyl, Lozol, others
Formula: $C_{19}H_{16}ClNO_4$
Monoisotopic protonated ion (*m/z*): 358.0841
Molecular weight: 357.79
CAS number: 53-86-1

Indomethacin, also known as indometacin, is one of the nonsteroidal anti-inflammatory drugs (NSAIDs) prescribed for reducing fever, pain, and swelling from inflammation such as that associated with rheumatoid arthritis (RA) [1]. It is a nonselective inhibitor of cyclooxygenases 1 and 2 (COX-1 and 2), enzymes that catalyze the production of prostaglandins.

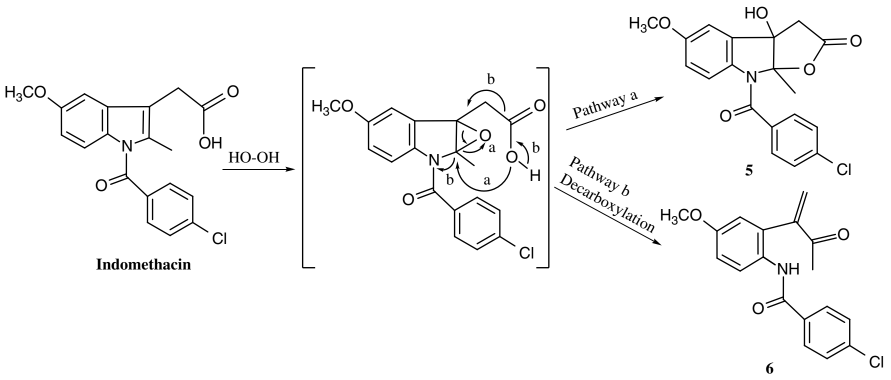
Indomethacin is an acylated indole derivative and its amide linkage is susceptible to hydrolytic degradation, leading to its two major degradants, 4-chlorobenzoic acid (**1**) and 5-methoxy-2-methylindoleacetic acid (**2**) (Scheme 227-1) [2, 3]. The activation energy for the hydrolysis was reported to be 97.6 kJ/mol in pH 7.4 phosphate buffer [2] and ~42 kJ/mol (~10 kcal/mol) in alkaline solutions [4], indicating it would be much more unstable under alkaline conditions. Degradant **2** could undergo decarboxylation to form degradant **3**. On the other hand, when crystalline indomethacin was heated to prepare its amorphous form, decarboxylated indomethacin (**4**) was produced [5].

In a forced oxidative degradation study by Li et al., two degradants (**5** and **6**) were found after indomethacin was treated with 0.23% hydrogen peroxide at 80°C. The structures of the degradants were elucidated based on MS and NMR measurement and their formation mechanisms were proposed (Scheme 227-2) [6]. The results of this study indicated that indomethacin, which is an acylated indole derivative and can be considered an embedded enamine, can still undergo nucleophilic oxidation.

Indomethacin was reported to be photochemically stable in crystalline form [7] but labile in solutions [8, 9]. In a suspension in benzene under irradiation by a medium-pressure mercury lamp, indomethacin was found to primarily undergo photo decarboxylation, leading to the formation of degradant **4** [8]. In another photodegradation study in organic solvents under irradiation by a low-pressure mercury lamp with a wavelength cutoff below 330 nm [9], degradant **4** and its tautomer



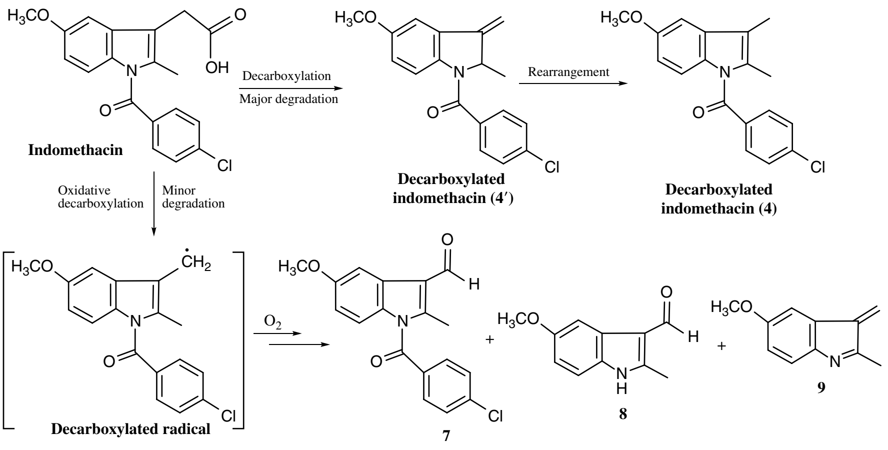
Scheme 227-1 Degradants from the hydrolysis and decarboxylation of indomethacin.



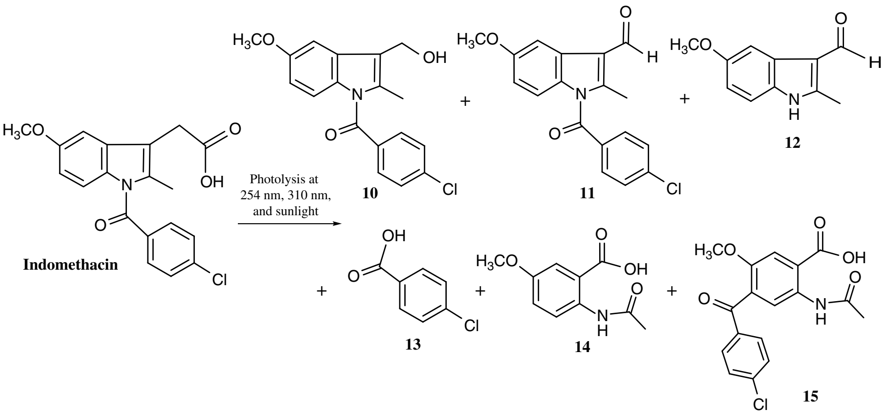
Scheme 227-2 Degradants from the oxidative degradation of indomethacin.

(**4′**) were observed as the major degradants, while a few minor degradants (**7**–**9**) were also present, among which **8** and **9** are photooxidative degradants (Scheme 227-3). Dabestani et al. also found that the photodegradation proceeded extremely slowly in more polar organic solvents, such as acetonitrile and ethanol [9].

In aqueous solutions, photodegradation products appeared to be more complex. In a photo stress study in aqueous solutions under irradiation at 254 nm and 310 nm, indomethacin was mostly decomposed to yield several photodegradants (**10**–**15**), among which **10** and **11** are oxidative degradants, **12** and **14** are oxidative and



Scheme 227-3 Photodegradation of indomethacin in organic solvents.



Scheme 227-4 Photodegradation of indomethacin in aqueous solutions.

hydrolytic degradants, **13** is hydrolytic degradant, and **15** is hydrolytic and photo-Fries degradant (Scheme 227-4) [10].

References

1 Indometacin. https://en.wikipedia.org/wiki/Indometacin (accessed December 23 2023).

2 Backensfeld, T., Muller, B.W., Wiese, M., and Seydel, J.K. (1990). Effect of cyclodextrin derivatives on indomethacin stability in aqueous solution. *Pharm. Res.* 7: 484–490.

3 Nováková, L., Matysová, L., Havlíková, L., and Solich, P. (2005). Development and validation of HPLC method for determination of indomethacin and its two degradation products in topical gel. *J. Pharm. Biomed. Anal.* 37: 899–905.

4 Hajratwala, B.R. and Dawson, J.E. (1977). Kinetics of indomethacin degradation I: presence of alkali. *J. Pharm. Sci.* 66: 27–29.

5 Shimada, Y., Komaki, H., Hirai, A. et al. (2018). Decarboxylation of indomethacin induced by heat treatment. *Int. J. Pharm.* 545: 51–56.

6 Li, M., Conrad, B., Maus, R.G. et al. (2005). A novel oxidative degradation pathway of indomethacin under the stressing by hydrogen peroxide. *Tetra. Lett.* 46: 3533–3536.

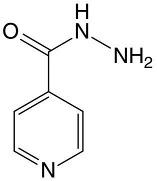
7 Ekiz-Guecer, N. and Reisch, J. (1991). Photostability of indomethacin in the crystalline state. *Pharm. Acta Helv.* 66: 66–67.

8 Weedon, A.C. and Wong, D.F. (1991). The photochemistry of indomethacin. *J. Photochem. Photobiol. A: Chem.* 61: 27–33.

9 Dabestani, R., Sik, R.H., Dvis, D.G. et al. (1993). Spectroscopic studies of cutaneous photosensitizing agents. XVIII. Indomethacin. *Photochem. Photobiol.* 58: 367–373.

10 Temussi, F., Cermola, F., DellaGreca, M. et al. (2011). Determination of photostability and photodegradation products of indomethacin in aqueous media. *J. Pharm. Biomed. Anal.* 56: 678–683.

228: Isoniazid [WHO List of Essential Medicines]

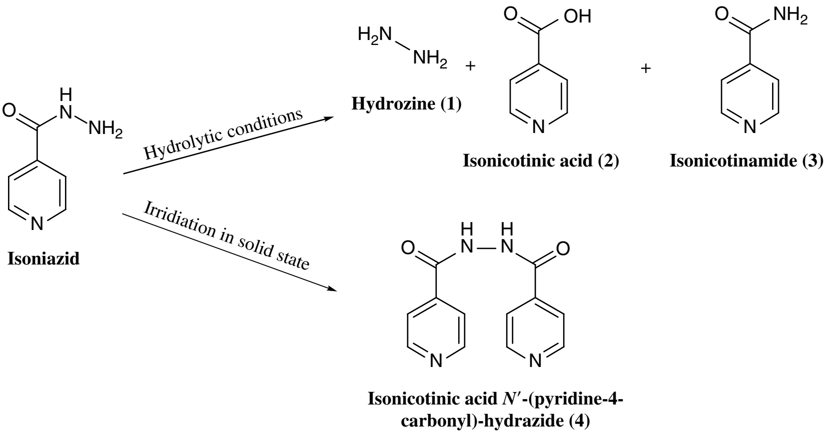


Chemical name: Pyridine-4-carbohydrazide
Trade name: Hydra, Hyzyd, Isovit, Rimifon, others
Formula: $C_6H_7N_3O$
Monoisotopic protonated ion (*m/z*): 138.0662
Molecular weight: 137.14
CAS number: 54-85-3

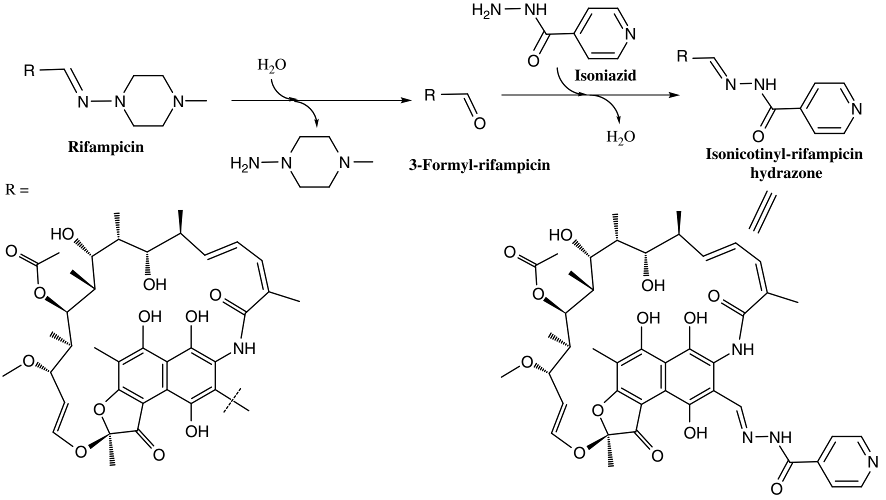
Isoniazid is an antibacterial drug used as a first-line treatment for both latent and active tuberculosis. For the latter category, it is often utilized in combination with other drugs such as rifampicin, pyrazinamide, and ethambutol [1]. The synthesis of the molecule was reported in 1912 and it was launched to treat tuberculosis in 1952 [2].

Isoniazid is an acylated hydrazine and, as such, it is susceptible to hydrolytic degradation, producing hydrazine (**1**) and isonicotinic acid (**2**) [3–6], both of which were detected in formulated products of isoniazid [3, 4]. In certain hydrolytic stress studies, isonicotinamide (**3**) was also obtained, in addition to isonicotinic acid (**2**) [5, 6]. In the forced degradation study by Bhutani et al. [5], **2** was the only degradant observed under acidic conditions (0.1 M HCl, 80°C), while both **2** (usually the major degradant) and **3** were formed in solutions of water, 0.1 M NaOH, or 30% hydrogen peroxide. In another forced degradation study, both **2** and **3** were reported to be present in comparable amounts under both acidic and alkaline conditions [6]. In solid state under irradiation by UV and fluorescent light, a dimeric degradant, *N′*-(pyridine-4-carbonyl)-hydrazide (**4**), was formed [5]. Degradant **4** was originally observed by Kakemi et al. under nonphotochemical degradation conditions [7], and later by Wollinger et al. in their drug-excipients compatibility study with certain colorants [8]. The degradation chemistry of isoniazid under hydrolytic and solid-state photodegradation conditions is summarized in Scheme 228-1.

The acylated hydrazine moiety of isoniazid is still quite nucleophilic and, as such, it is capable of reacting with carbonyl functionalities, particularly those more reactive ones such as aldehydes [9]. Rifampicin, another first-line anti-tuberculosis drug, contains a hydrazone moiety that can be hydrolyzed to produce the aldehyde degradant, 3-formyl-rifampicin. The latter can react with isoniazid in combination products of isoniazid and rifampicin to yield the isonicotinyl-hydrazone degradant (Scheme 228-2) [10, 11]. Likewise, isoniazid may be incompatible with excipients containing reducing sugar moieties, as it can form the corresponding isoniazid hydrazone degradants with these excipients (in such cases, these excipients would take the role of 3-formyl-rifampicin as shown in Scheme 228-2).



Scheme 228-1 The degradation chemistry of isoniazid under hydrolytic and solid state photodegradation conditions.



Scheme 228-2 Formation mechanism of isonicotinyl-rifampicin hydrazone degradant.

References

1 Isoniazid. https://en.wikipedia.org/wiki/Isoniazid (accessed September 18 2023).

2 Judge, V., Narasimhan, B., and Ahuja, M. (2012). Isoniazid: the magic molecule. *Med. Chem. Res.* 21: 3940–3957.

3 Matsui, F., Robertson, D.L., and Lovering, E.G. (1983). Determination of hydrazine in pharmaceuticals III: hydralazine and isoniazid using GLC. *J. Pharm. Sci.* 72: 948–951.

4 Faria, A.F., Vasconcelos, J.P., Goncalves, R.S.B. et al. (2012). Simultaneous analysis of isoniazid and its impurities by CZE. *Chromatographia* 75: 1335–1339.

5 Bhutani, H., Singh, S., Vir, S. et al. (2007). LC and LC–MS study of stress decomposition behaviour of isoniazid and establishment of validated stability-indicating assay method. *J. Pharm. Biomed. Anal.* 43: 1213–1220.

6 Prajapati, P. and Agrawal, Y.K. (2015). Development of a green method for separation and identification of the degradation impurity of Isoniazid by SFC-MS/MS. *Anal. Methods* 7: 7776–7783.

7 Kakemi, K., Sezaki, H., and Inoue, S. (1966). Stability of isoniazid and its related compounds. II. Base-catalyzed degradation of isoniazid (article in Japanese). *Yakugaku Zasshi* 86: 163–168.

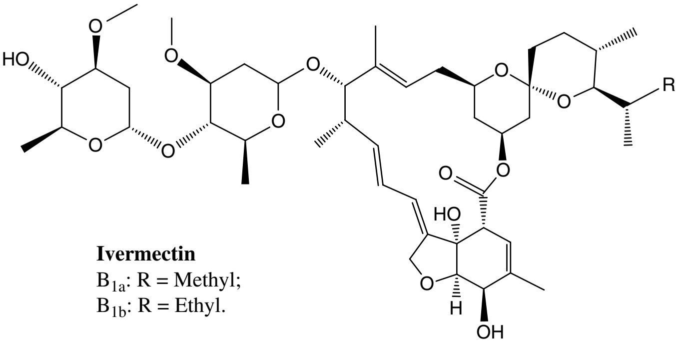
8 Wollinger, W., da Silva, R.A., da Nóbrega, A.B. et al. (2016). Assessing drug-excipient interactions in the formulation of isoniazid tablets. *J. Braz. Chem. Soc.* 27: 826–833.

9 Devani, M.B., Shishoo, C.J., Doshi, K.J., and Patel, H.B. (1985). Kinetic studies of the interaction between isoniazid and reducing sugars. *J. Pharm. Sci.* 74: 427–432.

10 Singh, S., Mariappan, T.T., Sharda, N. et al. (2000). The reason for an increase in decomposition of rifampicin in the presence of isoniazid under acid conditions. *Pharm. Pharmacol. Commun.* 6: 405–410.

11 Prasad, B. and Singh, S. (2010). LC–MS/TOF and UHPLC–MS/MS study of in vivo fate of rifamycin isonicotinyl hydrazone formed on oral co-administration of rifampicin and isoniazid. *J. Pharm. Biomed. Anal.* 52: 377–383.

229: Ivermectin [WHO List of Essential Medicines]



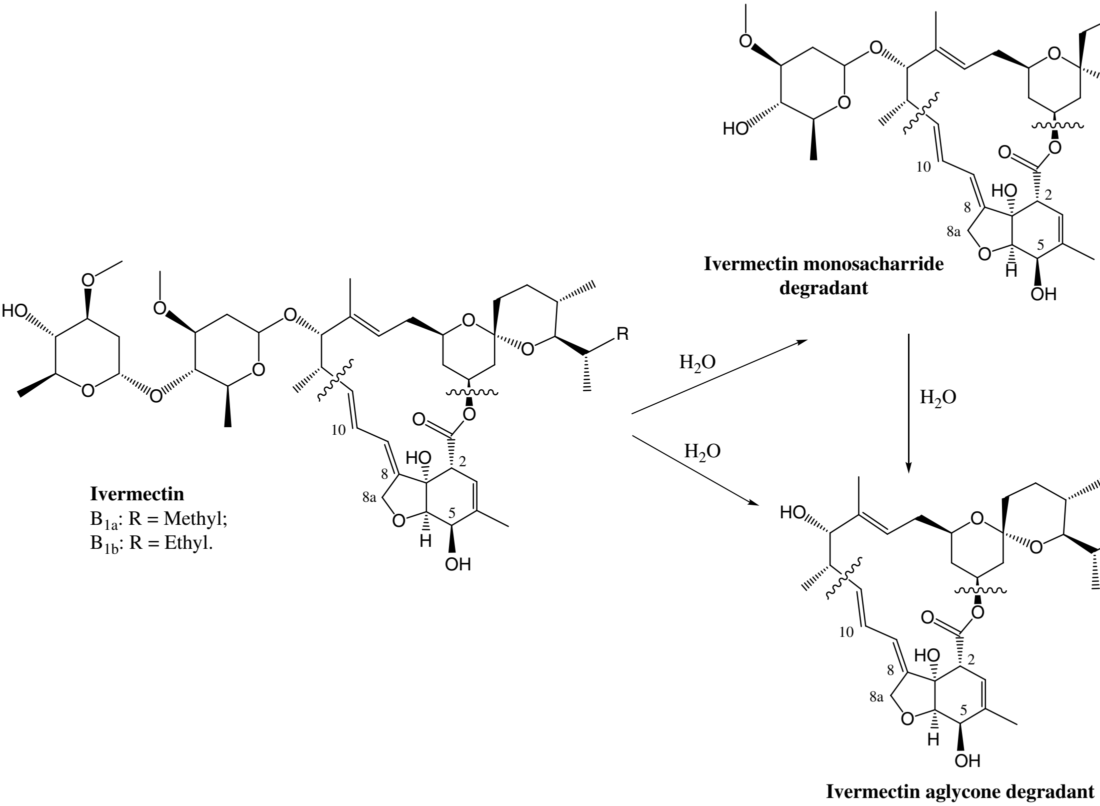
Ivermectin
B_{1a}: R = Methyl;
B_{1b}: R = Ethyl.

Chemical name: 22,23-Dihydroavermectin B_{1a} + 22,23-Dihydroavermectin B_{1b}
Trade name: Stromectol, Sklice, Mectizan, others
Formula: $C_{48}H_{74}O_{14}$; $C_{47}H_{72}O_{14}$
Monoisotopic protonated ion (*m/z*): 875.5151; 861.4995
Molecular weight: 875.11; 861.08
CAS number: 70288-86-7

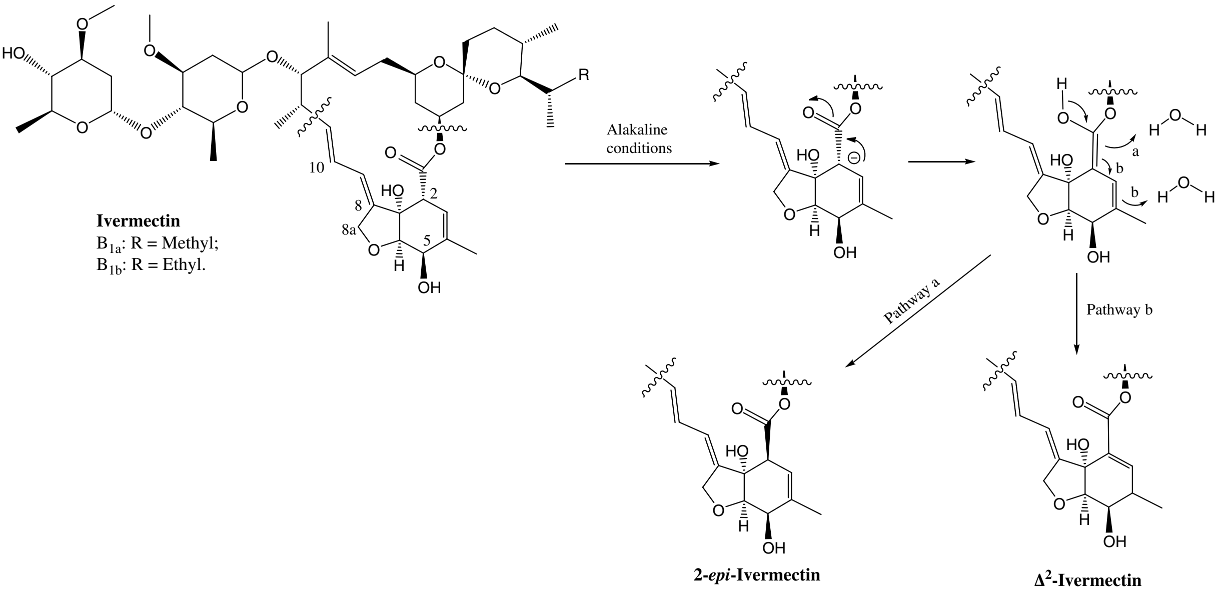
Ivermectin is an antiparasitic agent, which is a semi-synthetic compound prepared from the natural product, Avermectin. The latter was isolated by William Campbell of Merck Sharp & Dohme Research Laboratories from the sample provided by Satoshi Ōmura of Kitasato University [1]. The scientists at Merck modified the structure of avermectin to improve its pharmacologic property, which resulted in ivermectin, a mixture of two congeners (22,23-dihydroavermectin B_{1a} and 22,23-dihydroavermectin B_{1b}) with at least 80% B_{1a}. Ivermectin was initially approved for veterinary use to prevent and treat heartworm and acariasis, and later approved for human use to treat a number of infestations including river blindness. For the latter indication, Merck & Co., Inc. has established a donation program in Africa for several decades with the product trade name of Mectizan®.

Both components of ivermectin are disaccharide derivatives of a 16-membered macrocyclic lactone. Hence, ivermectin would be susceptible to hydrolytic degradation. Under acidic conditions, it was found to degrade initially as the monosaccharide degradant and then the aglycone degradant (Scheme 229-1) [2, 3]. Under alkaline conditions, the predominant degradation products observed were 2-epi-ivermectin and Δ^2-ivermectin, both of which were formed via base-catalyzed enolization at the position alpha to the lactone carbonyl group (Scheme 229-2) [2, 3]. At pH 6.3, ivermectin was found to be most stable [2].

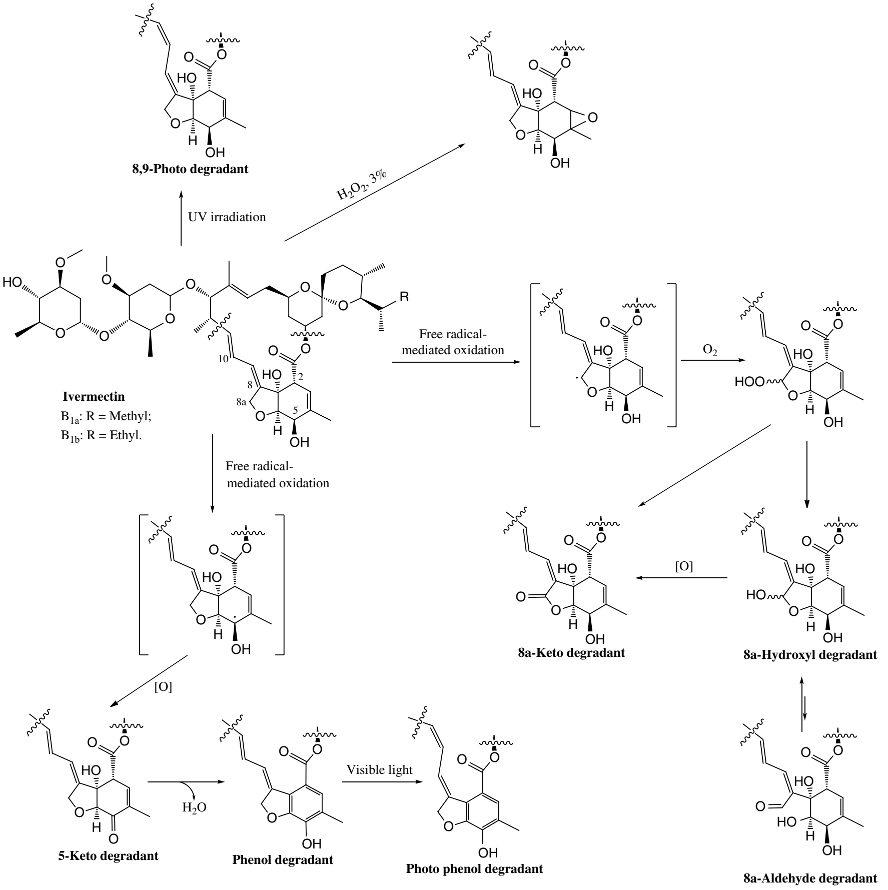
Ivermectin also contains structural moieties that are labile toward oxidative degradation. The 8a-methylene position, α to the butadiene moiety, and 5-position hydroxyl group would be expected to undergo oxidation under free-radical-mediated oxidative conditions to produce many oxidative degradants (Scheme 229-3) [2, 4, 5]. It would be reasonable to assume that the oxidation proceeds via a peroxide intermediate in both cases [6]. The free-radical-mediated oxidation can also take place



Scheme 229-1 Degradation of ivermectin under acidic conditions.



Scheme 229-2 Degradation of ivermectin under alkaline conditions.



Scheme 229-3 Degradation of ivermectin under free-radical-mediated oxidative and photo stress conditions.

under photo stress of ivermectin in the solid state [3]. On the other hand, the 3,4-olefnic double bond can be oxidized by forced degradation in 3% hydrogen peroxide solution to produce the corresponding peroxide degradant through a nucleophilic oxidation mechanism [3].

The butadiene functionality is also photochemically reactive, giving rise to C_8-C_9 and C_{10}-C_{11} geometric isomers under UV irradiation [2]. For the phenol degradant, the photochemical isomerization can even take place in the visible region due to the formation of the highly conjugated phenol chromophore (Scheme 229-3) [5].

References

1 Ivermectin. https://en.wikipedia.org/wiki/Ivermectin (accessed January 3 2024).
2 Fink, D.W. (1998). Ivermectin. *Anal. Prof. Drug Sub.* 17: 155–184.

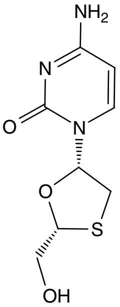
3 Adhikari, S. and Rustum, A.M. (2022). A comprehensive study to identify and characterize major degradation products of ivermectin drug substance including its degradation pathways using LC-HRMS and NMR. *J. Pharm. Biomed. Anal.* 214: 114730.

4 Stong, J.D.J., Pivnichny, V., Mrozik, H., and Waksmunski, F.S. (1992). Radical-induced oxidation of oxahydrindene (hexahydrobenzofuran) portion of 22,23-dihydroavermectin B_{1a}: identification of autooxidation products. *J. Pharm. Sci.* 81: 1000–1003.

5 Wang, Q., Stong, J.D., Demontigny, P. et al. (1996). Identification of the secondary degradates of L-648,548 in an animal health formulation. *J. Pharm. Sci.* 85: 446–450.

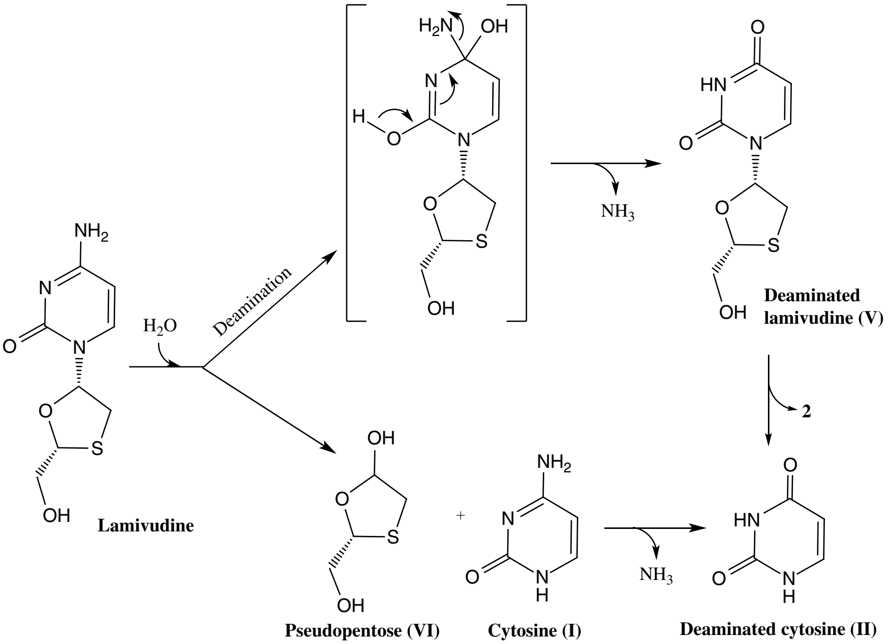
6 Li, M. (2012). Oxidative degradation. In: *Organic Chemistry of Drug Degradation*, 48–109. Cambridge, UK: RSC Publishing.

230: Lamivudine [WHO List of Essential Medicines]

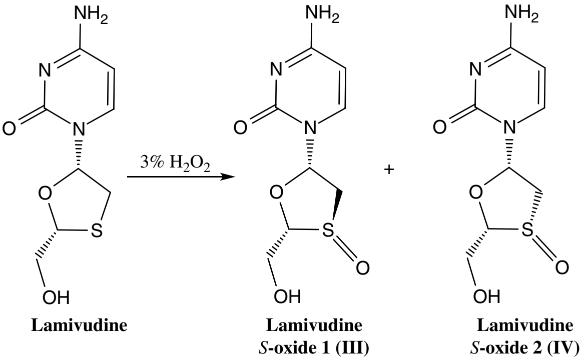


Chemical name: (*2R,5S*)-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1*H*)-pyrimidin-2-one
Trade name: Epivir, Zeffix, others
Formula: $C_8H_{11}N_3O_3S$
Monoisotopic protonated ion (*m/z*): 230.0594
Molecular weight: 229.25
CAS number: 134678-17-4

Lamivudine, also known as 3TC, is a reverse transcriptase inhibitor typically utilized for the treatment and prevention of AIDS. It is commonly used in combination with other anti-AIDS drugs such as zidovudine, dolutegravir, and abacavir [1]. It is



Scheme 230-1 Hydrolytic degradation of lamivudine. The numbering of the degradants follows that of Bedse et al. [2]. *Source:* Bedse et al. [2] / with permission of Elsevier.



Scheme 230-2 Oxidative degradation of lamivudine. The numbering of the degradants follows that of Bedse et al. [2]. *Source:* Bedse et al. [2] / with permission of Elsevier.

a nucleoside analog with a sulfur-containing pentose analog. Both its nucleobase (cytosine) and the glycosidic-like linkage would be susceptible to hydrolytic degradation to give cytosine (**I**), deaminated cytosine (**II**), deaminated lamivudine (**V**), and pseudopentose degradant (**VI**), as demonstrated in a forced degradation study by Bedse et al. [2]. The hydrolytic removal of the amino group of the cytosine moiety is also called a deamination process (Scheme 230-1). On the other hand, the thioether moiety can be oxidized under nucleophilic oxidative conditions to yield two isomeric degradants (**III** and **IV**, Scheme 230-2).

Wang and You studied thermal degradation of lamivudine in solid state using TG and DSC in both nitrogen and air and found that the activation energies were 126.5 kJ/mol and 133.0 kJ/mol, respectively, indicating lamivudine has good thermal stability [3].

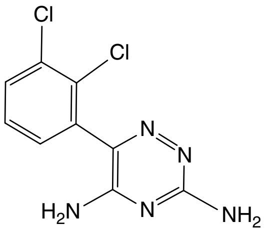
References

1 Lamivudine. https://en.wikipedia.org/wiki/Lamivudine (accessed January 5 2024).

2 Bedse, G., Kumar, V., and Singh, S. (2009). Study of forced decomposition behavior of lamivudine using LC, LC–MS/TOF and MS*n*. *J. Pharm. Biomed. Anal.* 49: 55–63.

3 Wang, X. and You, J. (2010). Thermal degradation and kinetics of lamivudine. *J. Shenyang Pharm. Univ.* 8: http://www.cnki.com.cn/Article/CJFDTotal-SYYD201008005.htm (accessed January 26 2014.

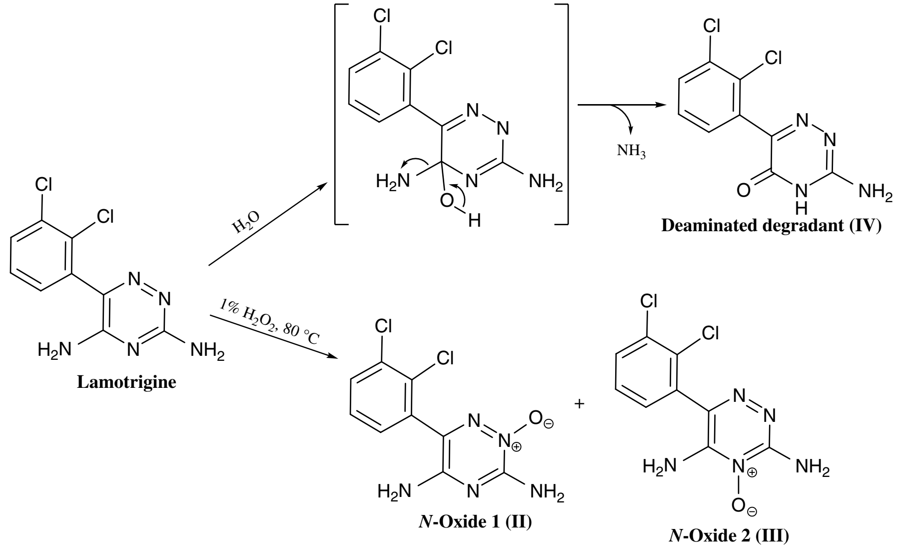
231: Lamotrigine [WHO List of Essential Medicines]



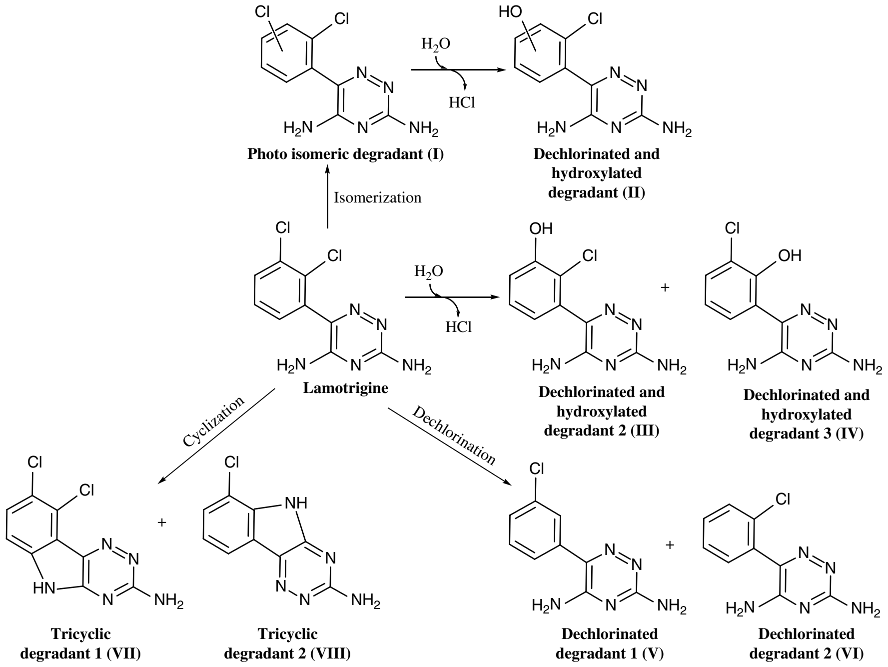
Chemical name: 6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine
Trade name: Lamictal, others
Formula: $C_9H_7Cl_2N_5$
Monoisotopic protonated ion (*m/z*): 256.0151
Molecular weight: 256.09
CAS number: 84057-84-1

Lamotrigine is a medicine used to treat epilepsy and stabilize mood in bipolar disorder, which was first approved for medical use in Ireland in 1991 [1]. Chemically, lamotrigine is a phenyltriazine derivative with two amino substituents on the triazine ring. Under forced hydrolytic stress conditions, either acidic (1 N HCl) or alkaline (1 N NaOH) at 80°C, the main degradation pathway is deamination of the 5-amino group, according to the forced degradation study by Mahajan et al. [2]. Under the alkaline stress condition, there was an early-eluting degradant (**I**), whose structure was not identified due to the inability to obtain its mass spectrometric data, in addition to the deaminated degradant (**IV**). Under the forced oxidative stress using 1% hydrogen peroxide at 80°C, two *N*-oxide degradants (**II** and **III**) were produced (Scheme 231-1). On the other hand, lamotrigine was found to be stable under the thermal stress (70°C for 3 days) and photolysis in the solid state [2].

In solution photolysis of lamotrigine with irradiation by either simulated sunlight [3] or UV light by both low- and medium-pressure mercury lamps [4], a number of photodegradants were observed, and the structures for the vast majority of these degradants were proposed based on high-resolution mass spectrometric data (Scheme 231-2) [3, 4]. The photodegradation pathways of lamotrigine can be categorized into four major types. The first one is photoisomerization, from which degradant I was formed. The second type is photo substitution, in which a chloro group was replaced by a hydroxyl group. Hence, further decomposition of degradant I via photo substitution resulted in degradant II. Likewise, direct photo substitution of lamotrigine gave rise to degradants III and IV. The third type is a straightforward dechlorination process, in which degradants V and VI were formed. The last one is a photo cyclization process, which produced degradants VII and VIII; such type of photo cyclization is not uncommon for bi-aryl compounds [5].



Scheme 231-1 Hydrolytic and oxidative degradation of lamotrigine under forced degradation conditions. The numbering of degradants follows that of Mahajan et al. [2]. *Source:* Mahajan et al. [2] /Taylor & Francis Group.



Scheme 231-2 Photochemical degradation pathways of lamotrigine.

References

1 Lamotrigine. https://en.wikipedia.org/wiki/Lamotrigine (accessed January 5, 2024).

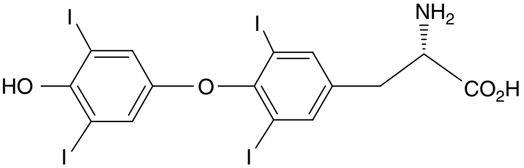
2 Mahajan, A.A., Thaker, A.K., Kale, S., and Mohanraj, K. (2012). LC, LC–MS/MS studies for identification and characterization of degradation products of lamotrigine and establishment of mechanistic approach towards degradation. *J. Liq. Chromat. Rel. Tech.* 35: 2255–2271.

3 Young, R.B., Chefetz, B., Liu, A. et al. (2014). Direct photodegradation of lamotrigine (an antiepileptic) in simulated sunlight – pH influenced rates and products. *Environ. Sci. Process. Impacts* 16: 848–857.

4 Keen, O.S., Ferrer, I., Thurman, E.M., and Linden, K.G. (2014). Degradation pathways of lamotrigine under advanced treatment by direct UV photolysis, hydroxyl radicals, and ozone. *Chemosphere* 117: 316–323.

5 Li, M. (2012). Section 6.2.5. Cyclization in polyaromatic ring systems. In: *Organic Chemistry of Drug Degradation*, 180–182. Cambridge, UK: RSC Publishing.

232: Levothyroxine [L-T4] [WHO List of Essential Medicines]



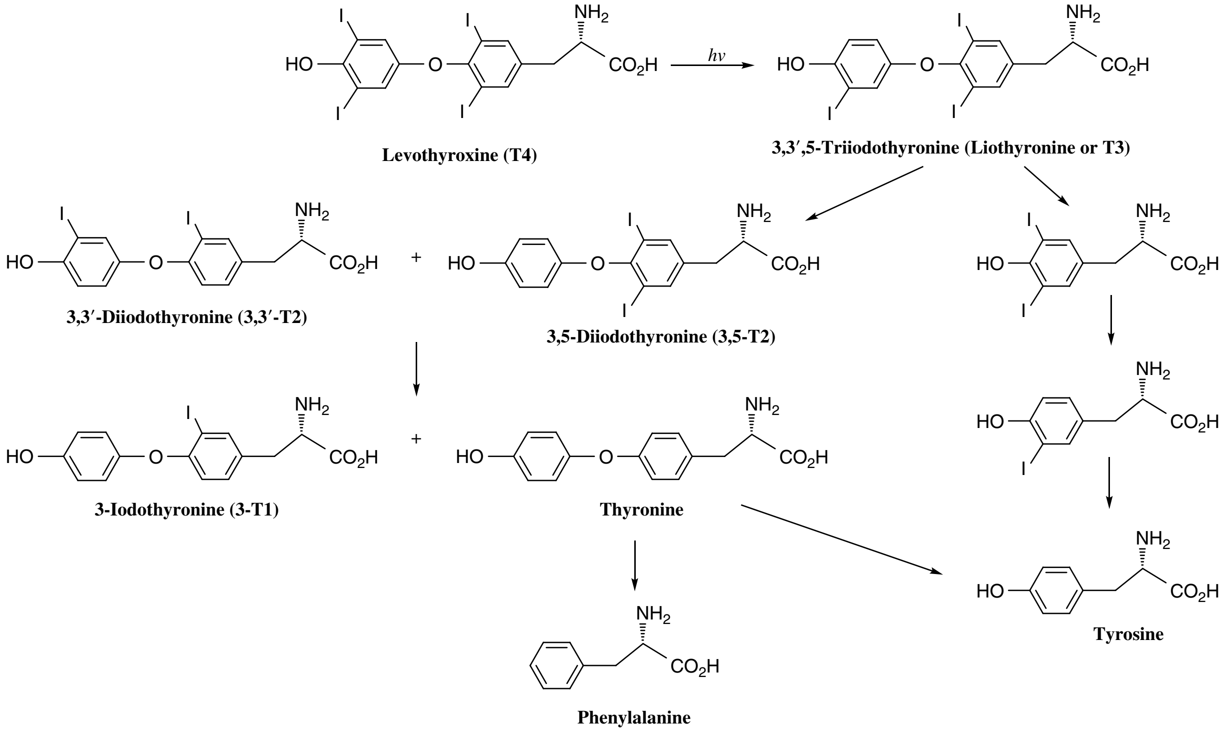
Chemical name: (*S*)-2-Amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoic acid
Trade name: Synthyroid, others
Formula: $C_{15}H_{11}I_4NO_4$
Monoisotopic protonated ion (*m/z*): 777.6940
Molecular weight: 776.87
CAS number: 51-48-9
Salt form: **Sodium salt**

Levothyroxine, or thyroxine, was isolated by E. C. Kendall from hog thyroid at the Mayo Clinic in 1914 [1, 2]. Its structure was determined by C.R. Harington in 1926, and subsequently it was synthesized by C.R. Harington and G. Barger in 1927 [3, 4]. Levothyroxine is used to treat thyroid hormone deficiency (hypothyroidism) and was the most prescribed medication in the United States in 2016 [5].

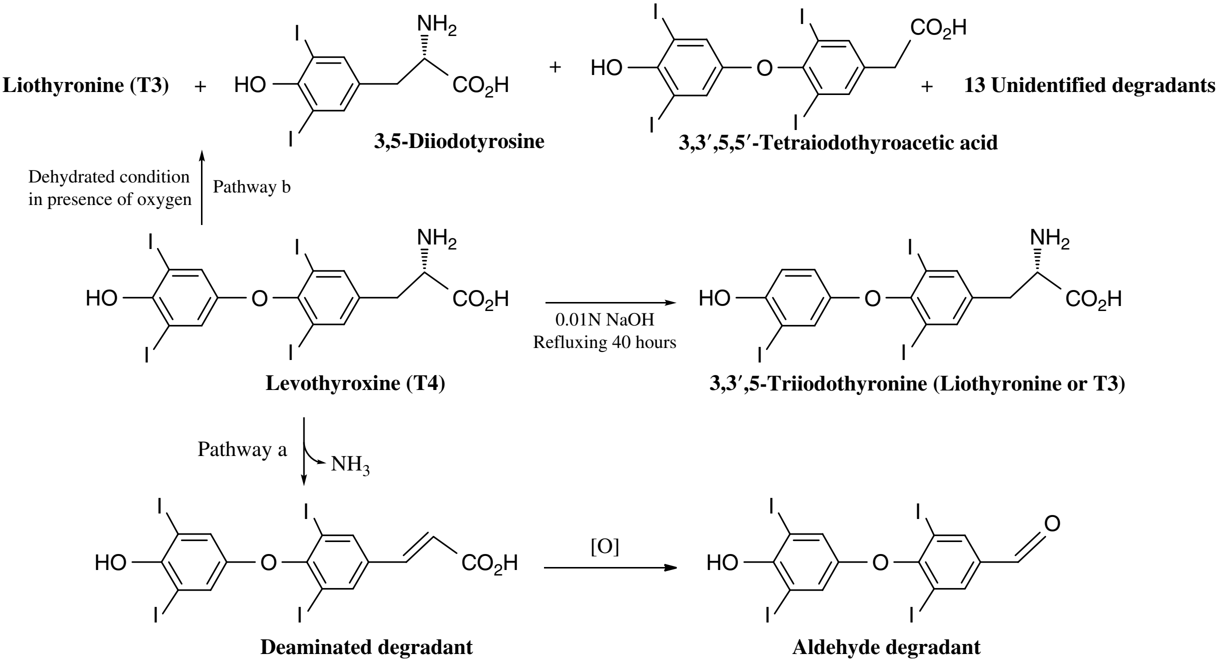
Chemically, thyroxine is the tetraiodo derivative of thyronine, and the latter is *O*-4-hydroxyphenyl-substituted tyrosine. The iodo substituents tend to fall off the substituted phenyl rings, i.e., they are prone to deiodination, particularly under photochemical conditions [6, 7]. The photo deiodination produces initially T3 and then a few T2 degradants and T1 degradant, upon further photolysis. Photo irradiation by UVA and UVB light can also cause the breakage of the biaryl ether linkage, leading to the formation of phenylalanine, tyrosine, and their derivatives (Scheme 232-1) [7]. Under irradiation with UV light at wavelength greater than 300 nm, no phenylalanine, tyrosine, or their derivatives were observed, suggesting that the cleavage of the biaryl ether linkage would require higher energy than that of deiodination.

In a forced degradation study of levothyroxine by Won, levothyroxine was reported to undergo nonphotochemical deiodination to produce T3 in 0.01 N NaOH solution under refluxing [8]. In solid state, the main degradation of levothyroxine was elimination of ammonia, followed by oxidative breakage of the carbon–carbon double bond, observed in a sample of levothyroxine sodium that was placed in the open dish at 60°C for 7 weeks (Scheme 232-2, Pathway a).

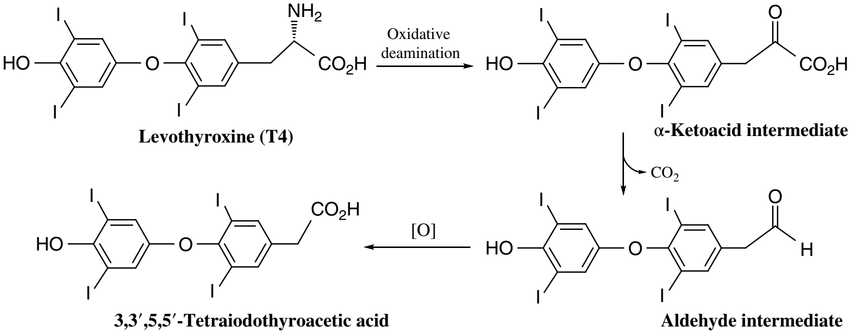
The stability of formulated levothyroxine products (with its sodium salt as the API), such as the tablets, seems to be a challenge ever since its market approval in the 1950s, as formulated levothyroxine products remain to be the most recalled products, primarily due to the chemical instability of the API [9, 10]. The instability of the formulated products was attributed to the dehydration of the API, in the form



Scheme 232-1 Photochemical degradation of levothyroxine.



Scheme 232-2 Nonphotochemical degradation of levothyroxine under forced degradation conditions. The identification of the three degradants, formed under dehydrated conditions in the presence of oxygen, was performed only based on retention time comparison with reference materials.



Scheme 232-3 Plausible mechanism for the degradation of levothyroxine to produce 3,3′,5,5′-tetraiodothyroacetic acid.

of pentahydrate, and subsequent oxidation of the dehydrated API [9, 11]. Shah et al. reported that a sample of levothyroxine drug substance, after exposing to a dehydrated condition in the presence of oxygen for a duration of 32 days, produced ~16 degradants, among which only 3 degradants were identified (3,5-diiodo-tyrosine, 3,3′,5-triiodo-thyronine, and 3,3′,5,5′-tetraiodothyroacetic acid) based on comparison to reference materials (Scheme 232-2, Pathway b) [11]. It needs to be pointed out that 3,3′,5,5′-tetraiodothyroacetic acid is also a metabolite of levothyroxine and its formation mechanism under the in vitro degradation conditions, which was not elaborated by Shah et al. [11], could be rationalized by the mechanism illustrated in Scheme 232-3, which is analogous to that proposed by Andre et al. for the thermal degradation of liothyronine sodium (T3 sodium salt) [12]. Most of the 16 degradants eluted after levothyroxine, as analyzed by a reversed-phase HPLC method with a C8 column.

References

1 Simoni, R.D., Hill, R.L., and Vaughan, M. (2002). The isolation of thyroxine and cortisone: the work of Edward C. *Kendall. J. Biol. Chem.* 277: 21–22.

2 Slater, S. (2010). The discovery of thyroid replacement therapy. Part 3: a complete transformation. *J. R. Soc. Med.* 104: 100–106.

3 Harington, C.R. (1926). Chemistry of thyroxine. II: constitution and synthesis of desiodo-thyroxine. *Biochem. J.* 20: 300–313.

4 Harington, C.R. and Barger, G. (1927). Chemistry of thyroxine. III: constitution and synthesis of thyroxine. *Biochem. J.* 21: 169–183.

5 Levothyroxine. https://en.wikipedia.org/wiki/Levothyroxine (accessed January 5 2024).

6 Van Der Walt, B. and Cahnmann, H.J. (1982). Synthesis of thyroid hormone metabolites by photolysis of thyroxine and thyroxine analogs in the near UV Proc. *Natl. Acad. Sci. USA.* 79: 1492–1496.

7 Kazemifard, A.G., Moore, D.E., and Aghazadeh, A. (2001). Identification and quantitation of sodium-thyroxine and its degradation products by LC using electrochemical and MS detection. *J. Pharm. Biomed. Anal.* 25: 697–711.

8 Won, C.M. (1992). Kinetics of degradation of levothyroxine in aqueous solution and in solid state. *Pharm. Res.* 9: 131–137.

9 Hamad, M.L., Engen, W., and Morris, K.R. (2013). Impact of hydration state and molecular oxygen on the chemical stability of levothyroxine sodium. *Pharm. Dev. Technol.* https://doi.org/10.3109/10.37450.2013.862635.

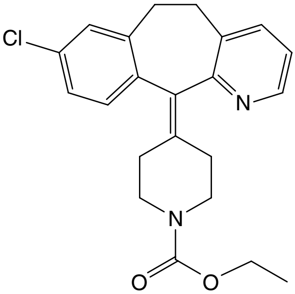
10 Rhodes, C. (1998). Regulatory aspects of the formulation and evaluation of levothyroxine tablets. *Clin. Res. Regul. Aff.* 15: 173–186.

11 Shah, H.S., Chaturvedi, K., Hamad, M. et al. (2019). New insights on solid-state changes in the levothyroxine sodium pentahydrate during dehydration and its relationship to chemical instability. *AAPS PharmSciTech.* 20: 39. https://doi.org/10.1208/s12249-018-1264-0.

12 Andre, M., Domanig, R., Riemer, E. et al. (1996). Identification of the thermal degradation products of G-triiodothyronine sodium (liothyronine sodium) by reversed-phase high-performance liquid chromatography with photodiode-array UV and mass spectrometric detection. *J. Chromat. A* 725: 287–294.

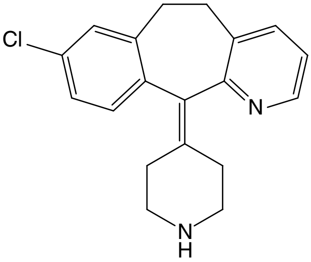
233–234: Loratadine, Desloratadine

233: Loratadine [WHO List of Essential Medicines]



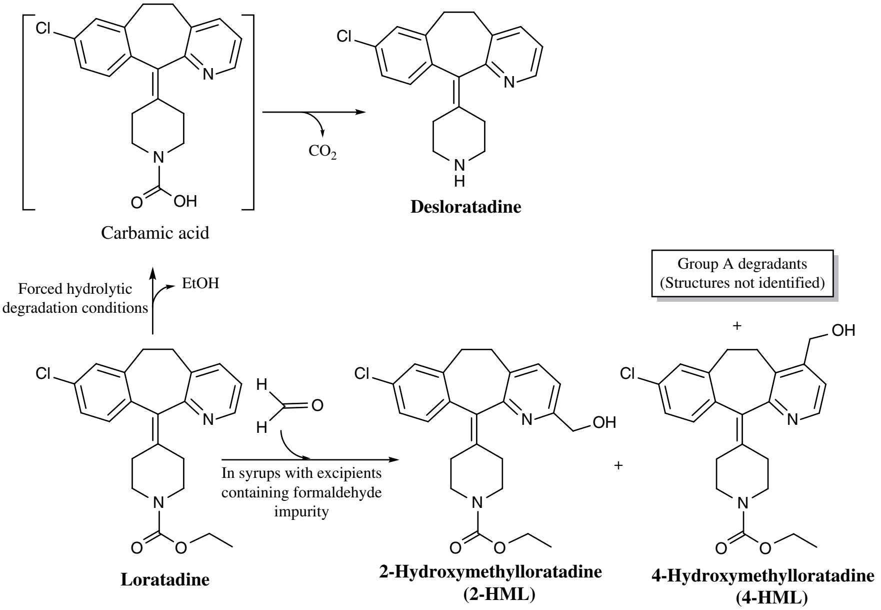
Chemical name: Ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-b] pyridin-11-ylidene)-1-piperidinecarboxylate
Trade name: Claritin
Formula: $C_{22}H_{23}ClN_2O_2$
Monoisotopic protonated ion (*m/z*): 383.1521
Molecular weight: 382.89
CAS number: 79794-75-5

234: Desloratadine



Chemical name: 8-Chloro-11-(4-piperidinylidene)-6,11-dihydro-5*H*-benzo[5,6] cyclohepta[1,2-b]pyridine
Trade name: Clarinex, Aerius
Formula: $C_{19}H_{19}ClN_2$
Monoisotopic protonated ion (*m/z*): 311.1310
Molecular weight: 310.82
CAS number: 100643-71-8

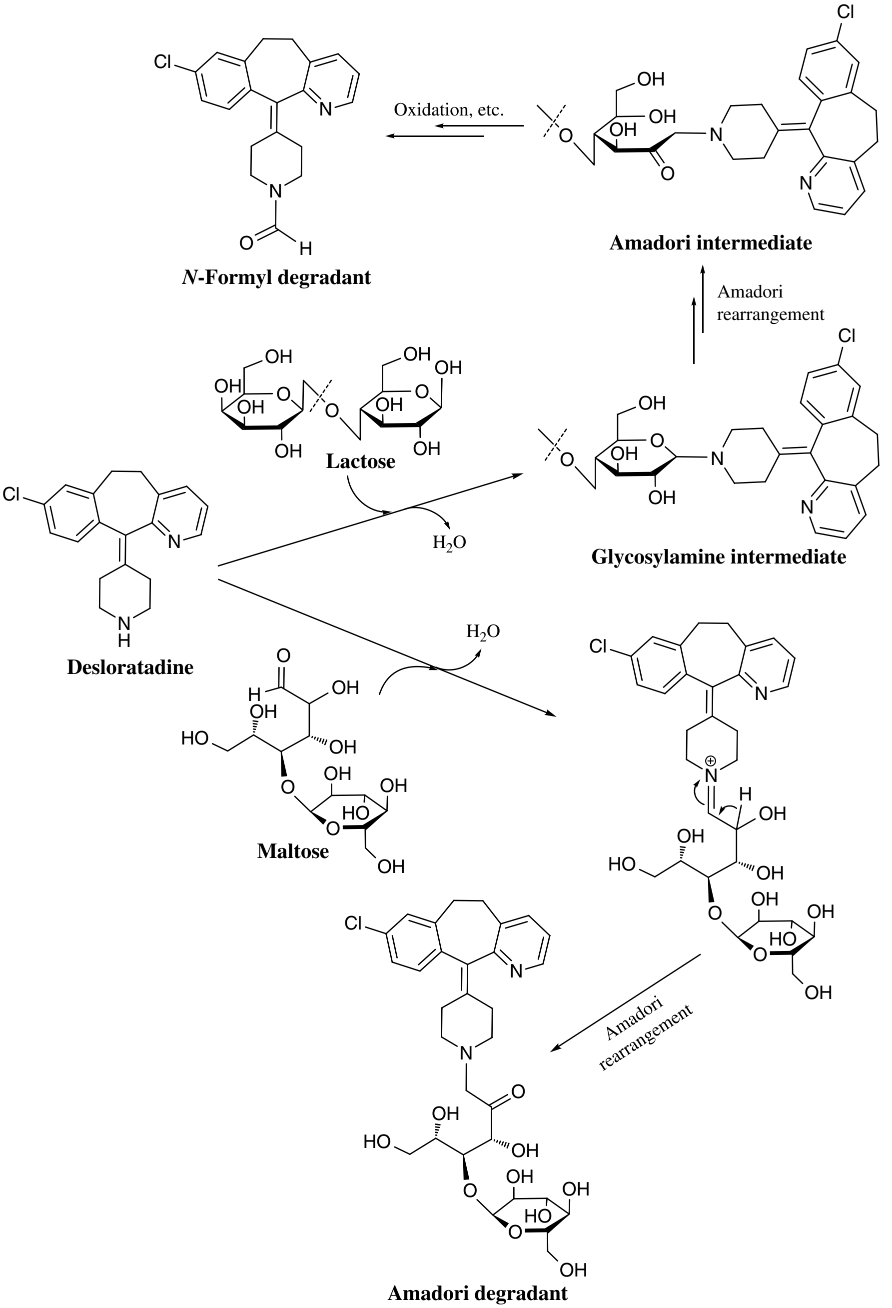
Loratadine is a second-generation antihistamine medication used to treat allergies. It was developed by Schering-Plough Corp. (later merged into Merck & Co., Inc.) and approved in 1988 [1]. It became an over-the-counter (OTC) drug after its patent expiration. Loratadine is a carbamate prodrug with a tricyclic ring that contains a fused pyridine moiety. In solid state, it is quite stable as a drug substance or in solid dosage forms [2]. In liquid formulations such as syrups containing sugar excipients,



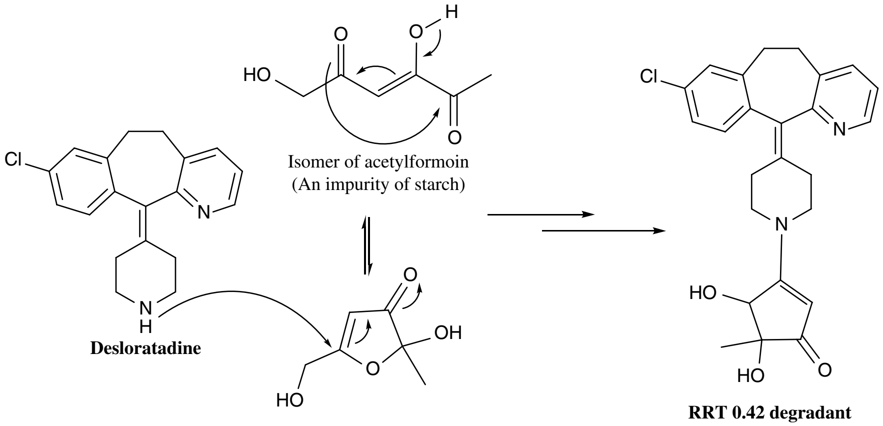
Scheme 233–234-1 Degradation of loratadine in syrups or under forced hydrolytic degradation conditions.

it was revealed that the pyridine moiety could react with certain impurities/degradants of these excipients such as formaldehyde, resulting in the formation of 2-hydroxymethylloratadine (2-HML), 4-hydroxymethylloratadine (4-HML), and other degradants (collectively known as "Group A impurities") (Scheme 233–234-1) [3]. The mechanism for the degradation is of oxidative nature, as many measures using either chelating agents (such as EDAT) [3] or antioxidants (such as ascorbic acid or phenol derivatives) [3, 4] can inhibit the formation of these degradants. Nevertheless, the exact mechanism remains unclear, although it was hypothesized as being mediated through a pyridine *N*-oxide intermediate [5]. Based on the known reactivity of pyridine, it could be a free-radical-mediated addition between loratadine and formaldehyde [6]. On the other hand, hydrolysis of the carbamate linkage does not appear to occur in appreciable amounts even in aqueous liquid formulations [3]. Therefore, desloratadine may not be a real degradant of loratadine, although desloratadine can be generated under forced hydrolytic degradation conditions [7].

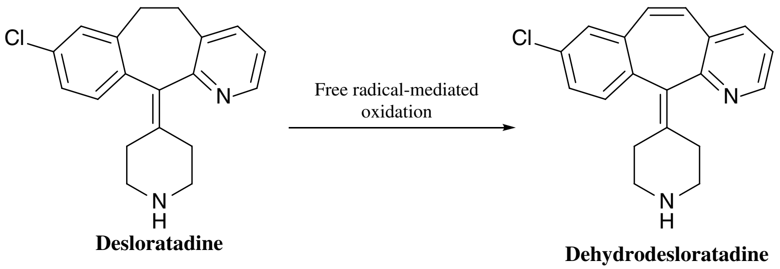
Desloratadine is the major metabolite of loratadine formed by hydrolysis of the carbamate group. The hydrolysis exposes the secondary amine functionality of the drug molecule and the secondary amine can react with reducing sugars, such as lactose, to undergo the Maillard reaction, which produces *N*-formyl desloratadine as one of the identified degradants (Scheme 233–234-2) [8, 9]. Yu et al. reported that desloratadine underwent the Maillard degradation with maltose, an impurity in starch, to yield an Amadori degradant during a compatibility study between desloratadine and starch [10]. The Maillard reaction consists of numerous individual



Scheme 233–234-2 Degradation of desloratadine due to the Maillard reaction.



Scheme 233–234-3 Degradation of desloratadine due to reaction with an impurity of starch. *Source:* Yu et al. [10] / with permission of Elsevier.



Scheme 233–234-4 Oxidative degradation of desloratadine to form dehydrodesloratadine [8].

reactions, and its early-stage degradants include glycosylamine and Amadori rearrangement products [11]. Further degradation from the Amadori degradants can occur, depending on the extent of a particular stability or forced degradation study.

In the same study, Yu et al. also found that the secondary amine group of desloratadine reacted with an isomer of acetylformoin, also an impurity of polysaccharide origin, to produce a novel degradant (Scheme 233–234-3). On the other hand, it was revealed that dehydrodesloratadine was identified in one of the experimental formulae [8]; this degradant was obviously resulting from the oxidation that occurred at either the 9- or 10-position of the tricyclic ring, both of which are benzylic-type positions and hence would be susceptible to free-radical-mediated oxidation (Scheme 233–234-4). Such an oxidation could also take place with loratadine, although it has not been reported in the literature.

References

1 Loratadine. https://en.wikipedia.org/wiki/Loratadine (accessed January 16 2024).

2 AlMasoud, N., Bakheit, A.H., Alshammari, M.F.M. et al. (2022). Loratadine. *Prof. Drug Sub. Excip. Rel. Methodol.* 47: 55–90.

3 Munayyer, F.J., Guazzo, F., Stupak, E.I. et al. (2003). Stabilized antihistamine syrup. US Patent 6,514,520 B2. Patent filed 05 July 2001. Patent granted 4 February 2003.

4 Dagar, A., Gudi, S., Opawale, F. (2007). Oral liquid loratadine formulations and methods. US Patent Application 2007/0286875 A1. Application filed 6 June 2007.

5 Eyjolfsson, R. (2003). Loratadine: hydroxymethylation in syrup. *Pharmazie* 58: 154.

6 Wang, G., Cao, J., Gao, L. et al. (2017). Metal-free synthesis of C-4 substituted pyridine derivatives using pyridine-boryl radicals via a radical addition/coupling mechanism: a combined computational and experimental study. *J. Am. Chem. Soc.* 139: 3904–3910.

7 El Ragehy, N.A., Badawey, A.M., and Khateeb, S.Z.-El. (2002). Stability indicating methods for the determination of loratadine in the presence of its degradation product. *J. Pharm. Biomed. Anal.* 28: 1041–1053.

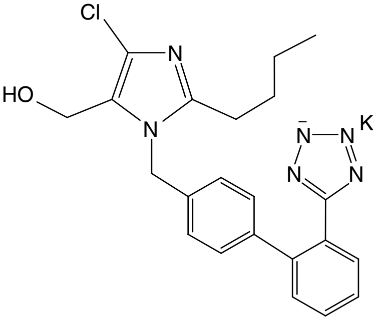
8 Rahul, G.S., Subbappa, P.K., Kodipyaka, R. et al. (2007). Stabilized loratadine compositions. US Patent Application 2007/0014855 A1. Application filed 12 July 2006.

9 Mehta, P.R., Bushan, I., Nasare, V.D. et al. (2006). Stabilized desloratadine composition. PCT Patent Application WO 2006/020534 A2. Application filed 8 August 2005.

10 Yu, X., Buevich, A.V., Li, M. et al. (2013). A compatibility study of a secondary amine active pharmaceutical ingredient with starch: identification of a novel degradant formed between desloratadine and a starch impurity using LC–MS^n and NMR spectroscopy. *J. Pharm. Sci.* 102: 717–731.

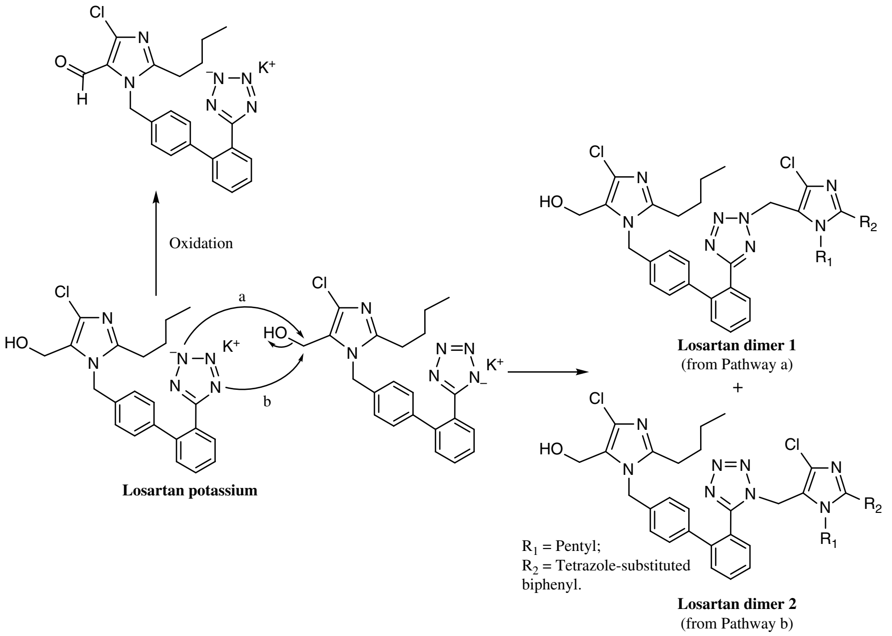
11 Li, M. (2012). Section 5.1.1. Degradation via the Maillard reaction. In: *Organic Chemistry of Drug Degradation*, 150–153. Cambridge, UK: RSC Publishing.

235: Losartan [WHO List of Essential Medicines]



Chemical name: (2-Butyl-4-chloro-1-{[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-imidazol-5-yl)methanol
Trade name: Cosaar
Formula: $C_{22}H_{23}ClN_6O$
Monoisotopic protonated ion (*m/z*): 423.1695
Molecular weight: 422.92
CAS number: 114798-26-4
Salt form: Potassium salt

Losartan is a nonpeptide angiotensin II receptor blocker (ARB) which is utilized, in the form of its potassium salt, for the treatment of hypertension as well as heart



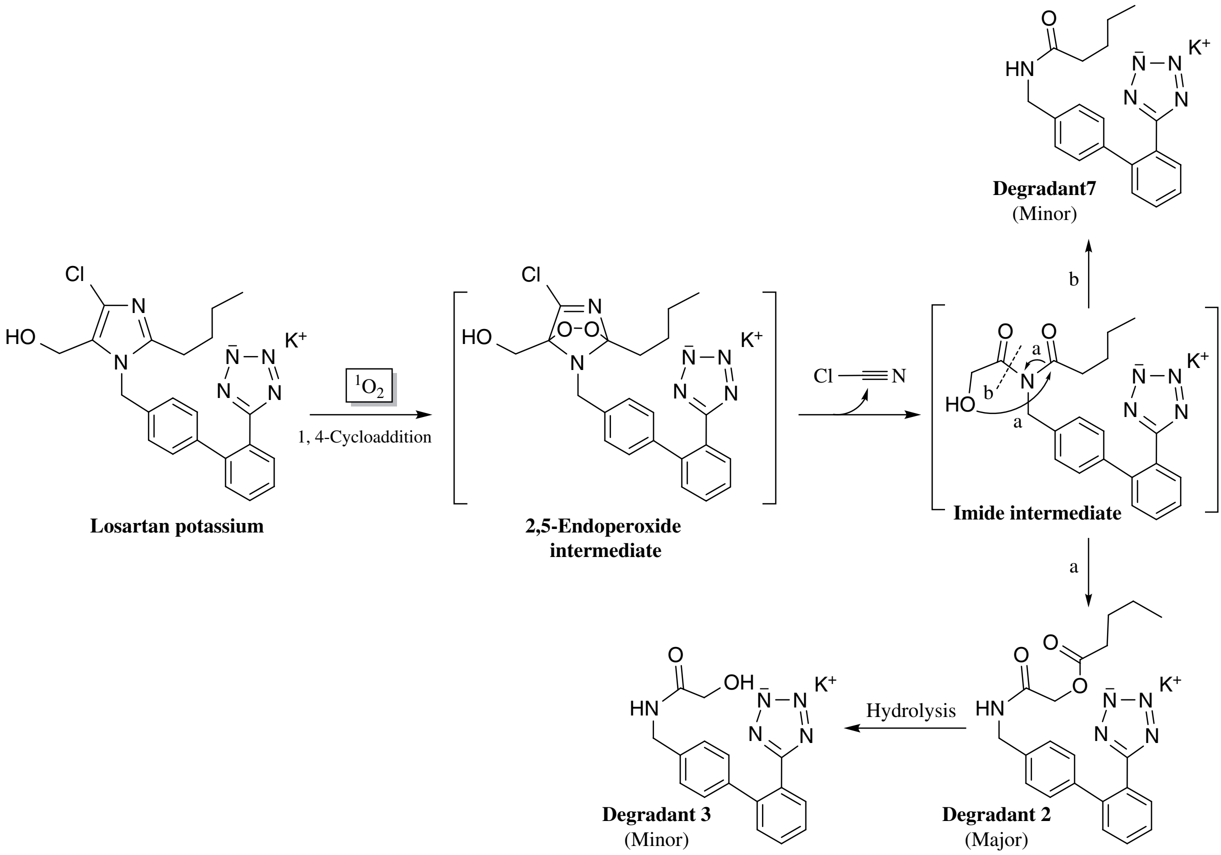
Losartan potassium

Losartan dimer 1
(from Pathway a)
+

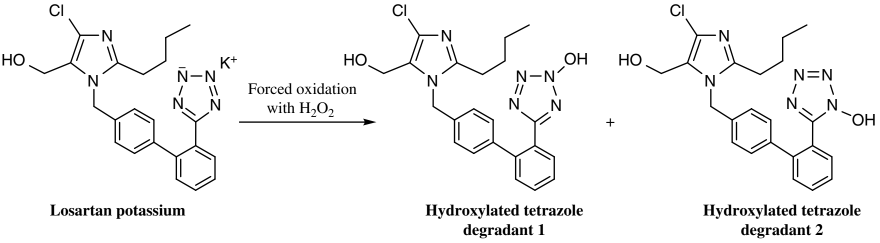
R$_1$ = Pentyl;
R$_2$ = Tetrazole-substituted biphenyl.

Losartan dimer 2
(from Pathway b)

Scheme 235-1 Oxidative and dimeric degradation pathways of losartan observed under long-term and accelerated stability studies [2, 3].



Scheme 235-2 Photo-sensitized oxidation of losartan. The numbering of degradants follows that of Seburg et al. [4]. *Source:* Seburg et al. [4] / with permission of Elsevier.



Scheme 235-3 Oxidation of losartan potassium with hydrogen peroxide.
Source: Singamsetti et al. [6] / John Wiley & Sons.

failure and left ventricular enlargement [1]. It was developed by Merck & Co., Inc. and approved in the United States in 1995.

Chemically, losartan is a highly substituted imidazole derivative, among which the 5-hydroxymethyl substituent could be susceptible to degradation. In a study of forced degradation of losartan tablets by Zhao et al., three degradants (≤0.1%) were observed in the severely stressed tablets, among which the aldehyde degradant resulted from oxidation of the hydroxymethyl group, while the two dimeric degradants were formed via SN2 nucleophilic substitution (Scheme 235-1). The two dimeric degradants were also observed during the stability study of losartan tablets at Merck [3].

During the development of a syrup formulation of losartan, a major degradant was observed, which was determined as a photo-sensitized degradant (Scheme 235-2) [4]. Although the species responsible for the photo-sensitization was not identified, it was clearly demonstrated by the authors that the degradation was mediated via singlet oxygen, a process known as "type II photosensitized oxidation" [5], which led to the destruction of the imidazole ring.

More recently, there have been a few forced degradation studies of losartan potassium. The vast majority of the degradants formed in these studies appear to be artificial degradants. For example, Singamsetti et al. reported two novel *N*-hydroxyl degradants produced under oxidative stress conditions (Scheme 235-3) [6]. Pandey et al. observed three novel dimeric degradants, which were formed via double cross-linkage between the two losartan molecules [7].

References

1 Losartan. https://en.wikipedia.org/wiki/Losartan (accessed January 28 2024).
2 Zhao, Z., Wang, Q., Tsai, E.W. et al. (1999). Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS. *J. Pharm. Biomed. Anal.* 20: 129–136.
3 McCarthy, K.E., Wang, Q., Tsai, E.W. et al. (1998). Determination of losartan and its degradates in COZAAR® tablets by reversed-phase high-performance thin-layer chromatography. *J. Pharm. Biomed. Anal.* 17: 671–677.
4 Seburg, R.A., Ballard, J.M., Hwang, T.-L., and Sullivan, C.M. (2006). Photosensitized degradation of losartan potassium in an extemporaneous suspension formulation. *J. Pharm. Biomed. Anal.* 42: 411–422.

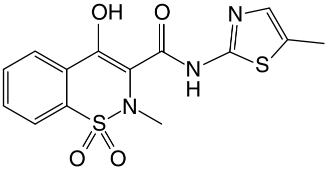
5 Li, M. (2012). Photochemical degradation. In: *Organic Chemistry of Drug Degradation*. Cambridge, UK: RSC Publishing.

6 Singamsetti, J.M., Gandham, H., Geereddy, M.K.R. et al. (2022). Isolation and structural characterization of two novel oxidative degradation products of losartan potassium active pharmaceutical ingredient using advanced analytical techniques. *Rapid Comm. Mass Spec.* 37: e9432.

7 Pandey, A.K., Rapolu, R., Raju, C.K. et al. (2016). The novel acid degradation products of losartan: isolation and characterization using Q-TOF, 2D-NMR and FTIR. *J. Pharm. Biomed. Anal.* 120: 65–71.

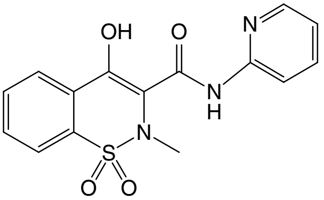
236–237: Meloxicam, Piroxicam

236: Meloxicam



Chemicalname:4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide
Trade name: Metacam, Mobic, others
Formula: $C_{14}H_{13}N_3O_4S_2$
Monoisotopic protonated ion (*m/z*): 352.0420
Molecular weight: 351.40
CAS number: 71125-38-7

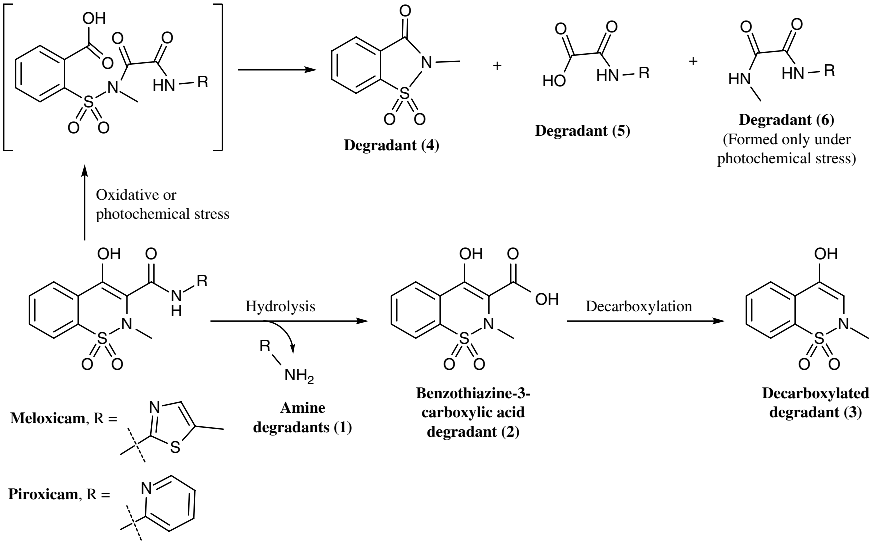
237: Piroxicam



Chemical name: 4-Hydroxy-2-methyl-*N*-(2-pyridinyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide
Trade name: Feldene
Formula: $C_{15}H_{13}N_3O_4S$
Monoisotopic protonated ion (*m/z*): 332.0700
Molecular weight: 331.35
CAS number: 36322-90-4

Meloxicam and piroxicam are nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit cyclooxygenase (COX) enzymes, particularly COX-2, a member of the prostaglandin family that triggers inflammation. Clinically, they are used to treat pain and inflammation in rheumatic diseases and osteoarthritis [1, 2]. Meloxicam was developed by Boehringer Ingelheim and approved in the United States in 2000, while piroxicam was developed by Pfizer and approved for clinical use in 1982.

Both drug molecules contain a common 4-hydroxybenzothiazine core, which connects to their heterocyclic amine moieties via an amide linkage, respectively. The amide linkage would be susceptible to hydrolytic degradation, leading to the formation of the corresponding amines (**1**) and the benzothiazine-3-carboxylic acid degradant (**2**, Scheme 236–237) [3]. Under forced oxidative and photochemical degradation conditions, both meloxicam and piroxicam produced degradants (**4**–**6**)



Scheme 236–237 Degradation pathways of meloxicam and piroxicam.

resulting from the destruction of the benzothiazine core, besides the acid degradants (**2**), while the acid degradant (**2**) could undergo decarboxylation in neutral aqueous solution at 80°C [4]. It is worth noting that the sulfonamide bond, which is usually very stable under forced hydrolytic degradation conditions, was cleaved under the photochemical degradation conditions to yield Degradant 6.

References

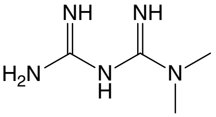
1 Meloxicam. https://en.wikipedia.org/wiki/Meloxicam (accessed January 30 2024).

2 Piroxicam. https://en.wikipedia.org/wiki/Piroxicam (accessed January 30 2024).

3 Taha, E.A., Salama, N.N., and Fattah, L.S.A. (2006). Stability-indicating methods for determination of meloxicam and tenoxicam in the presence of their degradation products. *Spectr. Lett.: An Int. J. Rapid Comm.* 35: 501–516.

4 Modhave, D.T., Handa, T., Shah, R.P., and Singh, S. (2011). Successful characterization of degradation products of drugs using LC-MS tools: application to piroxicam and meloxicam. *Anal. Methods* 3: 2864–2872.

238: Metformin [WHO List of Essential Medicines]

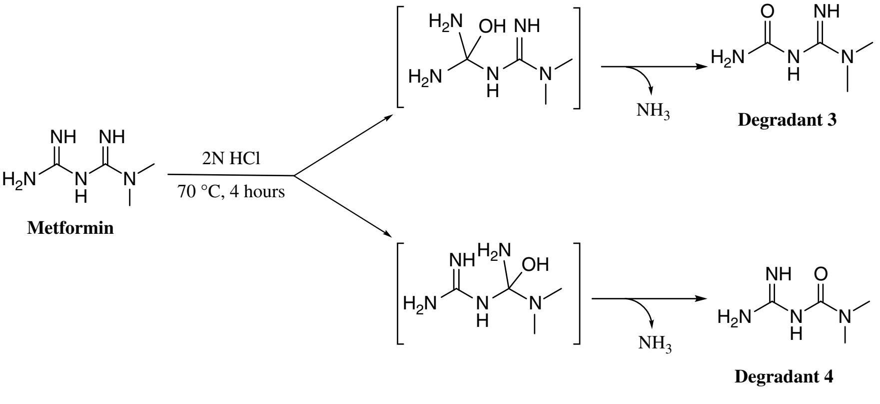


Chemical name: *N,N*-Dimethylimidodicarbonimidic diamide
Trade name: Glucophage
Formula: $C_4H_{11}N_5$
Monoisotopic protonated ion (*m/z*): 130.1087
Molecular weight: 129.17
CAS number: 657-24-9
Salt form: HCl salt

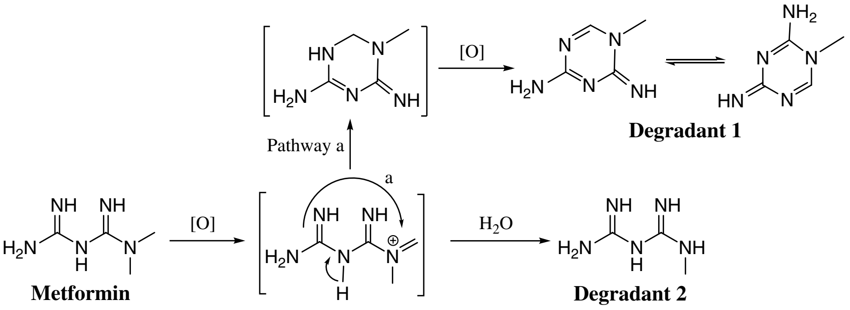
Metformin is the major first-line treatment for type II diabetes, and in 2021, it was the second most prescribed medication in the United States [1]. It was synthesized in the 1920s based on the lead compound, galegine, a natural product present in the medicinal herb *Galega officinalis,* with its usage since medieval times [2]. The medical use of metformin was first introduced in France in 1957, which is long before its introduction into the United States in 1995 [1]. Despite its long clinical history, during which time it has been demonstrated that metformin enhances hepatic insulin sensitivity, its exact molecular mechanism of action remains debatable [2].

Chemically, metformin is a nearly symmetric biguanide consisting of two coupled guanidine moieties. Its main degradation pathway is hydrolytic deamination. In a forced degradation study, metformin was found to produce two degradants under the acidic condition of 2 N HCl/70°C/4 hours (Scheme 238-1), while being stable in the alkaline condition of 2 N NaOH/70°C/2 hours [3].

Under a harsh oxidative stress condition (30% H_2O_2, 70°C, 2 hours), Degradants **1** and **2** were identified by Luo et al.; nevertheless, no formation mechanism was proposed by the original authors [3]. It might be possible that the two degradants were



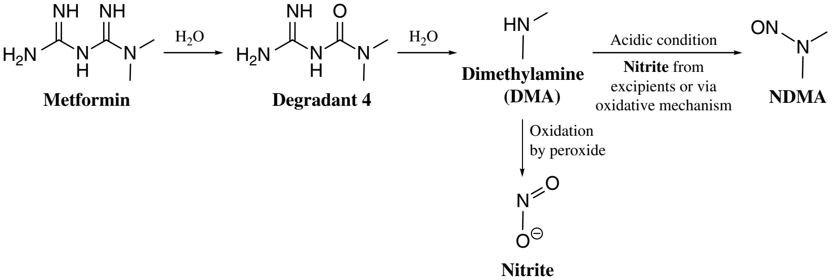
Scheme 238-1 Hydrolytic deamination of metformin. The numbering of the degradants follows that of Luo et al. [3]. *Source:* Luo et al. [3] / with permission of Elsevier.



Scheme 238-2 Forced degradation of metformin in 30% H_2O_2 at 70°C for 2 hours. The numbering of the degradants follows that of Luo et al. [3]. *Source:* Luo et al. [3] / with permission of Elsevier.

formed via a common iminium intermediate (Scheme 238-2). Both degradants were previously reported by Collin et al. in their experiments of gamma-irradiation of metformin solutions [4, 5]. In the accelerated stability study of metformin drug substance (40°C/75%RH) performed by Luo et al., Degradants **2** and **3** were produced, indicating they are more likely real degradants [3].

Recently, metformin drug products in extended release formulations were found to contain trace amounts of *N*-nitrosodimethylamine (NDMA) [6], a genotoxic nitrosamine impurity with an allowable intake (AI) of 96 ng/day [7]. Various investigations have indicated that NDMA is both a process impurity and degradant in the extended release formulations [8–12]. With regard to the formation of NDMA in metformin drug products, a study by Schlingemann et al. showed that metformin drug substance was not a significant source for NDMA and that the majority of NDMA was formed during the manufacturing steps that introduced heat and residual nitrite [9]. Their study also showed that the levels of NDMA increased during the storage of the formulated products, but the increase was minor and not shelf-life limiting for the products in their study. In another study of probing NDMA formation in metformin drug products, solutions and film-coated tablets with no obvious source of nitrosating agents were examined for the plausible formation mechanism of NDMA [11]. The results suggested that in solutions, dimethylamine, a degradant of metformin, could be converted into nitrite in the presence of hydrogen peroxide, and the nitrite



Scheme 238-3 Possible mechanisms for the formation of NDMA in metformin finished drug products.

formed could then turn dimethylamine into NDMA at acidic pH. Although the observation that metformin can be oxidized by hydrogen peroxide to produce ppm-levels of NDMA has been confirmed independently [12], this oxidative mechanism only contributed to less than 10% NDMA formation in the solid dosage of film-coated tablets according to Jires et al. [11]. The latter authors also found that spiking sodium carbonate into the excipients during the tablet manufacturing process could produce tablets with no quantifiable level of NDMA, even in tablet batches where dimethylamine and hydrogen peroxide were intentionally added. The inhibitory effect of an alkaline micro-environment in solid dosage achieved by sodium carbonate was also reported by Hao et al. [12]. The possible mechanisms of the formation of NDMA in metformin drug products are presented in Scheme 238-3.

References

1 Metformin. https://en.wikipedia.org/wiki/Metformin (accessed January 31 2024).

2 Rena, G., Hardie, D., and G., Pearson, E.R. (2017). The mechanisms of action of metformin. *Diabetologia* 60: 1577–1585.

3 Luo, M., Zheng, L., Wang, S. et al. (2021). Characterization of four major degradation products in metformin by 2D LC-QTOF/MS/MS. *J. Pharm. Biomed. Anal.* 192: 113662.

4 Collin, F., Khoury, H., Bonnefont-Rousselot, D. et al. (2004). Liquid chromatographic/electrospray ionisation mass spectrometric identification of the oxidation end-products of metformin in aqueous solutions. *J. Mass. Spectrom.* 39: 890–902.

5 Khouri, H., Collin, F., Bonnefont-Rousselot, D. et al. (2004). Radical-induced oxidation of metformin. *Eur. J. Biochem.* 271: 4745–4752.

6 FDA US FDA's search list of recalled metformin products. https://www.fda.gov/drugs/drug-safety-and-availability/search-list-recalled-metformin-products (accessed February 2 2024).

7 US FDA Guidance Control of nitrosamine impurities in human drugs. https://www.fda.gov/regulatory-information/search-fda.guidancedocuments/control-nitrosamine-impurities-human-drugs (accessed February 2 2024).

8 Nasr, N.E.H., Metwaly, M.G., Ahmed, E.O. et al. (2021). Investigating the root cause of *N*-nitrosodimethylamine formation in metformin pharmaceutical products. *Expert Opin. Drug Saf.* 20: 855–862.

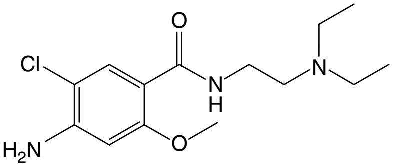
9 Schlingemann, J., Boucley, C., Hickert, S. et al. (2022). Avoiding *N*-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite. *Int. J. Pharm.* 620: 121740.

10 Jireš, J., Kalášek, S., Gibala, P. et al. (2021). Insight into the formation of *N*-nitrosodimethylamine in metformin products. *J. Pharm. Biomed. Anal.* 195: 113877.

11 Jireš, J., Dousa, M., Gibala, P., and Kubelka, T. (2022). *N*-Nitrosation in the absence of nitrosating agents in pharmaceuticals? *J. Pharm. Biomed. Anal.* 218: 114872.

12 Hao, G., Hu, R., Wang, X. et al. (2022). *N*-Nitrosodimethylamine formation in metforminhydrochloride sustained-release tablets: effects of metformin and hypromellose used in drug product formulation. *J. Pharm. Biomed. Anal.* 222: 115066.

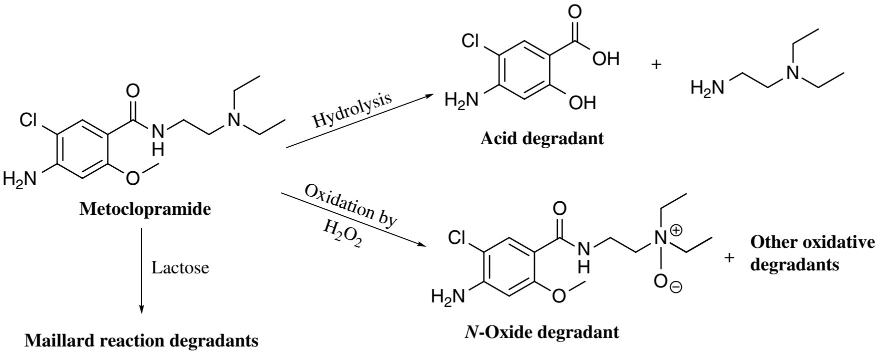
239: Metoclopramide [WHO List of Essential Medicines]



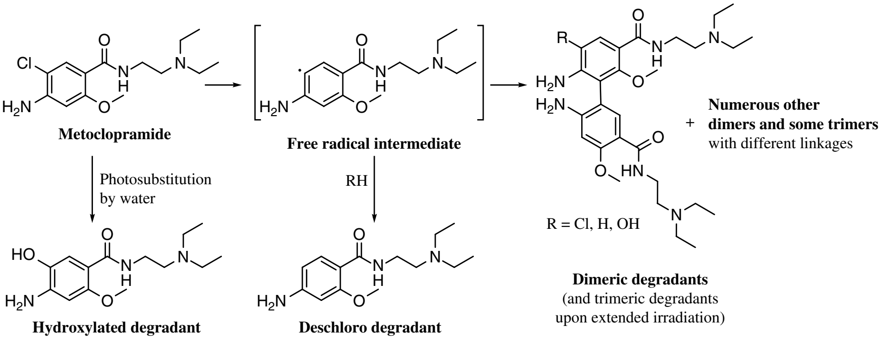
Chemical name: 4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide
Trade name: Primperan, Reglan, others
Formula: $C_{14}H_{22}ClN_3O_2$
Monoisotopic protonated ion (*m/z*): 300.1473
Molecular weight: 299.80
CAS number: 364-62-5
Salt form: HCl salt

Metoclopramide is an antagonist for the dopamine D_2 receptor and 5-HT_3 receptor, and an agonist for the 5-HT_4 receptor. Clinically, it is used as an antiemetic agent to treat and prevent nausea and vomiting, as well as to treat migraine headaches [1]. It was developed and approved for medical use in the 1960s by Laboratoires Delagrange, a company later acquired by SynthoLab and eventually became part of Sanofi.

Metoclopramide contains three functional groups susceptible to degradation: the amide, tertiary amino, and aniline amino group. In a forced degradation study by Sowjanya et al. [2], limited hydrolysis of the amide linkage was observed under both acidic and alkaline stress conditions (1 N HCl or 1 N NaOH solution, 80°C, 5 hours), seemingly with more hydrolysis under the alkaline condition than the acidic one (Scheme 239-1). The results of the hydrolytic forced degradation suggested that metoclopramide would be quite resistant to hydrolytic degradation in its formulated products. Under the oxidative stress condition with hydrogen peroxide (6% H_2O_2, 40°C, 6 hours), numerous degradants were observed, among which two were large



Scheme 239-1 Hydrolytic and oxidative degradation of metoclopramide.



Scheme 239-2 Photochemical degradation of metoclopramide.

degradants and two were medium degradants, while the rest were quite small. Among all the degradants, only one of the two major ones was identified as the *N*-oxide degradant. It could be reasonably assumed that the other oxidative degradants may be largely due to the oxidation of the aniline moiety. On the other hand, the aniline group could undergo the Maillard reaction with reducing sugars. In a compatibility study with lactose by Qiu et al. [3], metoclopramide was degraded in solid mixtures with lactose under heat and moisture. The degradant was presumed to be the corresponding ketosamine (i.e., the glycosylamine degradant) by the authors, which may not be entirely correct, as the initially formed ketosamine degradant could undergo the Amadori rearrangement to produce further degradants [4]. A pseudo-second-order kinetic model was developed for the solid-state degradation, and the activation energy was found to be 53.8 kcal/mol.

Metoclopramide is a substituted benzamide drug that is susceptible to photochemical degradation and thus requires protection from light [5, 6]. In aqueous matrices, metoclopramide was found to decompose rather easily under irradiation of either UV or sunlight [7, 8]. In the photo stress study by Maquille and Jiwan in which UV irradiation at 254 nm was used [7], the initial primary degradation appeared to be a free-radical-mediated dechlorination process, which was followed by abstraction of a hydrogen radical, substitution by a water molecule, and numerous dimerization products (a few trimers were also observed, particularly upon extended irradiation), respectively (Scheme 239-2). The dimers and trimers are linked together through the phenyl group of the drug molecule.

References

1 Metoclopramide. https://en.wikipedia.org/wiki/Metoclopramide (accessed February 4, 2024).

2 Sowjanya, P., Shanmugasundaram, P., Naidu, P., and Singamsetty, S.K. (2013). Novel validated stability-indicating UPLC method for the determination of metoclopramide and its degradation impurities in API and pharmaceutical dosage form. *J. Pharm. Res.* 6: 765–773.

3 Qiu, Z., Stowell, J.G., Morris, K.R. et al. (2005). Kinetic study of the Maillard reaction between metoclopramide hydrochloride and lactose. *Int. J. Pharm.* 303: 20–30.

4 Li, M. (2012). Section 5.1.1. Degradation via the Maillard reaction. In: *Organic Chemistry of Drug Degradation*, 150–153. Cambridge, UK: RSC Publishing.

5 Weclawska, K. and Siminska, E. (1985). The effect of visible and ultraviolet radiation on metoclopramide solutions. *Acta Pol. Pharm.* 42: 555–560.

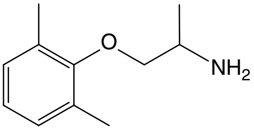
6 Sánchez-Quiles, I., Nájera-Pérez, M.D., Espuny-Miró, A., and Titos-Arcos, J.C. (2011). Review of the stability of photosensitive medication. *Farm. Hosp.* 35: 204–215.

7 Maquille, A. and Jiwan, J.-L.H. (2009). LC–MS characterization of metoclopramide photolysis products. *J. Photochem. Photobiol. A: Chem.* 205: 197–202.

8 Dabić, D., Hanževački, M., Škorić, I. et al. (2022). Photodegradation, toxicity and density functional theory study of pharmaceutical metoclopramide and its photoproducts. *Sci. Total Environ.* 807: 150694.

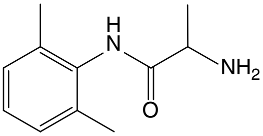
240–241: Mexiletine, Tocainide

240: Mexiletine



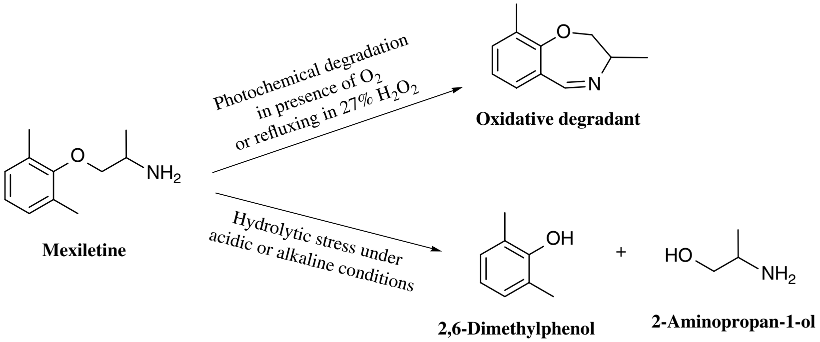
Chemical name: (*R,S*)-1-(2,6-dimethylphenoxy)propan-2-amine
Trade name: Mexitil, NaMuscla
Formula: $C_{11}H_{17}NO$
Monoisotopic protonated ion (*m/z*): 180.1383
Molecular weight: 179.26
CAS number: 31828-71-4
Salt form: HCl salt

241: Tocainide



Chemical name: *N*-(2,6-dimethylphenyl)alaninamide
Trade name: Tonocard
Formula: $C_{11}H_{16}N_2O$
Monoisotopic protonated ion (*m/z*): 193.1335
Molecular weight: 192.26
CAS number: 41708-72-9

Mexiletine is a nonselective voltage-gated sodium channel blocker, which is clinically used for treating arrhythmias, chronic pain, and myotonia [1]. Chemically, mexiletine is a phenylether-substituted primary amine. Although there were some forced degradation studies, it appears only one of them performed structural elucidation of degradation products formed under photochemical or oxidative conditions [2]. Irradiation of mexilentine in solid or solution by either sunlight or UV produced a cyclized degradant as the main degradation product (Scheme 240–241). This degradant was proven to be an oxidative degradant, as it could not be produced in the absence of oxygen during the photolysis. Furthermore, it could also be generated under nonphotochemical oxidative conditions, such as using hydrogen peroxide or potassium permanganate. Interestingly, tocainide, a structural analog of mexiletine, was found to be stable under irradiation by sunlight over an extended period [2]. In two forced degradation studies in which mexiletine was subjected to both acidic and alkaline conditions, significant degradation occurred in both cases [3, 4]. In a stability study of extemporaneous solutions of mexiletine, degradation was also observed [5]. Nevertheless, the structural elucidation of these



Scheme 240–241 Degradation pathways of mexilentine.

degradants was not attempted [3, 4] or was not successful [5]. It can be surmised, however, that one of the expected degradants would be 2,6-dimethylphenol, a related substance listed in US Pharmacopeia and British Pharmacopeia [4].

References

1 Mexiletine. https://en.wikipedia.org/wiki/Mexiletine (accessed February 4 2024).

2 Takács, M., Vámos, J., Tóth, G., and Mikó-Hideg, Z. (2000). Photochemical and chemical oxidation of mexiletine and tocainide. Structure elucidation of the major products. *Arch. Pharm. Pharm. Med. Chem.* 333: 48–52.

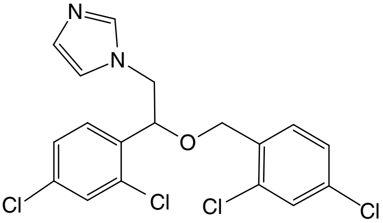
3 Kaushik, S. and Alexander, K.S. (2003). A modified reverse-phase HPLC method for the analysis of mexiletine hydrochloride. *J. Liq. Chromat. Rel. Tech.* 26: 1287–1296.

4 Belal, T.S., Haggag, R.S., and Shaalan, R.A. (2008). Selective and stability-indicating methods for the simultaneous determination of mexiletine hydrochloride and/or its related substance: 2,6-dimethylphenol. *J. AOAC Int.* 91: 720–730.

5 Alexander, K.S. and Kaushik, S. (2004). Extemporaneous formulation and stability testing of mexilentine hydrochloride solution. *Int. J. Pharm. Comp.* 8: 147–152.

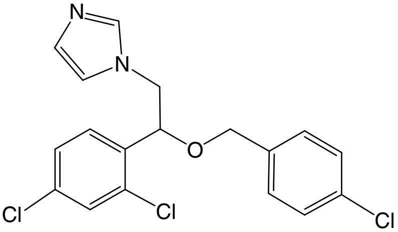
242–243: Miconazole, Econazole

242: Miconazole [WHO List of Essential Medicines]



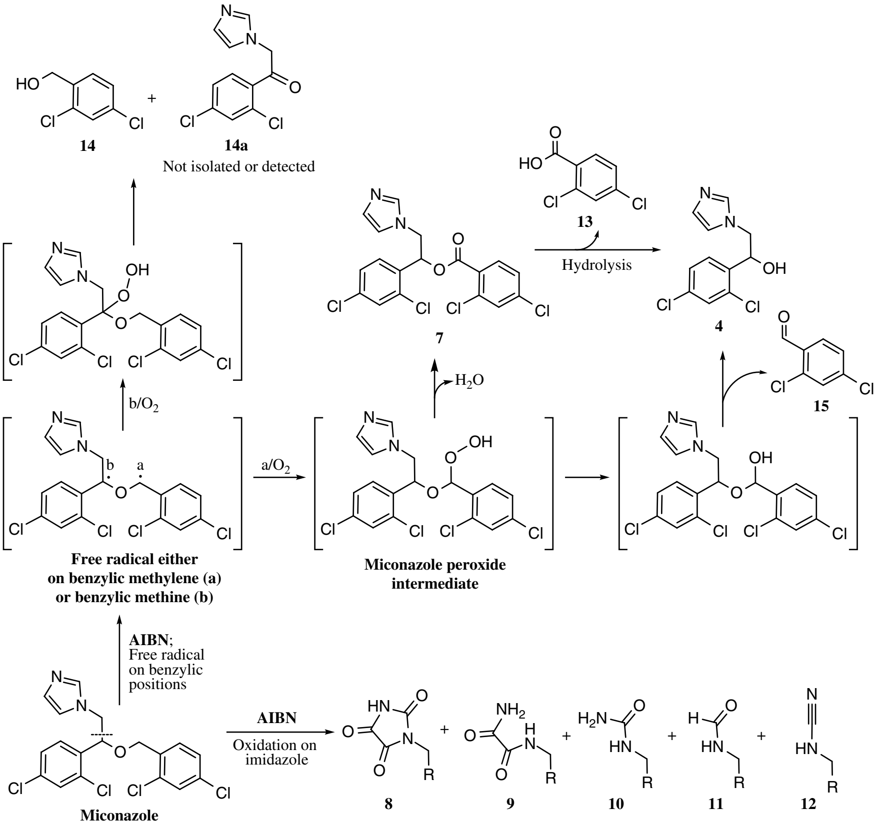
Chemical name: (*R,S*)-1-(2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-1 *H*-imidazole
Trade name: Monistat, others
Formula: $C_{18}H_{14}Cl_4N_2O$
Monoisotopic protonated ion (*m/z*): 414.9933
Molecular weight: 416.12
CAS number: 22916-47-8
Salt form: Nitrate

243: Econazole



Chemical name: (*R,S*)-1-{2-[(4-Chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl}-1 *H*-imidazole
Trade name: Spectrazole, others
Formula: $C_{18}H_{15}Cl_3N_2O$
Monoisotopic protonated ion (*m/z*): 381.0323
Molecular weight: 381.68
CAS number: 27220-47-9
Salt form: Nitrate

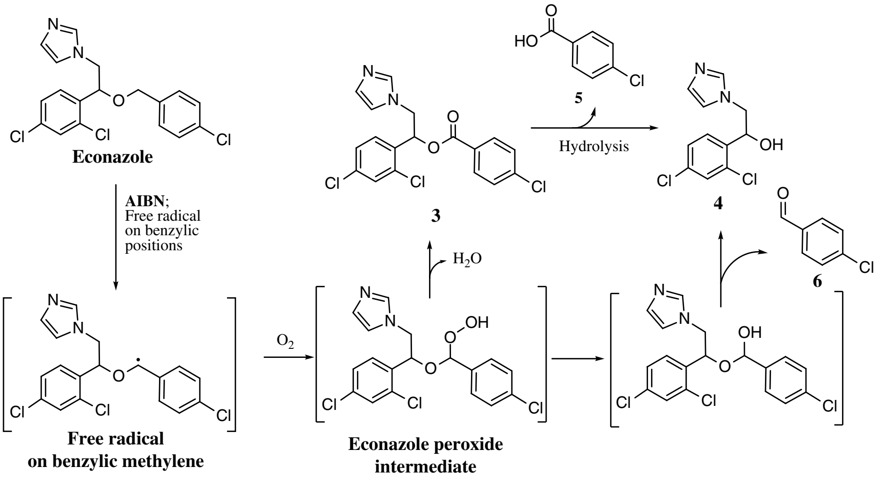
Miconazole is a first-generation antifungal drug in the imidazole family [1], which is typically in topical formulations and clinically used to treat ring worm, pityriasis versicolor, and yeast infections of skin and vagina [2]. It was developed by Janssen and approved for medical use in 1969 [3]. Chemically, miconazole is a substituted dibenzylic ether with a distinctive imidazole group. Both the ether and imidazole moieties were found to undergo autooxidative degradation in the presence of



Scheme 242–243-1 Autooxidative degradation of miconazole by catalysis of AIBN, a free-radical initiator. The numbering of the degradants follows that of Oyler et al. [4]. *Source:* Oyler et al. [4] / with permission of Elsevier.

free-radical initiator, AIBN [4]. A great number of degradants were isolated and their structures identified, which resulted from the oxidation of the benzylic positions and imidazole, respectively (Scheme 242–243-1). The radical formation occurred mostly on the benzylic methylene position (a, Scheme 242–243-1), probably due to its less steric hindrance, followed by the formation of the hydroperoxide intermediate, which resulted in the formation of degradants **7**, **13**, and **15**, respectively. The formation of the other hydroperoxide intermediate on the more steric benzylic methine position (b, Scheme 242–243-1) led to the production of degradant **14** . According to this formation pathway (b), degradant **14a** should also be expected to form; nevertheless, this degradant was not reported in the study [4].

Under the autooxidative degradation condition with AIBN, another set of oxidative degradants (**8**–**12**) was also isolated and identified, which resulted from the oxidation on the imidazole ring. Such degradation usually occurred through reaction with singlet oxygen; a mechanism with triplet oxygen was proposed by the authors [4].



Scheme 242–243-2 Autooxidative degradation of econazole by catalysis of AIBN, a free-radical initiator. The numbering of the degradants follows that of Oyler et al. [4]. *Source:* Shafiei et al. [3] and Oyler et al. [4].

The same autooxidative degradation conditions were applied to econazole, a closely related analogous antifungal drug with one less chloro substituent than miconazole, which was also developed by Janssen and marketed in 1974 [1]. The oxidation that occurred on the benzylic positions was similar to that of miconazole (Scheme 242–243-2). However, no degradants resulting from the oxidation on the imidazole ring were isolated or identified; the authors speculated that some of these analogous degradants might be among the unknown peaks not isolated for identification [4].

Other first-generation imidazole antifungal drugs, such as isoconazole and dapaconazole, would be expected to undergo the same autooxidative degradation.

References

1 Shafieia, M., Peytonb, L., Hashemzadehc, M., and Foroumadi, A. (2020). History of the development of antifungal azoles: a review on structures, SAR, and mechanism of action. *Bioorg. Chem.* 104: 104240.

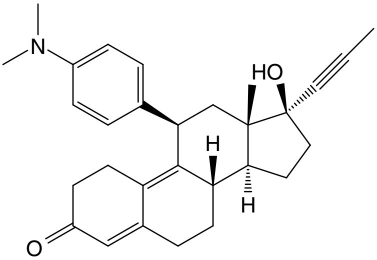
2 Miconazole. https://en.wikipedia.org/wiki/Miconazole (accessed February 4 2024).

3 Shafiei, M., Peyton, L., Hashemzadeh, M., and Foroumadi, A. (2020). History of the development of antifungal azoles: a review on structures, SAR, and mechanism of action. *Bioorg. Chem.* 104: 104240.

4 Oyler, A.R., Naldi, R.E., Facchine, K.L. et al. (1991). Characterization of autoxidation products of the antifungal compounds econazole nitrate and miconazole nitrate. *Tetrahedron* 47: 6549–6560.

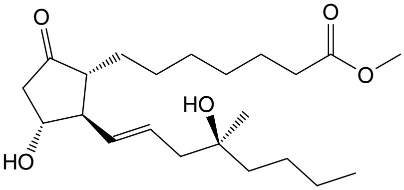
244–245: Mifepristone, Misoprostol

244: Mifepristone [WHO List of Essential Medicines]



Chemical name: (*8S,11R,13S,14S,17S*)-11-[4-(Dimethylamino)phenyl]-17-hydroxy-13-methyl-17-prop-1-ynyl-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta[a] phenanthren-3-one
Trade name: Mifegyne, Mifeprex, Korlym, others
Formula: $C_{29}H_{35}NO_2$
Monoisotopic protonated ion (*m/z*): 430.2741
Molecular weight: 429.60
CAS number: 84371-65-3

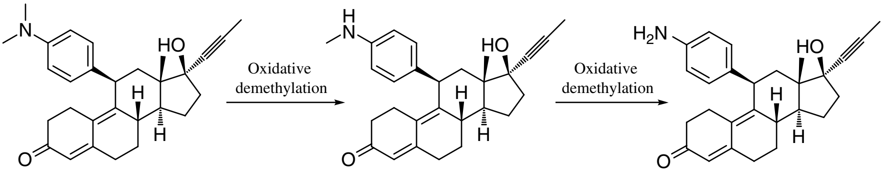
245: Misoprostol [WHO List of Essential Medicines]



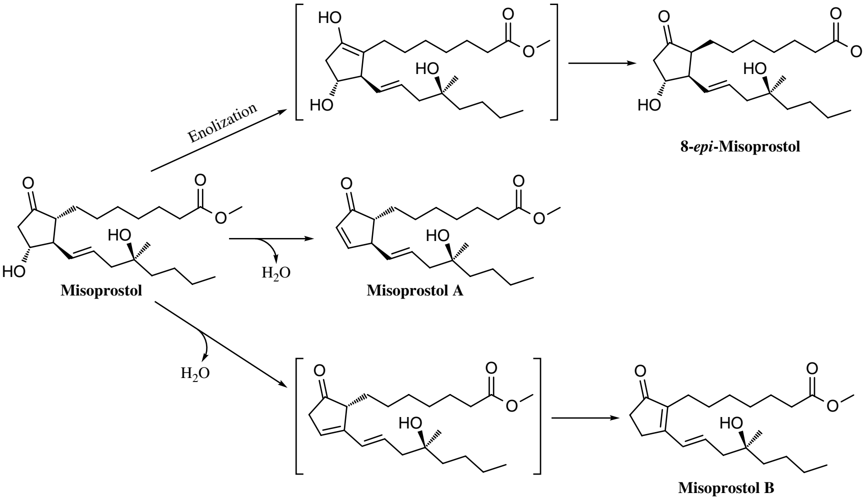
Chemical name: Methyl 7-((*1R,2R,3R*)-3-hydroxy-2-((*S,E*)-4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl)heptanoate
Trade name: Cytotec, Misodel, others
Formula: $C_{22}H_{38}O_5$
Monoisotopic protonated ion (*m/z*): 383.2792
Molecular weight: 382.54
CAS number: 59122-46-2

Mifepristone is also known as RU-486, produced in 1980 at the French pharmaceutical company Roussel-Uclaf with the original compound code RU-38486 [1]. It is an antiprogestogen and is clinically used for inducing medical abortion and managing early-stage miscarriage. Literature search indicated that the major degradation site is the dimethyl-amino moiety, which would be subject to oxidative demethylation to form the *N*-monodemethylated mifepristone as the major degradant and perhaps to a much less degree, the *N,N*-didemethylated mifepristone (Scheme 244–245-1) [2, 3].

Mifepristone is often used in combination with misoprostol, which is a prostaglandin analog, and when utilized as a single therapeutic agent, misoprostol is



Scheme 244–245-1 Oxidative demethylation of mifepristone. *Source:* Misoprostol [2] and Shi et al. [3].



Scheme 244–245-2 Degradation pathways of misoprostol per Hagen et al. [5] with modification. *Source:* Hagen et al. [5] / PLOS / CC BY 4.0.

indicated for treating post-partum hemorrhage (PPH), stomach and duodenal ulcers, among others [4, 5]. Misoprostol can undergo two types of degradation: epimerization and dehydration. The former degradation should lead to the formation of 8-*epi*-misoprostol, most likely mediated via enolization. Dehydration of the hydroxyl group on the 5-membered ring could take place in two directions: one leading to the formation of misoprostol A, while the other one to misoprostol B. In the paper by Hagen et al. [5], misoprostol B was proposed as forming from misoprostol A via isomerization. Nevertheless, it appears that the pathway proposed in Scheme 244–245-2 would be more probable from a mechanistic perspective.

References

1 Mifepristone. https://en.wikipedia.org/wiki/Mifepristone (accessed June 14 2024).

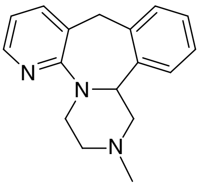
2 Misoprostol. https://en.wikipedia.org/wiki/Misoprostol (accessed June 14 2024).

3 Shi, J., Che, B.-Q., Zheng, Y. et al. (2014). HPLC determination of related substances in mifepristone capsules from different sources. (Original in Chinese). *Chin. J. Pharm. Anal.* 34: 480–484.

4 Iyer, J., Morgan, L.M., Harrison, P. et al. (2023). Applying material science principles to chemical stability: modelling solid state autoxidation in mifepristone containing different degrees of crystal disorder. *J. Pharm. Sci.* 112: 2463–2482.

5 Hagen, N., Bizimana, T., Kayumba, P.C. et al. (2020). Stability of misoprostol tablets collected in Malawi and Rwanda: importance of intact primary packaging. *PLoS ONE* 15 (9): e0238628. https://doi.org/10.1371/journal.pone.0238628.

246: Mirtazapine



Chemical name: 2-Methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-a]pyrido[2,3-c][2]benzazepine

Trade name: Remeron, others

Formula: $C_{17}H_{19}N_3$

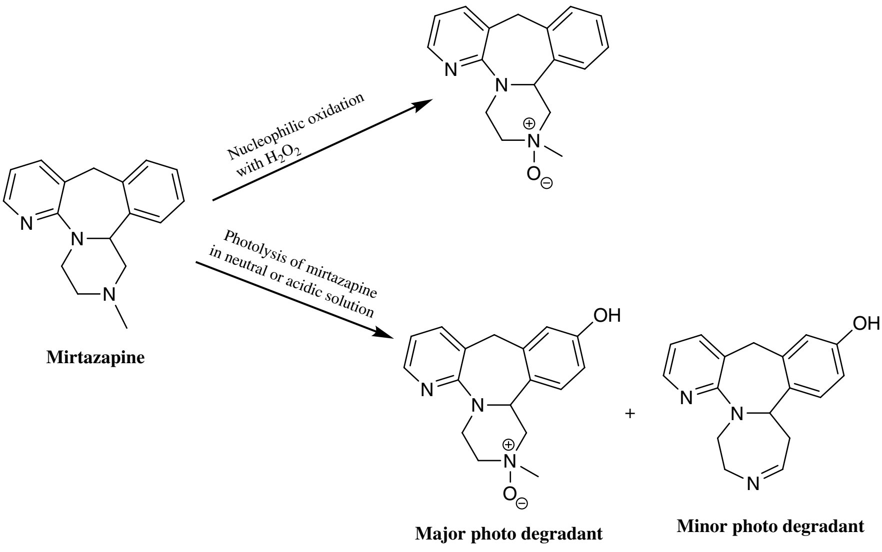
Monoisotopic protonated ion (*m/z*): 266.1652

Molecular weight: 265.36

CAS number: 85650-52-8

Salt form: HCl salt

Mirtazapine is a tricyclic antidepressant, which is often utilized in major depressive cases that are complicated by anxiety and insomnia [1]. Chemically, it is a racemic mixture of tetracyclic piperazine-azepine that contains a methylpyrazine moiety. The latter is a tertiary amine functionality and thus would be susceptible to nucleophilic oxidative degradation. Indeed, in a forced degradation study by Rao and Raju [2], mirtazapine *N*-oxide was found to form after treating mirtazapine in 3% hydrogen peroxide [2]. Under other forced degradation conditions, such as acidic, alkaline, thermal, and solid phase photolysis, mirtazapine was found to be stable. Nevertheless, in neutral and acidic solutions, photolysis led to the formation of a major and a minor photo-oxidative degradant (Scheme 246) [3]. The major



Scheme 246 Nucleophilic and photolytic oxidation of mirtazapine.

degradant was identified as a hydroxylated mirtazapine *N*-oxide, with the hydroxyl group being assigned to the phenyl group based on mass spectrometric data. The minor degradant was proposed to form via *N*-oxidation followed by ring expansion.

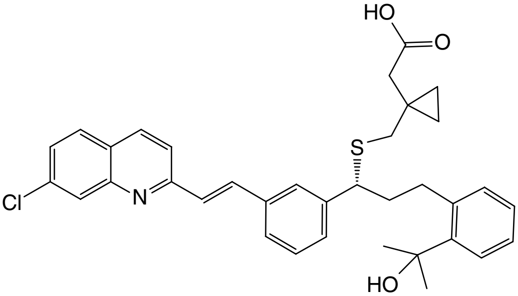
References

1 Mirtazapine. https://en.wikipedia.org/wiki/Mirtazapine (accessed February 4 2024).

2 Rao, R.N. and Raju, A.N. (2009). Development and validation of a reversed-phase HPLC method for separation and simultaneous determination of process-related substances of mirtazapine in bulk drugs and formulations. *J. Chromat. Sci.* 47: 223–230.

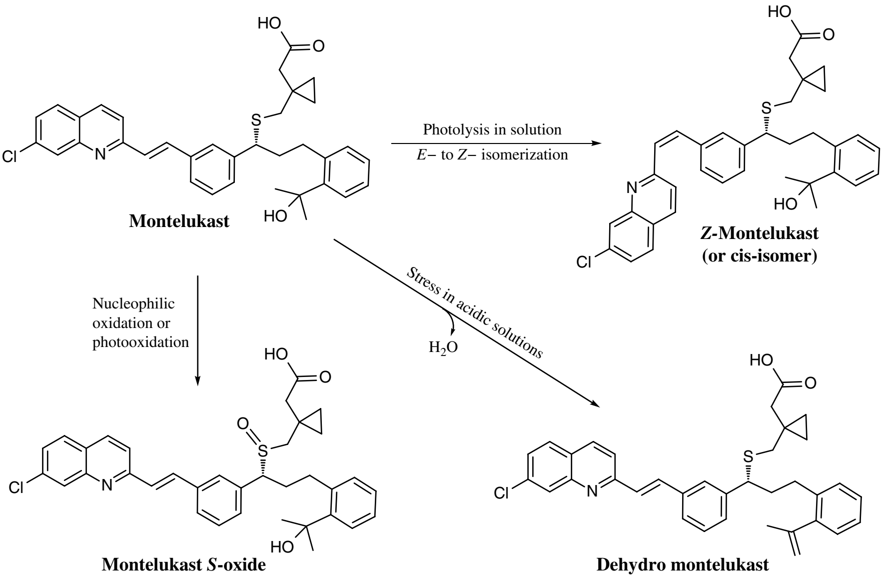
3 Anand, A. and Bansal, G. (2011). LC-UV and LC-MS studies on mirtazapine to characterize its degradation products. *J. Pharm. Educ. Res.* 2: 66–72.

247: Montelukast



Chemical name: (*E*)-2-(1-((1-(3-(2-(7-Chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl)acetic acid
Trade name: Singulair
Formula: $C_{35}H_{36}ClNO_3S$
Monoisotopic protonated ion (*m/z*): 586.2177
Molecular weight: 586.19
CAS number: 158966-92-8
Salt form: Sodium salt

Montelukast is a medication used in the maintenance treatment of asthma, but is not effective for acute asthma attack [1]. It is a leukotriene receptor antagonist that causes reduced inflammation and relaxation of smooth muscle. Montelukast was developed by the former Merck Frosst Centre for Therapeutic Research in Montreal,



Scheme 247 Degradation pathways of montelukast.

Canada [2], which was part of the Merck Research Laboratories. It was approved for medical use as its sodium salt in the United States in 1998. Chemically, montelukast is a stilbene-like derivative, and hence, its central carbon–carbon double bond would be susceptible to photochemical isomerization to produce the *Z*- or *cis*-isomer. Several papers have reported this isomeric degradation pathway (Scheme 247) [3–5]. Interestingly, it appeared that the *E*- to *Z*-isomerization (or *trans*- to *cis*- isomerization) only occurred in solution. In a photolysis study of uncoated chewable tablets, the major degradant was found to be the sulfoxide degradant, resulting from photooxidation of the thioether moiety of montelukast. The sulfoxide degradant was also the major degradation product observed in an accelerated stability study at 40°C/75%RH for 6 months [3]. Forced degradation studies under other degradation conditions showed that montelukast was stable in alkaline solutions, while in acidic solutions, it seemed to produce dehydro-montelukast (Scheme 247). On the other hand, the stress of montelukast in hydrogen peroxide solutions yielded the *S*-oxide degradant as expected [3, 5]. In principle, two diastereomers of the *S*-oxide degradant should be formed due to the presence of the sulfur lone pair electrons; nevertheless, it appears that only one peak of *S*-oxide was observed in the studies reported [3, 5], which may be attributed to the co-elution of the two isomers.

References

1 Montelukast. https://en.wikipedia.org/wiki/Montelukast (accessed February 4, 2024).

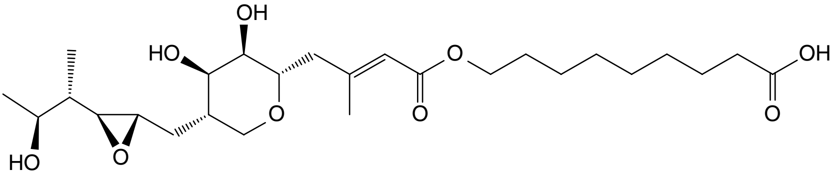
2 Young, R.N. (2001). Discovery of montelukast: a once-a-day oral antagonist of leukotriene D_4 for the treatment of chronic asthma. *Prog. Med. Chem.* 38: 249–277.

3 Al Omari, M.M., Zoubi, R.M., Hasan, E.I. et al. (2007). Effect of light and heat on the stability of montelukast in solution and in its solid state. *J. Pharm. Biomed. Anal.* 45: 465–471.

4 Roman, J., Breier, A.R., and Steppe, M. (2011). Stability indicating LC method to determination of sodium montelukast in pharmaceutical dosage form and its photodegradation kinetics. *J. Chromat. Sci.* 49: 540–546.

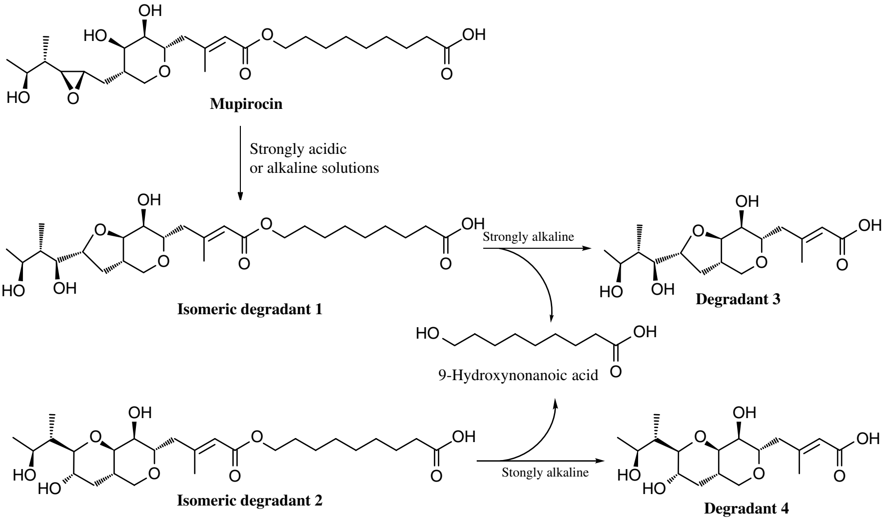
5 Tiwari, S.K., Singh, D.K., Ladumor, M.K. et al. (2018). Study of degradation behaviour of montelukast sodium and its marketed formulation in oxidative and accelerated test conditions and prediction of physicochemical and ADMET properties of its degradation products using ADMET Predictor™. *J. Pharm. Biomed. Anal.* 158: 106–118.

248: Mupirocin [WHO List of Essential Medicines]



Chemical name: 9-[(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl]-oxynonanoic acid
Trade name: Bactroban, others
Formula: $C_{26}H_{44}O_9$
Monoisotopic protonated ion (*m/z*): 501.3058
Molecular weight: 500.63
CAS number: 12650-69-0

Mupirocin is a natural product isolated from *Pseudomonas fluorescens* in 1971. It is used as a topical antibacterial for skin infections, particularly for treating methicillin-resistant *Staphylococcus aureus* (MRSA) in cases of impetigo [1]. Its mode of action is believed to be the inhibition of bacterial protein and RNA synthesis by inhibiting the bacterial isoleucyl-transfer RNA, which leads to cell death [2]. Chemically, it belongs to the pseudomonic acid family, with the predominant component being pseudomonic acid A, which accounts for more than 90% of the mixture. It contains an epoxide group which would react with the adjacent hydroxyl group on the six-membered ring in strongly acidic or alkaline solutions, leading to the formation of two isomeric degradants (**1** and **2**) [3, 4], In strongly alkaline solutions, the ester



Scheme 248 Degradation of mupirocin in strongly acidic or alkaline solutions.

functionality could be hydrolyzed to produce degradant **3** by eliminating 9-hydroxynonanoic acid. The degradation pathways of mupirocin are summarized in Scheme 248. In the pH range of 4–9, mupirocin was found to be reasonably stable [3].

References

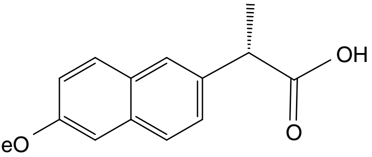
1 Mupirocin. https://en.wikipedia.org/wiki/Mupirocin (accessed February 4 2024).

2 Erwin, D.Z. and Chen, P. Mupirocin. https://www.ncbi.nlm.nih.gov/books/NBK599499/ (accessed February 27 2024).

3 Clayton, J.P., Oliver, R.S., Rogers, N.H., and King, T.J. (1979). The chemistry of pseudomonic acid. Part 3. The rearrangement of pseudomonic acid A in acid and basic solution. *J. Chem. Soc. Perkin Trans.* 1: 838–846.

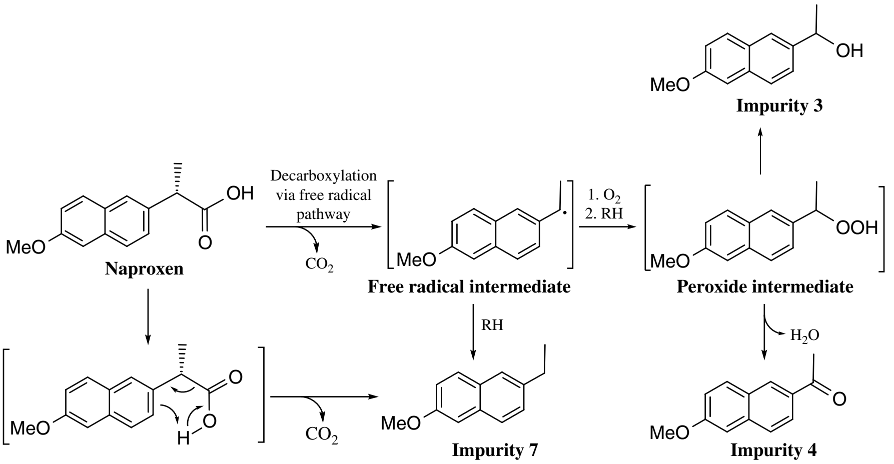
4 Scott, R.W., Murphy, A.C., Wu, J. et al. (2011). Mupirocin F: structure elucidation, synthesis and rearrangements. *Tetrahedron* 67: 5098–5106.

249: Naproxen

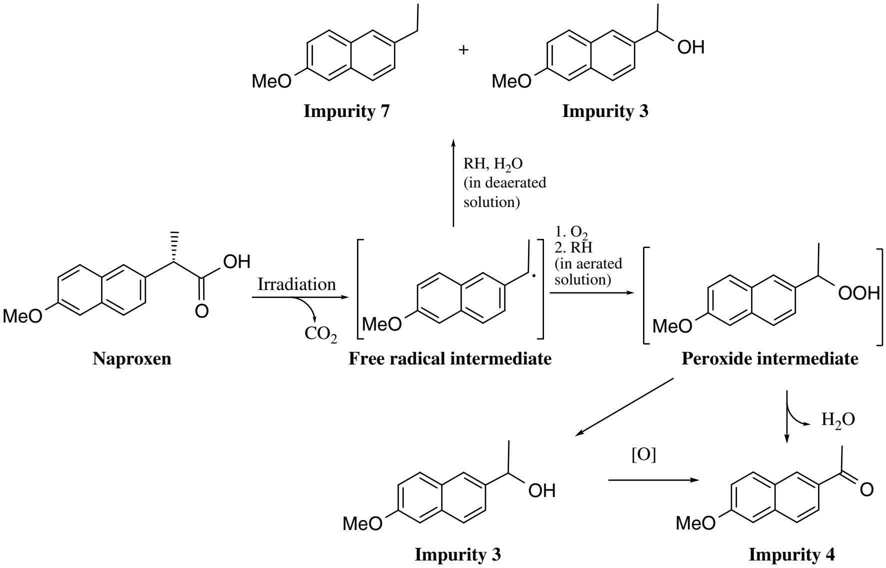


Chemical name: (+)-(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid
Trade name: Naprosyn, Aleve, others
Formula: $C_{14}H_{14}O_3$
Monoisotopic protonated ion (*m/z*): 231.1016
Molecular weight: 230.26
CAS number: 22204-53-1
Salt form: sodium salt

Naproxen is a nonselective COX enzyme inhibitor, which belongs to the family of nonsteroidal anti-inflammatory drugs (NSAIDs), and is indicated for the treatment of pain and inflammatory diseases such as rheumatoid arthritis, gout, and fever [1]. Patented by the pharmaceutical company Sythex in 1967 [2], it was approved for medical use in the United States in 1976 as a prescription drug and became an over-the-counter medication in 1994 [3]. Chemically, naproxen is an 1-substituted aryl propionic acid. A forced degradation study of naproxen soft gel capsules suggested that the major degradation resulted from decarboxylation as well as subsequent oxidation (Scheme 249-1) [4]. Under acidic (5 M HCl, 25°C, 60 minutes), alkaline



Scheme 249-1 Degradation pathways of naproxen under forced acidic, alkaline, and oxidative degradation conditions. The numbering of impurities follows that of Reddy et al. [4]. There was some contradictory description in the original paper: while in the main text of the paper, one of the major degradants was described as Impurity 2, while in the summary table of the forced degradation study, this major degradant was written as Impurity 3. Based on the mechanistic analysis performed by the current author, Impurity 3 would be a more reasonable major degradant than Impurity 2. *Source:* Reddy et al. [4] / Taylor & Francis Group.



Scheme 249-2 Photochemical degradation pathways of naproxen. For consistency, the numbering of impurities follows that of Reddy et al. [4]. *Source:* Reddy et al. [4] / Taylor & Francis Group.

(5 M NaOH, 85°C, 120 minutes), and oxidative (30% H_2O_2, 25°C, 60 minutes) stress conditions, three degradants (**2**, **4**, and **7**) were observed. Under humid (90% RH, 25°C, 120 minutes) and thermal (60°C, 24 hours) conditions, the observed degradants were **2** and **7**, and **7**, respectively. There was no mechanistic explanation given for the forced degradation results in the original paper; the current author hence provided one as shown in Scheme 249-1 based on the photochemical degradation mechanism proposed by Moore and Chappuis [5].

A photochemical degradation study in solution was performed by Moore and Chappuis [5], naproxen was found to produce **Impurities 3** and **4** in aerated solution, while the degradants were **Impurities 3** and **7** in deaerated solution (Scheme 249-2). It is generally believed that naproxen first underwent intramolecular decarboxylation at an excited triplet state to produce the decarboxylated intermediate, from which the photodegradants were formed [6]. The photodegradation profiles were similar to those under nonphotochemical conditions.

References

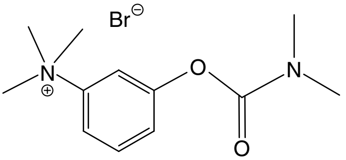
1 Naproxen. https://en.wikipedia.org/wiki/Naproxen (accessed February 4 2024).
2 Naproxen sodium. https://www.osc-ortho.com/blog/naproxen-sodium/ (accessed February 29 2024).
3 Naproxen. https://www.ncbi.nlm.nih.gov/books/NBK525965/ (accessed February 29 2024).

4 Reddy, R.S., Krishna, R.M., Vekaria, N.A. et al. (2016). Determination of potential impurities of naproxen sodium in soft gelatin capsules dosage by using ultra performance liquid chromatography. *Anal. Chem. Lett.* 6: 55–69.

5 Moore, D.E. and Chappuis, P.P. (1998). A comparative study of the photochemistry of the non-steroidal anti-inflammatory drugs, naproxen, benoxaprofen and indomethacin. *Photochem. Photobiol.* 47: 173–180.

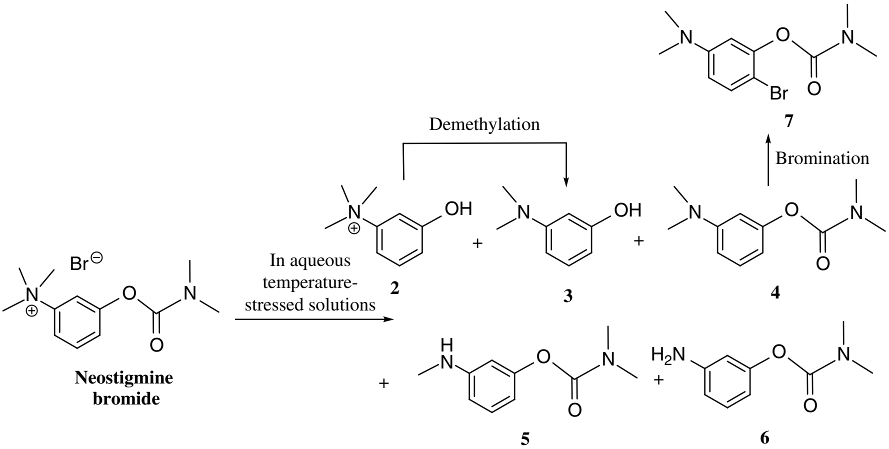
6 Tu, N., Liu, Y., Li, R. et al. (2019). Experimental and theoretical investigation on photodegradation mechanisms of naproxen and its photoproducts. *Chemosphere* 227: 142–150.

250: Neostigmine Bromide [WHO List of Essential Medicines]



Chemical name: 3-{[(Dimethylamino)carbonyl]oxy}-*N,N,N*-trimethylbenzenaminium
Trade name: Bloxiverz
Formula: $C_{12}H_{19}N_2O_2^+$
Monoisotopic protonated ion (*m/z*): 223.1441 (excluding bromide)
Molecular weight: 303.20
CAS number: 59-99-4
Salt form: Bromide

Neostigmine is a medication for the treatment of myasthenia gravis (an autoimmune disorder manifested by generalized muscle weakness), Ogilvie syndrome (a rare disorder of colonic dilation without anatomic obstruction), and urinary retention without the presence of a blockage [1]. It was synthesized by Aeschlimann and Reinert at Hoffmann La Roche in 1931 and was patented by Aeschlimann in 1933. Chemically, it is a phenylcarbamate as well as a quaternary amine of aniline derivative. Both moieties would be susceptible to degradation reactions, which are of hydrolytic and nucleophilic nature. In a stress study of neostigmine bromide in aqueous solutions at elevated temperature, six degradation products were observed and identified (Scheme 250) [2]. Degradant **2** was due to the carbamate linkage hydrolysis, while further degradation of **2** via demethylation produced degradant **3**.



Scheme 250 Degradation of neostigmine bromide in aqueous temperature-stressed solutions.

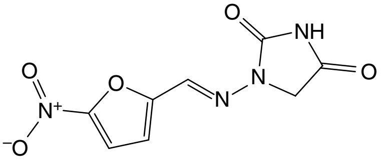
Degradants **4** to **6** stemmed from different levels of demethylation, while the carbamate linkage remained intact. Bromination of **4** would yield degradant **7**.

References

1 Neostigmine. https://en.wikipedia.org/wiki/Neostigmine (accessed February 4 2024).

2 Porst, H. and Kny, L. (1985). The structure of degradation products of neostigmine bromide. *Pharmazie* 40: 325–328.

251: Nitrofurantoin [WHO List of Essential Medicines]



Chemical name: (*E*)-1-[(5-Nitro-2-furyl)methylideneamino]imidazolidine-2,4-dione

Trade name: Macrobid, Macrodantin, Macpac, others

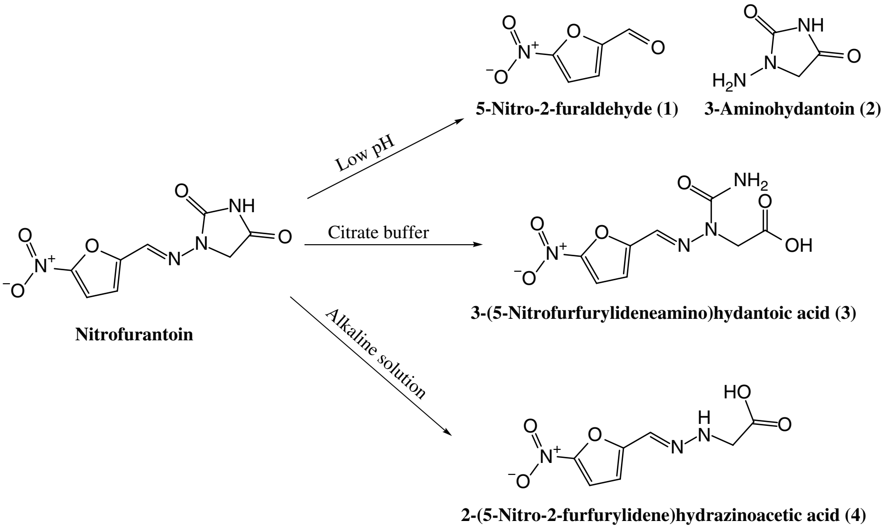
Formula: $C_8H_6N_4O_5$

Monoisotopic protonated ion (*m/z*): 239.0411

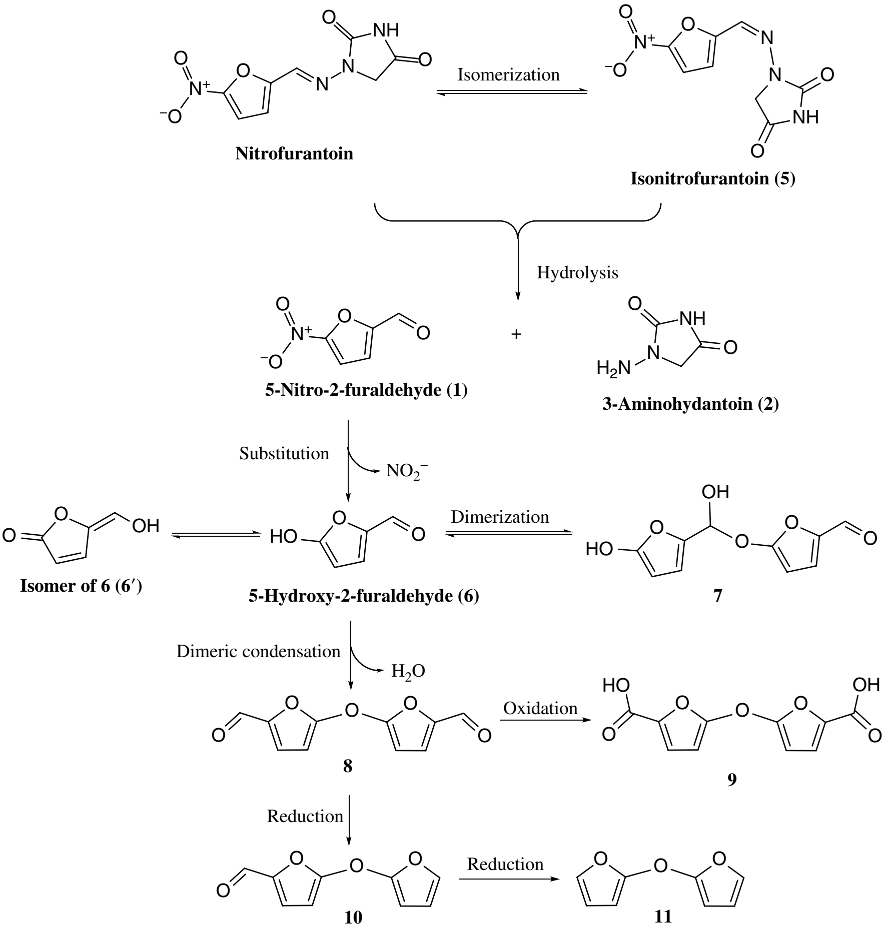
Molecular weight: 238.16

CAS number: 67-20-9

Nitrofurantoin is an antibacterial drug in the nitrofuran family and clinically used primarily for the treatment of urinary tract and bladder infections. It was invented by Kenyon J. Hayes of the pharmaceutical company Eaton Laboratories and was approved for medical use in 1953 [1]. Synthetically, it is made by a condensation reaction between 5-nitro-2-furaldehyde (**1**) and 3-aminohydantoin (**2**). Hence, nitrofurantoin would be susceptible to hydrolytic degradation to revert back to the two starting materials. Indeed, in solutions of low pH, these two compounds were observed as the major degradants (Scheme 251-1) [2], while in citrate buffer, the hydrolytic degradation occurred on the hydantoin ring, yielding 3-(5-nitrofurfurylideneamino)hydantoic acid (**3**) as the primary degradant [3]. In alkaline solutions, the hydantoin ring further degraded to produce



Scheme 251-1 Hydrolytic degradation of nitrofurantoin in solutions of different pH.



Scheme 251-2 Photochemical degradation pathways of nitrofurantoin in aqueous solution. The structural assignment of the degradants was based upon mass spectrometric data and thus considered tentative. *Source:* Szabó-Bárdos et al. [6] / with permission of Elsevier.

2-(5-nitro-2-furfurylidene)hydrazinoacetic acid (**4**) [4]. Biosic et al. studied the hydrolytic degradation kinetics of nitrofurantoin in solutions of pH 4, 7, and 9 and found that the half-lives at 40°C were 68.0, 10.9, and 4.2 days, respectively, indicating that the hydrolysis rate was the slowest at pH 4 [5].

In a photolysis study of nitrofurantoin in aqueous solutions with irradiation between 375 and 400 nm, which is near the maximum UV absorption of nitrofurantoin, nitrofurantoin first underwent rapid *trans*- to *cis*-transformation to produce isonitrofurantoin (**5**, Scheme 251-2) [6]. Both isomers were subsequently hydrolyzed to yield degradants **1** and **2**, and **1** was further degraded via photosubstitution to generate 5-hydroxy-2-furaldehyde (**6**). The latter degradant (**6**) could further degrade into a few more degradation products (**7**–**11**).

References

1 Molecule of the week archive, Nitrofurantoin. https://www.acs.org/molecule-of-the-week/archive/n/nitrofurantoin.html (accessed March 1 2024).

2 Paul, H.E., Ells, V.R., Kopko, F., and Bender, R.C. (1960). Metabolic degradation of the nitrofurans. *J. Med. Pharm. Chem.* 2: 563–584.

3 Juenge, E.C., Kreienbaum, M.A., and Gurka, D.F. (1985). Assay of nitrofurantoin oral suspensions contaminated with 3-(5-nitrofurfurylideneamino)hydantoic acid. *J. Pharmacy Sci.* 74: 100–102.

4 Ertan, G., Karasulu, Y., and Guneri, T. (1993). Degradation and gastrointestinal stability of nitrofurantoin in acidic and alkaline media. *Int. J. Pharm.* 96: 243–248.

5 Biosic, M., Skoric, I., Beganovic, J., and Babic, S. (2017). Nitrofurantoin hydrolytic degradation in the environment. *Chemosphere* 186: 660–668.

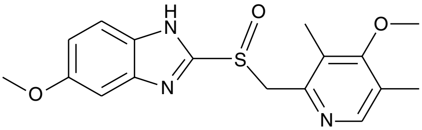
6 Szabó-Bárdos, E., Cafuta, A., Hegedűs, P. et al. (2020). Photolytic and photocatalytic degradation of nitrofurantoin and its photohydrolytic products. *J. Photochem. Photobiol. A: Chem.* 386: 112093.

8

Omeprazole and Related Azoles to Primaquine

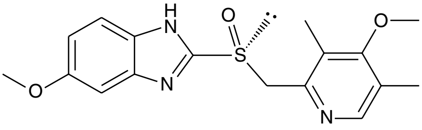
252–257: Omeprazole and Related Azoles

252: Omeprazole



Chemical name: (*R,S*)-5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) methanesulfinyl]-1*H*
-benzimidazole
Trade name: Prilosec, Losec
Formula: $C_{17}H_{19}N_3O_3S$
Monoisotopic protonated ion (*m/z*): 346.1220
Molecular weight: 345.42
CAS number: 73590-58-6
Common salt form: Magnesium, Sodium salts

253: Esomeprazole

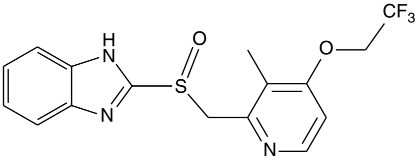


Chemical name: (*S*)-(−)-5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) methanesulfinyl]-1*H*
-benzimidazole
Trade name: Nexium, Neksium
Formula: $C_{17}H_{19}N_3O_3S$
Monoisotopic protonated ion (*m/z*): 346.1220
Molecular weight: 345.42

Compendium of Drug Degradation Pathways, First Edition. Min Li.

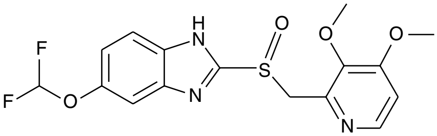
CAS number: 119141-88-7
Common salt form: Magnesium, Sodium, Strontium salts

254: Lansoprazole



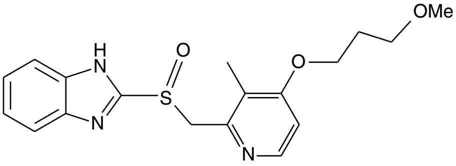
Chemical name: (*R,S*)-2-([3-Methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl] methylsulfinyl)-1
H-benzo[d]imidazole
Trade name: Agopton, Ogast, others
Formula: $C_{16}H_{14}F_3N_3O_2S$
Monoisotopic protonated ion (*m/z*): 370.0832
Molecular weight: 369.36
CAS number: 103577-45-3

255: Pantoprazole



Chemical name: (*R,S*)-6-(Difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl) methylsulfinyl]-1
H-benzo[d]imidazole
Trade name: Protonix
Formula: $C_{16}H_{15}F_2N_3O_4S$
Monoisotopic protonated ion (*m/z*): 384.0824
Molecular weight: 383.37
CAS number: 102625-70-7
Common salt form: Magnesium, Sodium salts

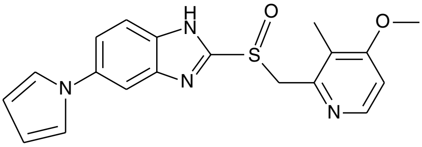
256: Rabeprazole



Chemical name: (*R,S*)-2-([4-(3-Methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl)-1
H-benzo[d]imidazole
Trade name: Aciphex

Formula: $C_{18}H_{21}N_3O_3S$
Monoisotopic protonated ion (*m/z*): 360.1376
Molecular weight: 359.44
CAS number: 117976-89-3
Common salt form: Sodium salt

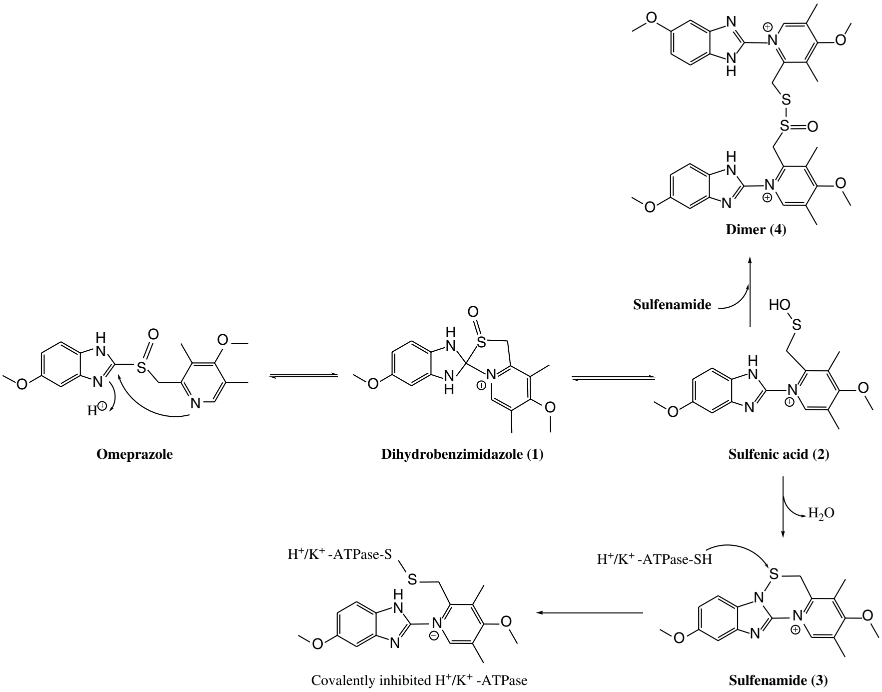
257: Ilaprazole



Chemicalname: 2-[(*R*,*S*)-[(4-methoxy-3-methylpyridin-2-yl)methyl]sulfinyl]-5-(1*H*-pyrrol-1-yl)-1*H*-benzimidazole
Trade name: Noltec
Formula: $C_{19}H_{18}N_4O_2S$
Monoisotopic protonated ion (*m/z*): 367.1223
Molecular weight: 366.44
CAS number: 172152-36-2

Omeprazole is a medication that is used for treating gastroesophageal reflux disease (GERD), peptic ulcer disease, and Zollinger–Ellison syndrome [1]. It is a specific, covalent irreversible inhibitor of H^+/K^+-ATPase, the enzyme controlling the secretion of gastric acid; hence, this class of drugs is also known as the proton pump inhibitors (PPIs). Omeprazole is the first clinically approved PPI [2], and it is a racemic mixture due to the chirality of the sulfoxide moiety. Chemically, it is a benzoimidazole-substituted sulfinylmethyl pyridine derivative. While it is reasonably stable in neutral and alkaline pH, under acidic conditions (particularly at pH 5 or below), omeprazole undergoes rather facile intramolecular rearrangement to form the isomeric sulfenic acid derivative (**2**), which is followed by dehydration to produce the cyclized sulfenamide (**3**) [3–6]. Either the sulfenic acid or the sulfenamide was thought to be the active form of the drug [5]. This transformation process is also its degradation pathway under acidic conditions, under which a dimeric degradant (**4**) would also be formed (Scheme 252–257-1) [5, 6] in addition to numerous other degradants, many of which may be of transient nature [5]. Because of its acidic lability, omeprazole is typically formulated with an enteric coating to prevent its degradation when passing through the stomach.

Roberts et al. studied acid-induced degradation of omeprazole in 18 mM HCl solution at 10°C for up to 48 hours [7]. It was found that the vast majority of omeprazole was decomposed, and ~10 degradants were identified, among which the major one was the sulfhydryl degradant (**6**, Scheme 252–257-2), which was also observed in the original study of Brandstrom et al. [5]. Another monomeric degradant identified was the des-sulfur degradant and its structure was proposed as **7** by Roberts et al. Nevertheless, a more reasonable structure for the des-sulfur degradant might be **7′**, as it can be more rationally accounted for by an elimination mechanism from dimer **4**. Furthermore, **7′** was also identified as an acidic degradant of

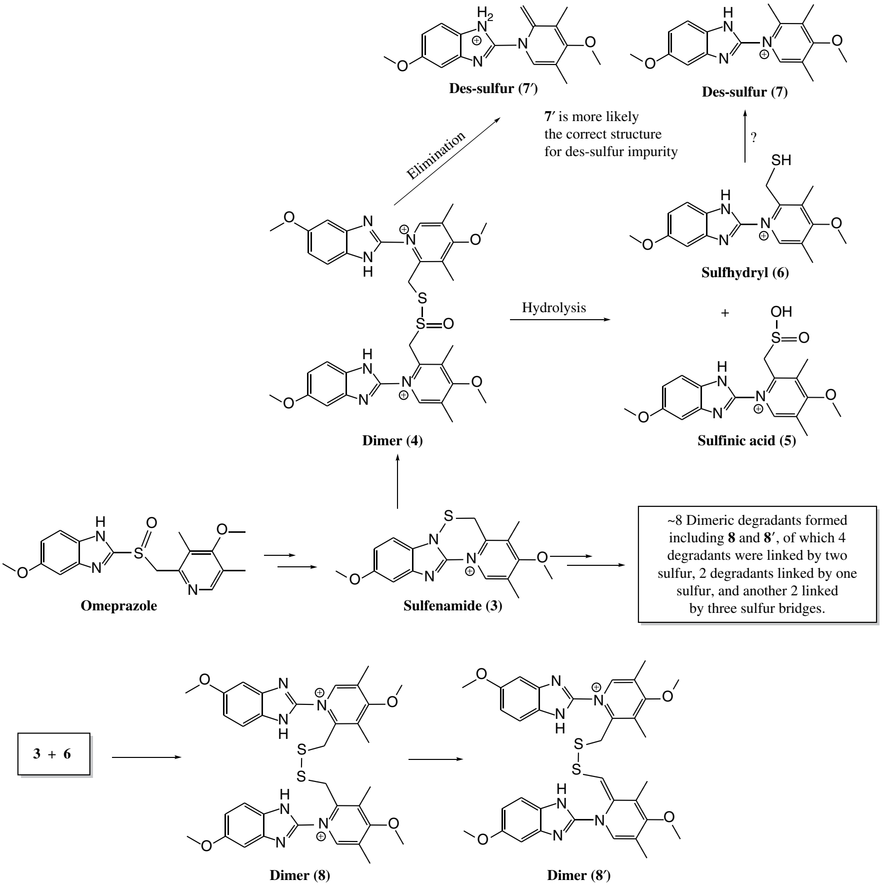


Scheme 252–257-1 Acidic degradation pathway of omeprazole per the studies of Brandstrom et al. [5] and Qaisi et al. [**6**] and its mode of action [4, 5]. *Source:* Brandstrom et al. [5] and Qaisi et al. [6].

omeprazole by Shankar et al. [8]. All other identified minor degradants were dimeric degradants, which were linked together by either mono-, di- (such as **8** and **8′**), or tri-sulfide bridges. Brandstrom et al. also reported many disulfide-linked dimeric degradants [5].

In the forced degradation study by Shankar et al. [8], omeprazole was also found to be very susceptible to acidic degradation; in 10 mM HCl at room temperature for 30 minutes, the majority of omeprazole decomposed to form three major degradants (**7′**,**9**–**10**, Scheme 252–257-3). The structural elucidation of these degradants was based on LC–MS and LC–MS/MS analysis. In their study, Shankar et al. proposed the structure of degradant **10**; nevertheless, due to the pseudo-symmetry of the molecule, the structure of **10′** would also be possible, as analogous degradants were present in sets of two isomers [5]. It would also be possible that both **10** and **10′** might be present, but could not be separated by the LC–MS method used in their study [8]. In studying degradation behaviors of omeprazole and lansoprazole in aquatic environments, the sulfide degradant (**9**) was observed as the major degradant for both drugs in aqueous solutions under either dark or simulated sunlight [9]; its formation mechanism remained unclear despite a few proposed ones.

The hydrolytic stress in neutral and alkaline solutions was carried out under quite harsh conditions, during which numerous degradants were generated [8].



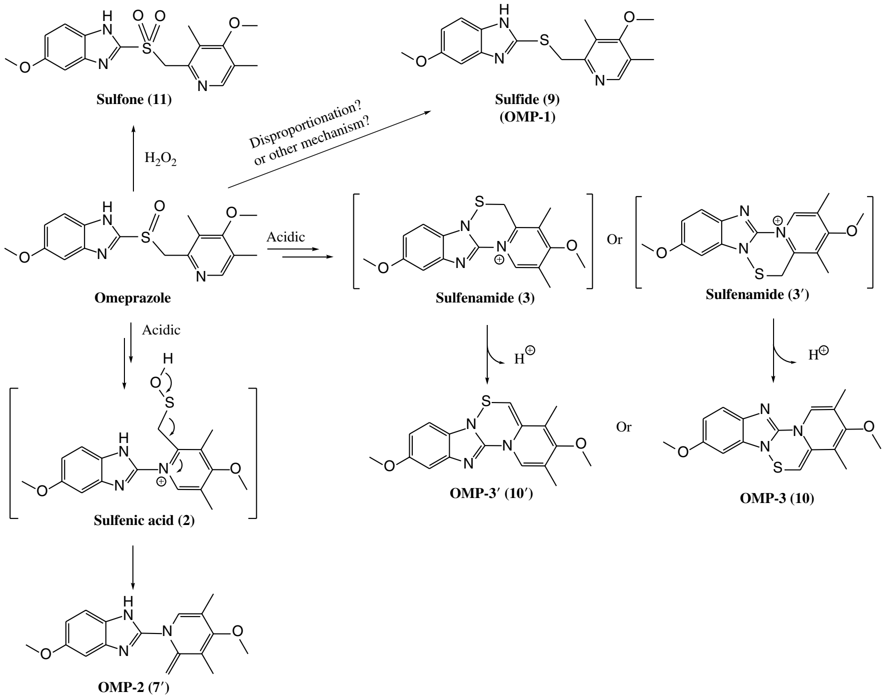
7′ is more likely the correct structure for des-sulfur impurity

~8 Dimeric degradants formed including **8** and **8′**, of which 4 degradants were linked by two sulfur, 2 degradants linked by one sulfur, and another 2 linked by three sulfur bridges.

Scheme 252–257-2 Acidic degradation of omeprazole in 18 mM HCl solution at 10°C for up to 48 hours [7]. The formation mechanism of **6** is compiled by the present author, which is also partly based on the original study of Brandstrom et al. *Source:* Brandstrom et al. [5] and Roberts et al. [7].

Nevertheless, these degradation profiles would not appear to be relevant to the real degradation scenario of omeprazole. In oxidative stress with 3% hydrogen peroxide at room temperature for 24 hours, the predominant degradant was omeprazole sulfone (**11**).

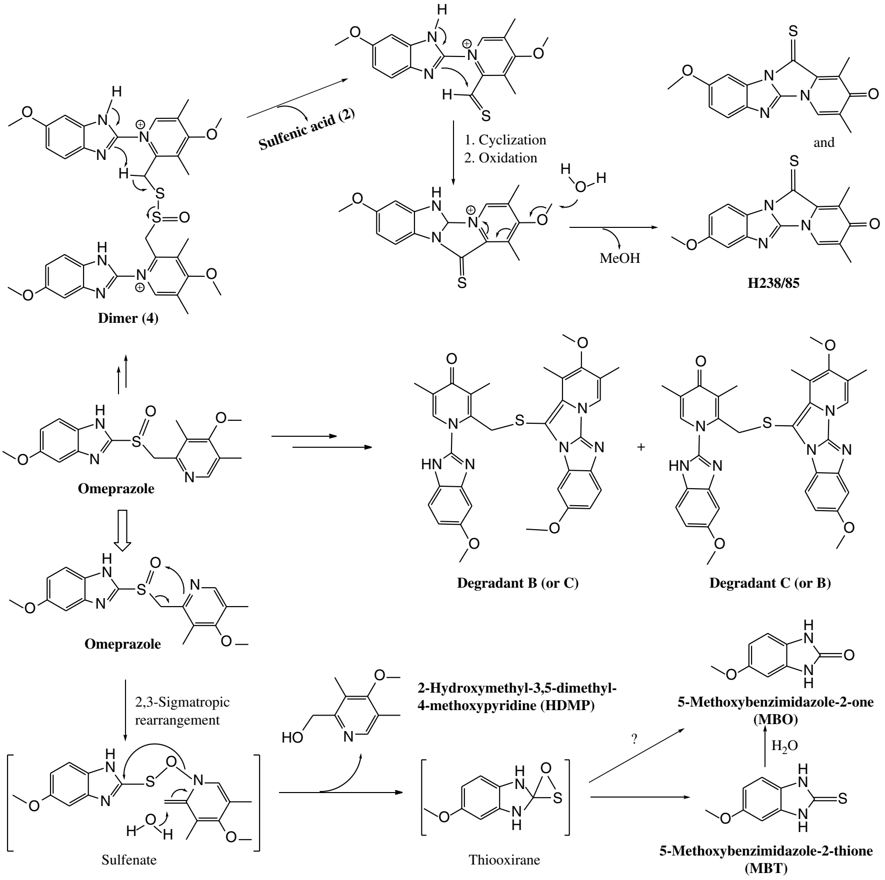
In solid state formulations, the degradation pathways of omeprazole appear to be different from those in the solution state as described above. For example, in an omeprazole sample subjected to accelerated stability condition, two sets of isomers (H238/85 and degradants B/C) were observed as the major degradants and identified by the original developer of the drug (Scheme 252–257-4) [10]. The isomeric degradants H238/85 were also seen as the major degradants in enteric-coated pellets that were stored under the accelerated stability condition of 40°C/75%RH for 120 days [4]. Yet in another stability study of a commercially available omeprazole formulated product, Rajab et al. reported the observation of a number of



Scheme 252–257-3 Forced degradation of omeprazole performed by Shankar et al. under acidic (10 mM HCl at room temperature for 30 minutes) and oxidative conditions (3% hydrogen peroxide at room temperature for 24 hours), respectively [8]. The nomenclature of the degradants, OMP-1, OMP-2, and OMP-3, follows that of Shankar et al. The mechanisms of the formation of these three major acidic degradants (OMP-1, OMP-2, and OMP-3) are different from those proposed by Shankar et al. The intermediates **2** and **3** were previously reported by Qaisi et al. [6]; by analogy, sulfenamide 3′ might also be formed, and its deprotonation should give OMP-3. *Source:* Qaisi et al. [6] / with permission of Elsevier.

degradants, of which at least two major degradants had not been reported before [11]. These two major degradants, HDMP and MBT (Scheme 252–257-4), resulted from the cleavage of the omeprazole molecule, which was proposed to proceed through a thiooxirane and a pseudo-carbenoid species (not shown). According to the present author, the formation of the thiooxirane intermediate might be better explained by a 2,3-sigmatropic rearrangement mechanism, which is well-known for the transformation of allylic sulfoxides to allylic sulfenates [12]. From the thiooxirane intermediate, the oxygen-containing analogous degradant (MBO) could also be formed; as a matter of fact, MBO was observed from the degradation of lansoprazole in the solid state [10] as well as in an aquatic environment [9].

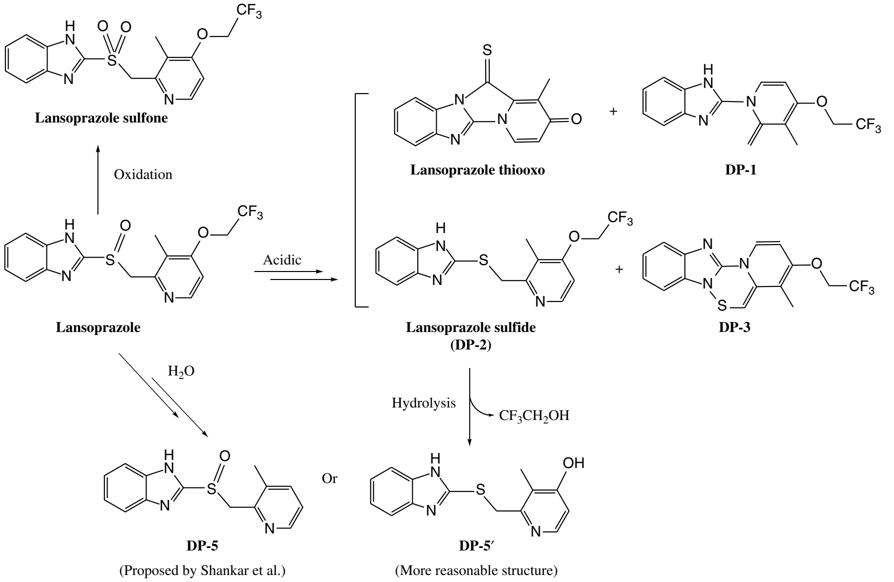
Overall, it appears that the degradation pathways of omeprazole are very complex. Even under similar acidic conditions [5–8], different temperatures and durations of the degradation could produce vastly different degradation profiles. Furthermore, the degradation profile in the solid state differed from that in the solution state.



Scheme 252–257-4 Degradation pathways of omeprazole in solid state [4,10, 11]. The early stage of the mechanism for the formation of HDMP and MBT is different from that of Rajab et al. [11], who proposed the involvement of a thiooxirane and a pseudo-carbenoid species (the latter is not shown).

Esomeprazole, approved for medical use in 2000, is the *S*-isomer of omeprazole, which is the chiral switch of the original racemic drug, omeprazole, to improve its pharmacokinetic properties [13]. Its degradation chemistry would be expected to be identical to that of omeprazole.

Lansoprazole is another PPI, which was developed by Takeda and approved in 1991 [14]. Its degradation chemistry is highly similar to that of omeprazole (Scheme 252–257-5) [9, 11, 15–17]. One acidic degradant reported for lansoprazole was designated as DP-5, and its structure was proposed by Shankar et al. [17]. It appears that the proposed structure of **DP-5** would be difficult to rationalize from a mechanistic perspective. On the other hand, the formation of **DP-5′**, an isomer of **DP-5**, could be easily explained through the hydrolysis of the trifluoroethoxy side chain of lansoprazole sulfide degradant (**DP-2**). For the family of the PPI drug molecules, hydrolysis of this alkoxy side chain (either a methoxy or trifluoroethoxy) is a

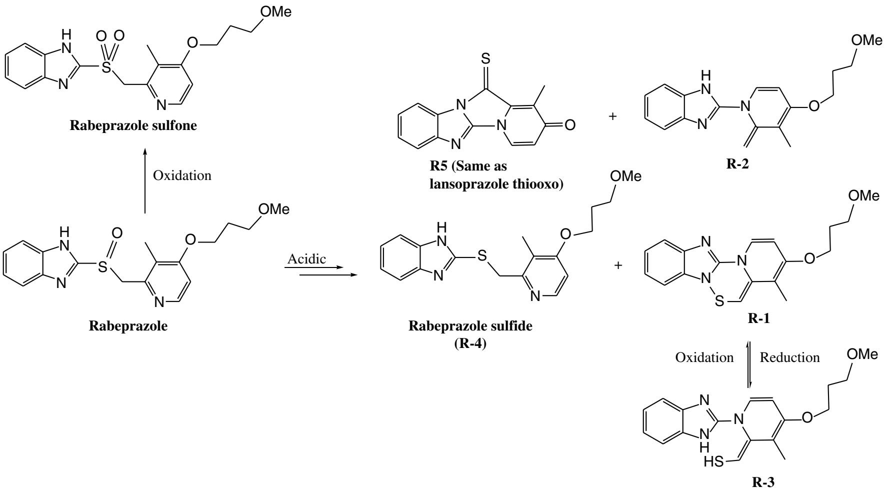


Scheme 252–257-5 Degradation pathways of lansoprazole under acidic, neutral, and oxidative conditions. The nomenclature of DP-1, DP-2, DP-3, and DP-5 follows that of Shankar et al. [17]. *Source:* Shankar et al. [17] / John Wiley & Sons.

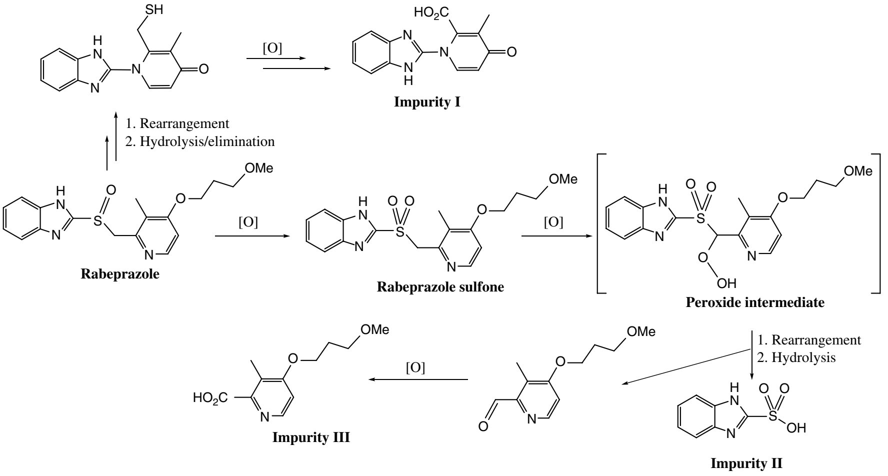
well-known degradation pathway [4, 5, 10, 17]. Hence, the real degradant could likely be **DP-5′**, rather than **DP-5**.

The other members of the PPI family include pantoprazole, rabeprazole, and ilaprazole, which were discovered and/or developed respectively by Byk Gulden Lomberg Chemische Fabrik/Smith Kline & French [18], Eisai [19], and Il-Yang Pharmaceutical (a Korean pharmaceutical company) [20]. The degradation chemistry of these PPIs would be expected to be similar to that of omeprazole and lansoprazole, particularly under acidic conditions [5–9, 11, 15–17]. For example, Bhandi et al. reported that rabeprazole decomposed to yield hydrolytic and oxidative degradants in neutral, acidic, and hydrogen peroxide solutions, respectively (Scheme 252–257-6) [21]. In an accelerated study of a formulated rabeprazole product, Dev et al. identified three novel degradants using LC–MS and LC-NMR (Scheme 252–257-7) [22]. Not only were these degradants not reported for rabeprazole and its formulated products before, but also the types of these degradants were not reported for other PPI drug products. Although no specific formation mechanisms were provided by the original researchers, the formation of Impurity II and III could be rationalized through Baeyer-Villiger oxidation of the sulfone degradant, which seems to be present in the accelerated stability study. Nevertheless, a drawback for this mechanism is that no Baeyer-Villiger oxidation of a sulfone has been reported. On the other hand, Baeyer-Villiger oxidation of a particular sulfoxide has been reported [23].

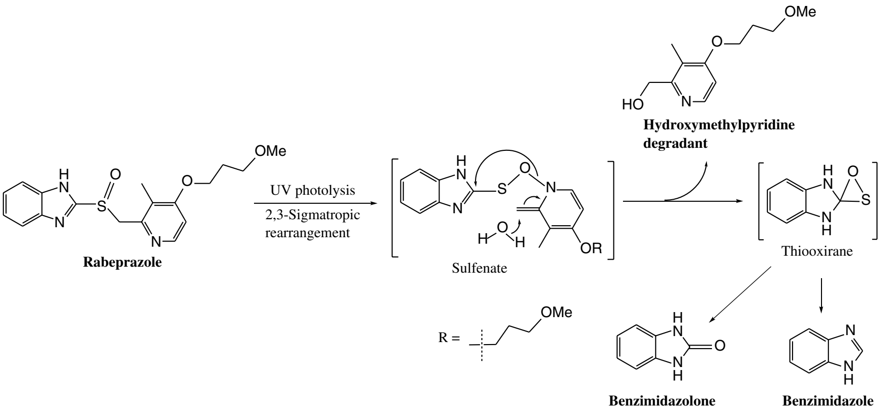
In a photo-stress degradation of rabeprazole in methanol solution, it was found to degrade into the hydroxymethylpyridine degradant, benzimidazolone, and benzimidazole degradants. The formation of these degradants might go through a



Scheme 252–257-6 Degradation pathways of rabeprazole under acidic, neutral, and oxidative conditions. The nomenclature of R-1, R-2, R-3, R-4, and R-5 follows that of Bhandi et al. [21]. *Source:* Bhandi et al. [21] / Royal Society of Chemistry.



Scheme 252–257-7 Degradation pathway of rabeprazole in a formulated product.



Scheme 252–257-8

thiooxirane intermediate (Scheme 252–257-8), and the latter type of intermediate was proposed in the nonphotochemical degradation of omeprazole in the solid state [4, 10, 11].

References

1 Omeprazole. https://en.wikipedia.org/wiki/Omeprazole (accessed March 3 2024).

2 Strand, D.S., Kim, D., and Peura, D.A. (2017). 25 years of proton pump inhibitors: a comprehensive review. *Gut Liver* 11: 27–37.

3 Bruton, L.L. (1996). *Goodman and Gilmans: The Pharmacological Basis of Therapeutics*, 901. New York: McGraw-Hill.

4 Storpirtis, S. and Rodrigues, D. (1998). In vitro evaluation of dissolution properties and degradation products of omeprazole in enteric-coated pellets. *Drug Dev. Ind. Pharm.* 24: 1101–1107.

5 Brandstrom, A., Lindberg, P., Bergman, N.-A. et al. (1989). Chemical reactions of omeprazole and omeprazole analogues. I. A survey of the chemical transformations of omeprazole and its analogues. *Acta Chem. Scand. B* 43: 536–548.

6 Qaisi, A.M., Tutunji, M.F., and Tutunji, L.F. (2006). Acid decomposition of omeprazole in the absence of thiol: a differential pulse polarographic study at the static mercury drop electrode (SMDE). *J. Pharm. Sci.* 95: 384–391.

7 Roberts, J., McNaughtan, M.L., MacLachlan, J. et al. (2018). Identification of the acid-induced degradation products of omeprazole and 5-hydroxyomeprazole by high-resolution mass spectrometry. *Rapid Commun. Mass Spectrom.* 32: 929–941.

8 Shankar, G., Borkar, R.M., Udutha, S. et al. (2019). Identification and structural characterization of stress degradation products of omeprazole using Q-TOF-LC-ESI-MS/MS and NMR experiments: evaluation of toxicity of degradation products. *New J. Chem.* 43: 7294–7306.

9 DellaGreca, M., Iesce, M.R., Previtera, L. et al. (2006). Degradation of lansoprazole and omeprazole in the aquatic environment. *Chemosphere* 63: 1087–1093.

10 Jansson, S.-O. and Vessman, J. (1991). Trends in product analysis for research in the pharmaceutical industry. *Mikrochim. Acta* II: 103–112.

11 Rajab, A., Touma, M., Rudler, H. et al. (2013). Slow, spontaneous degradation of lansoprazole, omeprazole and pantoprazole tablets: isolation and structural characterization of the toxic antioxidants 3H-benzimidazole-2-thiones. *Pharmazie* 68: 749–754.

12 Colomer, I., Velado, M., Fernández de la Pradilla, R., and Viso, A. (2017). From allylic sulfoxides to allylic sulfenates: fifty years of a never-ending [2,3]-sigmatropic rearrangement. *Chem. Rev.* 117: 14201–14243.

13 Kalaitzakis, E. and Björnsson, E. (2007). A review of esomeprazole in the treatment of gastroesophageal reflux disease (GERD). *Ther. Clin. Risk Manag.* 3: 653–663.

14 Lansoprazole. https://en.wikipedia.org/wiki/Lansoprazole (accessed March 9 2024).

15 Ramulu, K., Rao, B.M., and Rao, N.S. (2013). Identification, isolation and characterization of potential degradation product in lansoprazole drug substance. *Raysayan J. Chem.* 6: 274–283.

16 Battu, S. and Pottabathini, V. (2015). Hydrolytic degradation study of lansoprazole, identification, isolation and characterisation of base degradation product. *Am. J. Anal. Chem.* 6: 145–155.

17 Shankar, G., Borkar, R.M., Udutha, S. et al. (2017). Forced degradation studies of lansoprazole using LC-ESI HRMS and ^{1}HNMR experiments: in vitro toxicity evaluation of major degradation products. *J. Mass Spectrom.* 52: 459–471.

18 Pantoprazole. https://en.wikipedia.org/wiki/Pantoprazole (accessed March 9 2024).

19 Rabeprazole. https://en.wikipedia.org/wiki/Rabeprazole (accessed March 9 2024).

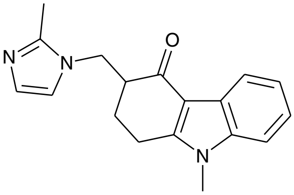
20 Ilaprazole. https://en.wikipedia.org/wiki/Ilaprazole (accessed March 9 2024).

21 Bhandi, M.M., Borkar, R.M., Shankar, G. et al. (2016). Identification and characterization of stressed degradation products of rabeprazole using LC-ESI/MS/MS and H-NMR experiments: in vitro toxicity evaluation of major degradation products. *RSC Adv.* 6: 10719–10735.

22 Dev, R.V., Kiran, G.S.U., Subbaiah, B.V. et al. (2009). Identification of degradation products in stressed tablets of Rabeprazole sodium by HPLC-hyphenated techniques. *Magn. Reson. Chem.* 47: 443–448.

23 Ma, X., Liu, Y., Du, L. et al. (2020). Post-functionalization of dibenzothiophene to functionalized biphenyls via a photoinduced thia-Baeyer-Villiger oxidation. *Nat. Commun.* 11: 914. https://doi.org/10.1038/s41467-020-14522-7.

258: Ondansetron [WHO List of Essential Medicines]

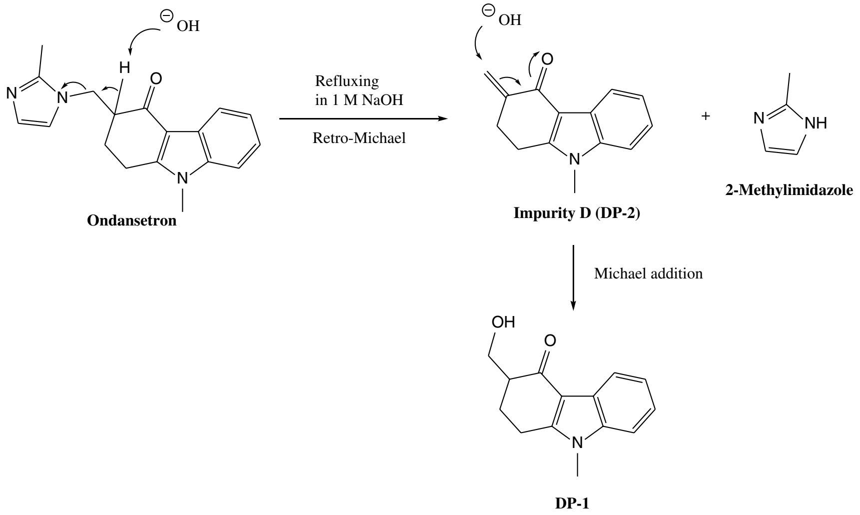


Chemical name: (*R,S*)-9-Methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-2,3-dihydro-1*H*-carbazol-4(9*H*)-one
Trade name: Zofran
Formula: $C_{18}H_{19}N_3O$
Monoisotopic protonated ion (*m/z*): 294.1601
Molecular weight: 293.37
CAS number: 99614-02-5
Common salt form: HCl salt

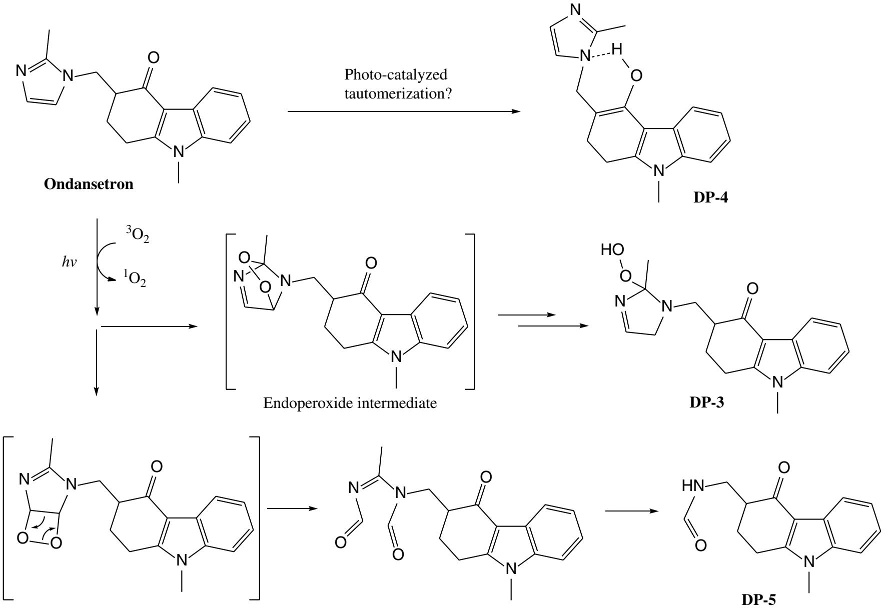
Ondansetron, a highly selective serotonin 5-HT_3 receptor antagonist, is clinically used for the prevention of nausea and vomiting induced by chemotherapy, radiation, surgery, and other causes [1]. Chemically, it is a fused indole derivative with a conjugated keto moiety and an imidazole-*N*-methyl substituent alpha to the keto. The alpha-substituted keto part of the drug molecule would be susceptible to base-catalyzed retro-Michael addition, producing Impurity D (USP) and 2-methylimidazole (Scheme 258-1) [2]. The forced degradation was conducted by refluxing ondansetron in 1 M NaOH solution (water/methanol, 1/1, v/v) for 30 hours. Under this rather harsh condition, the majority of the drug substance was degraded and Impurity D was formed as the largest degradant (33.9% yield), in addition to the other four degradants which were not identified in the study. On the other hand, Talluri et al. also performed a forced degradation of ondansetron in 1 M NaOH solution, in which they identified another degradant (DP-1) [3], which apparently resulted from the Michael addition of Impurity D by hydroxide ion.

The same researchers also conducted photochemical stress of ondansetron in solution and identified three photodegradants (Scheme 258-2), DP-3, DP-4, and DP-5. DP-3 and DP-5 were proposed as degradants with oxidation occurring on the imidazole ring by singlet oxygen, while DP-4 was proposed as the enol form of ondansetron due to tautomerization.

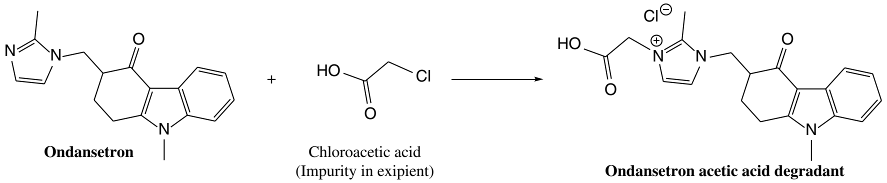
In a stability study of ondansetron tablets, a novel degradant was observed, and the subsequent investigation with API-excipient compatibility study revealed that this impurity was caused by the reaction of the API with chloroacetic acid, an impurity in excipient sodium starch glycolate (Scheme 258-3) [4].



Scheme 258-1 Base-catalyzed degradation of ondansetron via retro-Michael addition.



Scheme 258-2 Photochemical degradation of ondansetron in solution. The nomenclature of the degradants follows that of Talluri et al. [3]. *Source:* Talluri et al. [3] /John Wiley & Sons.



Scheme 258-3 Degradation of ondansetron tablets that contained sodium starch glycolate as one of the excipients.

References

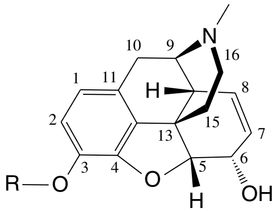
1 Ondansetron. https://en.wikipedia.org/wiki/Ondansetron (accessed March 9 2024).

2 Kristiana, E., Saefumillah, A., and Budianto, E. (2020). Degradation of ondansetron: isolation and characterization impurity D ondansetron as A candidate reference standard impurity in drug. *AIP Conf. Proc.* 2242: 040054. https://doi.org/10.1063/5.0010657.

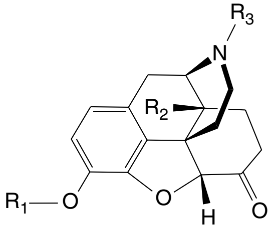
3 Talluri, M.V.N.K., Keshari, K.K., Kalariya, P.D., and Srinivas, R. (2015). Selective separation and characterization of the stress degradation products of ondansetron hydrochloride by liquid chromatography with quadrupole time-of-flight mass spectrometry. *J. Sep. Sci.* 38: 1625–1632.

4 Gandhi, H.M., Gollapalli, N.R., and Lilakar, J.K.D. (2018). Identification, isolation and characterization of unknown impurity in ondansetron drug product. *Int. J. Adv. Res. Ideas Innov. Tech.* 4: 1264–1271.

259–265: Opioids



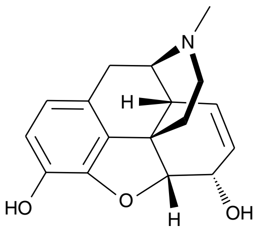
Morphine, R = H;
Codeine, R = Me.



Oxymorphone, R_1 = H, R_2 = OH, R_3 = Me;
Oxycodone, R_1 = Me, R_2 = OH, R_3 = Me;
Hydromorphone, R_1 = H, R_2 = H, R_3 = Me;
Hydrocodone, R_1 = Me, R_2 = H, R_3 = Me;
Naloxone, R_1 = H, R_2 = OH, R_3 = Propen-3-yl.

Opium has been used as a pain reliever for millennia, long before morphine, its main ingredient, was isolated in the early 1800s. Today, morphine is utilized not only as an active pharmaceutical ingredient but also as a starting material for a number of its analogous drugs. Among the opioids that are covered in this monograph, they may be divided into different structural categories, e.g., those with 3-hydroxyl group versus those with 3-methoxyl group. The ones with 3-hydroxyl group would be susceptible to oxidative coupling to yield the corresponding dimeric degradants, e.g., the so-called pseudomorphine, while the ones with 3-methoxyl group would not undergo such oxidative degradation due to the blockage of the phenolic hydroxyl group, which prevents the formation of the phenolic hydroxyl free radical that eventually leads to the dimerization. On the other hand, those opioids with a 6-keto moiety could undergo a different type of dimerization process – aldol condensation, as in the case of oxycodone. Aside from their difference, all the opioids contain a tertiary amine functional group, which could be oxidized to form the corresponding *N*-oxide degradants, particularly under neutral to alkaline conditions.

259: Morphine [WHO List of Essential Medicines]

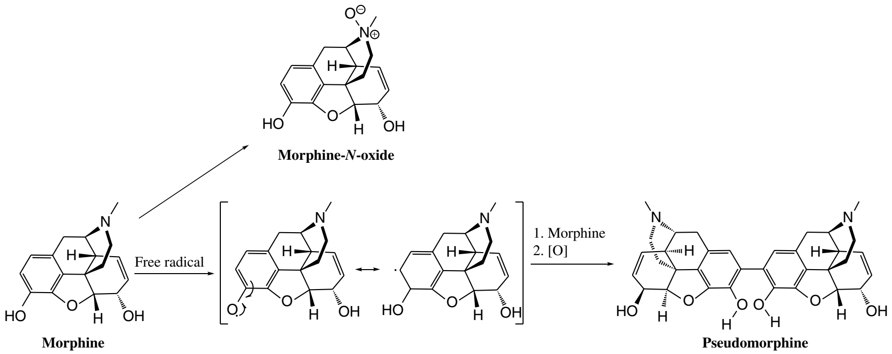


Chemical name: (*4R,4aR,7S,7aR,12bS*)-3-Methyl-2,3,4,4a,7,7a-hexahydro-1*H*-4,12-methano[1]benzofuro[3,2-*e*]isoquinoline-7,9-diol
Formula: $C_{17}H_{19}NO_3$
Monoisotopic protonated ion (m/z): 286.1438

Molecular weight: 285.34
CAS number: 57-27-2
Common Salt form: Sulfate, HCl salt

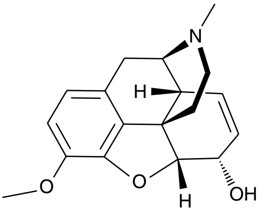
Morphine was isolated in 1804 from the opium poppy plant by Friedrich Sertürner, a German pharmacist who named the substance *morphium* after the Greek god of dreams, Morpheus, due to its effect of causing sleepiness [1]. On the other hand, opium has been known for approximately four millennia as a powerful pain reliever [2]. Today, morphine remains one of the most utilized analgesics for treating acute and chronic severe pain [3]. Several functional groups of morphine, such as the phenolic hydroxyl, the hydroxyl alpha to the vinyl moiety, and the tertiary amino group, are susceptible to oxidative degradation. More specifically, the oxidation of the phenolic functionality leads to the formation of pseudomorphine, a dimeric degradant via oxidative coupling, as the major degradant, and morphine-*N*-oxide (also known as genomorphine) as the minor degradant (Scheme 259–265-1) [4, 5]. The study by Vermeire and Remon also showed that the presence of oxygen and higher pH accelerated the degradation of morphine in solution [5].

Miolo et al. studied the photodegradation of morphine in solution and solid state [6], and found that while it was stable toward UVA irradiation, morphine decomposed significantly under UVB irradiation in solid state and solutions. In aqueous solution, degradation due to hydrolysis and photooxidation appeared to be the major photodegradation pathways, in addition to photo-reductive products. In methanol solution, three major degradants were observed, with each of them incorporating two oxygen atoms. Tentative structures for the degradants formed in both solutions were proposed solely based on accurate mass spectrometric analysis.



Scheme 259–265-1 Oxidative degradation of morphine.

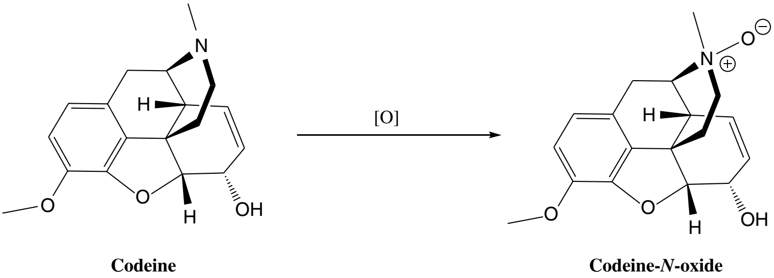
260: Codeine [WHO List of Essential Medicines]



Chemical name: (5*α*,6*α*)-7,8-Didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol
Formula: $C_{18}H_{21}NO_3$
Monoisotopic protonated ion (*m/z*): 300.1594
Molecular weight: 299.37
CAS number: 76-57-3
Common Salt form: Sulfate, HBr, HCl salt

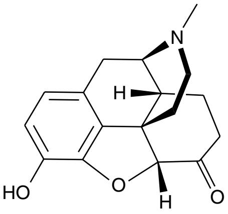
Codeine is a naturally occurring methylated morphine, which was discovered by Pierre Jean Robiquet, a French pharmacist and chemist who also had many other pioneering findings including the isolation and characterization of asparagine, the first amino acid to be identified [7, 8]. Codeine accounts for ~2% of opium and is the most commonly used opiate. Pharmacologically, it is a prodrug of morphine and it converts to morphine via in vivo demethylation. Due to the blocking of the 3-hydroxyl group by methylation, codeine would not be expected to undergo free-radical-mediated oxidation to form the corresponding dimeric degradant, dimethylpseudomorphine. Hence, its main oxidative degradant would be codeine-*N*-oxide (Scheme 259–265-2) [9].

Powell studied the degradation kinetics and pH-stability profile of codeine and found that it had a wide pH range of maximum stability between pH ~1 and 9 [10]. The study also confirmed the previous observations that phosphate buffer destabilized codeine: it decomposed ~20 times faster in a 50 mM, pH 7 phosphate buffer than in an unbuffered solution.



Scheme 259–265-2 Oxidative degradation of codeine.

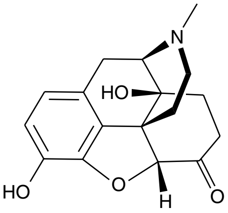
261: Hydromorphone [WHO List of Essential Medicines]



Chemical name: 4,5-α-Epoxy-3-hydroxy-17-methylmorphinan-6-one
Trade name: Dilaudid, Palladone, others
Formula: $C_{17}H_{19}NO_3$
Monoisotopic protonated ion (*m/z*): 286.1438
Molecular weight: 285.34
CAS number: 466-99-9
Common Salt form: HCl salt

Hydromorphone is an isomer of morphine, with the 6-hydroxy group oxidized to 6-keto and the 7,8-double bond reduced to a 1,2-substituted ethane moiety [11]. It can be made from morphine either by an isomerization or by a reductive/oxidative process. Because its two oxidizable functional groups, i.e., the 3-hydroxy and tertiary amino groups, are identical to those of morphine, its degradation chemistry would be expected to be the same or very similar to that of morphine (refer to Scheme 259–265-1) [12].

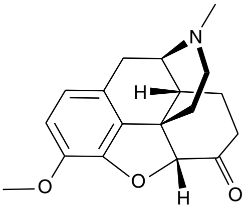
262: Oxymorphone



Chemical name: 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one
Trade name: Numorphan, Numorphone, Opana, others
Formula: $C_{17}H_{19}NO_4$
Monoisotopic protonated ion (m/z): 302.1387
Molecular weight: 301.34
CAS number: 76-41-5
Common Salt form: HCl salt

Oxymorphone was approved for medical use in 1959, and some of its extended-release forms were removed from the market at the request of the FDA due to illicit use of the dosage forms [13]. Chemically, it is a hydroxylated derivative of hydromorphone, and thus its degradation chemistry would be expected to be the same or very similar to that of hydromorphone and morphine (refer to Scheme 259–265-1).

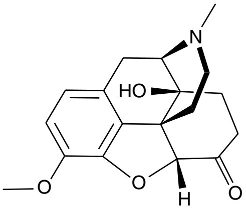
263: Hydrocodone



Chemical name: 4,5α-Epoxy-3-methoxy-17-methylmorphinan-6-one
Formula: $C_{18}H_{21}NO_3$
Monoisotopic protonated ion (*m/z*): 300.1594
Molecular weight: 299.37
CAS number: 125-29-1
Common Salt form: Bitartrate, HCl salt

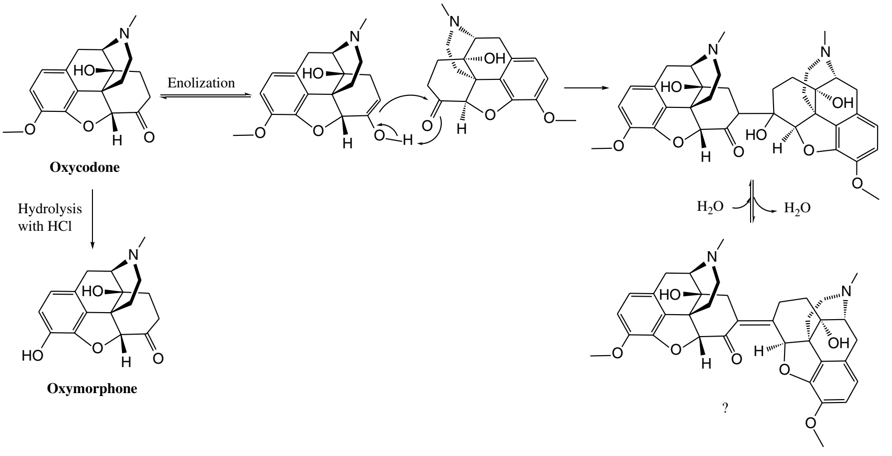
Hydrocodone, also known as dihydrocodone, is a semi-synthetic opioid drug usually made from codeine. In addition to treating pain, it is also used as a cough suppressant, typically in combination with other medicines for similar indications [14]. Due to its structural similarity to codeine, its degradation chemistry would be expected to be similar to that of codeine (refer to Scheme 259–265-2).

264: Oxycodone [WHO List of Essential Medicines]



Chemical name: (*5R,9R,13S,14S*)-4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one
Trade name: Roxicodone, OxyContin
Formula: $C_{18}H_{21}NO_4$
Monoisotopic protonated ion (*m/z*): 316.1543
Molecular weight: 315.37
CAS number: 76-42-6
Common Salt form: HCl salt

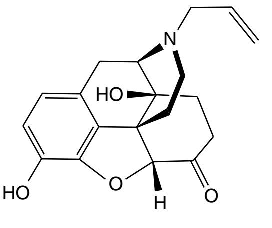
Oxycodone is a semi-synthetic opioid drug, which is a methylated derivative of hydromorphone and a hydroxylated derivative of hydrocodone. Due to the blockage of the phenolic hydroxyl at the 3-position by the methyl group, oxidative dimerization would not be expected for oxycodone, which is the same scenario for hydrocodone. On the other hand, it can still undergo oxidation on its tertiary amino group



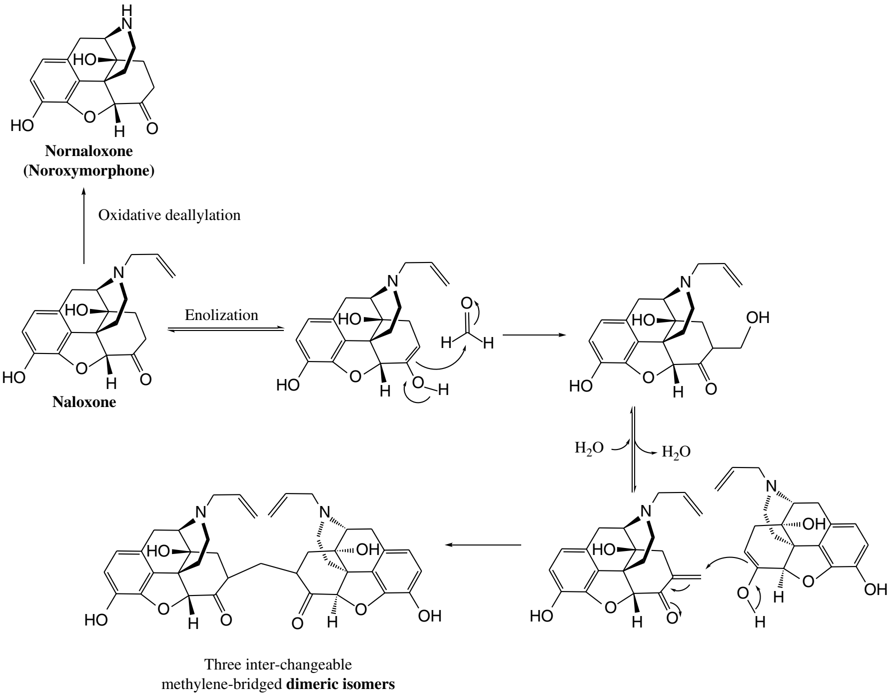
Scheme 259–265-3 Degradation pathways of oxycodone.

to produce the corresponding *N*-oxide [15]. Furthermore, Sitasuwan et al. reported that the 3-methoxyl moieties in opioids, such as oxycodone, hydrocodone, and codeine, could be hydrolyzed to produce the corresponding 3-hydroxyl derivatives by hydrochloric acid (Pathway a, Scheme 259–265-3) [16]. Nevertheless, such 3-O-demethylation would not proceed easily under nonenzymatic conditions, and thus, it would not be expected to be a meaningful degradation pathway under real-life scenarios. Even though 3-*O*-methylated opioids such as oxycodone would not undergo oxidative dimerization, a dimeric degradant of oxycodone was observed in a long-term stability study of an oxycodone hydrochloride injectable product [17]. The formation of this dimeric degradant was attributed to an aldol condensation reaction between two drug molecules under weakly acidic conditions (Pathway b, Scheme 259–265-3).

265: Naloxone [WHO List of Essential Medicines]



Chemicalname:(*4R,4aS,7aR,12bS*)-4a,9-Dihydroxy-3-(prop-2-en-1-yl)-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methano[1]benzofurano[3,2-e]isoquinolin-7(7a*H*)-one
Trade name: Narcan, Evzio, Nyxoid, others
Formula: $C_{19}H_{21}NO_4$
Monoisotopic protonated ion (*m/z*): 328.1543
Molecular weight: 327.38
CAS number: 465-65-6
Common Salt form: HCl salt



Scheme 259–265-4 Dealkylation and formaldehyde-triggered dimerization of naloxone.

In contrast to the opioids discussed in this monograph, naloxone is an opioid antagonist and as such, it is used clinically to reverse or reduce the pharmacological effects of opioids [18]. It was developed in the 1960s and approved for treating opioid overdose in the United States in 1971. Its structure is identical to oxymorphone, except that the *N*-methyl group of the latter is replaced by an allyl group. Thus, its degradation chemistry would be expected to be similar to that of oxymorphone, as well as hydromorphone and morphine with regard to the phenolic hydroxyl and tertiary amino moiety (refer to Scheme 259–265-1). Furthermore, this replacement seems to create another degradation pathway by which nornaloxone (noroxymorphone) is formed, resulting from the dealkylation of the tertiary amino moiety. Nornaloxone appeared to be the only degradant in certain expired naloxone products from first-responders' supplies [19]. In another study, a methylene-bridged dimeric degradant of naloxone was observed, the formation of which was attributed to formaldehyde present in an experimental oral film formulation of naloxone with buprenorphine (Scheme 259–265-4) [20].

References

1 Morphine. https://en.wikipedia.org/wiki/Morphine (accessed March 3 2024).

2 Norn, S., Kruse, P.R., and Kruse, E. (2005). History of opium poppy and morphine [in Danish]. *Dan Medicinhist Arbog.* 33: 171–184.

3 Raspopin, Y.S., Shifman, E.M., and Feoktistova, D.A. (2024). History and prospects for the use of morphine in clinical practice: literature review [in Russian]. *Reg. Manag. Anesth. Acute Pain Manag.* 18: 33–40. https://doi.org/10.17816/RA624003.

4 Vermeire, A. and Remon, J.P. (1997). The solubility of morphine and the stability of concentrated morphine solutions in glass, polypropylene syringes and PVC containers. *Int. J. Pharm.* 146: 213–223.

5 Vermeire, A. and Remon, J.P. (1999). Stability and compatibility of morphine. *Int. J. Pharm.* 187: 17–51.

6 Miolo, G., Tucci, M., Mazzoli, A. et al. (2016). Photostability of 6-MAM and morphine exposed to controlled UV irradiation in water and methanol solution: HRMS for the characterization of transformation products and comparison with the dry state. *J. Pharm. Biomed. Anal.* 126: 48–59.

7 Codeine. https://en.wikipedia.org/wiki/Codeine (accessed May 4 2024).

8 Pierre Jean Robiquet. https://en.wikipedia.org/wiki/Pierre_Jean_Robiquet (accessed May 4 2024).

9 Stubberud, K.P. and Åström, O. (1998). Separation of ibuprofen, codeine phosphate, their degradation products and impurities by capillary electrophoresis: II. *Validation. J. Chrom. A.* 826: 95–102.

10 Powell, M.F. (1986). Enhanced stability of codeine sulfate: effect of pH, buffer, and temperature on the degradation of codeine in aqueous solution. *J. Pharm. Sci.* 75: 901–903.

11 Hydromorphine. https://en.wikipedia.org/wiki/Hydromorphine (accessed May 5 2024).

12 Hildebrand, K.R., Elsberry, D.E., and Anderson, V.C. (2001). Stability and compatibility of hydromorphone hydrochloride in an implantable infusion system. *J. Pain Symp. Manag.* 22: 1042–1047.

13 Oxymorphone. https://en.wikipedia.org/wiki/Oxymorphone (accessed May 5 2024).

14 Hydrocodone. https://en.wikipedia.org/wiki/Hydrocodone (accessed May 5 2024).

15 Sonar, V.N., Parkin, S., and Crooks, P.A. (2012). Oxycodone *N*-oxide. *Acta Cryst.* C68: o436–o438.

16 Sitasuwan, P., Melendez, C., Marinova, M. et al. (2016). Degradation of opioids and opiates during acid hydrolysis leads to reduced recovery compared to enzymatic hydrolysis. *J. Anal. Toxicol.* 40: 601–607.

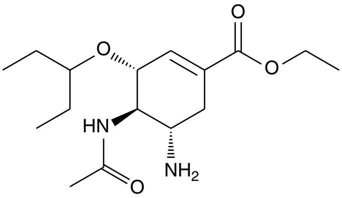
17 Zhuang, T., Jin, J., Ma, Y. et al. (2021). Structural elucidation and synthesis of a dimeric degradation impurity during long-term stability studies of oxycodone hydrochloride injection. *New J. Chem.* 45: 18651–18658.

18 Naloxone. https://en.wikipedia.org/wiki/Naloxone (accessed May 5 2024).

19 Pruyn, S., Frey, J., Baker, B. et al. (2019). Quality assessment of expired naloxone products from first-responders' supplies. *Prehosp. Emerg. Care* 23: 647–653.

20 Schmidt, P., Kolb, C., Reiser, A. et al. (2022). Isolation, identification and structural verification of a methylene-bridged naloxone "dimer" formed by formaldehyde. *J. Pharm. Sci.* 111: 1682–1689.

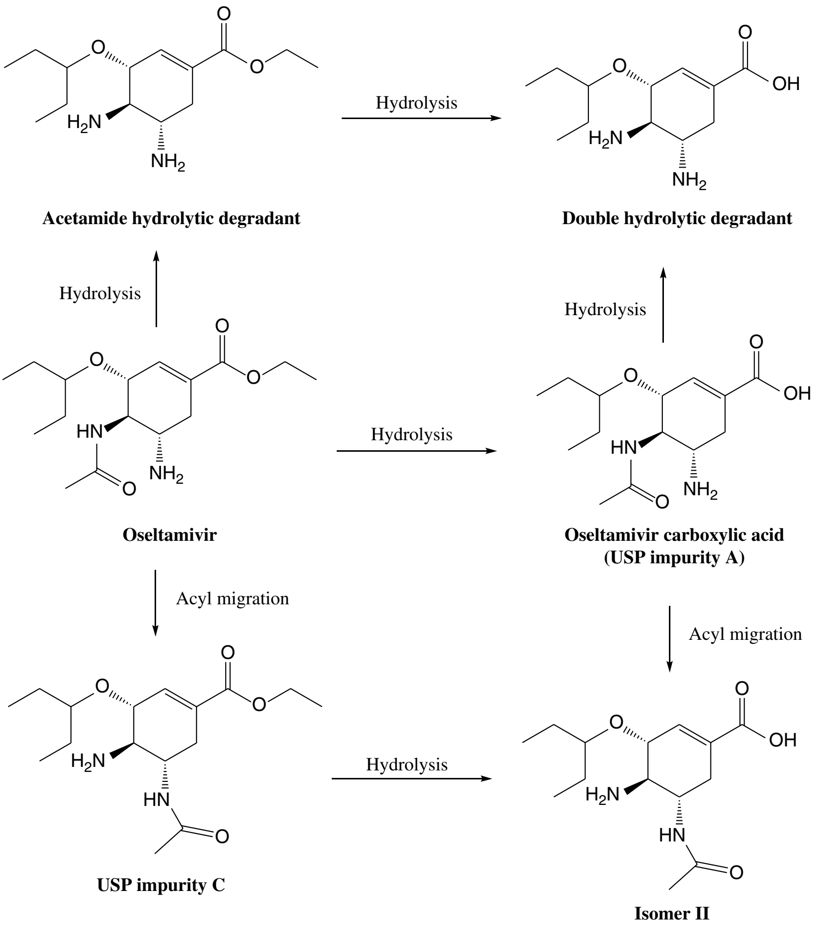
266: Oseltamivir



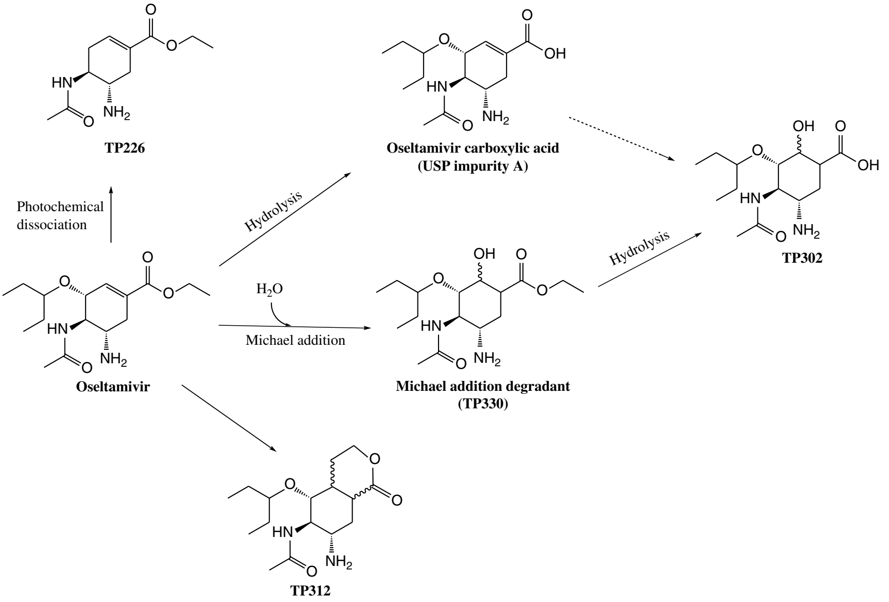
Chemical name: Ethyl (*3R,4R,5S*)-5-amino-4-acetamido-3-(pentan-3-yloxy)-cyclohex-1-ene-1-carboxylate
Trade name: Tamiflu
Formula: $C_{16}H_{28}N_2O_4$
Monoisotopic protonated ion (*m/z*): 313.2122
Molecular weight: 312.41
CAS number: 196618-13-0
Salt form: Phosphate

Oseltamivir was discovered by Gilead Sciences and then licensed to Roche in 1996 [1]. It is an inhibitor of neuraminidase, a viral surface enzyme that enables the virus to bud from infected cells and thus spread in the body [2]; it is used to treat and prevent influenza A and B, the viruses that cause influenza. Oseltamivir contains several potential hot spots susceptible to various types of degradation: the ethyl ester (hydrolysis), Michael acceptor (potentially Michael addition, but steric hindrance should decrease or suppress the reactivity), primary amine group (may subject to the Maillard reaction if excipients contain reducing sugars), and acetamide (hydrolysis and intra-molecular migration). In a forced degradation and kinetics study of oseltamivir in aqueous solution at 70°C, Oliyai et al. observed three degradants, oseltamivir carboxylic acid, isomer I and II, resulting respectively from hydrolysis of the ethyl ester, acyl migration, and both hydrolysis and migration (Scheme 266-1) [3]. In another study examining the stability of extemporaneously prepared oral solutions, Albert and Bockshorn only found oseltamivir carboxylic acid and isomer I as the degradants [4]. In another stress study, two additional degradants were formed [5], which are secondary hydrolytic degradants and hence would be unlikely to form under regular stability storage conditions.

In a photochemical degradation study under UV and simulated sunlight, oseltamivir was found to undergo photochemical Michael addition as the major photodegradation pathway, in addition to other pathways (Scheme 266-2) [6]. The formation of the photodegradant, TP312, seems to be rather unusual; the structure was proposed based on high-resolution LC–MS/MS data.



Scheme 266-1 Degradation pathways of oseltamivir.

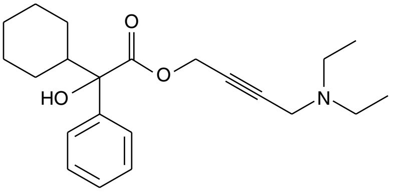


Scheme 266-2 Photodegradation pathways of oseltamivir.

References

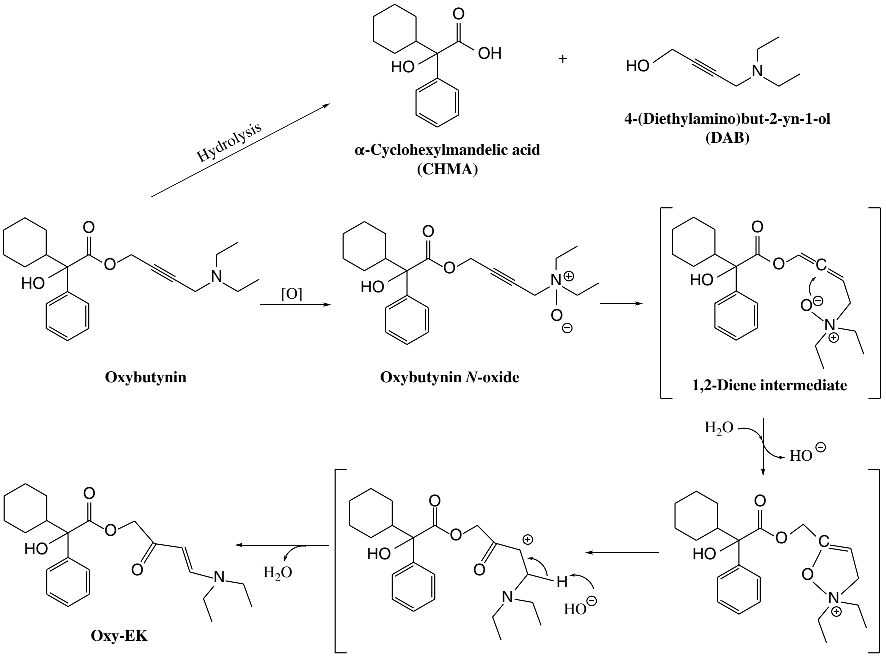
1. Oseltamivir. https://en.wikipedia.org/wiki/Oseltamivir (accessed May 8 2024).
2. Air, G.M. and Laver, W.G. (1989). The neuraminidase of influenza virus. *Proteins* 6: 341–356.
3. Oliyai, R., Yuan, L.C., Dahl, T.C. et al. (1998). Biexponential decomposition of a neuraminidase inhibitor prodrug (GS-4104) in aqueous solution. *Pharm. Res.* 15: 1300–1304.
4. Albert, K. and Bockshorn, J. (2007). Chemical stability of oseltamivir in oral solutions. *Pharmazie* 62: 678–682.
5. Junwal, M., Sahu, A., Handa, T. et al. (2012). ICH guidance in practice: degradation behaviour of oseltamivir phosphate under stress conditions. *J. Pharm. Biomed. Anal.* 62: 48–60.
6. Goncalves, C., Perez, S., Osorio, V. et al. (2011). Photofate of oseltamivir (Tamiflu) and oseltamivir carboxylate under natural and simulated solar irradiation: kinetics, identification of the transformation products, and environmental occurrence. *Environ. Sci. Technol.* 45: 4307–4314.

267: Oxybutynin



Chemical name: 4-Diethylaminobut-2-ynyl 2-cyclohexyl-2-hydroxy-2-phenylethanoate
Trade name: Ditropan, Gelnique, others
Formula: $C_{22}H_{31}NO_3$
Monoisotopic protonated ion (*m/z*): 358.2377
Molecular weight: 357.49
CAS number: 5633-20-5
Salt form: HCl salt

Oxybutynin is an anticholinergic agent, which is used primarily as the first-line treatment for urinary incontinence due to an overactive bladder [1]. It contains an ester and tertiary amine moieties, thus rendering it susceptible to hydrolytic and oxidative degradation, respectively (Scheme 267) [2–4]. In acidic to neutral pH at 37°C, oxybutynin was quite stable, but became increasingly unstable as pH increased



Scheme 267 Degradation pathways of oxybutynin.

at the alkaline pH region [2]. Furthermore, due to the presence of the alkyne functionality alpha to the tertiary amine (i.e., a tertiary propargylamine), Canavesi et al. found that oxybutynin *N*-oxide, the initially formed oxidative degradant in the presence of hydrogen peroxide, could undergo a rearrangement to produce the degradant (Oxy-EK) that contains an enamino ketone functionality [4]. Interestingly, the Oxy-EK degradant was also formed in the presence of a free-radical initiator, but the *N*-oxide degradant was absent in the reaction solution. As no specific formation mechanism was proposed by the original authors, a plausible mechanism is presented in Scheme 267 by the present author. The key for this mechanism would be the 1,2-diene intermediate, which could be formed via deprotonation of the methylene group next to the ester functionality.

References

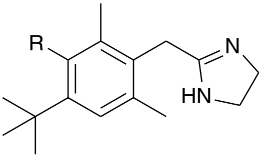
1 Oxybutynin. https://en.wikipedia.org/wiki/Oxybutynin (accessed May 9 2024).

2 Miyamoto, E., Kawashima, S., Murata, Y. et al. (1994). Physico-chemical properties of oxybutynin. *Analyst* 119: 1489–1492.

3 El-Gindy, A. (2005). High performance liquid chromatographic determination of oxeladin citrate and oxybutynin hydrochloride and their degradation products. *Il Farmaco* 60: 689–699.

4 Canavesi, R., Aprile, S., Giovenzana, G.B. et al. (2016). New insights in oxybutynin chemical stability: identification in transdermal patches of a new impurity arising from oxybutynin *N*-oxide rearrangement. *Eur. J. Pharm. Sci.* 84: 123–131.

268–269: Oxymetazoline, Xylometazoline



Oxymetazoline, R = –OH;
Xylometazoline, R = –H.

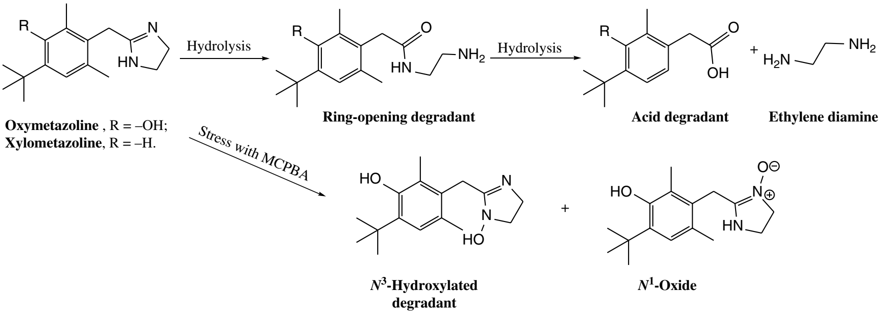
268: Oxymetazoline

Chemical name: 3-(4,5-Dihydro-1*H*-imidazol-2-ylmethyl)-2,4-dimethyl-6-tert-butylphenol
Trade name: Afrin, others
Formula: $C_{16}H_{24}N_2O$
Monoisotopic protonated ion (*m/z*): 261.1961
Molecular weight: 260.38
CAS number: 1491-59-4
Salt form: HCl salt

269: Xylometazoline [WHO List of Essential Medicines]

Chemical name: 2-[(4-tert-Butyl-2,6-dimethylphenyl)methyl]-4,5-dihydro-1*H*-imidazole
Trade name: Otrivin, Otrivine, others
Formula: $C_{16}H_{24}N_2$
Monoisotopic protonated ion (*m/z*): 245.2012
Molecular weight: 244.38
CAS number: 526-36-3
Salt form: HCl salt

Oxymetazoline and xylometazoline are sympathomimetic drugs and clinically used as topical decongestants and vasoconstrictors through stimulation of the α-receptors on blood vessels [1, 2]. Both drugs are usually available as a nasal spray or drops for treating nasal congestion, as well as eye drops for the treatment of drooping eyelids or allergic conjunctivitis. Both oxymetazoline and xylometazoline are imidazoline drugs containing a 4,5-dihydroimidazole ring, which are susceptible to typical hydrolytic degradation of amidines, particularly in the alkaline pH region (Scheme 268–269) [3–6]. Under severe hydrolytic conditions, further hydrolysis of the resulting amide linkage could occur [3]. A kinetics study by Stanisz showed that xylometazoline was most stable in pH 2.0–4.0 solutions [5]. On the other hand, a forced degradation study of oxymetazoline using 3-chloroperoxybenzoic acid (MCPBA) generated two isomeric oxidative degradants: the N^3-hydroxylated



Scheme 268–269 Hydrolytic and oxidative degradation pathways of oxymetazoline and xylometazoline.

degradant and *N*^{1}-oxide, with the former as the major one, which was observed in a severely stressed commercial sample [6].

Challier et al. studied the photodegradation behaviors of oxymetazoline and xylometazoline in the presence of riboflavin (vitamin B_2), which is a photosensitizer and also a natural constituent of the eye [7]. Oxymetazoline was found to degrade photochemically four orders of magnitude faster than xylometazoline, apparently due to the presence of the hydroxyl group that renders oxymetazoline much more labile toward the reactive oxygen species (ROS) generated in the photosensitization by riboflavin.

References

1 Oxymetazoline. https://en.wikipedia.org/wiki/Oxymetazoline (accessed April 18 2024).

2 Xylometazoline. https://en.wikipedia.org/wiki/Xylometazoline (accessed April 18 2024).

3 Golander, Y. and DeWitte, W.J. (1985). Xylometazoline hydrochloride. *Anal. Prof. Drug Sub.* 14: 135–156.

4 Prajapati, N., Dalal, M., and Raj, H.A. (2016). Stability indicating liquid chromatographic method for estimation of xylometazoline hydrochloride in pharmaceutical dosage form. *Int. J. Pharm. Chem. Anal.* 3: 124–132.

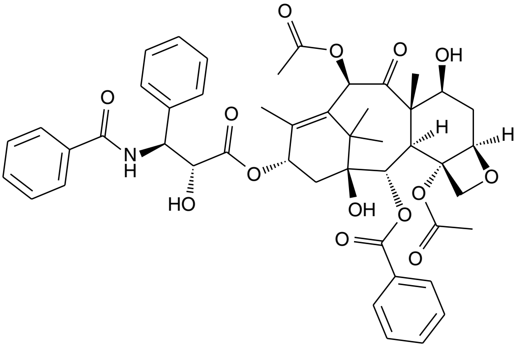
5 Stanisz, B. (2003). Kinetics of hydrolysis of xylometazoline (XYL) in aqueous solution. *React. Kinet. Catal. Lett.* 80: 67–73.

6 Leader, I.P., Halket, J., and Finch, P. (2004). Synthesis and identification of two potential oxidation degradants of oxymetazoline. *Rapid Commun. Mass Spectrom.* 18: 1645–1654.

7 Challier, C., Mártire, D.O., García, N.A., and Criado, S. (2017). Visible light-mediated photodegradation of imidazoline drugs in the presence of riboflavin: possible undesired effects on imidazoline-based eye drops. *J. Photochem. Photobiol. A: Chem.* 332 (1): 399–405.

270–271: Paclitaxel, Docetaxel

270: Paclitaxel [WHO List of Essential Medicines]



Chemicalname: (*1S,2S,3R,4S,7R,9S,10S,12R,15S*)-4,12-Bis(acetyloxy)-1,9-dihydroxy-15-({(*2R,3S*)-2-hydroxy-3-phenyl-3-[(phenylcarbonyl)amino]propanoyl}oxy)-10,14,17,17-tetramethyl-11-oxo-6-oxatetracyclo[11.3.1.0^{3,10}.0^{4,7}]eptadic-13-en-2-yl benzoate

Trade name: Taxol

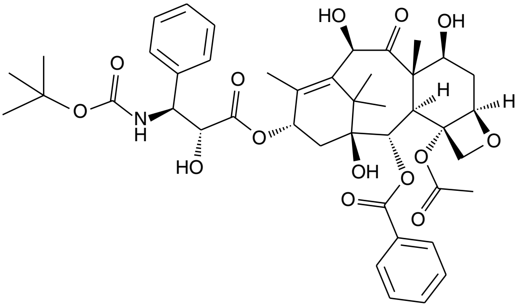
Formula: $C_{47}H_{51}NO_{14}$

Monoisotopic protonated ion (*m/z*): 854.3382

Molecular weight: 853.92

CAS number: 33069-62-4

271: Docetaxel [WHO List of Essential Medicines]



Chemical name: (*2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS*)-12b-Acetoxy-9-(((*2R,3S*)-3-((tert-butoxycarbonyl)amino)-2-hydroxy-3-phenylpropanoyl)oxy)-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1*H*-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-12-yl benzoate

Trade name: Taxotere, Docecad, Docefrez, others

Formula: $C_{43}H_{53}NO_{14}$

Monoisotopic protonated ion (*m/z*): 808.3539

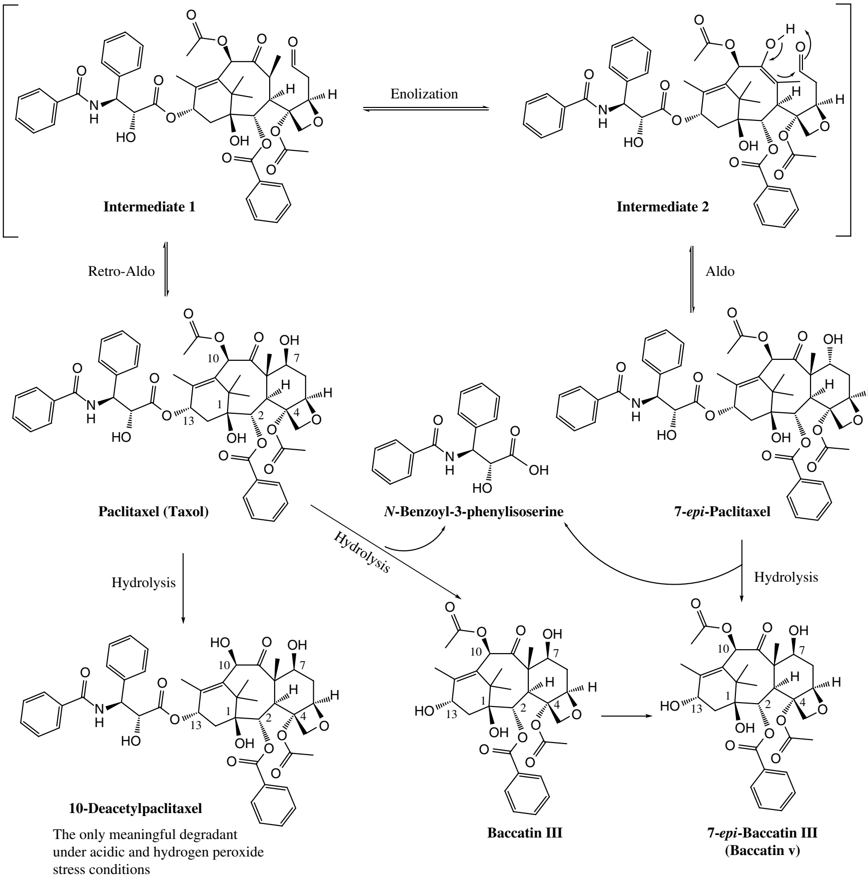
Molecular weight: 807.89

CAS number: 114977-28-5

Paclitaxel, originally known as taxol, is a chemotherapeutic drug, which is widely used to treat cancers of various types, including refractory ovarian cancer, the first indication approved by the US FDA in 1992 [1], as well as numerous other cancers such as esophageal cancer, breast cancer, lung cancer, cervical cancer, and pancreatic cancer [2]. The efforts leading to the discovery of taxol started in August 1962 when the USDA scientist Arthur Barclay collected samples from the bark of a Pacific yew tree, *Taxus brevifolia*, as part of a National Cancer Institute (NCI)-funded screening program headed by Jonathan Hartwell [1, 3]. After the extract of the bark showed anti-cancer activity in a KB cell assay, Barclay went back to the original collection site to collect 30 pounds of the bark 2 years later and sent it to Research Triangle Institute (RTI) at North Carolina where Monroe Wall and Mansukh Wani used a process of "bioactivity-directed fractionation" to identify bioactive compounds [1]. A crystalline compound was isolated from the bark extract in 1966, which was named taxol by Wall. In 1971, Wall, Wani, and their colleagues published the complete structure of taxol. In 1979, Susan Horwitz's group at Albert Einstein College of Medicine determined the anti-cancer mechanism of taxol, showing that taxol induces cell death by promoting the assembly of cell microtubules. Under a NCI's Cooperative Research and Development Agreement, Bristol Myers-Squibb took over the clinical development of taxol in 1989 and finally brought the drug into market in 1992 [1–4], which became a game changer for millions of cancer patients [5].

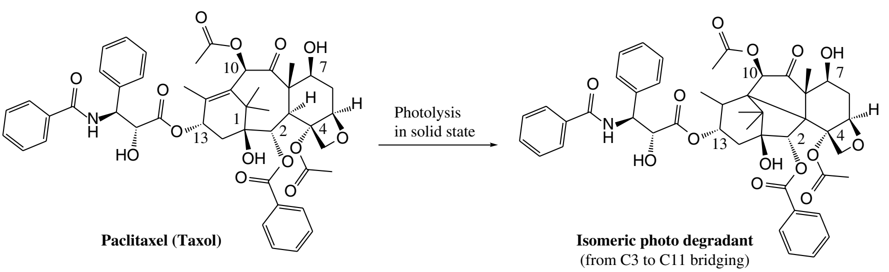
Paclitaxel has a complex tricyclic core structure with four relatively simple side chains linked via ester linkage. It is primarily converted to 7-*epi*-taxol, the thermodynamically more stable isomer due to epimerization, in both solid and solution states upon heating [6]. The epimerization at the 7-position is reversible through a retro-aldol/aldol process (Scheme 270–271-1) [7], which can be catalyzed by base but not acid [8]. Removal of the 10-acetyl group accelerated the epimerization process. On the other hand, all the four ester bonds are susceptible to hydrolytic degradation, with the C-13 ester bond being the most labile one toward hydrolysis in alkaline pH, yielding baccatin III, *N*-benzoyl-3-phenylisoserine, and 7-*epi*-baccatin III as the major hydrolytic degradants, in addition to minor hydrolytic degradants resulting from slower parallel and sequential hydrolytic degradation pathways (Scheme 270–271-1) [9]. Volk et al. performed a forced degradation study of paclitaxel under alkaline, oxidative (hydrogen peroxide), and photolytic conditions [10]. While the alkaline stress generated degradants that were consistent with those reported in the literature, 10-deacetylpaclitaxel was observed as the only degradant in hydrogen peroxide solution (Scheme 270–271-1), and an isomeric degradant was formed as the predominant photodegradant (Scheme 270–271-2), respectively [11]. It was found that amorphous paclitaxel, formulated in polymeric micelles based on hydrophobized hyaluronic acid, was more susceptible to the photoisomerization process [12].

Unlike paclitaxel, a natural product, docetaxel is a semi-synthetic compound made from 10-deacetylbaccatin III, the noncytotoxic precursor extracted from the needles of the European yew, *Taxus baccata*, which is more abundant than the Pacific yew [13]. Approved by the US FDA in 1994, it has the same mode of action as paclitaxel and thus, very similar indications for various cancers [14]. Chemically,

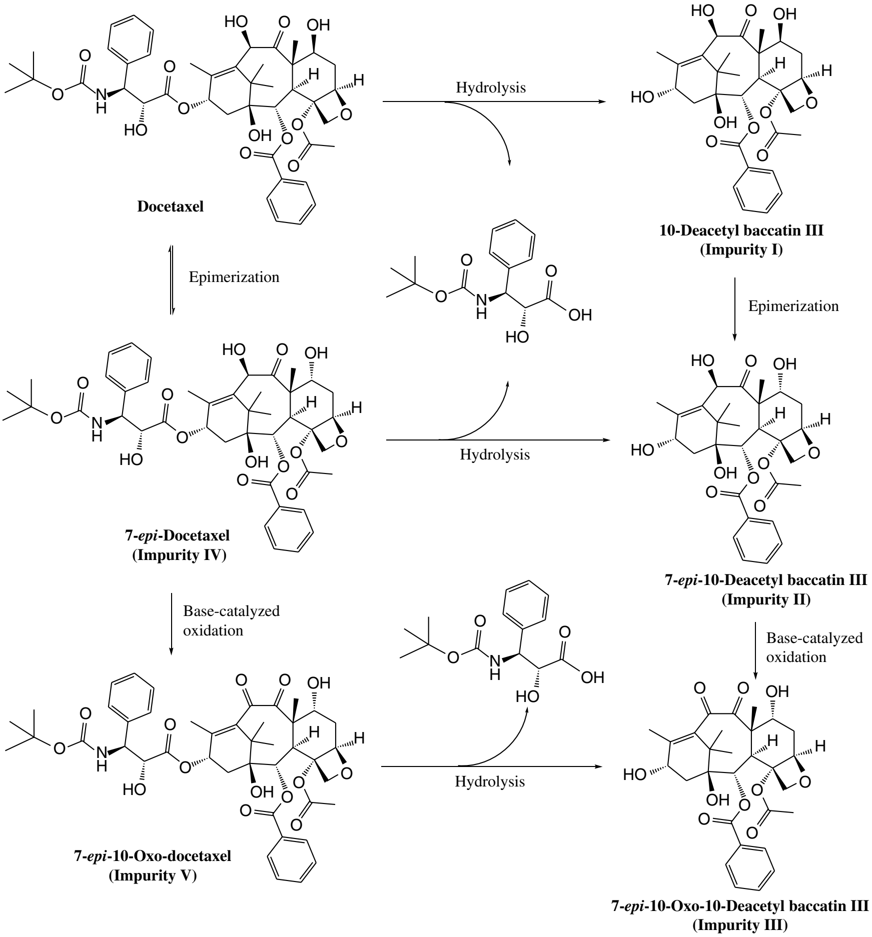


The only meaningful degradant under acidic and hydrogen peroxide stress conditions

Scheme 270–271-1 Degradation pathways of paclitaxel under alkaline (50 mM NaCO3 solution), acidic (240 minutes in 2.4 M HCl solution), and oxidative (15 minutes in 7.5% hydrogen peroxide solution) stress conditions.



Scheme 270–271-2 Photodegradation pathway of paclitaxel in the solid state.



Scheme 270–271-3 Degradation pathways of docetaxel under forced degradation conditions; the pathways are proposed by the present author based on the degradation products identified by Kumar et al., and the numbering of the degradants follows that of Kumar et al. [15]. *Source:* Kumar et al. [15] / with permission of Elsevier.

docetaxel has the 10-deacetylbaccatin III core structure with an *N*-tert-BOC-3-phenylisoserine side chain on the 13-hydroxyl group. Due to the similarity between the two drug molecules, their degradation chemistry would be expected to be quite similar, except for the now exposed 10-hydroxyl group on the core of docetaxel. Indeed, in a forced degradation study, major degradation products were observed under the alkaline stress condition in which similar hydrolytic degradation and epimerization were observed (Scheme 270–271-3) [15]. Nevertheless, the exposed 10-hydroxyl group of docetaxel was found to undergo base-catalyzed oxidation to produce the corresponding 10-oxo degradants.

References

1 National Historic Chemical Landmark. Discovery of camptothecin ad taxol. https://www.acs.org/education/whatischemistry/landmarks/camptothecintaxol.html (accessed June 8 2024).

2 Paclitaxel. https://en.wikipedia.org/wiki/Paclitaxel (accessed May 18 2024).

3 Wani, M.C. and Horwitz, S.B. (2014). Nature as a remarkable chemist: a personal story of the discovery and development of Taxol®. *Anticancer Drugs* 25: 482–487.

4 Gallego-Jara, J., Lozano-Terol, G., Sola-Martínez, R.A. et al. (2020). A compressive review about Taxol®: history and future challenges. *Molecules* 25: 5986.

5 Arnst, J. (2020). When Taxol met tubulin. *J. Biol. Chem.* 295: 13994–13995.

6 MacEachern-Keith, G.J., Wagner Butterfield, L.J., and Incorvia Mattina, M.J. (1997). Paclitaxel stability in solution. *Anal. Chem.* 69: 72–77.

7 McLaughlin, J.L., Miller, R.W., Powell, R.G., and Smith, C.R. Jr. (1981). 19-Hydroxybaccatin III, 10-deacetylcephalomannine, and 10-deacetyltaxol: new antitumor taxanes from *Taxus wallichiana*. *J. Nat. Prod.* 44: 312–319.

8 Tian, J. and Stella, V.J. (2008). Degradation of paclitaxel and related compounds in aqueous solutions I: epimerization. *J. Pharm. Sci.* 97: 1224–1235.

9 Tian, J. and Stella, V.J. (2008). Degradation of paclitaxel and related compounds in aqueous solutions II: nonepimerization degradation under neutral to basic pH conditions. *J. Pharm. Sci.* 97: 3100–3108.

10 Volk, K.J., Hill, S.E., Kerns, E.H., and Lee, M.S. (1997). Profiling degradants of paclitaxel using liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry substructural techniques. *J. Chrom. B* 696: 99–115.

11 Chen, S.H., Combs, C.M., Hill, S.E. et al. (1992). The photochemistry of taxol: synthesis of a novel pentacyclic taxol isomer. *Tetrahedron Lett.* 33: 7679–7680.

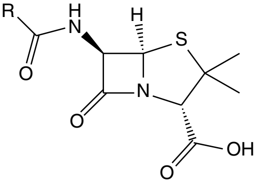
12 Šmejkalová, D., Nešporová, K., Hermannová, M. et al. (2014). Paclitaxel isomerisation in polymeric micelles based on hydrophobized hyaluronic acid. *Int. J. Pharm.* 466: 147–155.

13 Dev, R.V., Babu, J.M., Vyas, K. et al. (2006). Isolation and characterization of impurities in docetaxel. *J. Pharm. Biomed. Anal.* 40: 614–622.

14 Docetaxel. https://en.wikipedia.org/wiki/Docetaxel (accessed May 18 2024).

15 Kumar, D., Tomar, R.S., Deolia, S.K. et al. (2007). Isolation and characterization of degradation impurities in docetaxel drug substance and its formulation. *J. Pharm. Biomed. Anal.* 43: 1228–1235.

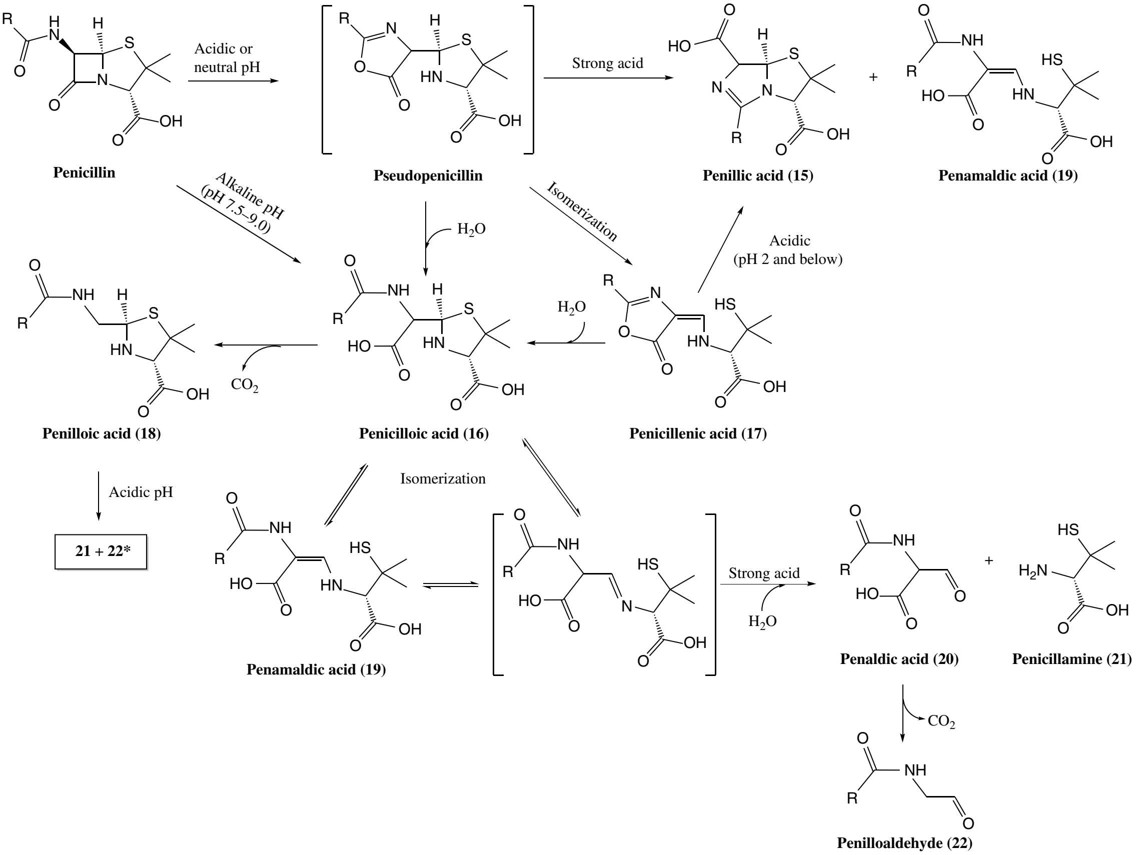
272–277: Penicillins



General structure of penicillins

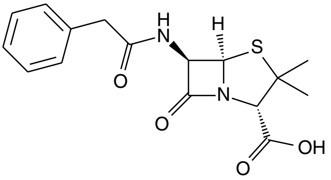
The penicillin family of antibiotics was initially discovered “accidentally” by Alexander Fleming during August–September of 1928 when he noticed a blue-green mold inhibiting the growth of the bacteria *S. aureus* [1]. It took another 14 years, during which time Howard Florey, Ernst Chain, and their colleagues scaled up the penicillin preparation in the laboratory, which enabled them to perform various in vitro and in vivo experiments to demonstrate the efficacy of penicillin extract. The scale-up preparation also enabled Fleming to perform the most important clinical test in 1942, in which a patient with a fatal infection in the nervous system was cured [1]. During the period of 1943–1945, collaborative research aiming to elucidate the structure of penicillin was carried out by research groups in the United Kingdom and the United States, resulting in the finding that several different forms of penicillin exist, dependent upon the strain of the mold used and/or the cultural conditions. The original penicillin prepared by Florey and Chain at Oxford University was named penicillin F (or penicillin I) to commemorate the discovery of penicillin by Fleming. Another penicillin isolated from a different *Penicillium* mold was penicillin G, which turned out to be much easier to isolate and more stable than penicillin F [1, 2]. Penicillin G is still clinically used today, while all of the other natural penicillins are no longer used [3]. Since the elucidation of 6-aminopenicillanic acid (6-APA) as the core of the penicillin family [4], various semi-synthetic penicillins have been developed for clinical use [5, 6], including those covered in this monograph. The discovery and eventual mass production of penicillins is a landmark for modern medicines, for which Fleming, Florey, and Chain were awarded the Nobel Prize in Physiology or Medicine in 1945.

The structures of penicillins feature a fused bicyclic core (a highly constrained thiazolidine) that contains a constrained four-membered β-lactam ring. The β-lactam is unstable and its reactivity is the basis for the mode of action of penicillins. On the other hand, this reactivity is also the main reason for the degradation of penicillins, which starts with the breakage of the β-lactam. Deshpande et al. performed a survey of the degradation of β-lactam antibiotics [7]. Under acidic or neutral pH, the main degradation pathway is isomerization to produce pseudopenicillins (Scheme 272–277-1). Under alkaline pH, the β-lactam is hydrolyzed directly, which is followed by decarboxylation. Various further degradants could form depending on specific pH, temperature, and duration of the degradation.



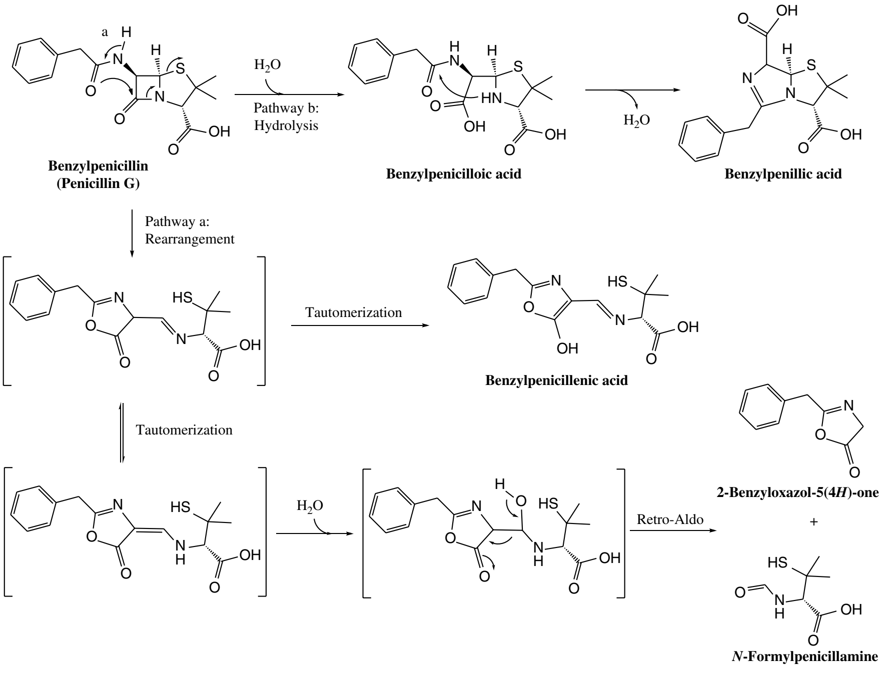
Scheme 272–277-1 Degradation pathways of penicillin in acidic and alkaline pH [7]. *Source:* Adapted from Deshpande et al. [7].

272: Benzylpenicillin [WHO List of Essential Medicines]



Chemical name: (*2S,5R,6R*)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Formula: $C_{16}H_{18}N_2O_4S$
Monoisotopic protonated ion (*m/z*): 335.1060
Molecular weight: 334.39
CAS number: 61-33-6
Salt form: Potassium salt, sodium salt

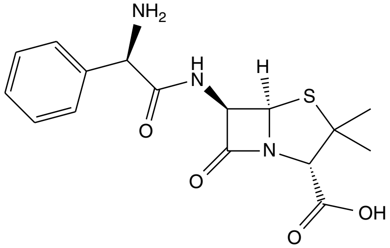
Benzylpenicillin, also called penicillin G, penicillin II, or benzylpenicillinic acid, is the first natural penicillin antibiotic that is mass produced and distributed for antibacterial indications, particularly for Gram-positive infections [1, 8]. The main degradation pathway of benzylpenicillin is the hydrolytic cleavage of the β-lactam to produce benzylpenicilloic acid. At pH 2.5, benzylpenicilloic acid could undergo intramolecular condensation to give benzylpenillic acid (Scheme 272–277-2) [9]. Decomposition of benzylpenicillin at pH 2.5 could also proceed via rearrangement



Scheme 272–277-2 Degradation pathways of benzylpenicillin in acidic pH solutions [7, 8].

and subsequent tautomerization, hydration, and retro-aldol degradation to yield benzylpenicillenic acid, *N*-formylpenicillamine, and presumably 2-benzyloxazol-5 (4*H*)-one. Benzylpenicillenic acid and its analogous degradants are believed to be the main determinants for causing the allergic reactions of benzylpenicillin and its analogs, through bio-conjugation with the lysine ε-amino groups of extracellular proteins [8]. Bird et al. reported that several penicillins decomposed to give *N*-formylpenicillamine, and at pH 5 benzylpenicillin would degrade to yield 30% *N*-formylpenicillamine [10].

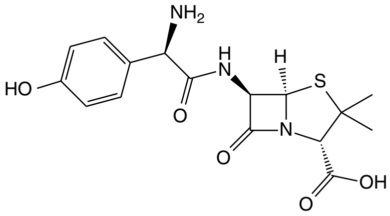
273: Ampicillin [WHO List of Essential Medicines]



Chemicalname:(*2S,5R,6R*)-6-([(*2R*)-2-Amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Trade name: Principen, others
Formula: $C_{16}H_{19}N_3O_4S$
Monoisotopic protonated ion (*m/z*): 350.1169
Molecular weight: 349.41
CAS number: 69-53-4
Salt form: Sodium salt

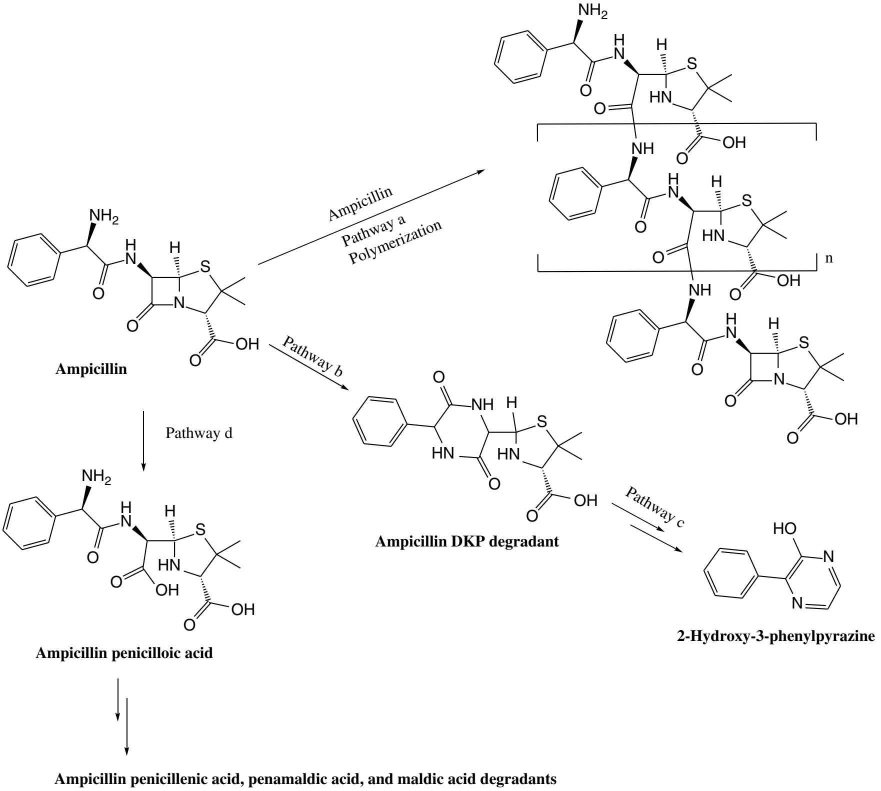
Ampicillin, introduced in 1961, is an amino derivative of benzylpenicillin, and because the amino group helps the drug to transport through the membrane of Gram-negative bacteria, it is also effective for treating Gram-negative bacterial infections [11]. Furthermore, the presence of the amino group also significantly enhances the bioavailability of the drug, rendering it available in oral dosage forms. On the other hand, the amino group causes additional degradation pathways (Scheme 272–277-3); other than that, the degradation pathways of ampicillin are similar to those outlined in Scheme 272–277-1 and Scheme 272–277-2 [12].

274: Amoxicillin [WHO List of Essential Medicines]

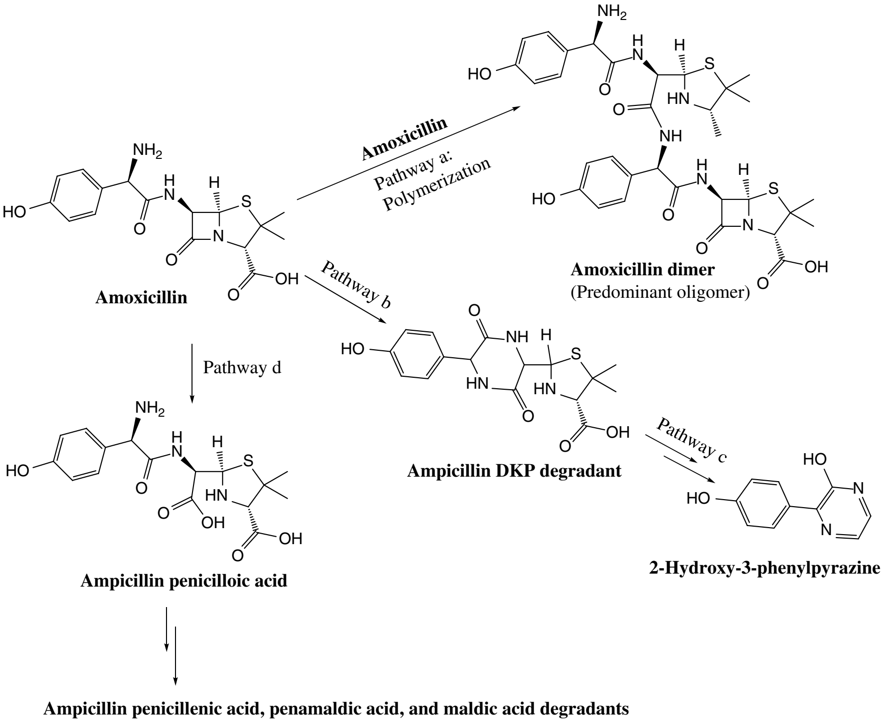


Chemical name: (*2S,5R,6R*)-6-{[(*2R*)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Trade name: Amoxil, Trimox, others
Formula: $C_{16}H_{19}N_3O_5S$
Monoisotopic protonated ion (*m/z*): 366.1118
Molecular weight: 365.40
CAS number: 26787-78-0
Salt form: Sodium salt

Amoxicillin, developed by Beecham in the United Kingdom and approved for medical use in 1972, is a widely used oral antibiotic [13] and also one of the most commonly prescribed antibiotics for children [14]. It is a hydroxyl derivative of ampicillin, with the hydroxyl group on the phenyl ring. In its formulated drug products, it is present in both the acid form (in the form of trihydrate) and the sodium salt form. Nowadays, it is often used in combination with clavulanic acid, a β-lactamase inhibitor, to overcome drug resistance toward penicillins [15]. Due to the structural similarity to ampicillin, the degradation chemistry of



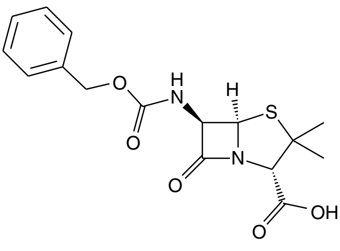
Scheme 272–277-3 Degradation pathways of ampicillin, based on those of Robinson-Fuentes et al. [12] with modification. *Source:* Adapted from Robinson - Fuentes et al. [12].



Scheme 272–277-4 Degradation pathways of ampicillin [18–20]. For the core structures of penicillenic acid, penamaldic acid, and maldic acid degradants, refer to Scheme 272–277-1.

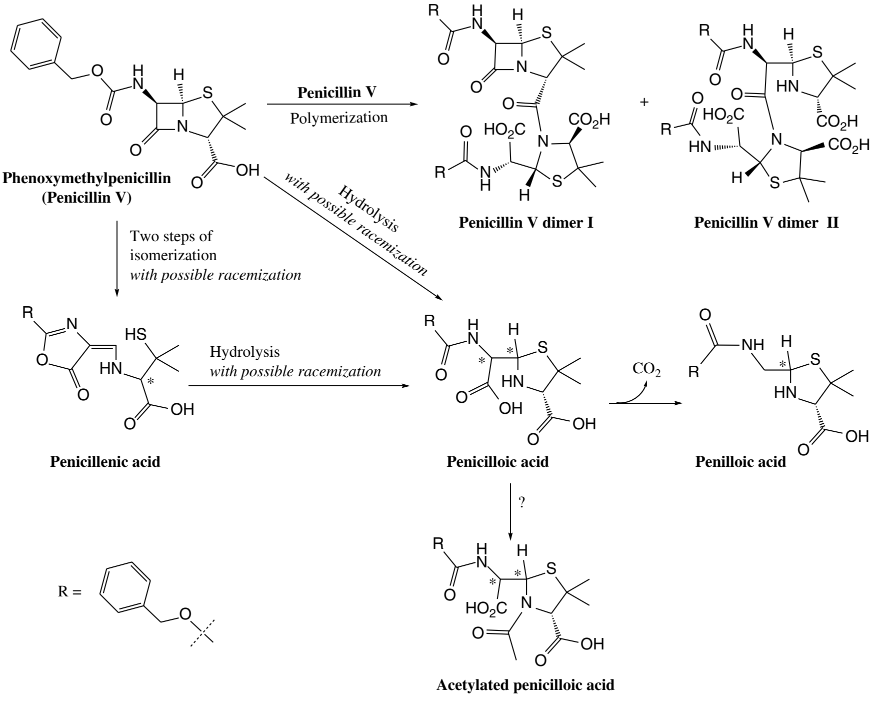
amoxicillin would be expected to be similar to that of ampicillin, particularly under stress conditions in which analogous penicilloic acid, penilloic acid, DKP degradants, as well as their diastereomers were observed [16, 17]. In API samples of amoxicillin trihydrate, amoxicilloic acid (penicilloic acid) was found to be the main degradant, while in API samples of amoxicillin sodium, DKP degradant (piperazine-2,5-dione) and a dimeric degradant were also observed (Scheme 272–277-4) [18, 19]. In a photo stress degradation study in aqueous solutions, the majority of the degradants described above were observed during different stages of the photolysis [20].

275: Phenoxymethylpenicillin [WHO List of Essential Medicines]



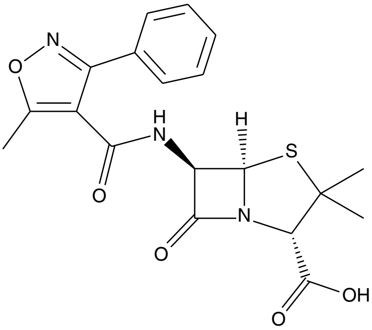
Chemical name: 3,3-Dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid
Trade name: Veetids, Apocillin, others
Formula: $C_{16}H_{18}N_2O_5S$
Monoisotopic protonated ion (*m/z*): 351.1009
Molecular weight: 350.39
CAS number: 87-08-1
Salt form: Potassium salt

Phenoxymethylpenicillin, also known as penicillin V and penicillin VK, was developed by Eli Lilly in 1948. It is a penicillin antibiotic indicated for several bacterial infections, particularly strep throat, otitis media, and cellulitis [21]. Due to its structural similarity to benzylpenicillin, their degradation chemistry would be expected to be very similar. In a study performed by Du et al. [22], penicilloic acid (3 diastereomers), penilloic acid (2 diastereomers), penicillenic acid, and two dimeric degradants were detected in commercial drug substance samples (Penicillin V for suspension) (Scheme 272–277-5).



Scheme 272–277-5 Degradation products detected in one batch of penicillin raw material and three batches of Penicillin V for suspension per the study by Du et al. [22]. The source of acetylation was not clear. *Source:* Du et al. [22] / with permission of Elsevier.

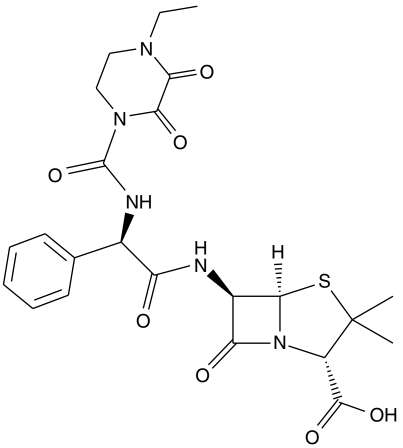
276: Oxacillin



Chemical name: 3,3-Dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid
Trade name: Veetids, Apocillin, others
Formula: $C_{19}H_{19}N_3O_5S$
Monoisotopic protonated ion (*m/z*): 402.1118
Molecular weight: 401.44
CAS number: 87-08-1
Salt form: Sodium salt

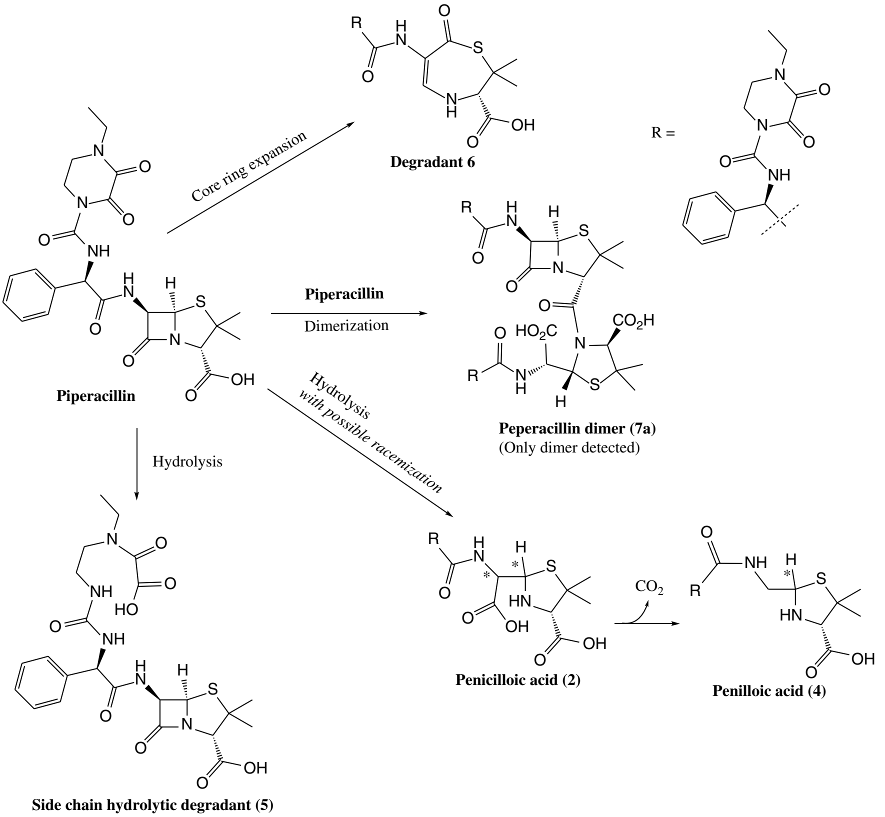
Oxacillin is a penicillinase-resistant β-lactam antibiotic, which was developed by Beecham and approved for medical use in 1962 [23]. It is usually used in its sodium salt form in its formulated products. Due to its structural similarity with the penicillins that have already been described in this monograph, the degradation chemistry of oxacillin would be expected to be similar to the penicillins [24, 25]. For example, Ashline et al. reported the isolation and structural elucidation of novel thietan-2-one degradants of sodium nafcillin and sodium oxacillin, respectively, which turned out to be the corresponding penicillenic acid degradants (refer to Scheme 272–277-1).

277: Piperacillin [WHO List of Essential Medicines]



Chemical name: (*2S,5R,6R*)-6-{[(*2R*)-2-[(4-Ethyl-2,3-dioxo-piperazine-1-carbonyl) amino]-2-phenyl-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylicacid
Trade name: Pipracil
Formula: $C_{23}H_{27}N_5O_7S$
Monoisotopic protonated ion (*m/z*): 518.1704
Molecular weight: 517.56
CAS number: 61477-96-1
Salt form: Sodium salt

Piperacillin is a β-lactam antibiotic of the ureidopenicillin class and it is typically used in combination with tazobactam, a β-lactamase inhibitor, particularly for treating serious, healthcare-associated infections [26]. It is a derivative of ampicillin and the incorporation of the ureido substituent on the side chain amino group enhances its activity against Gram-negative bacterial infections. Piperacillin was synthesized by Toyama Chemical of Japan in 1976, which was then introduced to the world market by Lederle [27]. Although the degradation chemistry of piperacillin resembles that of the structurally similar beta-lactam antibiotics, such as oxacillin, the ureido substituent could introduce new degradation pathways. In a stability study of a piperacillin/tazobactam combination by Viola et al., the ureido side chain was found to be hydrolyzed, while the rest of the drug molecule remained intact [28]. Furthermore, a degradant resulting from the ring expansion of the penicillin core was also detected. Among the more common degradants, a dimeric degradant, penicilloic acid, penicillenic acid, and penilloic acid degradants of piperacillin were also observed (Scheme 272–277-6).



Scheme 272-277-6 Degradation pathways of piperacillin per study of Viola et al. [28]. The numbering of degradants follows that of Viola et al. *Source:* Viola et al. [28] / with permission of Elsevier.

References

1 Lalchhandama, K. (2021). History of penicillin. *Wiki J. Med.* 8: 3. https://doi.org/10.15347/wjm/2021.003.

2 Robinson, F.A. (1947). Chemistry of penicillin. *Analyst* 72 (856): 274.

3 Penicillin. https://en.wikipedia.org/wiki/Penicillin (accessed June 15 2024).

4 Hodgkin, D.C. (1949). The X-ray analysis of the structure of penicillin. *Adv. Sci.* 6: 85–89.

5 Sheehan, J.C. and Logan, K.R.H. (1959). A general synthesis of the penicillins. *J. Am. Chem. Soc.* 81: 5838–5839.

6 Batchelor, F.R., Doyle, F.P., Nayler, J.H., and Rolinson, G.N. (1959). Synthesis of penicillin: 6-aminopenicillanic acid in penicillin fermentations. *Nature* 183: 257–258.

7 Deshpande, A.D., Baheti, K.G., and Chatterjee, N.R. (2004). Degradation of β-lactam antibiotics. *Cur. Sci.* 87: 1684–1695.

8 Canzani, D. and Aldeek, F. (2017). Penicillin G's function, metabolites, allergy, and resistance. *J. Nutr. Hum. Health* 1: 28–40.

9 Kessler, D.P., Cushman, M., Ghebre-Sellassie, I. et al. (1983). Investigation of a proposed penicillin G acidic degradation scheme using high-pressure liquid chromatography and optimization techniques and mechanistic considerations. *J. Chem. Soc. Perkin Trans.* 2: 1699–1703.

10 Bird, A.E., Jennings, K.R., and Marshall, A.C. (1986). *N*-Formylpenicillamine and penicillamine as degradation products of penicillins in solution. *J. Pharmacy Pharmacol.* 38: 913–917.

11 Ampicillin. https://en.wikipedia.org/wiki/Ampicillin (accessed June 14 2024).

12 Robinson-Fuentes, V.A., Jefferies, T.M., and Branch, S.K. (1997). Degradation pathways of ampicillin in alkaline solutions. *J. Pharm. Pharmacol.* 49: 843–851.

13 de Haen, P. (1975). New products parade 1973–1974, USA. *Drug Intelli. Clin. Pharm.* 9: 504–512.

14 Amoxicillin. https://en.wikipedia.org/wiki/Amoxicillin (accessed June 14 2024).

15 Geddes, A.M., Klugman, K.P., and Rolinson, G.N. (2007). Introduction: historical perspective and development of amoxicillin/clavulanate. *Int. J. Antimicrob. Agents Suppl.* 2: S109–S112.

16 Nägele, E. and Moritz, R. (2005). Structure elucidation of degradation products of the antibiotic amoxicillin with ion trap MS^n and accurate mass determination by ESI TOF. *J. Am. Soc. Mass Spectrom.* 16: 1670–1676.

17 Perez-Parada, A., Aguera, A., del Mar Gomez-Ramos, M. et al. (2011). Behavior of amoxicillin in wastewater and river water: identification of its main transformation products by liquid chromatography/electrospray quadrupole time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 25: 731–742.

18 De Pourcq, P., Hoebus, J., Roets, E. et al. (1985). Quantitative determination of amoxicillin and its decomposition products by high-performance liquid chromatography. *J. Chrom. A.* 321: 441–449.

19 Roets, E. and De Pourcq, P. (1984). Isolation and structure elucidation of ampicillin and amoxicillin oligomers. *J. Chrom. A.* 303: 117–129.

20 Arsand, J.B., Hoff, R.B., Jank, L. et al. (2018). Transformation products of amoxicillin and ampicillin after photolysis in aqueous matrices: identification and kinetics. *Sci. Total Environ.* 642: 954–967.

21 Phenoxymethylpenicillin. https://en.wikipedia.org/wiki/Phenoxymethylpenicillin (accessed June 14 2024).

22 Du, J., Chang, Y., Zhang, X., and Hu, C. (2020). Development of a method of analysis for profiling of the impurities in phenoxymethylpenicillin potassium based on the analytical quality by design concept combined with the degradation mechanism of penicillins. *J. Pharm. Biomed. Anal.* 186: 113309.

23 Oxacillin. https://en.wikipedia.org/wiki/Oxacillin (accessed June 14 2024).

24 Ashline, K.A., Attrill, R.P., Chess, E.K. et al. (1990). Isolation, structure elucidation, and synthesis of novel penicillin degradation products: thietan-2-ones. *J. Chem. Soc. Perkin Trans.* 2: 1559–1566.

25 Wu, Q.-Q., Liu, Y.-M., and Ni, M.-X. (2011). Analyzing impurities degraded by alkali in oxacillin sodium. *Chin. J. Pharm. Anal.* 31: 1102–1105.

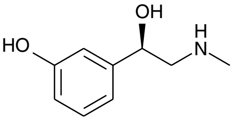
26 Piperacillin. https://en.wikipedia.org/wiki/Piperacillin (accessed June 14 2024).

27 Kumazawa, J. and Yagisawa, M. (2002). The history of antibiotics: the Japanese story. *J. Infect. Chemother.* 8: 125–133.

28 Viola, A., Ferrazzano, L., Martelli, G. et al. (2019). Novel insights into the chemistry of an old medicine: a general degradative pathway for penicillins from a piperacillin/tazobactam stability study. *Eur. J. Pharm. Sci.* 136: 104957.

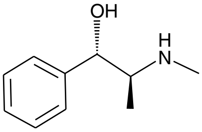
278–280: Phenylephrine, Pseudoephedrine, Ephedrine

278: Phenylephrine



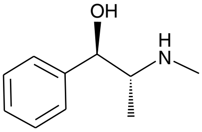
Chemical name: (*R*)-3-[-1-Hydroxy-2-(methylamino)ethyl]phenol
Trade name: Neo-synephrine, others
Formula: $C_9H_{13}NO_2$
Monoisotopic protonated ion (*m/z*): 168.1019
Molecular weight: 167.21
CAS number: 59-42-7
Salt form: HCl salt, Bitartrate

279: Pseudoephedrine



Chemical name: (*1S,2S*)-2-Methylamino-1-phenylpropan-1-ol
Formula: $C_{10}H_{15}NO$
Monoisotopic protonated ion (*m/z*): 166.1226
Molecular weight: 165.24
CAS number: 90-82-4
Salt form: HCl salt, Sulfate

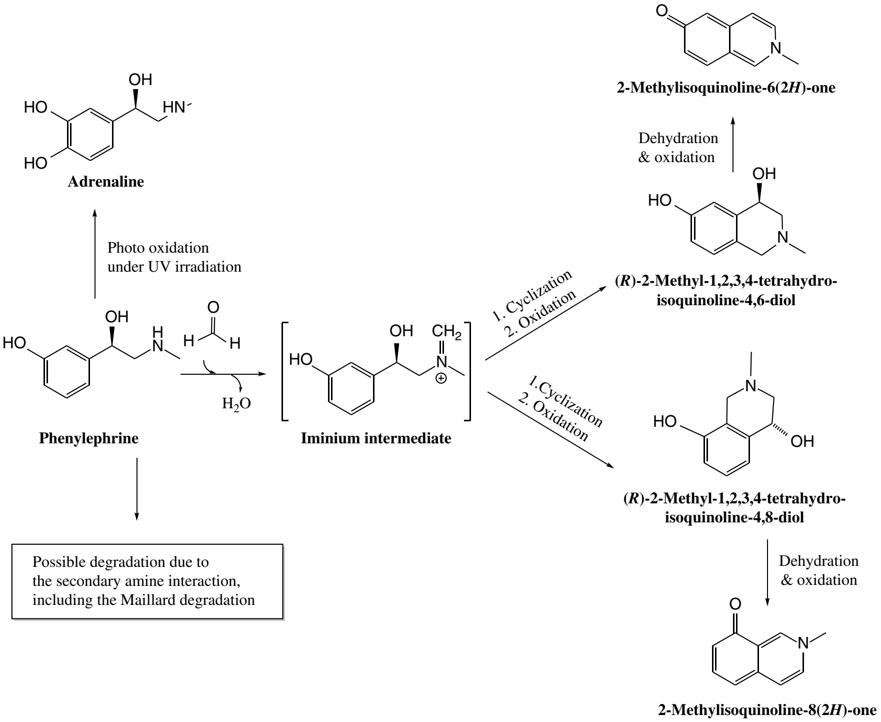
280: Ephedrine [WHO List of Essential Medicines]



Chemical name: (*1R,2S*)-2-(Methylamino)-1-phenylpropan-1-ol
Formula: $C_{10}H_{15}NO$
Monoisotopic protonated ion (*m/z*): 166.1226
Molecular weight: 165.24
CAS number: 299-42-3
Salt form: HCl salt, Sulfate

Phenylephrine is a sympathomimetic drug primarily used as a nasal decongestant. Although it can be taken orally or as a nasal spray for that indication, a recent study by an independent advisory committee to the US FDA concluded that there is insufficient evidence to show that the oral administration of phenylephrine is effective in relieving nasal congestion [1]. Phenylephrine came into medical use in the 1930s, and more recently, it has become a substitute for pseudoephedrine, a nasal decongestant that has the potential for abuse. Despite its long history of medical use, there has been limited literature regarding the degradation chemistry of phenylephrine. The phenol ring of the drug molecule is an electron-rich moiety, and as such, it would be labile toward further oxidation. In a photodegradation study, phenylephrine was oxidized to form adrenaline (Scheme 278–280-1) [2]. In a stability study by Millard et al., phenylephrine was found to yield two isoquinolinone degradants [3, 4], which were most likely preceded by the two tetrahydroisoquinolinediols. The latter two intermediary degradants were also observed in the formulated products containing phenylephrine and drug molecules with similar structural features via phenolic cyclization [5]. The phenolic cyclization process consists of two steps: cyclization, in which the iminium intermediates are formed between the drug molecules and formaldehyde, a common impurity and contaminant [6], and the subsequent spontaneous oxidation.

The secondary amine functionality of phenylephrine would also chemically interact with other excipients (or their impurities) or counter ions of a drug molecule that is co-formulated with phenylephrine. For example, in tablet formulations of phenylephrine with aspirin, three acetylated phenylephrine degradants

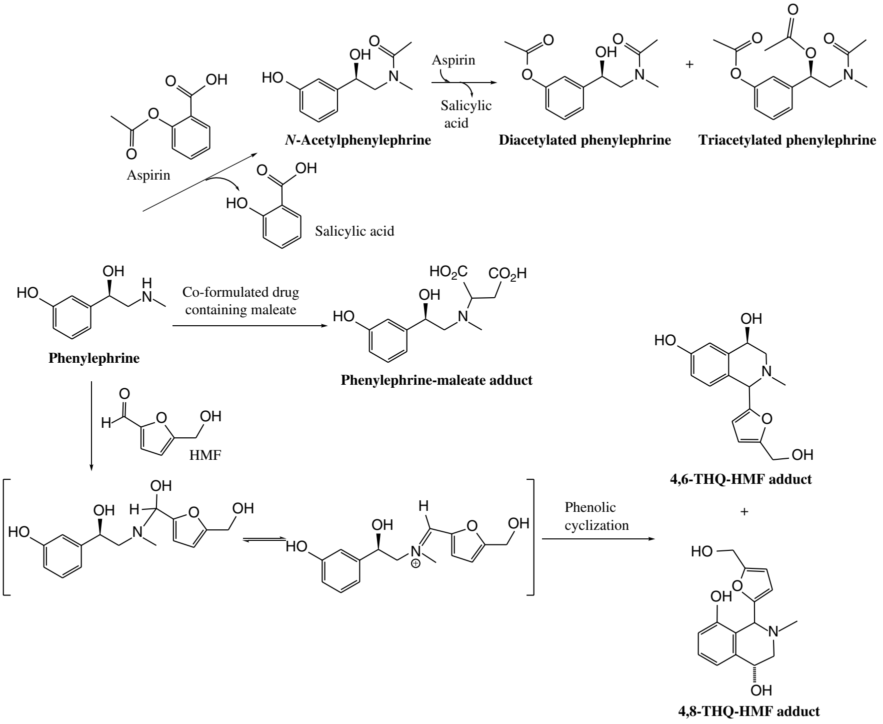


Scheme 278–280-1 Degradation pathway of phenylephrine (Part 1) [2–5].

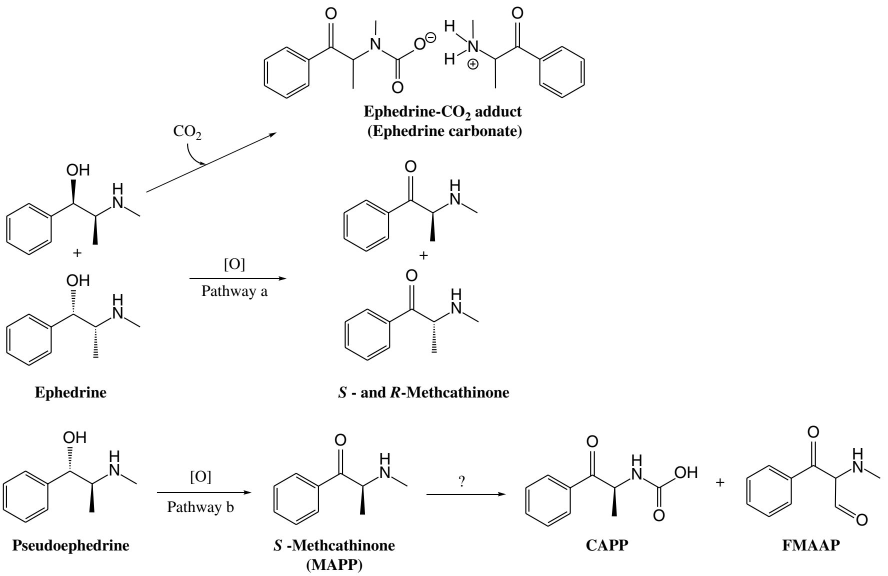
were detected. At room temperature, it was primarily *N*-acetylphenylephrine, while at elevated temperatures, acetylation also occurred onto the phenylephrine's phenolic and hydroxyl groups, resulting in the formation of di- and tri-acetylated degradants, respectively (Scheme 278–280-2) [7]. In a combination product containing chlorpheniramine maleate, phenylephrine was found to react with the counter ion maleate [8], and the correct structure of this degradant was reported by Wong et al. as resulting from the Michael addition [9]. In another combination tablet formation containing acetaminophen (paracetamol), Dousa et al. reported the identification of two novel degradants formed between the drug molecule and 5-hydroxymethyl-2-furaldehyde (5-HMF), an aldehyde impurity commonly present in polysaccharides such as starch [10].

Pseudoephedrine is a natural product that is present in *Ephedra sinica*, also known as Ma Huang, along with ephedrine, its diastereomer. The medical use of Ma Huang dates back to over 5,000 years in China and over 2,000 years in the Middle East for treating fever, cough, and common colds among others [11]. The use of pure pseudoephedrine in modern times for treating nasal and sinus congestion started in the 1920s [12]. Due to the risk of potential abuse and the ease of converting to methcathinone, a controlled substance [13], its over-the-counter (OTC) status has been changed to behind-the-counter in the United States, and it is being partially replaced by phenylephrine, its structural analog.

On the other hand, ephedrine, which is a diastereomer of pseudoephedrine and available as a racemic mixture, was first isolated in 1885 and came into commercial use in 1926 [14]. Today, its sulfate salt is often used as a ready-to-use injectable for the prevention of low blood pressure during anesthesia. Its oral dosage forms are used for the temporary relief of shortness of breath, chest tightness, and wheezing due to asthma. Due to their structural similarity to phenylephrine, the degradation chemistry of pseudoephedrine and ephedrine would be expected to be similar to that of phenylephrine, particularly with regard to the reactivity of the secondary amine moieties (refer to Scheme 278–280-1 and -2). On the other hand, it has long been known that ephedrine and pseudoephedrine are susceptible to oxidative degradation [15–17], resulting in the formation of *R,S*- and *S*-methcathinone, respectively, as the primary degradants (Scheme 278–280-3). In a forced degradation study of a pseudoephedrine tablet formulation by Wu et al. [17], two additional degradants were reported, which would result from rather unusual oxidation of each methyl group, respectively. Typically, an *N*-carboxylic acid such as the degradant CAPP would not be very stable, particularly in isolated form. In the original study of ephedrine stability in solution performed in 1931, a 2:1 adduct between ephedrine and carbon dioxide (referred to as ephedrine carbonate by the authors) was isolated, and it would decompose on standing at room temperature to give back ephedrine [15]. Although no specific structure was provided in the 1931 study, a plausible structure is presented in Scheme 278–280-3, where the first ephedrine molecule covalently interacts with carbon dioxide from the air to yield the *N*-carboxylic acid, and then the latter forms a salt with the second ephedrine molecule.



Scheme 278–280-2 Degradation pathway of phenylephrine (Part 2) [7–10].



Scheme 278–280-3 Degradation pathways of ephedrine and pseudoephedrine. The nomenclature for the degradants MAPP, CAPP, and FMAAP follows that of Wu et al. [17]. *Source:* Wu et al. [17] / with permission of Elsevier.

References

1 Phenylephrine. https://en.wikipedia.org/wiki/Phenylephrine (accessed May 18 2024).

2 Luduena, F.P., Snyder, A.L., and Lands, A.M. (1963). Effect of ultra-violet irradiation of phenylephrine solutions. *J. Pharm. Pharmacol.* 15: 538–543.

3 Millard, B.J., Priaulx, D.J., and Shotton, E. (1973). The stability of aqueous solutions of phenylephrine at elevated temperatures: identification of the decomposition products. *J. Pharm. Pharmacol.* 25 (Suppl): 24P–31P.

4 Luduena, F.P., Snyder, A.L., and Lands, A.M. (1963). Effect of ultra-violet irradiation of phenylephrine solutions. *J. Pharm. Pharmacol.* 15: 538–543.

5 Chafetz, L., Turdiu, R., and R. (1987). Phenolic cyclization of epinephrine, metaproterenol, metaraminol, phenylephrine, and terbutaline with formaldehyde. *Pharm. Res.* 4: 158–161.

6 Li, M. (2012). Section 5.2. Degradation caused by impurity of excipients. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

7 Troup, A.E. and Mitchner, H. (1964). Degradation of phenylephrine hydrochloride in tablet formulations containing aspirin. *J. Pharm. Sci.* 53: 375–379.

8 Marin, A., Espada, A., Vidal, P., and Barbas, C. (2005). Major degradation product identified in several pharmaceutical formulations against the common cold. *Anal. Chem.* 77: 471–477.

9 Wong, J., Wiseman, L., Al-Mamoon, S. et al. (2006). Major degradation product identified in several pharmaceutical formulations against the common cold. *Anal. Chem.* 78: 7891–7895.

10 Dousa, M., Gibala, P., Havlícek, J. et al. (2011). Drug-excipient compatibility testing – identification and characterization of degradation products of phenylephrine in several pharmaceutical formulations against the common cold. *J. Pharm. Biomed. Anal.* 55: 949–956.

11 Głowacka, K. and Wiela-Hojenska, A. (2021). Pseudoephedrine – benefits and risks. *Int. J. Mol. Sci.* 22: 5146.

12 Kenny, K. (2017). Pseudoephedrine: uses, indications, and adverse effects. *Pharmacy Times* 83 (11): https://www.pharmacytimes.com/view/pseudoephedrine-uses-indications-and-adverse-effects (accessed July 10 2024.

13 American Chemical Society. Pseudoephedrine, molecule of the week archive. https://www.acs.org/molecule-of-the-week/archive/p/pseudoephedrine.html (accessed July 10 2024).

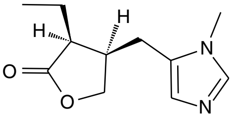
14 Ephedrine. https://en.wikipedia.org/wiki/Ephedrine (accessed July 14 2024).

15 Moore, E.E. and Moore, M.B. (1931). Effect of sunlight on ephedrine solutions. *Ind. Eng. Chem.* 23: 21–23.

16 Beckett, A.H., Jones, G.R., and Hollingsbee, D.A. (1978). Degradation of (-)-ephedrine in solution and during extraction with diethyl ether. *J. Pharm. Pharmac.* 30: 15–19.

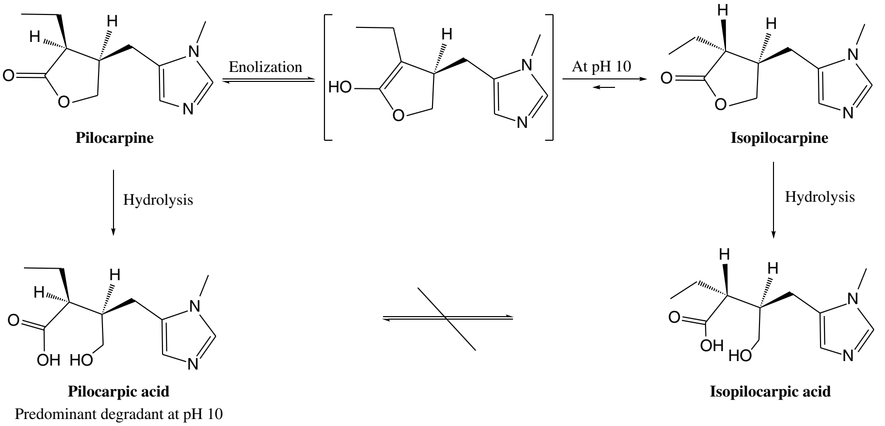
17 Wu, N., Feng, W., Lin, E. et al. (2002). Quantitative and structural determination of pseudoephedrine sulfate and its related compounds in pharmaceutical preparations using high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* 30: 1143–1155.

281: Pilocarpine [WHO List of Essential Medicines]



Chemical name: (*3S,4R*)-3-Ethyl-4-((1-methyl-1*H*-imidazol-5-yl)methyl) dihydrofuran-2(3*H*)-one
Trade name: Isopto Carpine, Salagen, Vuity, others
Formula: $C_{11}H_{16}N_2O_2$
Monoisotopic protonated ion (*m/z*): 209.1285
Molecular weight: 208.26
CAS number: 92-13-7
Salt form: HCl salt, Nitrate

Pilocarpine is a histidine alkaloid that was originally isolated from *Pilocarpus jaborandi*, a South American plant. It is an agonist of the cholinergic M-receptor and clinically used for treating glaucoma in the form of eye drops [1], and for treating dry mouth in the form of oral dosages [2]. The main degradation pathways (Scheme 281) of pilocarpine are epimerization (at the methylene position α to the carbonyl group) and hydrolysis (of the lactone ring) [3–8]. At pH 10, Bundgaard and Hansen found that the predominant degradant was pilocarpic acid, and while the epimerization was found to be reversible, the equilibrium was strongly in favor of isopilocarpine [4]. Although it might not be clearly described in the literature, the epimerization of pilocarpine would most likely proceed through enolization of the lactone ring. On the other hand, pilocarpic acid would not epimerize to isopilocarpic acid [4].



Scheme 281 Degradation pathways of pilocarpine.

References

1 Lin, Y.-H., Fang, L.-H., and Du, G.-H. (2018). Pilocarpine. In: *Natural Small Molecule Drugs from Plants*. Singapore: Springer https://doi.org/10.1007/978-981-10-8022-7_51.

2 Pilocarpine. https://en.wikipedia.org/wiki/Pilocarpine (accessed May 18 2024).

3 O'Donnell, J.J., Sandman, R., and Drake, M.V. (1980). Measurement of pilocarpine and its degradation products by high-performance liquid chromatography. *J. Pharm. Sci.* 69: 1096–1097.

4 Bundgaard, H. and Hansen, S.H. (1982). Hydrolysis and epimerization kinetics of pilocarpine in basic aqueous solution as determined by HPLC. *Int. J. Pharm.* 10: 281–289.

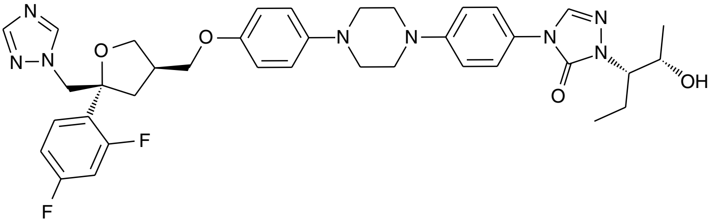
5 Yoshioka, S., Aso, Y., Shibazaki, T., and Uchiyama, M. (1986). Stability of pilocarpine ophthalmic formulations. *Chem. Pharm. Bull.* 34: 4280–4286.

6 Sternitzke, K.D., Fan, T.Y., and Dunn, D.L. (1992). High-performance liquid chromatographic determination of pilocarpine hydrochloride and its degradation products using a β-cyclodextrin column. *J. Chrom. A* 589: 159–164.

7 Fawcett, J.P., Tucker, I.G., Davies, N.M., and Ferguson, M.M. (1994). Formulation and stability of pilocarpine oral solution. *Int. J. Pharm. Pract.* 3: 14–18.

8 Fan, T.Y., Wall, G.M., Sternitzke, K. et al. (1996). Improved high-performance liquid chromatographic determination of pilocarpine and its degradation products in ophthalmic solutions. Importance of octadecylsilane column choice. *J. Chromatogr.* 740: 289–295.

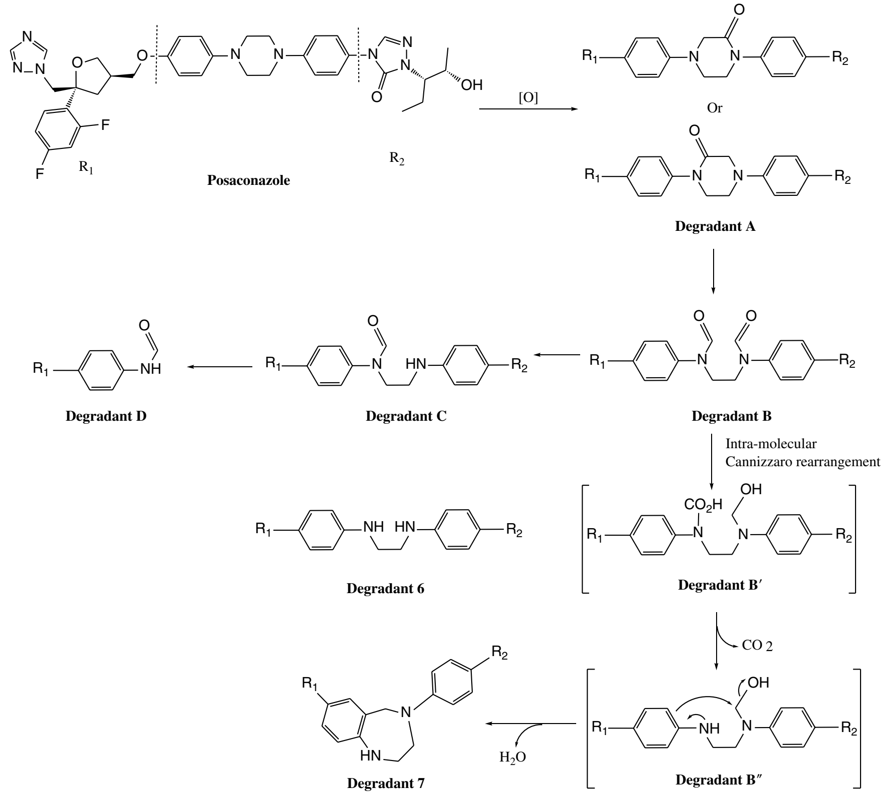
282: Posaconazole



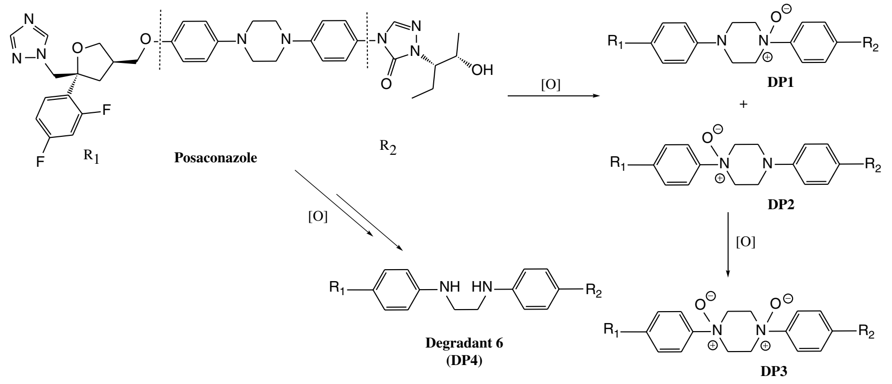
Chemicalname: 4-[4-[4-[4-[[(*3R,5R*)-5-(2,4-Difluorophenyl)tetrahydro-5-(1*H*-1,2,4-triazol-1-ylmethyl)-3-furanyl]methoxy]phenyl]-1-piperazinyl]phenyl]-2-[(*1S,2S*)-1-ethyl-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one
Trade name: Noxafil, Posanol
Formula: $C_{37}H_{42}F_2N_8O_4$
Monoisotopic protonated ion (*m/z*): 701.3370
Molecular weight: 700.79
CAS number: 171228-49-2

Posaconazole is a triazole antifungal medication developed in the mid-2000s by Schering-Plough, which was merged into Merck & Co. in 2009. Its mode of action is to inhibit the fungal enzyme lanosterol 14-α-demethylase, which is vital for the formation of fungal cell walls [1]. Posaconazole is a long, somewhat linear molecule with a rigid central moiety consisting of an *N, N*-diphenyl-substituted piperazine building block. The substituted piperazine ring is susceptible to oxidative degradation. In 2000, a Schering-Plough research group published a forced degradation study of posaconazole in solid state, in which all four major degradants stemmed from the oxidative destruction of the piperazine ring (Scheme 282-1) [2, 3]. Later on, two more degradants were reported: one is a ring-broken degradant and the other ring-contracted degradant. The structure of the ring-contracted degradant (degradant **7**) was originally proposed by Zhong et al. based on high-resolution mass spectrometric data [4], and the corrected structure was shortly published by the same research group with comprehensive NMR analysis [5]. Due to the fact that no mechanism for the formation of degradant **7** was proposed by the original authors [5], a plausible mechanism is hereby proposed, which might proceed via an intramolecular Cannizzaro rearrangement (Scheme 282-1) [6].

In a forced degradation study of posaconazole in solution, both nitrogens of the substituted piperazine ring were found to be oxidized, resulting in the formation of the respective *N*-oxide degradants, as well as the *N,N*-dioxide degradant (Scheme 282-2) [7, 8]. Posaconazole was found to be stable under other conditions of forced degradation.



Scheme 282-1 Degradation of posaconazole in solid state under either a forced degradation condition (150°C, 12 hours) [2], which gave degradants A–D, or in a formulation under stability condition [4], which yielded degradants 6 and 7. The nomenclature of the degradants follows that of respective studies [2, 4]. The plausible formation mechanism of degradant 7 is proposed by the current author.



Scheme 282-2 Forced oxidative degradation of posaconazole in hydrogen peroxide solution [5, 7]. The nomenclature of the degradants follows that of Zhong et al. (Degradant 6) [5] and Yang et al. (DP1–DP4) [7], respectively. *Source:* Zhong et al. [5] and Yang et al. [7].

References

1 Greer, N.D. (2007). Posaconazole (Noxafil): a new triazole antifungal agent. *Proc. (Bayl Univ Med Cent).* 20: 188–196. https://doi.org/10.1080/08998280.2007.11928283.

2 Feng, W., Liu, H., Chen, G. et al. (2001). Structural characterization of the oxidative degradation products of an antifungal agent Scheme 56592 by LC-NMR and LC–MS. *J. Pharm. Biomed. Anal.* 25: 545–557.

3 Warrack, B.M., Goodenough, A.K., and Chen, G. (2010). Analysis of impurities and degradants in pharmaceuticals by high resolution tandem mass spectrometry and on-line H/D exchange LC/MS. *Am. Pharm. Rev.* 13: 20–27. https://www.americanpharmaceuticalreview.com/Featured-Articles/116627-Analysis-of-Impurities-and-Degradants-in-Pharmaceuticals-by-High-Resolution-Tandem-Mass-Spectrometry-and-On-line-H-D-Exchange-LC-MS/ (accessed July 13 2024).

4 Zhong, W., Yang, J., and Yang, X. (2011). Structural determination of an unknown drug product using CASI technology in a Fourier transform ion cyclotron resonance mass spectrometer. *Rapid Commun. Mass Spectrom.* 25: 3651–3655.

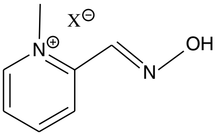
5 Zhong, W., Yang, X., Tong, W., and Martin, G.E. (2012). Structural characterization of a novel degradant of the antifungal agent posaconazole. *J. Pharm. Biomed. Anal.* 66: 40–49.

6 Li, M., Chen, B., Monteiro, S., and Rustum, A.M. (2009). Mechanism of base-catalyzed autooxidation of corticosteroids containing 20-keto-21-hydroxyl side chain. *Tetrahedron Lett.* 50: 4575–4581.

7 Yang, Y., Zhu, X., Zhang, F. et al. (2016). Stability-indicating HPLC method development and structural elucidation of novel degradation products in posaconazole injection by LC–TOF/MS, LC–MS/MS and NMR. *J. Pharm. Biomed. Anal.* 125: 165–177.

8 Ribeiro de Araújo, J.I., de Moura França, L., Soares, M.F.L.R. et al. (2019). Stability study and oxidative degradation kinetics of posaconazole. *Microchem. J.* 151: 104181.

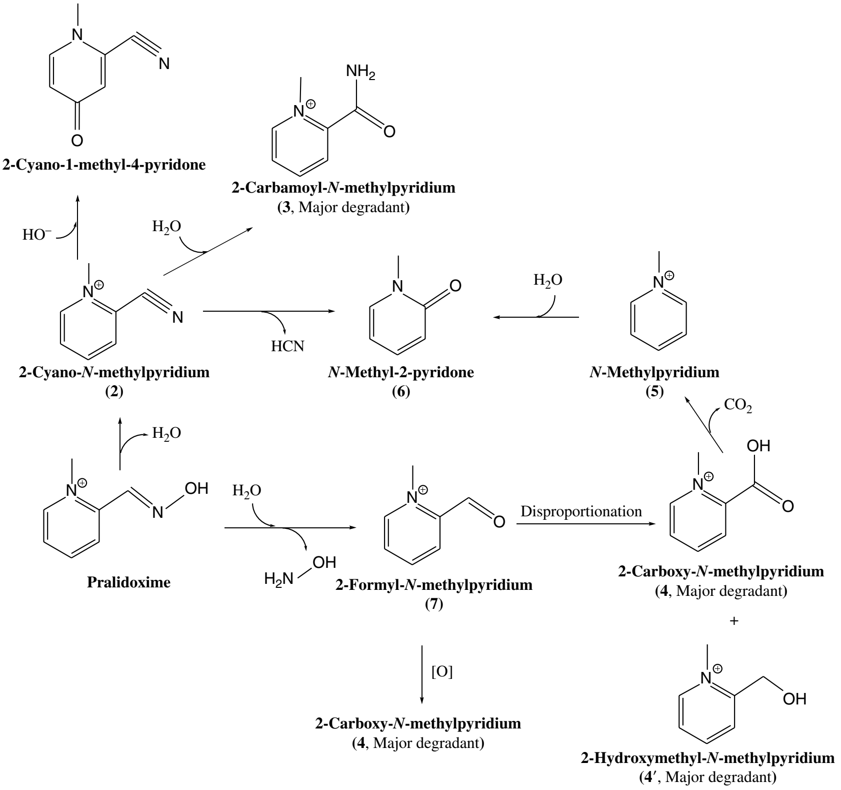
283: Pralidoxime



$X^- = Cl^-$, I^-, or $MeSO_3^-$

Chemical name: 2-[(Hydroxyimino)methyl]-1-methylpyridin-1-ium chloride (for the chloride salt)
Trade name: Atnaa, Duodote, Protopam
Formula: $C_7H_9N_2O^+$
Monoisotopic pyridinium ion (*m/z*): 137.070939
Molecular weight: 172.61 (for Pralidoxime chloride)
CAS number: 6735-59-7
Salt form: Chloride, Iodide, Mesylate

Pralidoxime is an antidote for the treatment of poisoning by organophosphates, typically in combination with atropine and diazepam or midazolam [1]. Organophosphates are covalent inhibitors of acetylcholinesterase, and pralidoxime reactivates the enzyme by cleaving the covalent linkage formed between the inhibitors and acetylcholinesterase. Pralidoxime is susceptible to degradation in aqueous solutions of both acidic and alkaline pH (Scheme 283) [2–5]. In a study of pralidoxime injection solution buffered at pH 2.5, six degradants were observed after storage at 80°C for 7—26 days, of which five were identified based on comparison to reference compounds, i.e., the major degradants **3**, **4**, **4′** and minor ones **2** and **6** [2]. Hydroxylamine and cyanide were detected in pralidoxime formulation [5]. Although a straightforward hydrolysis of pralidoxime should produce degradant **7** and hydroxylamine, degradant **7** was not observed in the studies performed by Prue et al. and Fyhr et al., respectively. It would be possible that degradant **7**, an aldehyde, would undergo disproportionation to yield simultaneously degradants **4** and **4′**, which appears to be consistent with the observation that both degradants **4** and **4′** are major degradation products [2].



Scheme 283 Degradation chemistry of pralidoxime in aqueous solutions of different pH. The numbering of the degradants follows that of Prue et al. [2]. The summary of the degradation pathways is based on the literature cited in this monograph, and the disproportionation mechanism is proposed by the present author. *Source:* Prue et al. [2] / John Wiley & Sons.

References

1 Pralidoxime. https://en.wikipedia.org/wiki/Pralidoxime (accessed March 3 2024).

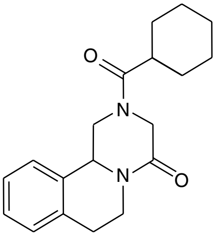
2 Prue, D.G., Johnson, R.N., and Kho, B.T. (1983). High-performance liquid chromatographic determination of pralidoxime chloride and its major decomposition products in injectable solutions. *J. Pharm. Sci.* 72: 751–756.

3 Utley, D. (1983). Determination of 2-hydroxyiminomethyl-1-methyl-pyridinium methanesulphonate (pralidoxime mesylate, P2S) and its degradation products in solution by liquid chromatography. *J. Chrom. A.* 265: 311–322.

4 Fyhr, P., Brodin, A., Ernerot, L., and Lindquist, J. (1986). Degradation pathway of pralidoxime chloride in concentrated acidic solution. *J. Pharm. Sci.* 75: 608–611.

5 Utley, D. (1990). Simultaneous determination of hydroxylamine and formulations containing pralidoxime salt by flow injection. *Analyst* 115: 1239–1242.

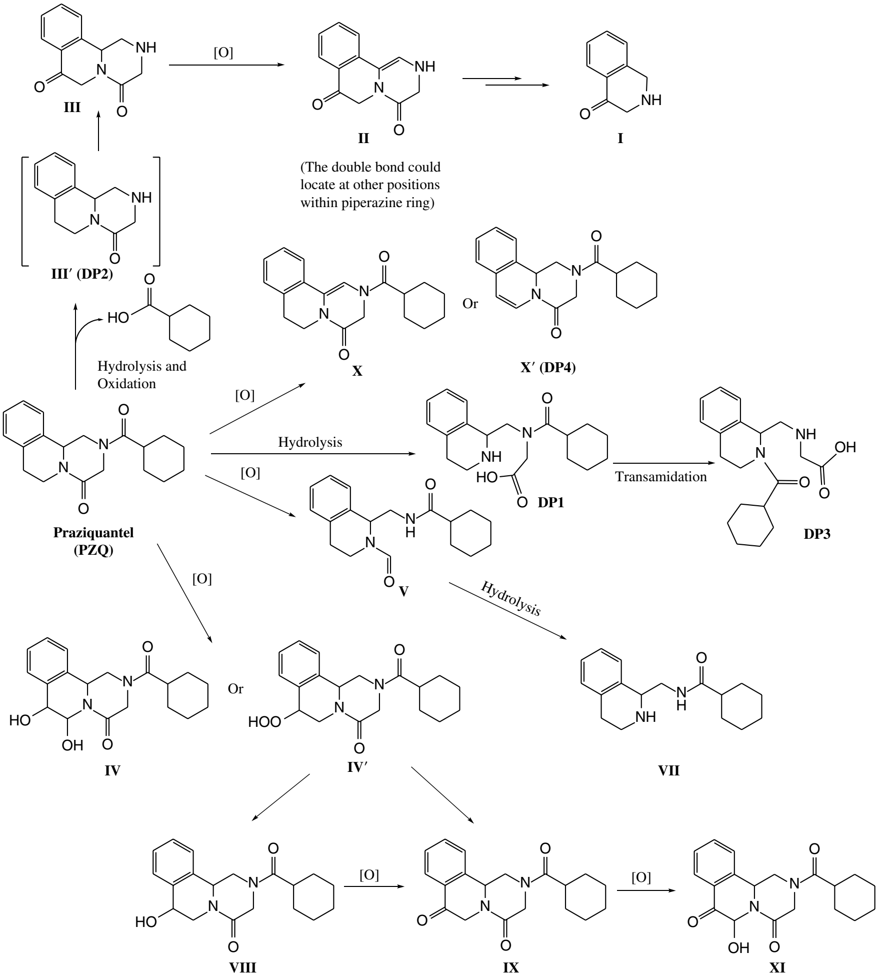
284: Praziquantel [WHO List of Essential Medicines]



Chemical name: (*R*,*S*)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one
Trade name: Biltricide
Formula: $C_{19}H_{24}N_2O_2$
Monoisotopic protonated ion (*m/z*): 313.1911
Molecular weight: 312.41
CAS number: 55268-74-1

Praziquantel is an anthelmintic medication for the treatment of the tropical diseases caused by parasitic worm infections [1–3]. It can be viewed as a piperazine derivative in which both nitrogens are acylated. While the two amide bonds are susceptible to hydrolytic degradation, particularly under forced degradation conditions [3], the main degradation pathways of praziquantel are of oxidative nature [2]. The structure of the drug molecule lacks polar functional groups, rendering it a biopharmaceutical classification system (BCS) class II drug, i.e., a drug with high permeability but low solubility [2]. In order to increase the solubility of the drug, various approaches have been tried, including using different cyclodextrins to form inclusion complexes [2] and mechanochemical activation [3]. During the process of evaluating the stability of praziquantel-cyclodextrin inclusion complexes by Spehar et al., a total of ten degradants were observed, among which seven had not been reported before (Scheme 284-1) [2]. The structure assignment of these degradants was primarily based on high-resolution mass spectrometric data, and as such, some of the proposed structures may be ambiguous. For example, degradant **X** may likely have the structure of **X'** instead, where the double bond is located at a different position, as proposed by Kumar and Chalannavar in their forced degradation study, in which it was called **DP4** [3]. Furthermore, Kumar and Chalannavar only observed four major degradants, i.e., **DP1–3** and **DP4**, among which **DP2** was not observed in the study by Spehar et al. [2]. Nevertheless, **DP2** could be an intermediate for degradant **III**. The fact that the forced degradation study projected only a very limited number of degradants may partially be attributed to the forced oxidative condition employed by Kumar and Chalannavar, where only hydrogen peroxide was used [3]. As hydrogen peroxide is usually a nucleophilic oxidative reagent, it apparently failed to produce the vast majority of the oxidative degradants that were likely formed via free-radical-mediated oxidation [4].

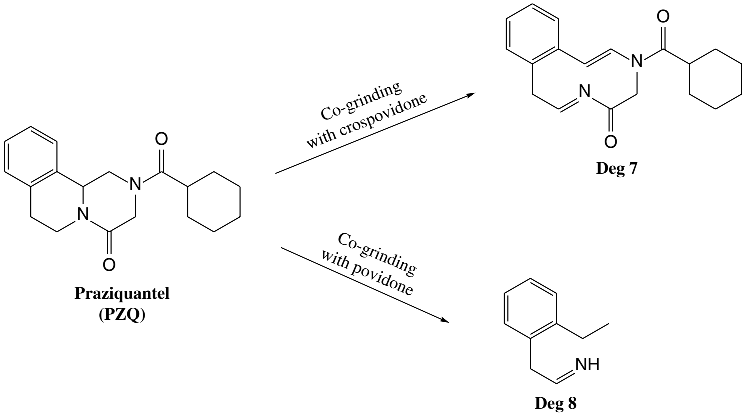
In another effort to improve the solubility of praziquantel, the drug substance was co-ground with either crospovidone or povidone to produce amorphous



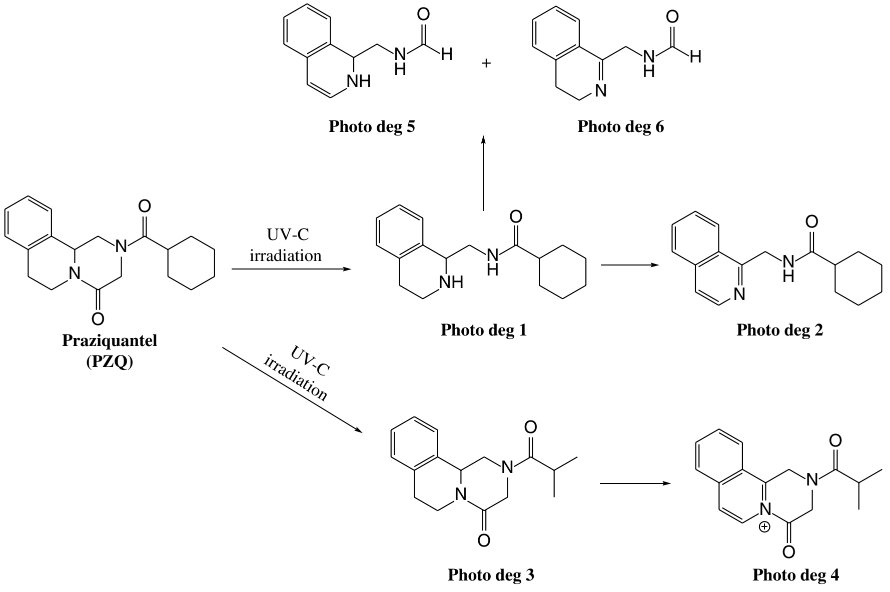
Scheme 284-1 Degradation products (I-XI) of praziquantel-cyclodextrin complexes under accelerated stability conditions, observed by Spehar et al. [2], and degradation products (DP1-DP4) of praziquantel in solutions under forced degradation conditions, observed by Kumar and Chalannavar [3]. The degradation pathways illustrated for degradants I-XI are proposed by the present author based on the results by Spehar et al. [2]. *Source:* Spehar et al. [2] and Kumar and Chalannavar [3].

dispersions [5]. Different degradation products were formed in the two processes, and two of the most prominent degradants were analyzed by GC–MS, LC–MS, and 1H NMR, and their structures proposed (Scheme 284-2).

In a photodegradation study of praziquantel with UV-C irradiation, a number of photodegradants were detected [6]; those that were formed in relatively early stages are presented in Scheme 284-3.



Scheme 284-2 Oxidative degradation of praziquantel during co-grinding with crospovidone or povidone. Only the structures of two major degradants were proposed based on the data from GC–MS, LC–MS, and 1H NMR analysis [5]. *Source:* Sagud et al. [5] / with permission of Elsevier.



Scheme 284-3 Photodegradation of praziquantel in air-purged aqueous solution with UV-C irradiation.

References

1 Praziquantel. https://en.wikipedia.org/wiki/Praziquantel (accessed March 10 2024).

2 Spehar, T.K., Pocrnic, M., Klaric, D. et al. (2021). Investigation of praziquantel/cyclodextrin inclusion complexation by NMR and LC-HRMS/MS: mechanism, solubility, chemical stability, and degradation products. *Mol. Pharmaceutics* 18: 4210–4223.

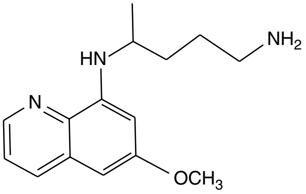
3 Kumar, A. and Chalannavar, R.K. (2022). Characterization of the major degradation products of the praziquantel API by mass spectrometry: development and validation of a stability-indicating reversed phase UPLC method. *J. Liq. Chrom. Related Tech.* 45: 130–142.

4 Li, M. (2012). Section 3.2. Free radical-mediated autooxidation. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

5 Sagud, I., Zanolla, D., Perissutti, B. et al. (2018). Identification of degradation products of praziquantel during the mechanochemical activation. *J. Pharm. Biomed. Anal.* 159: 291–295.

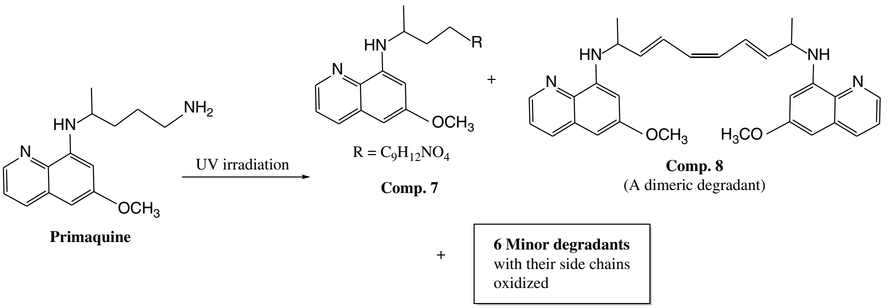
6 Cizmic, M., Ljubas, D., Curkovic, L. et al. (2017). Kinetics and degradation pathways of photolytic and photocatalytic oxidation of the anthelmintic drug praziquantel. *J. Hazardous Mat.* 323 (Part A): 500–512.

285: Primaquine [WHO List of Essential Medicines]



Chemical name: (*RS*)-*N*-(6-Methoxyquinolin-8-yl)pentane-1,4-diamine
Trade name: Neo-Quipenyl, Primachin
Formula: $C_{15}H_{21}N_3O$
Monoisotopic protonated ion (*m/z*): 260.1757
Molecular weight: 259.35
CAS number: 90-34-6
Salt form: Diphosphate

Primaquine is an anti-malaria drug that was first made by Robert Elderfield of Columbia University in the 1940s [1]. It is effective in treating malaria caused by the latent liver forms of *Plasmodium vivax* and *Plasmodium ovale*, which may survive after the treatment with chloroquine. Unlike the other actively used quinoline-based anti-malarial drugs, it is the only 8-amino-substituted derivative [2]. Furthermore, its side chain amino is a primary amine and thus, it would not undergo *N*-oxidation, as opposed to the tertiary amino groups in both chloroquine and hydroxychloroquine that would be oxidized to form the corresponding *N*-oxide degradants (refer to Monograph 221–222). In addition, the photochemical degradation of primaquine in the presence of oxygen was reported to occur exclusively at the side chain, in which the alkyl component of the side chain suffered various types of oxidative degradation along with deamination [2], resulting in the formation of two major degradants and six minor ones (Scheme 285). This photodegradation behavior is quite different from that of chloroquine and hydroxychloroquine, except for a similar type of dimeric degradants. In another study where the solution was purged with helium prior to UV irradiation, only two photodegradants were observed [3].



Scheme 285 Photooxidative degradation of primaquine.

References

1 Primaquine. https://en.wikipedia.org/wiki/Primaquine (accessed March 10 2024).

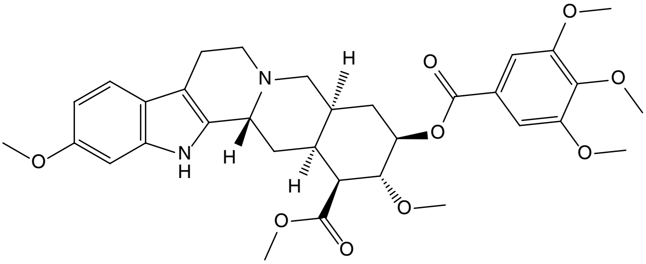
2 Kristensen, S., Grislingaas, A.-L., Greenhill, J.V. et al. (1993). Photochemical stability of biologically active compounds: V. Photochemical degradation of primaquine in an aqueous medium. *Int. J. Pharm.* 100: 15–23.

3 Kristensen, S., Nord, K., Orsteen, A.-L., and Tønnesen, H.H. (1998). Photoreactivity of biologically active compounds, XIV: influence of oxygen on light induced reactions of primaquine. *Pharmazie* 53: 98–103.

9

Reserpine to Zolpidem

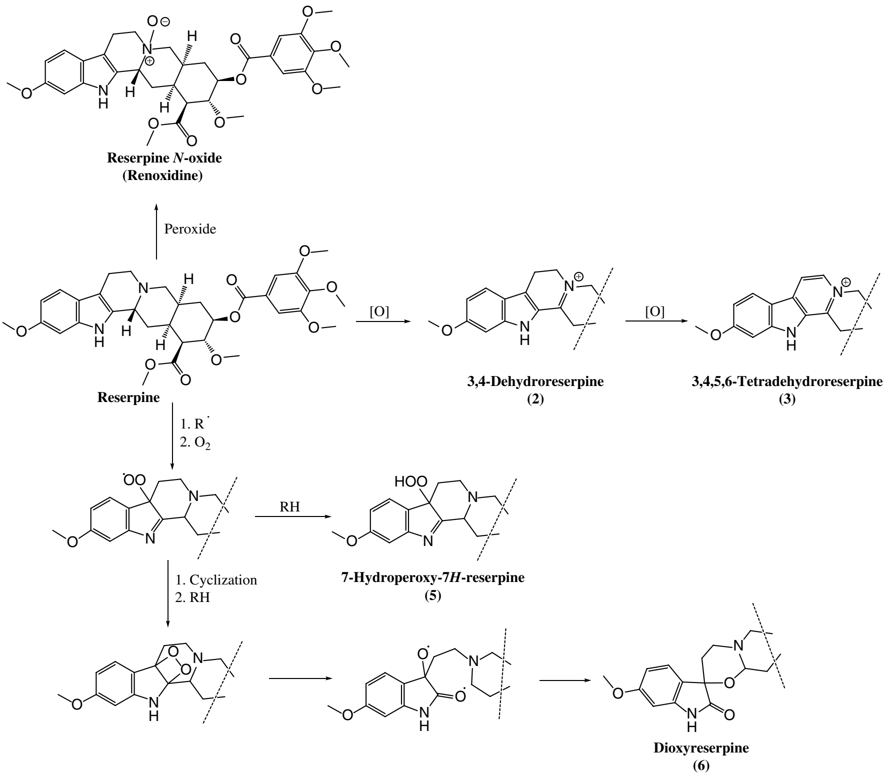
286: Reserpine



Chemical name: Methyl (3β,16β,17α,18β,20α)-11,17-dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]yohimban-16-carboxylate
Trade name: Serpasil, others
Formula: $C_{33}H_{40}N_2O_9$
Monoisotopic protonated ion (*m/z*): 609.2807
Molecular weight: 608.69
CAS number: 50-55-5

Reserpine is an indole alkaloid that was first isolated in 1952 by Muller et al. from the dried root of the medicinal plant *Rauwolfia serpentina*, commonly known as Indian snakeroot, according to the description by Slotkin [1]. One of its common ancient names means "madman's medicine," as the plant had been used for the treatment of insanity in India for centuries [2]. After its isolation in modern times, it was used as a first-generation antidepressant and played an important role in the treatment of schizophrenia [3]. Nowadays, it is mostly used for treating hypertension, particularly in combination with a thiazide diuretic, such as hydrochlorothiazide [2]. As a substituted indole derivative, both the 2-methine position and the double bond of the pyrrole moiety are quite susceptible to autooxidation. In aged solid dosage form of reserpine, 3,4-dehydroreserpine was found to be the major degradant (**2**, Scheme 286-1) [4]. Further oxidation of degradant **2** led to the formation of 3,4,5,6-tetradehydroreserpine, also known as lumireserpine (**3**). Degradants **2**

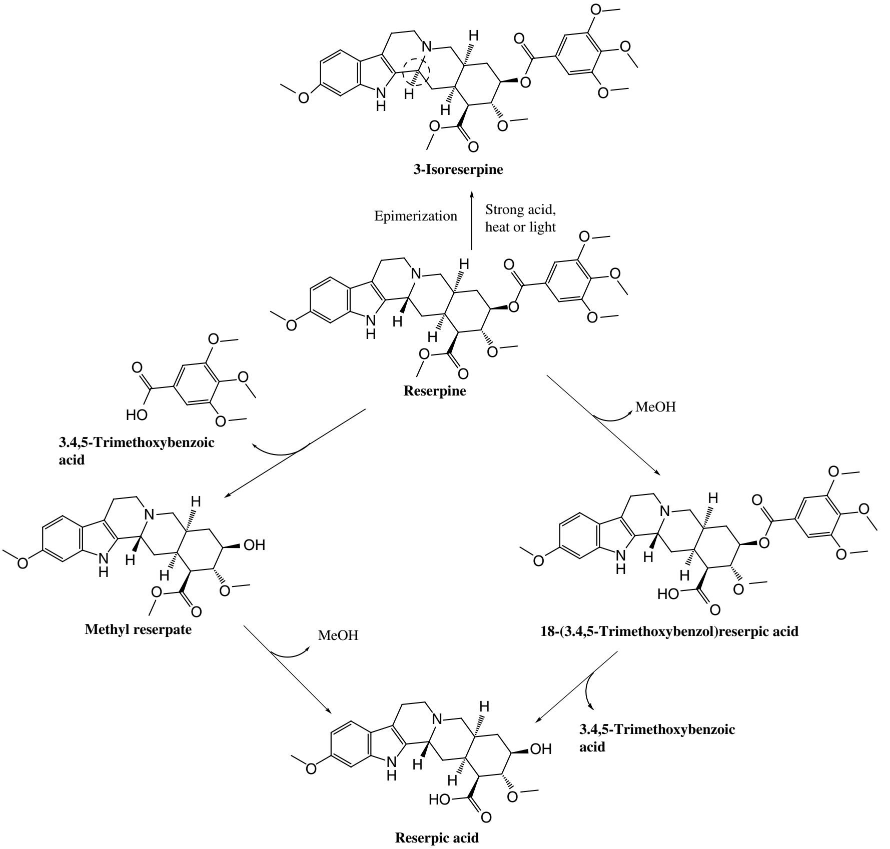
Compendium of Drug Degradation Pathways, First Edition. Min Li.



Scheme 286-1 Oxidative degradation of reserpine.

and **3** were also reported to be the main degradation products under photochemical degradation conditions [4, 5]. In another study by Awang et al., in which a solution of reserpine in chloroform was standing at room temperature and exposed to air, reserpine was found to form another two oxidative degradants (**5** and **6**) [6]. While the major degradant (**6**) has a rather unusual spiro structure, the minor one (**5**) was determined to be 7-hydroperoxy-7*H*-reserpine, a degradant resulting from the initial hydroperoxidation of reserpine. It was proposed by Awang et al. that both degradants **5** and **6** could be formed from a common hydroperoxidyl radical intermediate. Although the mechanism proposed by Awang et al. is mediated by free radicals, the same degradation pathway could also proceed via a non-free-radical-mediated mechanism. It is interesting to note that the *N*-oxide degradant of reserpine, also known as renoxidine, was not observed in the aforementioned autooxidation studies under various photochemical conditions. On the other hand, treatment of reserpine with peroxides produced reserpine *N*-oxide [7, 8].

Reserpine contains two ester functional groups, both of which are susceptible to hydrolytic degradation, with the hydrolysis of the trirmethoxybenzoate ester moiety being much more rapid than the methyl ester (Scheme 286-2) [9]. The final hydrolytic degradation product is reserpic acid. Reserpine was also reported to undergo epimerization at the 3-position to give rise to 3-isoreserpine in the presence of strong acid, heat, or light [10].



Scheme 286-2 Degradation of reserpine via hydrolysis and epimerization.

References

1 Slotkin, T.A. (1974). Reserpine. In: *Poisons of Plant Origin* (ed. L.L. Simpson and D.R. Curtis). Boston, MA: Springer https://doi.org/10.1007/978-1-4684-2943-5_1.

2 Reserpine. https://en.wikipedia.org/wiki/Reserpine (accessed March 10 2024).

3 López-Muñoz, F., Bhatara, V.S., Alamo, C., and Cuenca, E. (2004). Historical approach to reserpine discovery and its introduction in psychiatry. [Article in Spanish]. *Actas Esp. Psiquiatr.* 32: 387–395.

4 Vincent, A. and Awang, D.V.C. (1981). Determination of reserpine in pharmaceutical formulations by high performance liquid chromatography. *J. Liq. Chromotogr.* 4: 1651–1661.

5 Ljungberg, S. (1959). Effect of ultraviolet irradiation on reserpine. *J. Pharm. Belg.* 14: 115–125.

6 Awang, D.V.C., Dawson, B.A., Girard, M., and Vincent, A. (1990). The product of reserpine autoxidation. *J. Org. Chem.* 55: 4443–4448.

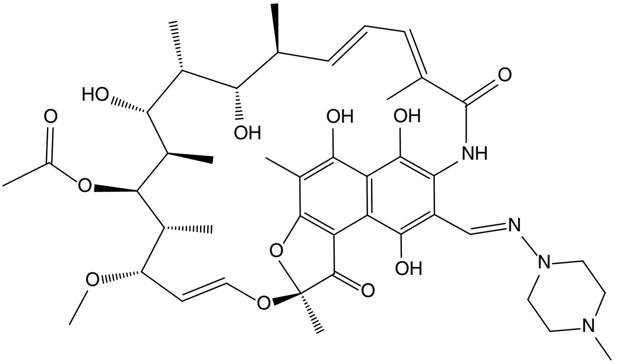
7 Court, W.E. and Timmins, P. (1975). The thin layer chromatographic-behaviour of some rauwolfia alkaloids. *Planta. Med.* 27: 319–329.

8 Dargel, E. and Mielck, J.B. (1990). HPLC-methods for separation and quantitation of reserpine and its main degradation products. *J. Liq. Chromotogr.* l3: 3973–3984.

9 Gaskell, A.J. and Joule, J.A. (1967). The acid catalyzed C3 epimerization of reserpine and deserpidine. *Tetrahedron* 23: 4053–4063.

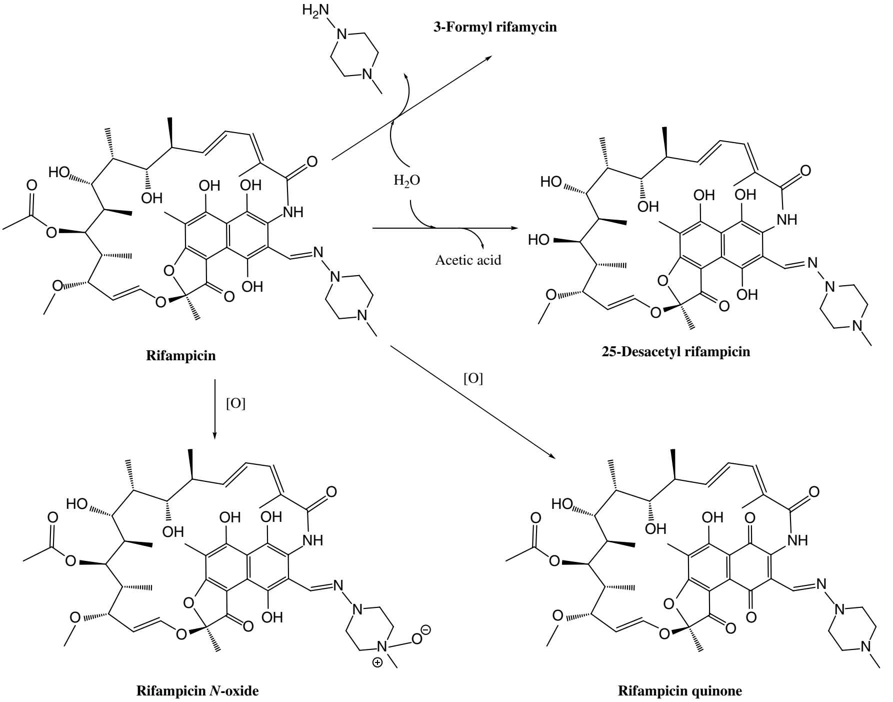
10 Schirmer, R.E. (1975). *Reserpine. Anal. Prof. Drug Sub.* 4: 384–430.

287: Rifampicin [WHO List of Essential Medicines]



Chemical name: (7*S*,9*E*,11*S*,12*R*,13*S*,14*R*,15*R*,16*R*,17*S*,18*S*,19*E*,21*Z*)-2,15,17,27,29-Pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-26-{(*E*)-[(4-methylpiperazin-1-yl)imino]methyl}-6,23-dioxo-8,30-dioxa-24-azatetracyclo[23.3.1.1^{4,7}.0^{5,28}]triaconta-1(28),2,4,9,19,21,25(29),26-octaen-13-yl acetate
Trade name: Rifadin, others
Formula: $C_{43}H_{58}N_4O_{12}$
Monoisotopic protonated ion (*m/z*): 823.4124
Molecular weight: 822.95
CAS number: 13292-46-1
Common salt form: Sodium salt

Rifampicin, also known as rifampin, is a semi-synthetic antibiotic that was developed at the Italian pharmaceutical company Dow-Lepetit Research Laboratories in the 1950s [1]. It is used as a first-line antituberculosis drug and most often in combination with other antituberculosis drugs such as isoniazid and pyrazinamide. It has two moieties that are susceptible to hydrolytic degradation. The hydrolysis of its hydrazone linkage yields 3-formylrifamycin (Scheme 287), which could react with isoniazid, another antituberculosis drug that is often co-formulated with rifampicin [2]. The interaction between 3-formylrifamycin and isoniazid has been described in the monograph for isoniazid in this book. On the other hand, the hydrolysis of the acetyl group of rifampicin gives rise to 25-desacetyl rifampicin [3]. Rifampicin also contains a highly substituted naphthalene ring and the vast majority of the substituents are electron-donating functional groups. Hence, the naphthalene moiety is electron-rich and thus susceptible to oxidative degradation to produce rifampicin quinone [3]; this degradation process can be inhibited by ascorbic acid [4]. In addition, the piperazine ring can also undergo oxidative degradation to give rifampicin *N*-oxide [5].



Scheme 287 Hydrolytic and oxidative degradation pathways of rifampicin. For the chemical interaction between 3-formyl rifamycin and isoniazid, refer to the monograph for isoniazid.

References

1 Sensi, P. (1983). History of the development of rifampin. *Rev. Infect. Dis.* 5 (Suppl 3): S402–S406.

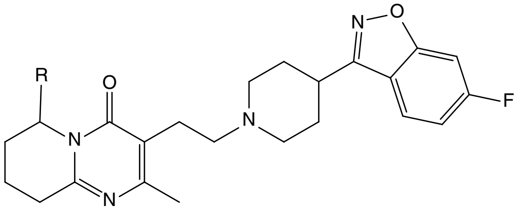
2 Singh, S., Mariappan, T.T., Sharda, N. et al. (2000). The reason for an increase in decomposition of rifampicin in the presence of isoniazid under acid conditions. *Pharm. Pharmacol. Commun.* 6: 405–410.

3 Mohan, B., Sharda, N., and Singh, S. (2003). Evaluation of the recently reported USP gradient HPLC method for analysis of anti-tuberculosis drugs for its ability to resolve degradation products of rifampicin. *J. Pharm. Biomed. Anal.* 31: 607–612.

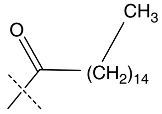
4 Maggi, N., Pasqualucci, C.R., Ballotta, R., and Sensi, P. (1966). Rifampicin: a new orally active rifamycin. *Chemother.* 11: 285–292.

5 Ripamonti, A., Ferrari, P., and Gallo, G.G. (1984). Structure identification of rifampicin *N*-oxide. Il Farmaco. *Ed. Sci.* 39: 806–809.

288–290: Risperidone, Paliperidone, Paliperidone Palmitate



Risperidone, R = H;
Paliperidone, R = OH;
Paliperidone palmitate, R =



288: Risperidone [WHO List of Essential Medicines]

Chemical name: 3-[2-[4-(6-Fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrimidin-4-one
Trade name: Risperdal, Okedi, others
Formula: $C_{23}H_{27}FN_4O_2$
Monoisotopic protonated ion (*m/z*): 411.2191
Molecular weight: 410.49
CAS number: 106266-06-2
Common salt form: Tartrate

289: Paliperidone

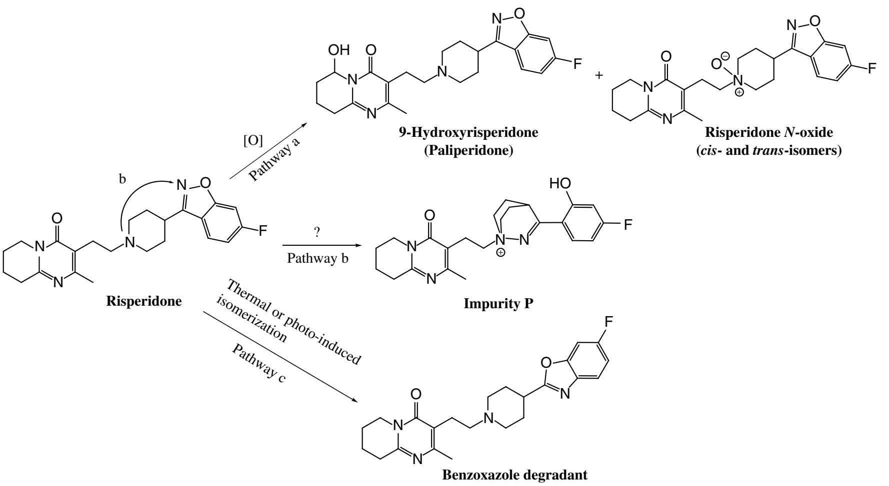
Chemical name: 3-{2-[4-(6-Fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl}-9-hydroxy-2-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrimidin-4-one
Trade name: Invega
Formula: $C_{23}H_{27}FN_4O_3$
Monoisotopic protonated ion (*m/z*): 427.2140
Molecular weight: 426.49
CAS number: 144598-75-4

290: Paliperidone Palmitate

Chemical name: 3-{2-[4-(6-Fluoro-1,2-benzoxazol-3-yl)-1-piperidinyl]ethyl}-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-a]pyrimidin-9-yl palmitate
Trade name: Invega Sustenna, Invega Trinza, Invega Hafyera
Formula: $C_{39}H_{57}FN_4O_4$
Monoisotopic protonated ion (*m/z*): 665.4437
Molecular weight: 664.91
CAS number: 199739-10-1

Risperidone is an atypical antipsychotic medication used primarily to treat schizophrenia and bipolar disorder. It was developed in Janssen Pharmaceutical Research in the late 1980s and was approved by the US FDA in 1993 [1, 2]. Risperidone is susceptible to oxidative degradation, particularly at its 9-position. In a tablet dosage form, 9-hydroxyrisperidone was found to be the major degradant after the tablets were stressed at 60°C for 1 month (Pathway a, Scheme 288–290-1) [3]. 9-Hydroxyrisperidone is also an active metabolite of risperidone and is designated as Impurity C in the European Pharmacopeia. It was later developed by Janssen into another antipsychotic drug, which is known as paliperidone [4]. Also present in the stressed tablets was the minor degradant, risperidone *N*-oxide [3], which has both the *cis*- and *trans*-forms [5] and the *cis*-form is specified in the US Pharmacopeia as Impurity Q. In another paper [6], Impurity P of risperidone, also known as bicyclorisperidone [7], was mentioned as a potential degradant as well, although it was not specified how this impurity was formed. Based on its structure, it appears that Impurity P could have been formed through an intramolecular rearrangement process (Pathway b, Scheme 288–290-1). Another degradation pathway via an intramolecular rearrangement was reported by Bharathi et al. [6], in which the benzisoxazole ring of risperidone was isomerized into benzoxazole (Pathway c, Scheme 288–290-1). This degradant was observed in a batch of risperidone tablets that were stored under the accelerated stability condition of 40°C/75%RH for 6 months. According to the authors, this degradant could also be formed under thermal stress conditions. This intramolecular isomerization is very similar to the photoisomerization of benzisothiazole–benzothiazole that has been reported for ziprasidone and lurasidone, respectively [8, 9].

Paliperidone is 9-hydroxyrisperidone, which was also developed by Janssen and approved by the US FDA for the treatment of schizophrenia in 2006 and later for schizoaffective disorder [10]. Due to its high structural similarity to risperidone, its

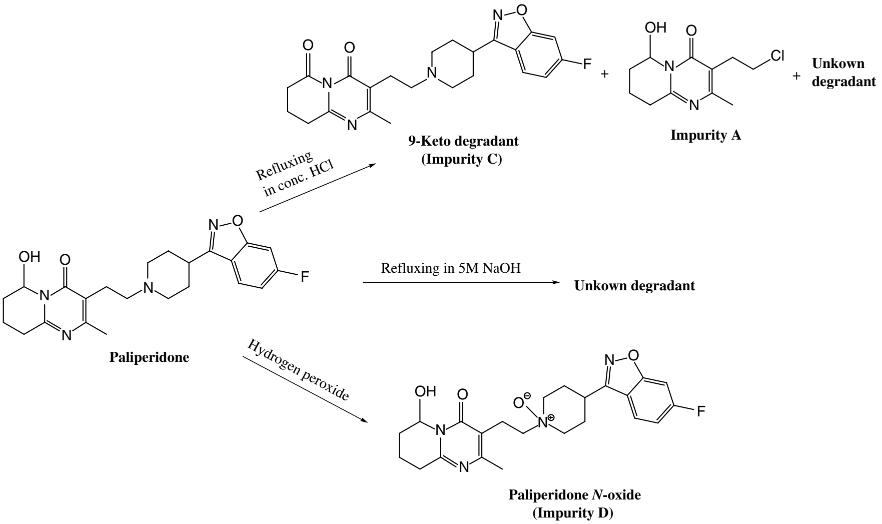


Scheme 288–290-1 Oxidative and isomerization degradation pathways of risperidone.

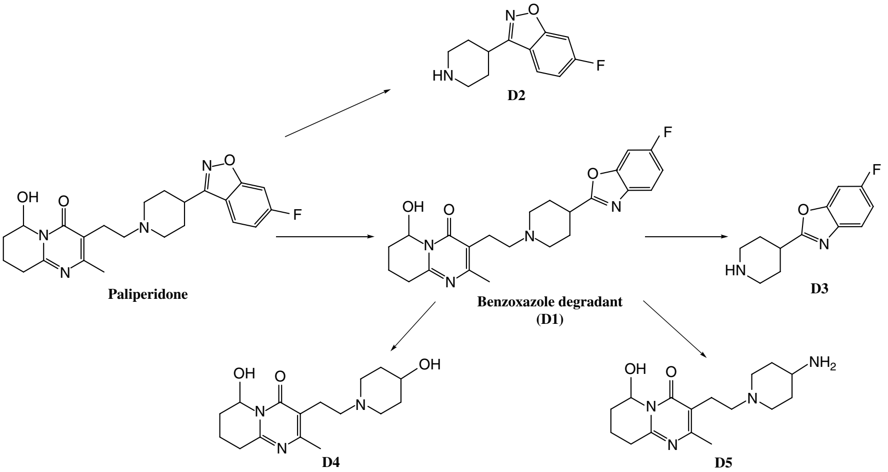
degradation chemistry would be expected to be largely similar. On the other hand, among the few reported forced degradation studies of paliperidone, two seem to be of reasonable quality. In a solution of concentrated HCl, which was subjected to 4 hours of refluxing, paliperidone was found to give the keto degradant (Impurity C), a specified impurity in the USP, and an unknown degradant as the major degradants (Scheme 288–290-2). It was surprising that an oxidative degradant (Impurity C) was formed under this acidic stress condition. Impurity A was also observed in this acidic stress, which is nonetheless an artificial degradant due to the use of the HCl solution [11]. While in a solution of 5 M NaOH, which was refluxed for 48 hours, an unknown impurity was found to be the major degradant. In the oxidative stress with 6% hydrogen peroxide at room temperature, the *N*-oxide degradant (Impurity D) was detected as the predominant degradant. In the apparent solid state forced degradation under thermal and ICH photostability conditions, no obvious degradation was observed.

In a photo stress study in solution with UVA and UVC irradiation, paliperidone was found to be converted to the dealkylated piperidine degradant (Impurity B) and its benzoxazole isomer [12]. The latter degradant was further degraded to form three additional degradants (Scheme 288–290-3).

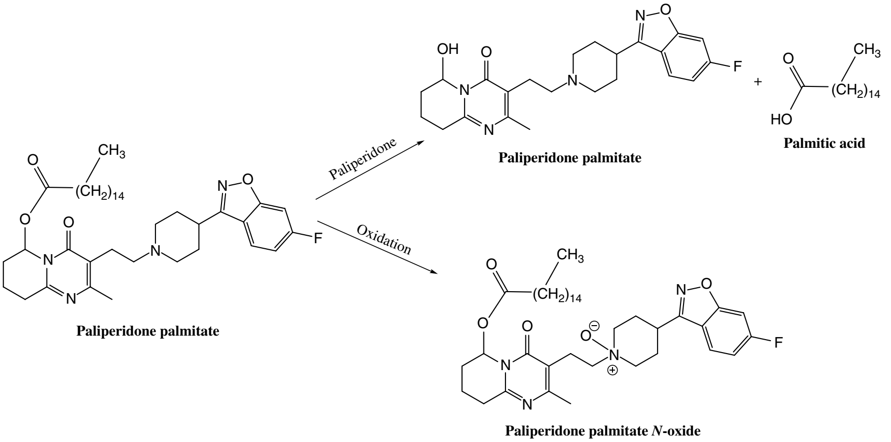
Paliperidone palmitate is a prodrug of paliperidone that enables a sustained release of the active ingredient over a period of up to several months [13]. As usual, in the case of prodrugs, the active drug paliperidone is a degradant, resulting from the hydrolytic degradation of paliperidone palmitate. Under the stress of hydrogen peroxide, paliperidone palmitate *N*-oxide was formed as another major degradant (Scheme 288–290-4) [14, 15]. The photochemical degradation of paliperidone palmitate would be expected to be quite similar to that of paliperidone and risperidone, as they contain nearly identical chromophores.



Scheme 288–290-2 Degradation of paliperidone in solutions under forced degradation conditions.



Scheme 288–290-3 Photochemical degradation of paliperidone under UVA and UVC irradiation. The nomenclature of the degradants follows that of Skibinski et al. [12]. *Source:* Skibinski et al. [12] /John Wiley & Sons.



Scheme 288–290-4 Hydrolytic and oxidative degradation of paliperidone palmitate.

References

1 Colpaert, F.C. (2003). Discovering risperidone: the LSD model of psychopathology. *Nat. Rev. Drug Discov.* 2: 315–320.

2 Risperidone. https://en.wikipedia.org/wiki/Risperidone (accessed March 10 2024).

3 Tomar, R.S., Joseph, T.J., Murthy, A.S.R. et al. (2004). Identification and characterization of major degradation products of risperidone in bulk drug and pharmaceutical dosage forms. *J. Pharm. Biomed. Anal.* 36: 231–235.

4 Wu, Y.-J. (2012). Heterocycles and medicine: A survey of the heterocyclic drugs approved by the U.S. FDA from 2000 to present. In: *Progress in Heterocyclic Chemistry*, vol. 24 (ed. G.W. Gribble and J.A. Joule). Elsevier, Oxford.

5 El-Sherif, Z.A., El-Zeany, B., and El-Houssini, O.M. (2005). High performance liquid chromatographic and thin layer densitometric methods for the determination of risperidone in the presence of its degradation products in bulk powder and in tablets. *J. Pharm. Biomed. Anal.* 36: 975–981.

6 Bharathi, C., Chary, D.K., Kumar, M.S. et al. (2008). Identification, isolation and characterization of potential degradation product in risperidone tablets. *J. Pharm. Biomed. Anal.* 46: 165–169.

7 Germann, D., Kurylo, N., and Han, F. (2012). Risperidone. *Prof. Drug Sub. Excip. Relat. Methodol.* 37: 313–361.

8 Sharp, T.R., Leeman, K.R., Bryant, D.E., and Horan, G.J. (2003). On the photoisomerization of the benzisothiazole portion of ziprasidone. *Tetrahedron Lett.* 44: 1559–1561.

9 Wang, K.C., Guo, Q., Kuang, Z. et al. (2022). Structural elucidation of two novel degradants of lurasidone and their formation mechanisms under free radical-mediated oxidative and photolytic conditions via liquid chromatography photodiode array/ultraviolet-tandem mass spectrometry and one-dimensional/two-dimensional nuclear magnetic resonance spectroscopy. *J. Mass Spectrom.* 57 (7): e4871.

10 Minwalla, H.D., Wrzesinski, P., Desforges, A. et al. (2021). Paliperidone to treat psychotic disorders. *Neurol. Int.* 13: 343–358.

11 Jadhav, S.A., Landge, S.B., Choudhari, P.M. et al. (2011). Stress degradation behavior of paliperidone, an antipsychotic drug, and development of suitable stability-indicating RP-LC method. *Chrom. Res. Int.* 2011: 256812. https://doi.org/10.4061/2011/256812.

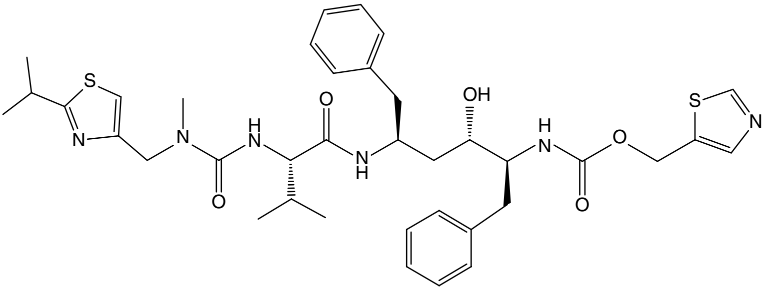
12 Skibinski, R., Komsta, L., and Inglot, T. (2016). Characterization of paliperidone photodegradation products by LC-Q-TOF multistage mass spectrometry. *Biomed. Chromatogr.* 30: 894–901.

13 Morris, M.T. and Tarpada, S.P. (2017). Long-acting injectable paliperidone palmitate: a review of efficacy and safety. *Psychopharmacol. Bull.* 47: 42–52.

14 Bindu, K.H., Dhekale, N.H., Suryanarayana, M.V., and Anjaneyulu, Y. (2012). A validated stability indicating UPLC method for simultaneous determination of assay, related substances, and degradation products of paliperidone palmitate active pharmaceutical ingredient and its pharmaceutical injection forms. *J. Liq. Chromat. Rel. Technol.* 35: 533–546.

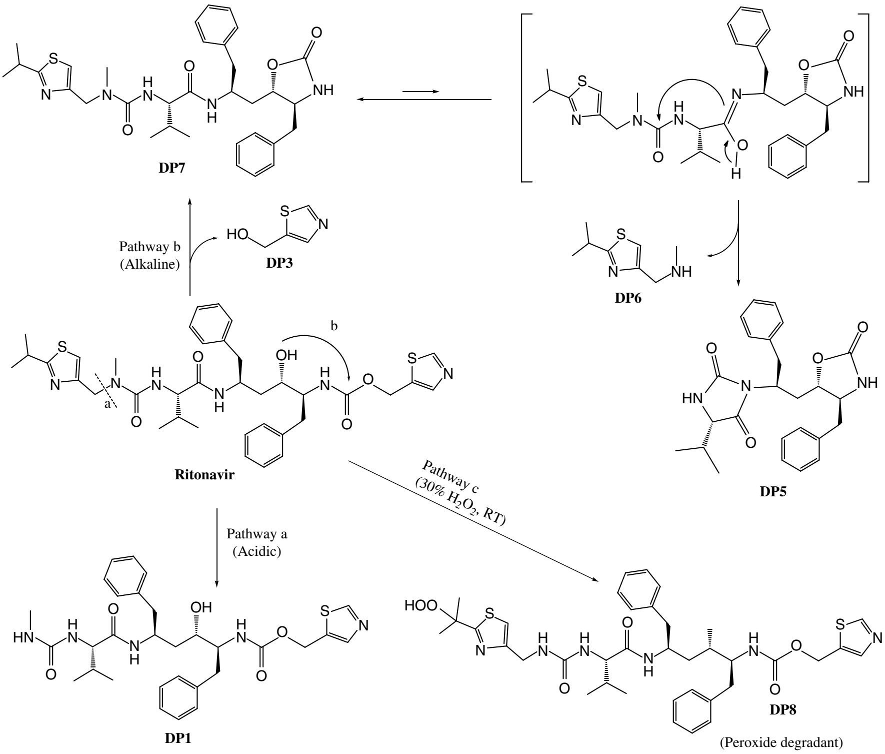
15 Trivedi, R.K., Jain, P., Patel, M.C. et al. (2013). A rapid, stability indicating RP-UPLC method for determination of paliperidone palmitate in a depot injectable formulation. *J. Appl. Pharm. Sci.* 3: 087–092.

291: Ritonavir [WHO List of Essential Medicines]



Chemical name: 1,3-Thiazol-5-ylmethyl *N*-[(2*S*,3*S*,5*S*)-3-hydroxy-5-[(2*S*)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate
Trade name: Norvir
Formula: $C_{37}H_{48}N_6O_5S_2$
Monoisotopic protonated ion (*m/z*): 721.3200
Molecular weight: 720.95
CAS number: 155213-67-5

Ritonavir is an inhibitor of the protease of HIV, the virus that causes AIDS. This anti-AIDS drug was developed by Abbott Laboratories, the predecessor of AbbVie, and approved by the US FDA in 1996 [1]. It is used in combination with other antiviral agents, and since it is a potent inhibitor of CYP3A4, quantitatively the most important metabolizing enzyme [2], nowadays ritonavir is more commonly utilized as a pharmacokinetic booster for its companion drugs [3]. The most recent example is the anti-COVID-19 drug, Paxlovid, which contains nirmatrelvir, an inhibitor of the main protease of SARS-CoV-2, and ritonavir [4]. Ritonavir is a peptidomimetic that contains an amide, carbamate, and urea moieties, all of which could be susceptible to hydrolytic degradation. A forced degradation study reported five major degradation products under acidic, alkaline, and oxidative conditions (Scheme 291) [5]. The structural elucidation of the degradants was only based on LC–MS analysis and thus, considered tentative. It is interesting to note that no degradant would be produced from the direct hydrolysis of three hydrolysable moieties. Two out of the five major degradants were formed from intramolecular cyclization of the carbamate moiety via the attack by the nearby hydroxyl group. Part of the problems associated with these forced degradation studies are either too much degradation (and thus the degradants may not represent the real ones observed in normal stability studies) [5] or the proposed structures are gas phase fragments (such as carbocation), rather than usual compounds [6].



Scheme 291 Degradation of ritonavir under acidic, alkaline, and oxidative (30% hydrogen peroxide) conditions. The mechanism illustrated is based on that of Rao et al. [5] with modification. *Source:* Rao et al. [5] / with permission of Elsevier.

References

1 Ritonavir. https://en.wikipedia.org/wiki/Ritonavir (accessed August 6 2024).

2 Werk, A.N. and Cascorbi, I. (2014). Functional gene variants of CYP3A4. *Clin. Pharmacol. Thera.* 96: 340–348.

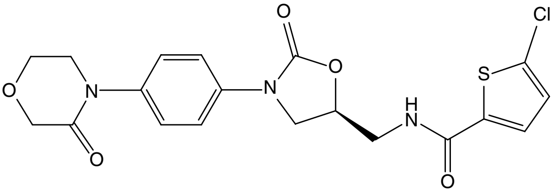
3 Jackson, A., Hill, A., Puls, R. et al. (2011). Pharmacokinetics of plasma lopinavir/ritonavir following the administration of 400/100 mg, 200/150 mg and 200/50 mg twice daily in HIV-negative volunteers. *J. Antimicrob. Chemothe.* 66: 635–640.

4 Lamb, Y.N. (2022). Nirmatrelvir plus ritonavir: first approval. *Drugs* 82: 585–591.

5 Rao, R.N., Ramachandra, B., Vali, R.M., and Raju, S.S. (2010). LC–MS/MS studies of ritonavir and its forced degradation products. *J. Pharm. Biomed. Anal.* 53: 833–842.

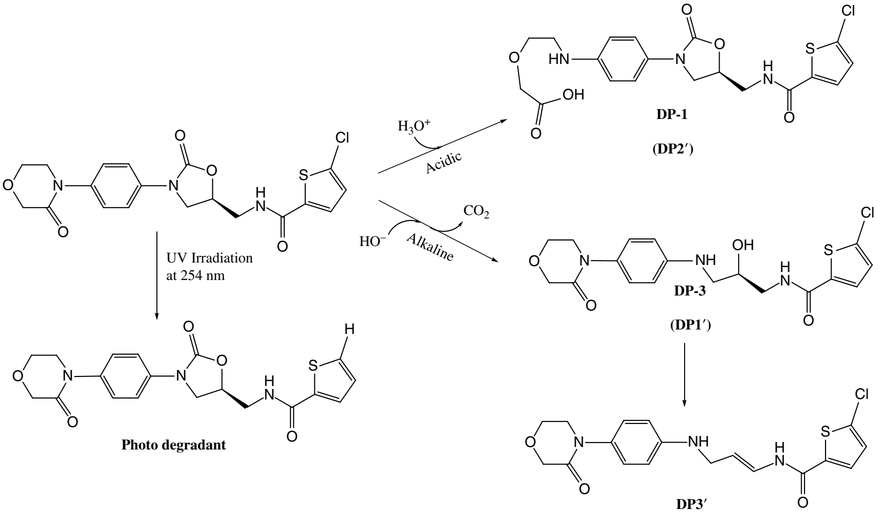
6 Tiwari, R.N. and Bonde, C.G. (2011). LC, LC-MS/TOF and MS^n studies for the separation, identification and characterization of degradation products of ritonavir. *Anal. Methods* 3: 1674–1681.

292: Rivaroxaban [WHO List of Essential Medicines]



Chemical name: (*S*)-5-Chloro-*N*-{[2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl] oxazolidin-5-yl]methyl}thiophene-2-carboxamide
Trade name: Xarelto
Formula: $C_{19}H_{18}ClN_3O_5S$
Monoisotopic protonated ion (*m/z*): 436.0728
Molecular weight: 435.88
CAS number: 366789-02-8

Rivaroxaban is an inhibitor of Factor Xa, a serine protease in the blood coagulation cascade, which activates prothrombin into thrombin, an event that eventually leads to clot formation and wound closure [1]. It was originally developed by Bayer and became the first approved direct and orally available Factor Xa inhibitor by regulatory agencies, first by Health Canada and the European Medicines Agency in 2008, and then by the US FDA in 2011 [2]. The degradation chemistry of rivaroxaban is primarily of hydrolytic nature, as demonstrated by a few forced degradation studies [3-6]. Among these studies, the 3-oxomorpholine and 2-oxo-oxazolidine rings were hydrolytically degraded under acidic and alkaline stress conditions to yield



Scheme 292 Degradation of rivaroxaban under forced acidic, alkaline, and photolytic degradation conditions.

DP-1 and DP-3 (subsequently to DP3’), respectively (Scheme 292) [3]. The structures of the degradants reported from different studies were somewhat different, among which the two structures proposed by Ramisetti and Kuntamukkala (**DP-1** and **DP-3**) [3] and the one by Arous et al. (**DP3’**) [4] based on MS and NMR data appeared more reliable than others. UV irradiation of a rivaroxaban solution in acetonitrile at 254 nm produced the des-chloro degradant [5].

References

1 Núñez-Navarro, N.E., Santana, F.M., Parra, L.P., and Zacconi, F.C. (2019). Surfing the blood coagulation cascade: insight into the vital Factor Xa. *Curr. Med. Chem.* 26: 3175–3200.

2 Rivaroxaban. https://en.wikipedia.org/wiki/Rivaroxaban (accessed March 10 2024).

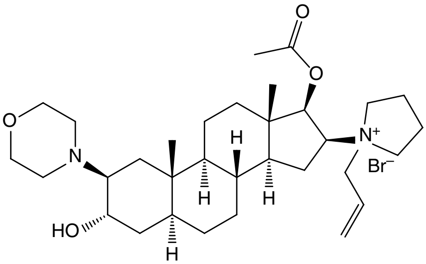
3 Ramisetti, N.R. and Kuntamukkala, R. (2014). Development and validation of a stability indicating LC-PDA-MS/MS method for separation, identification and characterization of process related and stress degradation products of rivaroxaban. *RSC Adv.* 4: 23155–23167.

4 Arous, B., Al-Mardini, M.A., Ghazal, H., and Al-Lahham, F. (2018). Stability-indicating method, for the determination of rivaroxaban and its degradation products, using LC-MS and TLC. *Res. J. Pharm. Tech.* 11: 1–9.

5 Wingert, N.R., dos Santos, N.O., Nunes, M.A.G. et al. (2016). Characterization of three main degradation products from novel oral anticoagulant rivaroxaban under stress conditions by UPLC-Q-TOF-MS/MS. *J. Pharm. Biomed. Anal.* 123: 10–15.

6 Nimje, H., Chavani, R., Pawari, S., and Deodhar, M. (2022). Development and validation of stability-indicating RP-HPLC method for rivaroxaban in tablet dosage form. *J. Res. Pharm.* 26: 1703–1712.

293: Rocuronium Bromide

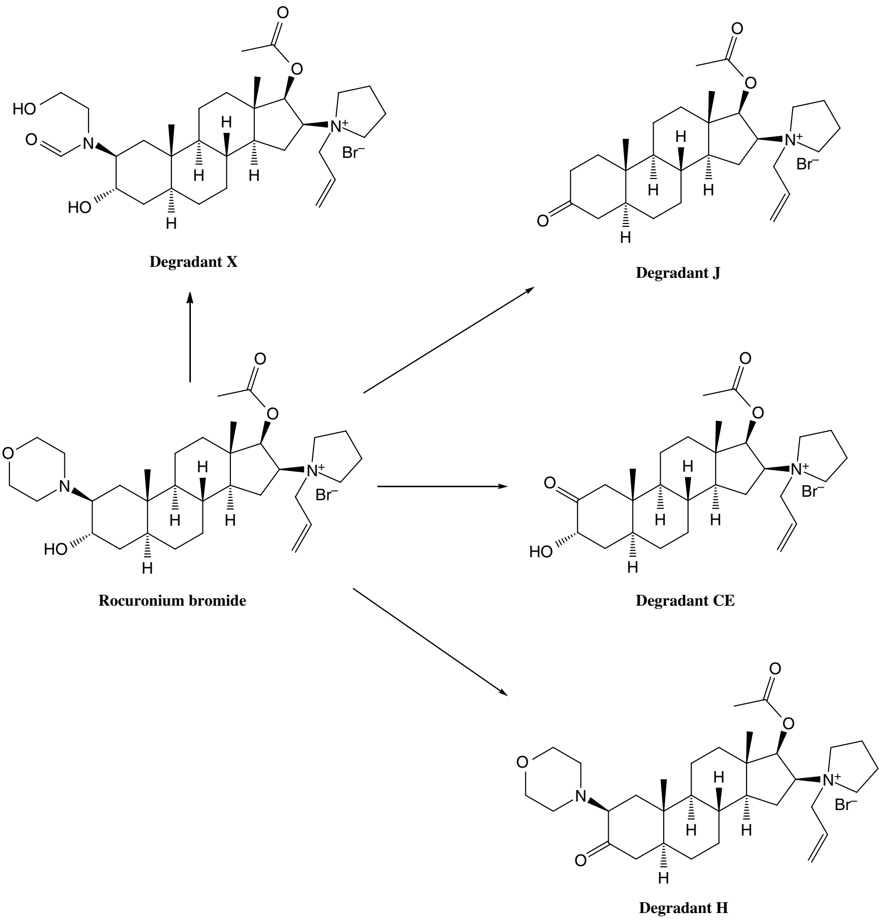


Chemical name: 1-((*2S,3S,5S,8R,9S,10S,13S,14S,16S,17R*)-17-Acetoxy-3-hydroxy-10,13-dimethyl-2-morpholinohexadecahydro-1*H*-cyclopenta[a]phenanthren-16-yl)-1-allylpyrrolidinium Bromide
Trade name: Zemuron, Esmeron
Formula: $C_{32}H_{53}BrN_2O_4$
Monoisotopic quaternary ammonium ion (*m/z*): 529.4000
Molecular weight: 609.69
CAS number: 119302-91-9

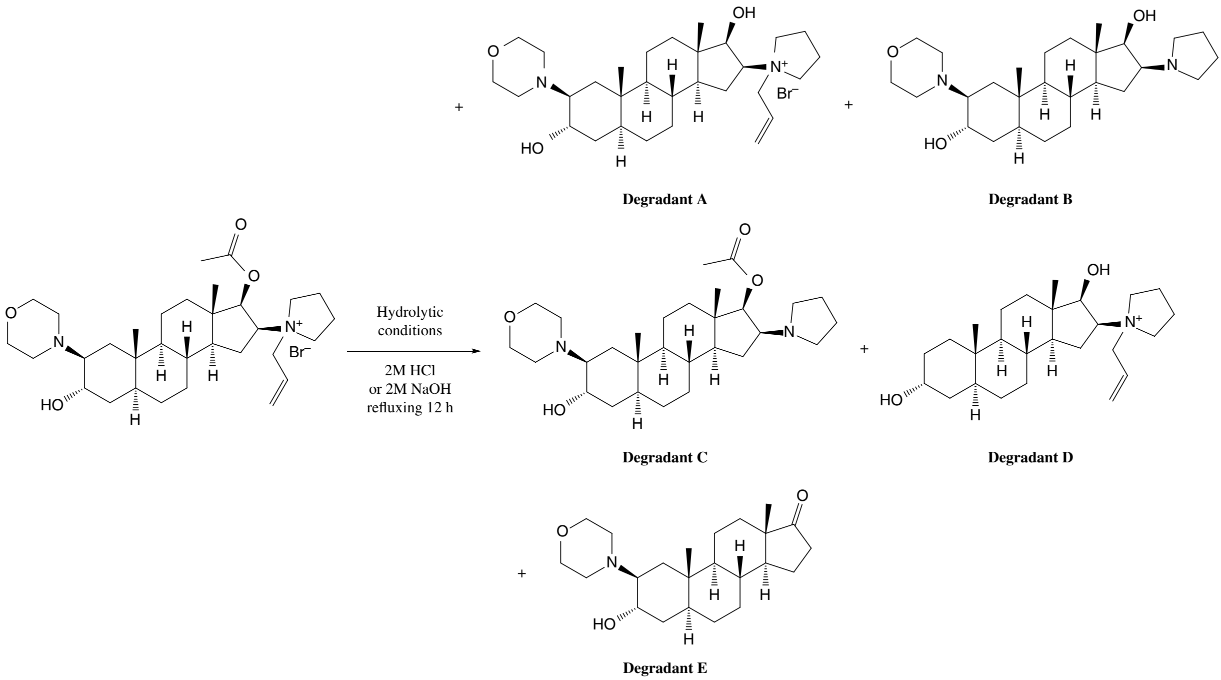
Rocuronium bromide is a nondepolarizing neuromuscular blocker, which is clinically used to provide skeletal muscle relaxation in general anesthesia or to maintain mechanical ventilation [1].

Designed to have a rapid onset and intermediate duration of action by Organon [2], it was approved by the US FDA in 1994. Chemically, it is an aminosteroid with four substituents at the 2-, 3-, 16-, and 17-positions, respectively. The 2-morpholine substituent was found to be susceptible to oxidative degradation, either under forced degradation conditions or normal storage conditions, leading to the formation of the degradants with partially or totally destructed morpholine ring (Scheme 293-1) [3–5].

El Houssini et al. performed a systematic forced degradation study on rocuronium bromide [4]. However, the hydrolytic and oxidative conditions used were quite harsh; hence, the majority of the degradants observed are likely artificial degradants, except perhaps for Degradant A (the deacetylated degradant). The latter was formed under both acidic and alkaline stress conditions, which was reported to be a potential metabolite of rocuronium, with 20 times less potency [6]. The majority of the degradants formed under harsh hydrolytic conditions are summarized in Scheme 293-2, in which the structure of Degradant E is different from that proposed by El Houssini et al.



Scheme 293-1 Oxidative degradation pathways of rocuronium bromide. Degradant X was elucidated by Wegener et al. [3], Degradants H and J reported by El Houssini et al. [4], and Degradant CE elucidated by Guo et al. [5]. *Source:* El Houssini et al. [4] and Guo et al. [5].



Scheme 293-2 Degradation of rocuronium bromide under hydrolytic stress conditions. The stress conditions were very harsh, and it seems that only Degradant A might be a real degradant that could be formed during a normal stability study or in storage.

References

1 Rocuronium bromide. https://en.wikipedia.org/wiki/Rocuronium_bromide (accessed August 27 2024).

2 Hunter, J.M. (1996). Rocuronium: the newest aminosteroid neuromuscular blocking drug. *Brit. J. Anaesth.* 76: 481–483.

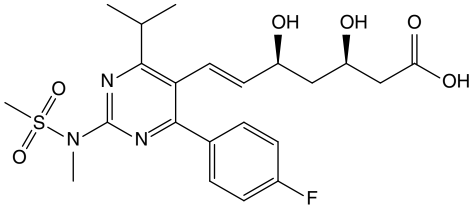
3 Wegener, O., Harms, G., Volmer, D.A., and Hayen, H. (2015). Structural characterization of a degradation product of rocuronium using nanoelectrospray-high resolution mass spectrometry. *Drug Test. Anal.* 7: 773–779.

4 El Houssini, O.M., El-Rahman, M.K.A., Fahem, D.K., and Zaazaa, H.E. (2022). Application of ICH guidelines for studying the degradation behavior of rocuronium bromide coupled with stability-indicating RP-LC method. *J. Chromat. Sci.* 60: 217–223.

5 Guo, Q., Kuang Z., Lin, J., Li, M. Unpublished results at the Center of Excellence for Modern Analytical Technologies (CEMAT) of Huahai Pharmaceutical Co., Ltd. Degradant CE occurred at a relative retention time (RRT) of 1.09 in a HILIC HPLC method and its structure was elucidated by LC-multi stage high resolution mass spectrometric analysis.

6 Muir, A.W., Houston, J., Green, K.L. et al. (1989). Effects of a new neuromuscular blocking agent (Org 9426) in anaesthetized cats and pigs and in isolated nerve-muscle preparations. *Brit. J. Anaesthesia* 63: 400–410.

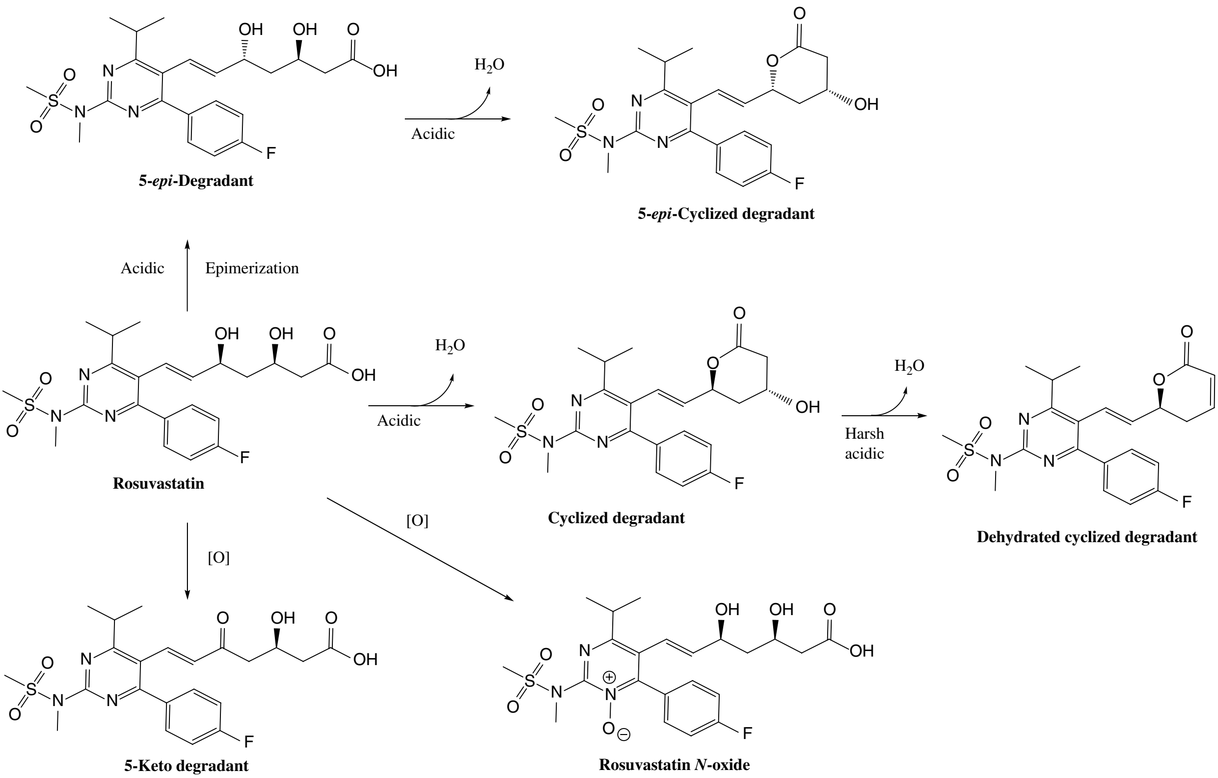
294: Rosuvastatin



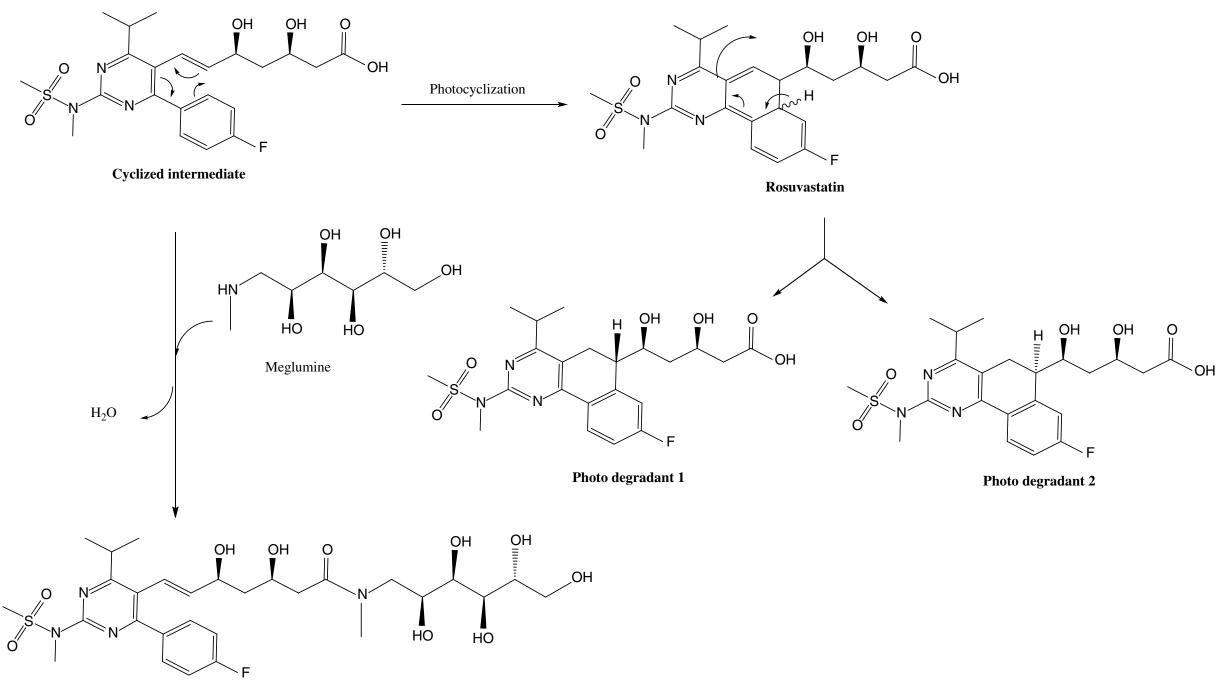
Chemical name: (*3R,5S,6E*)-7-[4-(4-Fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic Acid
Trade name: Crestor
Formula: $C_{22}H_{28}FN_3O_6S$
Monoisotopic protonated ion (*m/z*): 482.1756
Molecular weight: 481.54
CAS number: 287714-41-4
Common salt form: Calcium salt

Rosuvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (often abbreviated as HMG-CoA reductase), the rate-limiting enzyme in cholesterol biosynthesis. It is clinically used to treat high cholesterol (hyperlipidemia) and thus lowers the risk of heart attacks and strokes in patients at high risk. Rosuvastatin was developed by AstraZeneca and approved by the US FDA in 2003 [1]. All statin drugs have a chiral 3,5-dihydroxy-6-heptanoic acid side chain or its cyclized lactone form, which is essential for their activities. For statin drugs that adopt the open acid form, such as rosuvastatin and atorvastatin, the heptanoic acid side chain tends to cyclize to produce the corresponding 6-membered lactone ring (Scheme 294-1) [2, 3]. Further stress under harsh acidic condition led to the formation of the dehydrated cyclized degradant [4]. Rosuvastatin was reported to be relatively stable under alkaline and thermal forced degradation conditions [2].

A number of studies have demonstrated that rosuvastatin is quite susceptible to photochemical degradation, and the major degradants are a pair of cyclized epimers (Scheme 294-2) [2, 3, 5, 6]. In a formulation containing meglumine, a secondary amine excipient, rosuvastatin was found to react with meglumine to form an amide adduct [3].



Scheme 294-1 Hydrolytic and oxidative degradation pathways of rosuvastatin.



Scheme 294-2 Photochemical degradation of rosuvastatin and the adduct formation with meglumine, a secondary amine excipient.

References

1 Rosuvastatin. https://en.wikipedia.org/wiki/Rosuvastatin (accessed March 3 2024).

2 Khedr, A., Belal, F., Ibrahim, F., and Elawady, T. (2013). Analysis of rosuvastatin stress degradation behavior using liquid chromatography coupled to ultraviolet detection and electrospray ionization mass spectrometry. *Anal. Meth.* 5: 6494–6502.

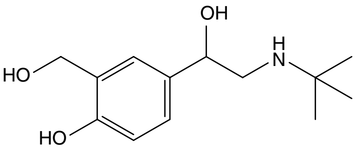
3 Kishore, C.R.P. and Mohan, G.V.K. (2017). Structural identification and estimation of rosuvastatin calcium related impurities in rosuvastatin calcium tablet dosage form. *Anal. Chem. Res.* 12: 17–27.

4 Mostafa, N.M., Badawey, A.M., Lamie, N.T. et al. (2014). Selective chromatographic methods for the determination of rosuvastatin calcium in the presence of its acid degradation products. *J. Liq. Chromat. Rel. Technol.* 37: 2182–2196.

5 Litvic, M., Smic, K., Vinkovic, V., and Filipan-Litvic, M. (2013). A study of photodegradation of drug rosuvastatin calcium in solid state and solution under UV and visible light irradiation: the influence of certain dyes as efficient stabilizers. *J. Photochem. Photobiol. A: Chem.* 252: 84–92.

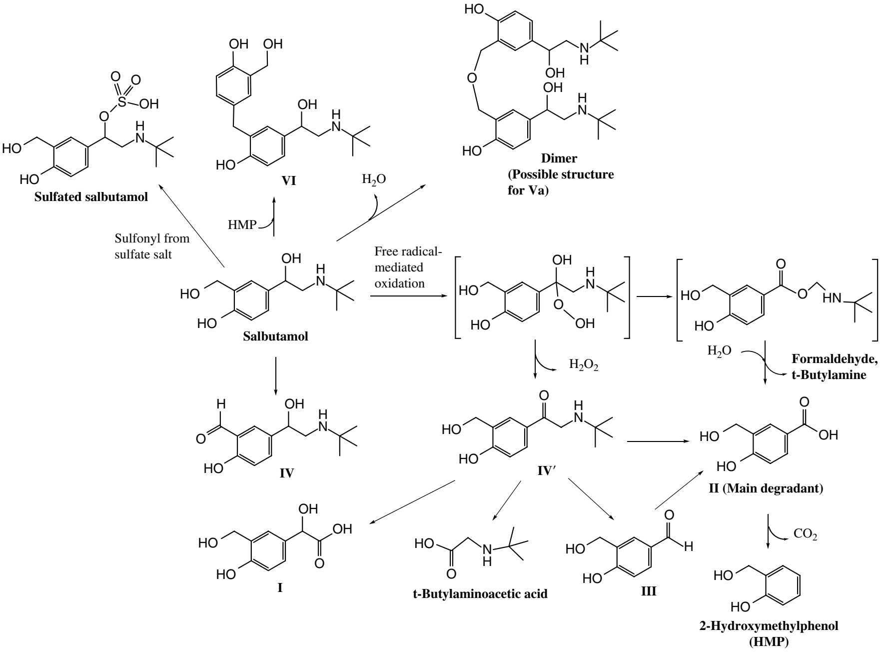
6 Borioni, A., Mammone, F.R., Risoluti, R. et al. (2023). Insight into the photolytic degradation products of Rosuvastatin: full chiral and structural elucidation and conversion kinetics by a combined chromatographic, spectroscopic and theoretical approach. *J. Pharm. Biomed. Anal.* 236: 115636.

295: Salbutamol [WHO List of Essential Medicines]



Chemical name: (*R,S*)-4-[2-(tert-Butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol
Trade name: Ventolin, Proventil, Others
Formula: $C_{13}H_{21}NO_3$
Monoisotopic protonated ion (*m/z*): 240.1594
Molecular weight: 239.32
CAS number: 18559-94-9
Common salt form: Sulfate

Salbutamol, also known as albuterol, is a short-acting β2-selective adrenergic receptor agonist, which causes the relaxation of airway smooth muscle [1]. It is clinically used for the treatment of asthma and chronic obstructive pulmonary disease (COPD). Salbutamol went onto the market in the United Kingdom in 1969 and was approved by the US FDA in 1982. It is a derivative of salicyl alcohol that contains a phenol core structure. Hence, the stability of salbutamol is pH dependent, and it was reported to be most stable at pH 3.5 [2, 3]. In a forced degradation study of salbutamol in a few acidic media, which were heated at 65°C or 85°C for various periods of time, approximately seven degradants were detected, the majority of which are oxidative degradants (Scheme 295). Although no specific mechanism was provided to rationalize the formation of these degradants in the original paper [2], it would be reasonable to presume that the formation of Degradants I, II, III, IV, and IV' was mediated by free-radical-mediated processes. For example, free-radical-mediated oxidation of salbutamol would lead to the Criegee intermediate [4], and its subsequent decomposition should give Degradant II, formaldehyde, and t-butylamine. Under elevated temperature, Degradant II could undergo decarboxylation to yield HMP. The latter could then couple with salbutamol to give Degradant VI, probably via the benzylic carbocation generated from salbutamol in acidic solutions. Degradant IV' was not mentioned in the original paper; instead Degradant IV was presented, which is an isomer of IV'. Mechanistically, Degradant IV' should be a reasonable intermediate leading to the formation of Degradants I, II, III, and t-butylaminoacetic acid. On the other hand, the dimeric degradant, although its structure was not proposed in the original paper, could be formed via the condensation of two molecules of salbutamol in acidic solutions. In an attempt to control the oxidative degradation of salbutamol, it seemed that the sulfonyl ester of salbutamol was formed as one of the degradants when sodium bisulfite or sodium metabisulfite was present in the solutions [3].



Scheme 295 Degradation pathways of salbutamol.

References

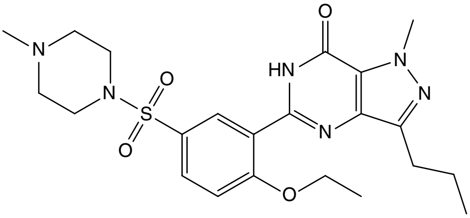
1 Salbutamol. https://en.wikipedia.org/wiki/Salbutamol (accessed October 25 2023).

2 Mälkki-Laine, L. and Bruins, A.P. (1994). Structural characterization of the decomposition products of salbutamol by liquid chromatography ionspray mass spectrometry. *J. Pharm. Biomed. Anal.* 12: 543–550.

3 Mälkki-Laine, L., Purra, K., Kähkönen, K., and Tammilehto, S. (1995). Decomposition of salbutamol in aqueous solutions. II. The effect of buffer species, pH, buffer concentration and antioxidants. *Int. J. Pharm.* 117: 189–195.

4 Li, M. (2012). Section 3.5.8. Oxidation of drugs containing alcohol, aldehyde, and ketone functionalities. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

296: Sildenafil

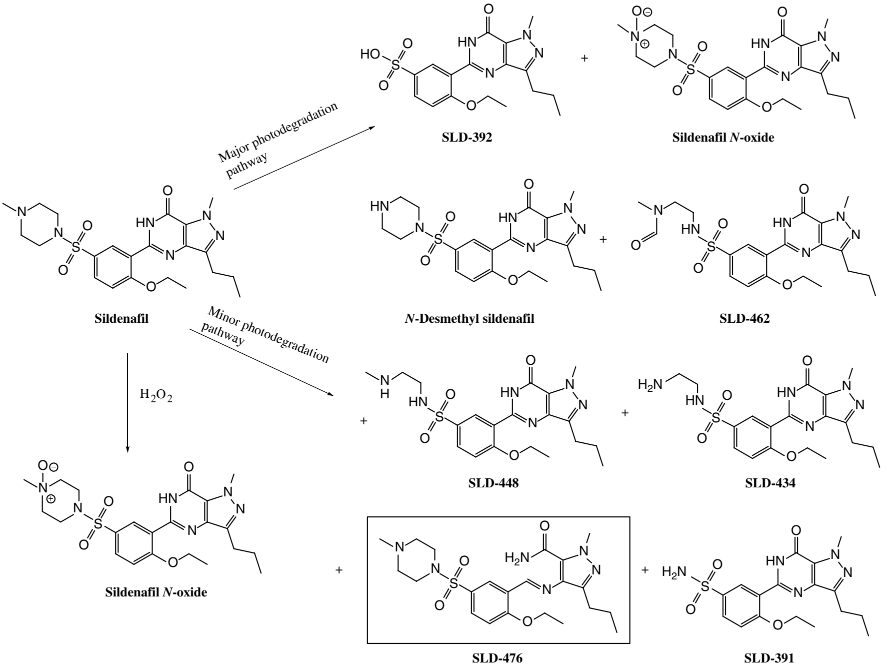


Chemical name: 5-{2-Ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}-1-methyl-3-propyl-1,6-dihydro-6*H*-pyrazolo[4,3-d]pyrimidin-7-one
Trade name: Viagra
Formula: $C_{22}H_{30}N_6O_4S$
Monoisotopic protonated ion (*m/z*): 475.2122
Molecular weight: 474.58
CAS number: 139755-83-2
Common salt form: Citrate

Sildenafil is a selective PDE5 inhibitor originally developed for treating angina by Pfizer. During clinical trials, it was found that sildenafil had limited efficacy for angina but a significant effect on erectile dysfunction. Hence, the drug was repurposed for treating erectile dysfunction, and this indication was approved by the US FDA in 1998. Since 2015, the indication of sildenafil has been expanded to include pulmonary arterial hypertension [1].

Although a few forced degradation studies have been reported, none seem to have performed in-depth structure elucidation of the degradants, except for the *N*-oxide degradant, which was fully characterized by LC–MS and NMR [1]. Two photochemical degradation studies were reported, in which the photodegradation occurred almost exclusively on the piperazine moiety, including its complete removal (Scheme 296) [2, 3]. On the other hand, it appeared that the pyrazolopyrimidine core of sildenafil is susceptible to hydrolysis under forced degradation conditions [4, 5]; nevertheless, the structures of the hydrolytic degradants were not elucidated.

The structure of vardenafil, another PDE5 inhibitor, is highly similar to that of sildenafil. Hence, its degradation chemistry would be expected to be very similar to that of sildenafil, particularly the photochemical and oxidative degradation pathways [3].



Scheme 296 Photochemical and oxidative degradation pathways of sildenafil [3]. The photochemical degradation occurred on the piperazine ring, except for SLD-476, where the pyrazolopyrimidine core was destroyed. The nomenclature for the SLD-series follows that of Herbert et al. in which the numeric numbers denote the nominal molecular weights of the corresponding degradants. *Source:* Herbert et al. [3] / with permission of Elsevier.

References

1 Abdel-Kader, M.S., Alam, P., Soliman, G.A. et al. (2021). Eco-friendly stability-indicating RP-HPTLC method for sildenafil analysis, characterization and biological evaluation of its oxidized stress degradation product. *Nature Sci. Rep.* 11: 15358.

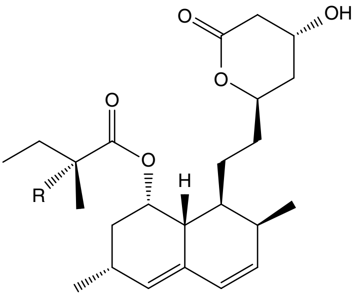
2 Eichhorn, P., Pérez, S., Aceña, J. et al. (2012). Identification of phototransformation products of sildenafil (Viagra) and its N-demethylated human metabolite under simulated sunlight. *J. Mass Spectrom.* 47 (6): 701–711.

3 Herbert, L.P., Becker-Krail, D.B., and Cory, W.C. (2015). Persistent phototransformation products of vardenafil (Levitra®) and sildenafil (Viagra®). *Chemosphere* 134: 557–562.

4 Segall, A.I., Vitale, M.F., Perez, V.L. et al. (2000). Revered-phase HPLC determination of sildenafil citrate in the presence of its oxidative-induced degradation products. *J. Liq. Chromat. Rel. Technol.* 23: 1377–1386.

5 Atipairin, A., Woradechakul, C., Chee, K.S. et al. (2014). Method validation for determination of sildenafil citrate in extemporaneous oral suspension. *Int. J. Pharm. Pharm. Sci.* 6: 131–136.

297–298: Simvastatin, Lovastatin



Simvastatin, R = Me;
Lovastatin, R = H.

297: Simvastatin

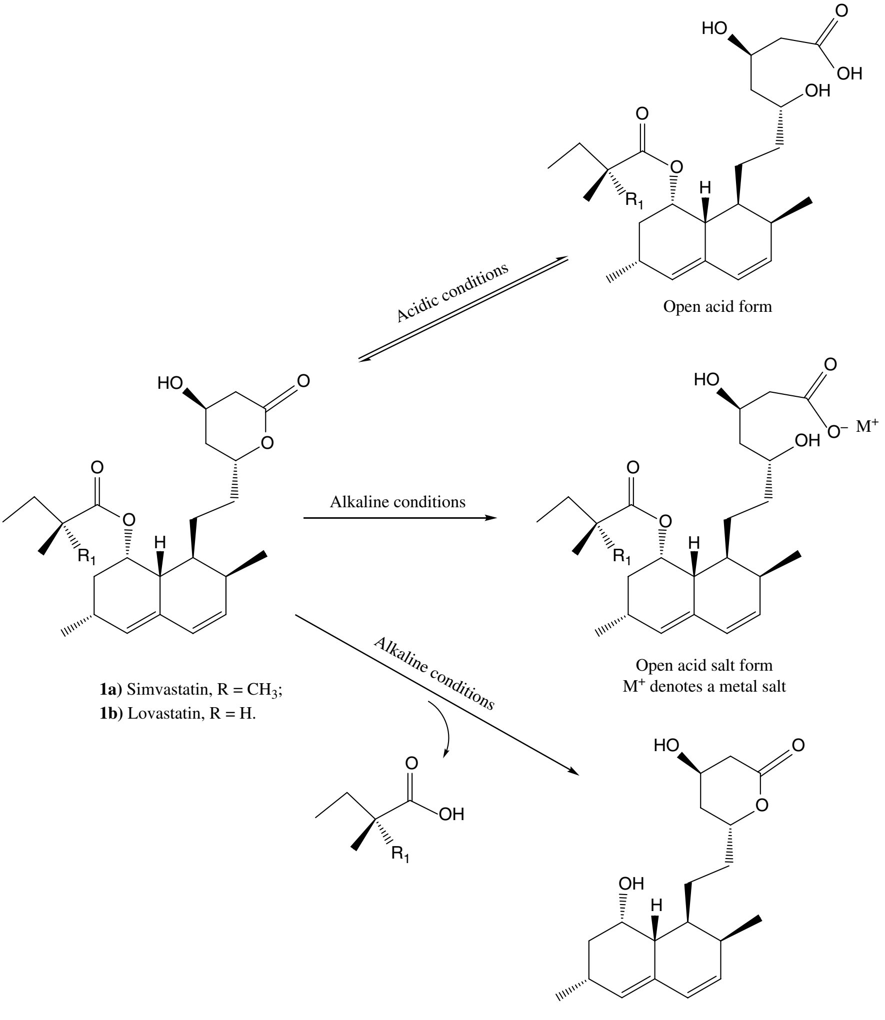
Chemical name: (*1S,3R,7S,8S,8aR*)-8-{2-[(*2R,4R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate
Trade name: Zocor
Formula: $C_{25}H_{38}O_5$
Monoisotopic protonated ion (*m/z*): 419.2792
Molecular weight: 418.57
CAS number: 79902-63-9

298: Lovastatin [Mevinolin] [WHO List of Essential Medicines]

Chemical name: (*1S,3R,7S,8S,8aR*)-8-{2-[(*2R,4R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (*2S*)-2-methylbutanoate
Trade name: Mevacor
Formula: $C_{24}H_{36}O_5$
Monoisotopic protonated ion (*m/z*): 405.2636
Molecular weight: 404.55
CAS number: 75330-75-5

Lovastatin, a natural product originally isolated from the fungus *Aspergillus terreus* by Merck Sharp & Dohme Research Laboratories [1], is the first specific HMG CoA reductase inhibitor approved for treating hypercholesterolemia in 1987 [2]. It was developed based on the pioneering finding of the lead compound, mevastatin (also known as compactin), another natural product isolated from *Penicillium citrium* by Akira Endo of Sankyo Co., Ltd. in Japan in 1973, which went into clinical study, but the program was terminated later [3].

Lovastatin is the 3-methylated derivative of mevastatin, and it appeared that the introduction of this extra methyl group enabled lovastatin to become an approvable drug. On the other hand, simvastatin is a methylated derivative of lovastatin with the methyl group on the pentanoyl side-chain. Due to the steric hindrance imposed by this extra methyl group, the ester functional group of simvastatin becomes more resistant to hydrolysis either in vitro or in vivo [4]. For the critical 3,5-dihydroxy-6-heptanoic acid side chain, both simvastatin and lovastatin take the form of its cyclized lactones, and hence the lactones are pro-drugs. Thus, the open ring acid forms, which are active drugs, are considered degradation impurities of the respective drug molecules (Scheme 297–298-1). According to Ellison et al. of Merck Sharp & Dohme Research Laboratories [5], in the acidic pH range, the

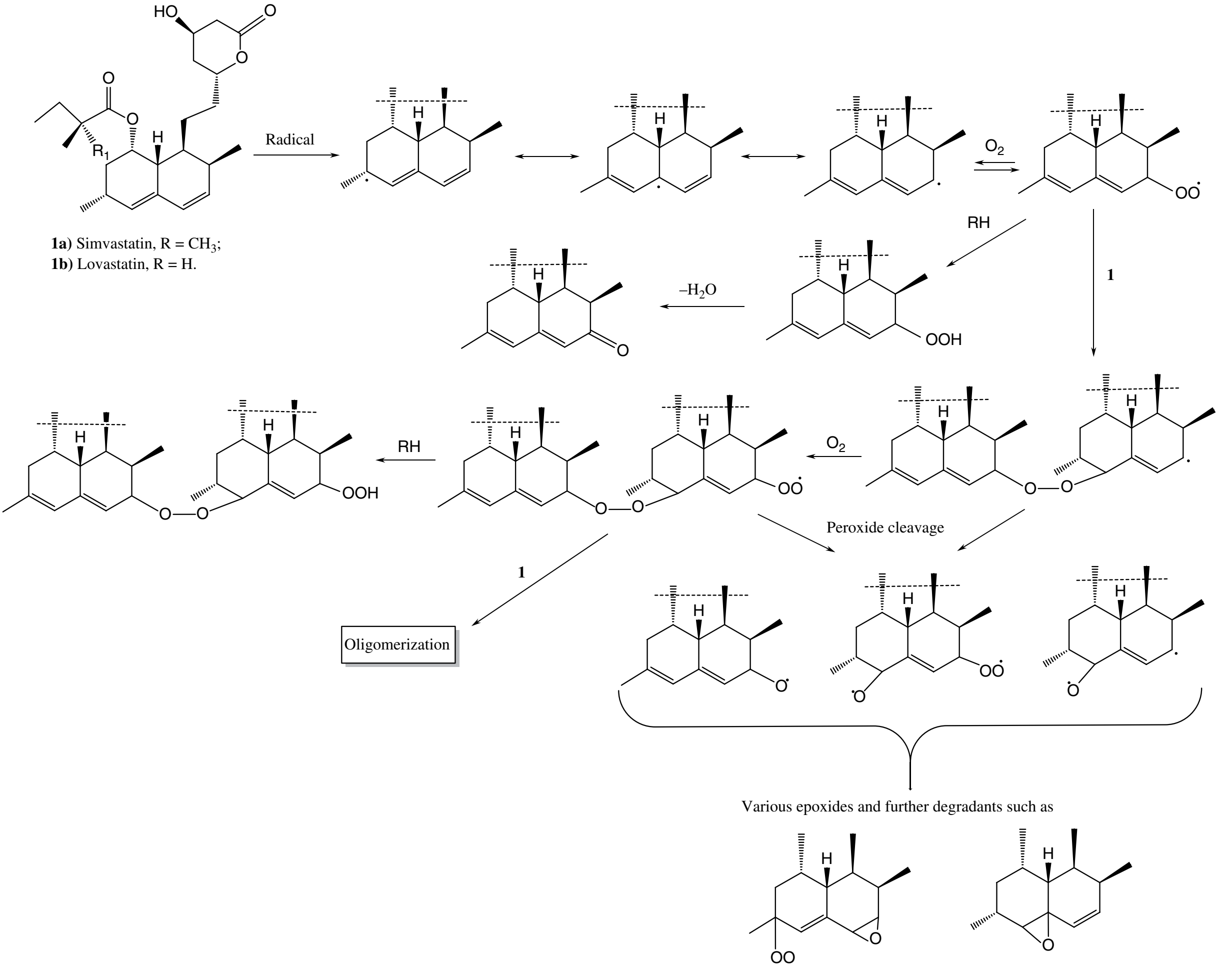


1a) Simvastatin, R = CH_3;
1b) Lovastatin, R = H.

Scheme 297–298-1 Hydrolytic degradation pathways of simvastatin and lovastatin.

lactone and the open acid forms are in equilibrium, while in alkaline pH, the hydrolytic ring opening of the lactone forms is quick and irreversible.

In addition to the two moieties that are susceptible to hydrolytic degradation as mentioned above, the major degradation of simvastatin and lovastatin is of oxidative nature, which initially occurs on the α-positions of the embedded butadiene functionality via a free-radical-mediated process (Scheme 297–298-2). The carbon-based free radicals formed should readily react with molecular oxygen to produce hydroperoxide intermediates, which are not stable and subsequently decompose to yield a large number of low-level degradants [6]. It was reported that the oxidative degradation could be prevented by storage in an inert atmosphere [5].



1a) Simvastatin, R = CH_3;
1b) Lovastatin, R = H.

Scheme 297–298-2 Oxidative degradation pathways of simvastatin and lovastatin.

References

1 Alberts, A.W., Chen, J., Kuron, G. et al. (1980). Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci. USA* 77: 3957–3961.

2 Lovastatin. https://en.wikipedia.org/wiki/Lovastatin (accessed March 3 2024).

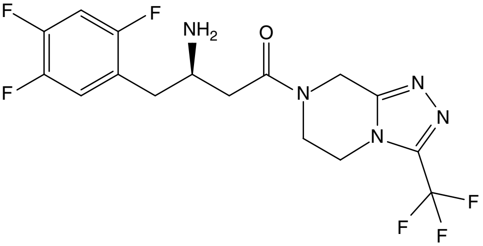
3 Endo, A., Kuroda, M., and Tsujita, Y. (1976). ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by *Penicillium citrinium*. *J. Antibio.* 29: 1346–1348.

4 Vree, T.B., Dammers, E., Ulc, I. et al. (2003). Differences between lovastatin and simvastatin hydrolysis in healthy male and female volunteers. *Sci. World J.* 3: 1332–1343.

5 Ellison, D.K., Moore, W.D., and Petts, C.R. (1993). Simvastatin. *Anal. Prof. Drug Sub. Excip.* 22: 359–388.

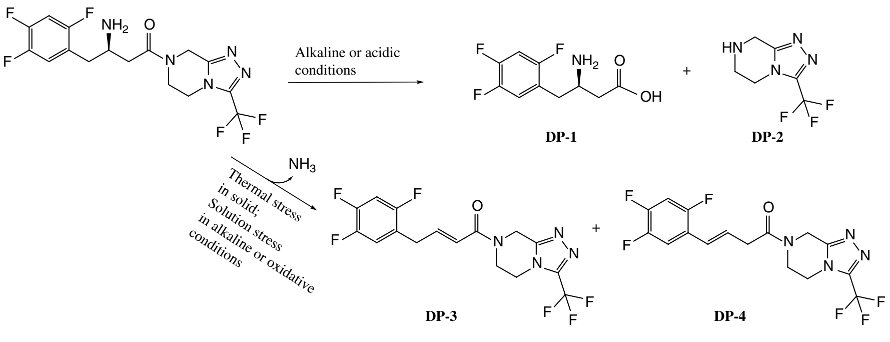
6 Smith, G.B., DiMichele, L., Colwell, L.F. Jr. et al. (1993). Autooxidation of simvastatin. *Tetrahedron* 49: 4447–4462.

299: Sitagliptin



Chemical name: (*R*)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine
Trade name: Januvia
Formula: $C_{16}H_{15}F_6N_5O$
Monoisotopic protonated ion (*m/z*): 408.1254
Molecular weight: 407.32
CAS number: 486460-32-6
Common salt form: Phosphate

Sitagliptin, developed by Merck & Co., Inc., was the first dipeptidyl peptidase IV (DPP4) inhibitor approved by the US FDA in 2006 to treat type II diabetes [1, 2]. It contains an amide linkage in the middle of the drug molecule, which is susceptible to hydrolytic degradation. The cleavage of the amide bond under both acidic and alkaline conditions was observed in several forced degradation studies, most of which reported both hydrolytic degradants, i.e., (*R*)-3-amino-4-(2,4,5-trifluorophenyl) butanoic acid (DP-1) and 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazine (DP-2) (Scheme 299) [3–8]. In a thermal stress study of sitagliptin in solid state, two deaminated degradants (DP-3 and DP-4) were detected [5], which were also observed in a compatibility study between sitagliptin and magnesium stearate, as well as in an alkaline stress solution and an oxidative stress solution of sitagliptin, respectively [8]. It is worth noting that most of the reported oxidative



Scheme 299 Degradation of sitagliptin via hydrolytic and deaminative pathways under stress conditions.

stress studies utilized hydrogen peroxide solutions, and the resulting solutions were heated at quite high temperatures, such as 70°C or 80°C, for extended periods [6]. Such conditions seem to be too harsh, which tend to produce artificial degradants.

Sitagliptin also possesses a primary amino group, which would be expected to interact chemically with excipients containing functional groups such as Michael acceptors and reducing sugars via Michael addition and Maillard degradation, respectively [9]. In the API-excipient compatibility study performed by Gumieniczek et al. [8], sitagliptin was found to show potent interaction with fumaric acid (a Michael acceptor) and lactose (a reducing sugar), based on infrared spectroscopic evidence. Sitagliptin is often used in fixed combination with metformin, e.g., the drug product Janumet®. Peraman et al. performed an open-dish stress on Janumet tablets and found that more degradants were observed than those combined from the individually stressed sitagliptin and metformin samples, of which only DP-1 and DP-2 were identified for sitagliptin per comparison with reference compounds [4].

References

1 Florentin, M., Kostapanos, M.S., and Papazafiropoulou, A.K. (2022). Role of dipeptidyl peptidase 4 inhibitors in the new era of antidiabetic treatment. *World J. Diabetes* 13: 85–96.

2 Sitagliptin. https://en.wikipedia.org/wiki/Sitagliptin (accessed March 12 2024).

3 El-Bagary, R.I., Elkady, E.F., and Ayoub, B.M. (2011). Liquid chromatographic determination of sitagliptin either alone or in ternary mixture with metformin and sitagliptin degradation product. *Talanta* 85: 673–680.

4 Peraman, R., Gowra, C.S., Reddy, Y.P., and Peruru, K.K. (2013). Stability-indicating RP-HPLC method for simultaneous determination of metformin hydrochloride and sitagliptin phosphate in dosage forms. *Chromatogr.* https://doi.org/10.1007/s10337-013-2525-4.

5 Sonune, D.P. and Mone, M.K. (2013). Isolation, characterization of degradation products of sitagliptin and development of validated stability-indicating HPLC assay method for sitagliptin API and tablets. *Int. J. Pharm. Sci. Res.* 4: 3494–3503.

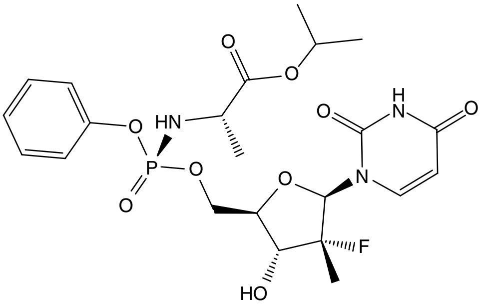
6 Vishnuvardhan, C., Radhakrishnanand, P., Navalgund, S.G., and Satheeshkumar, N. (2014). Liquid chromatography/electrospray ionisation tandem mass spectrometric study of sitagliptin and its stressed degradation products. *Drug Res.* 64: 668–674.

7 Lange, A.D.C., Miron, D., Sfair, L.L., and Schapoval, E.E.S. (2023). Sitagliptin phosphate: isolation and identification of two degradation products formed under acid conditions and determination of in vitro cytotoxicity. *Drug Anal. Res. Porto. Alegre.* 7: 41–45.

8 Gumieniczek, A., Berecka, A., Mroczek, T. et al. (2019). Determination of chemical stability of sitagliptin by LC-UV, LC-MS and FT-IR methods. *J. Pharm. Biomed. Anal.* 164: 789–807.

9 Li, M. (2012). Drug–excipient interactions and adduct formation. In: *Organic Chemistry of Drug Degradation*, 150–164. Cambridge: RSC Publishing.

300: Sofosbuvir [WHO List of Essential Medicines]

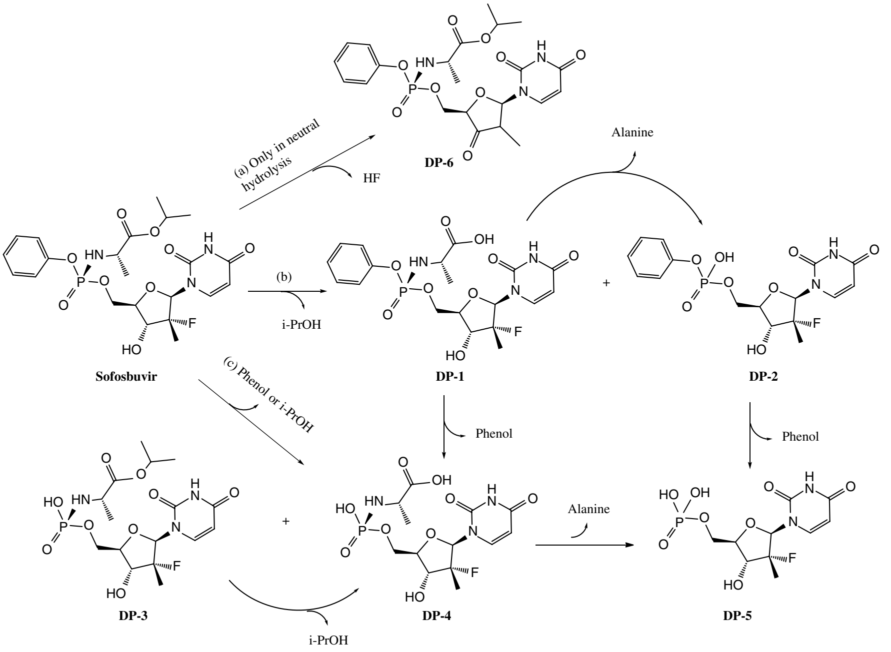


Chemical name: Isopropyl (2*S*)-2-{[(*S*)-{[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydro-1 (2*H*)-pyrimidinyl)-4-fluoro-3-hydroxy-4-methyltetrahydro-2-furanyl]methoxy} (phenoxy) phosphoryl]amino}propanoate
Trade name: Sovaldi
Formula: $C_{22}H_{29}FN_3O_9P$
Monoisotopic protonated ion (*m/z*): 530.1698
Molecular weight: 529.46
CAS number: 1190307-88-0

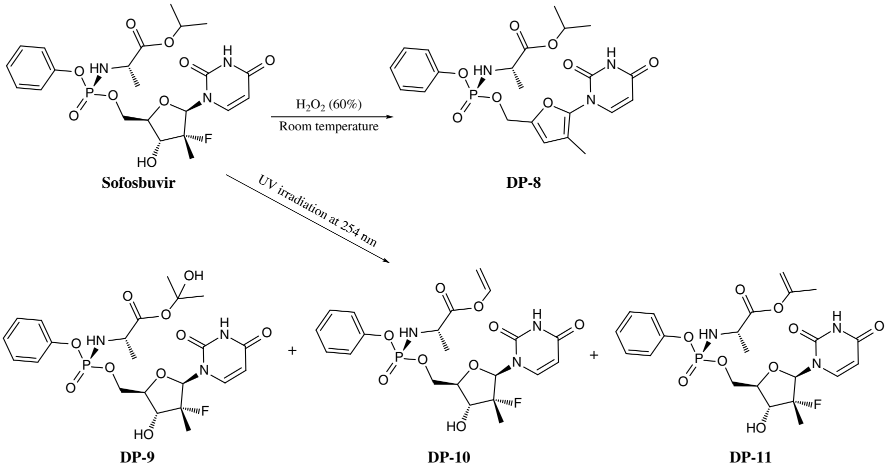
Sofosbuvir, also known as PSI-7977 or GS-7977, is a breakthrough medication for the treatment of hepatitis C virus (HCV) infection, with significantly higher curing rates than previous therapies but with much less side effects [1]. It is a prodrug and its active metabolite, GS-461203 (2′-deoxy-2′-α-fluoro-β-C-methyluridine-5′-triphosphate), is a defective substrate for HCV's NS5B RNA-dependent RNA polymerase (RdRp), thus inhibiting the viral RNA synthesis and viral replication [2, 3]. It was originally invented by Michael J. Sofia and his research team at Pharmasset, Inc. [3], a New Jersey biotech company that was acquired by Gilead Sciences for the antiviral asset in 2011. Sofosbuvir combination with ribavirin was first approved for oral dual therapy of HCV genotypes 2 and 3 [2]. Its combinations with other antiviral agents, e.g., ledipasvir and velpatasvir (both are NS5A inhibitors), were subsequently approved to cover other HCV genotypes [4].

The major degradation of sofosbuvir is of hydrolytic nature: the isopropyl ester, phenyl phosphate, and phosphoryl amide functionalities can be hydrolyzed respectively or successively to yield a number of hydrolytic degradants, depending on the pH of the media and duration of the hydrolysis (Scheme 300-1) [5–8]. The activation energies for hydrolytic degradation of sofosbuvir in solutions were found to be 77.9–79.5 kJ/mol [9]. On the other hand, the activation energy for the solid state decomposition was determined to be 115.3 kJ/mol, indicating that sofosbuvir has good thermal stability in the solid state under routine temperature [10].

In an oxidative stress of sofosbuvir in 6% hydrogen peroxide at room temperature for 19 days, Nebsen and Elzanfaly reported an oxidative degradant resulting from the oxidation of the sugar ring (Scheme 300-2) [6]. They also performed a photochemical forced degradation study and found that the photo reactions all occurred on the isopropyl ester side chain, which is somewhat surprising as the ester group is distant away from the chromophore (the phenol group).



Scheme 300-1 Degradation of sofosbuvir to produce the hydrolytic degradants (DP-1–DP-5) in either acidic or alkaline solutions [4–6, 8] and to produce the elimination degradant (DP-6) in neutral solution [7]. The occurrence of a specific pathway or the distribution of the degradants can vary depending on the pH of the media and or duration of the forced degradation.



Scheme 300-2 Degradation of sofosbuvir under oxidative and photochemical stress conditions.

References

1 McQuaid, T., Savini, C., and Seyedkazemi, S. (2015). Sofosbuvir, a significant paradigm change in HCV treatment. *J. Clin. Transl. Hepatol.* 3: 27–35.

2 Sofosbuvir. https://en.wikipedia.org/wiki/Sofosbuvir (accessed March 12 2024).

3 Sofia, M.J., Bao, D., Chang, W. et al. (2010). Discovery of a β-D-2′-Deoxy-2′-α-fluoro-2′-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J. Med. Chem.* 53: 7202–7218.

4 Greig, S.L. (2016). Sofosbuvir/Velpatasvir: a review in chronic hepatitis C. *Drugs* 76: 1567–1578.

5 Pottabathini, V., Gugulothu, V., Kaliyaperumal, M., and Battu, S. (2016). Identification, isolation and structure confirmation of forced degradation products of sofosbuvir. *Am. J. Anal. Chem.* 7: 797–815.

6 Nebsen, M. and Elzanfaly, E.S. (2016). Stability-indicating method and LC–MS-MS characterization of forced degradation products of sofosbuvir. *J. Chromatogr. Sci.* 54: 1631–1640.

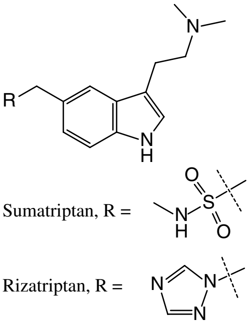
7 Swain, D., Samanthula, G., Shweta Bhagat, S. et al. (2016). Characterization of forced degradation products and *in silico* toxicity prediction of sofosbuvir: a novel HCV NS5B polymerase inhibitor. *J. Pharm. Biomed. Anal.* 120: 352–363.

8 Zaman, B. and Hassan, W. (2020). Development of stability indicating HPLC-UV method for determination of process impurities and degradation products in sofosbuvir and velpatasvir tablets. *Pharm. Chem. J.* 54: 1295–1305.

9 Petřík, J., Heřt, J., Řezanka, P. et al. (2020). Development of HPLC method for the purity test by design of experiments and determination of activation energy of hydrolytic degradation reactions of sofosbuvir. *Curr. Pharm. Anal.* 16: 976–987.

10 Wang, X.-J. and You, J.-Z. (2017). Study on the thermal decomposition of sofosbuvir. *J. Anal. Appl. Pyrolysis* 123: 376–384.

301–302: Sumatriptan, Rizatriptan



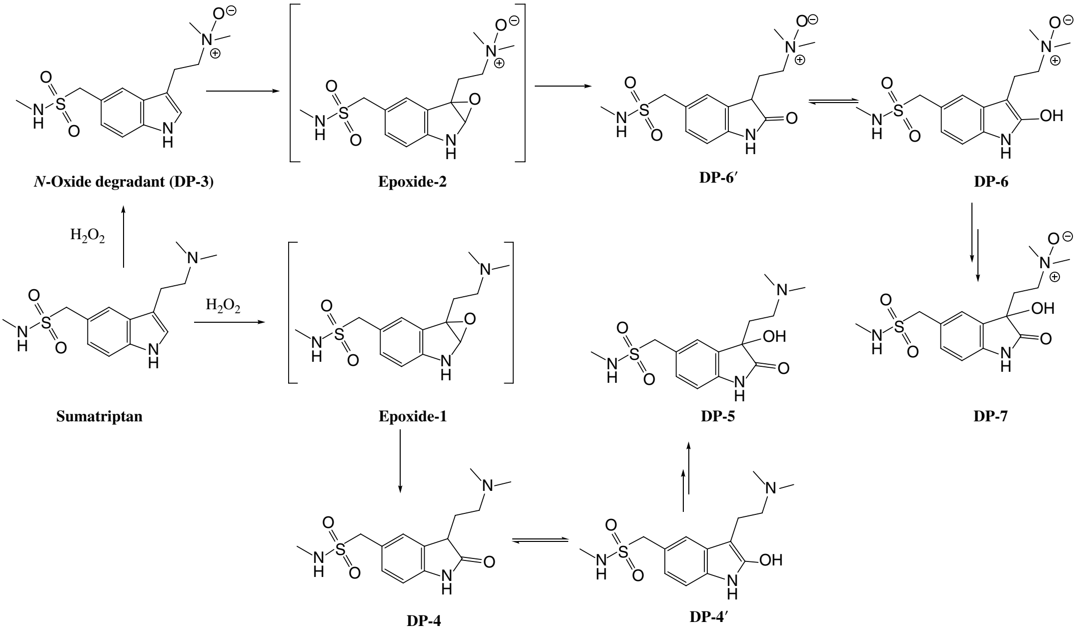
301: Sumatriptan [WHO List of Essential Medicines]

Chemical name: 1-[3-(2-Dimethylaminoethyl)-1*H*-indol-5-yl]-*N*-methyl-methane sulfonamide
Trade name: Imitrex, Onzetra, Tosymra
Formula: $C_{14}H_{21}N_3O_2S$
Monoisotopic protonated ion (*m/z*): 296.1427
Molecular weight: 295.40
CAS number: 103628-46-2
Common salt form: Hemisulfate, Succinate

302: Rizatriptan

Chemical name: *N,N*-Dimethyl-2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl] ethanamine
Trade name: Maxalt
Formula: $C_{15}H_{19}N_5$
Monoisotopic protonated ion (*m/z*): 270.1713
Molecular weight: 269.35
CAS number: 145202-66-0
Common salt form: Benzoate

Sumatriptan is a second-generation serotonin 5-$HT_{1B/1D}$ receptor agonist. Developed by Glaxo, it is the first triptan drug approved for clinical treatment of migraine headaches in 1991 [1]. It is now available in several dosage forms, including oral, nasal spray, and subcutaneous injectable forms. Sumatriptan is an indole derivative with a side chain tertiary amine and methylsulfamoyl groups. The indole ring and the tertiary amino group would be susceptible to oxidative degradation. In the forced degradation study by Xu et al. in 2001 [2], six main oxidative degradants were reported; nevertheless, based on the LC–MS/MS results and degradation chemistry principles, it was only possible for the authors to propose a definite structure (the *N*-oxide degradant) for the most abundant degradant (Scheme 301–302-1). The

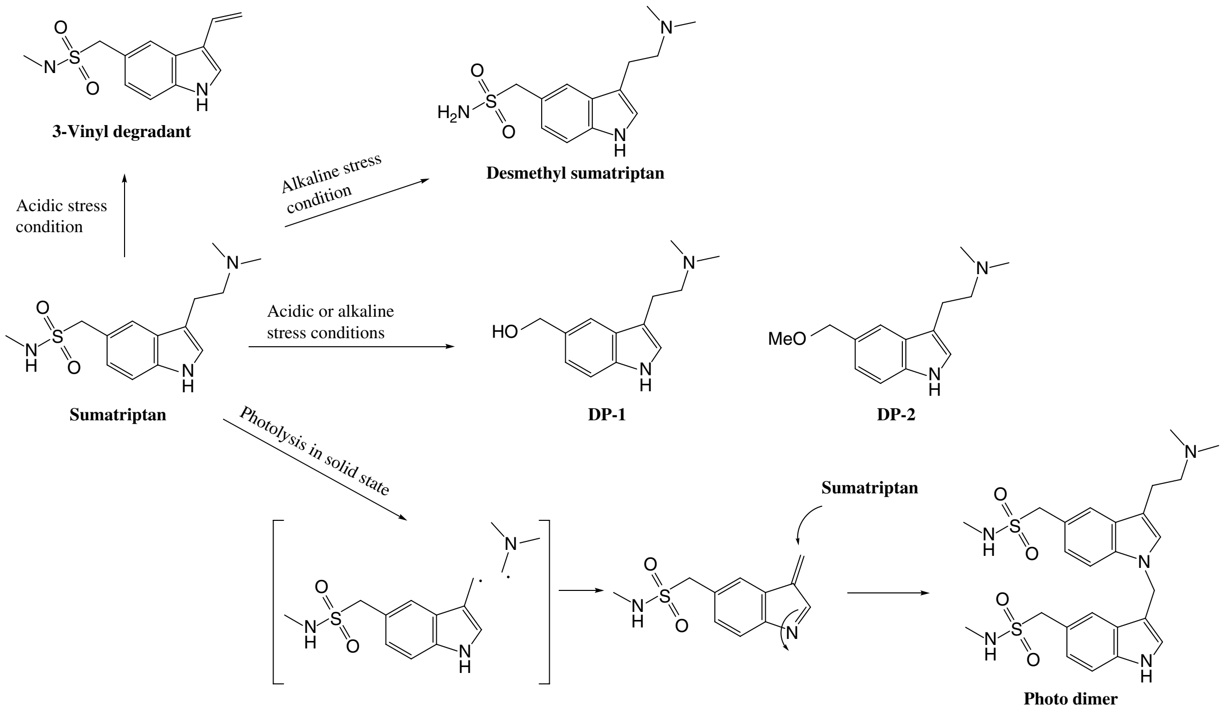


Scheme 301–302-1 Oxidative degradation pathways of sumatriptan. The nomenclature of DP-3 through DP-7 follows that of Udutha et al. [3]. The mechanism proposed here is somewhat different from Udutha et al. in that the epoxide intermediates were not mentioned in the original paper.

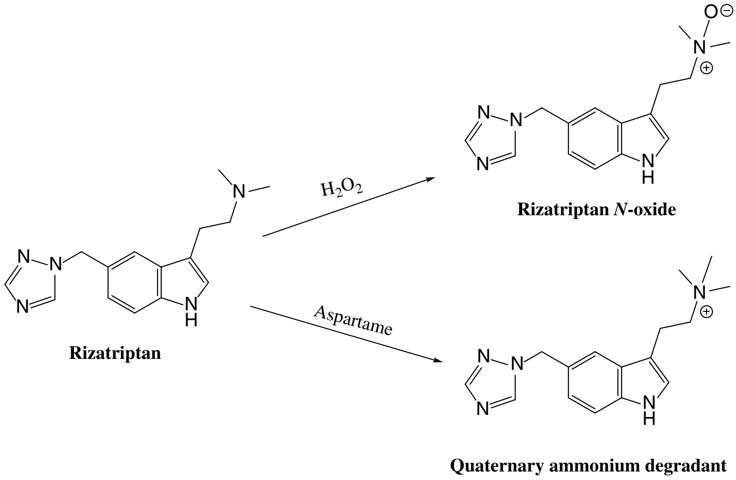
other oxidative degradants were found to contain one, two, or three extra oxygens, as compared to sumatriptan, and it appeared that all the extra oxygens would be on the indole ring according to their MS^2 fragmentation pathways. It needs to be noted that the oxidative stress condition used in this study was somewhat harsh (3%, 90°C, 30 minutes). In another oxidative stress study in 3% hydrogen peroxide at room temperature for 10 days, five oxidative degradants were reported with proposed structures [3]. Among the oxidative degradants, the most abundant one (DP-7) had three extra oxygens incorporated at both the tertiary amine and indole moieties. The structures of DP-7 and DP-3 (the *N*-oxide degradant) were further confirmed by NMR analysis. Although it was not mentioned by the original authors, it would be reasonable to assume that the oxidation of the indole ring would proceed via the two epoxide intermediates, which tend to be unstable at room temperature and should spontaneously rearrange to the corresponding 2-oxo degradants [4] (Scheme 301–302-1). The latter 2-oxo degradants are the tautomers of the corresponding 2-hydroxyindole degradants.

Other than the oxidative degradation, sumatriptan was found to be susceptible to degradation under somewhat harsh acidic or alkaline conditions. Most of the degradation occurred at the methylsulfamoyl side chain, yielding DP-1 and DP-2 in refluxing 1 N NaOH solution for up to 72 hours, [3] in 0.1 N NaOH at 90°C for 10 hours, or in 0.1 N HCl at 90°C for 10 hours (Scheme 301–302-2) [2]. Under the latter acidic condition, the 3-vinyl degradant was observed as the major degradant, resulting from the elimination of the side chain amino group [2]. In the stress solution of 0.1 N NaOH at 90°C for 10 hours, desmethyl degradant was detected as a minor degradant [2]. In a photolytic stress study of sumatriptan in solid state, a dimeric degradant (DP-8) was formed after exposure to $200\,\mathrm{Whm^{-2}}$ UV lighting in a photo stability chamber for 1 week [3].

Rizatriptan is another second-generation serotonin $5\text{-HT}_{1B/1D}$ receptor agonist, which was developed by Merck & Co., Inc. and approved in 1998. It is clinically used for the treatment of migraine through inhibiting inflammation and dural vasodilation [5, 6]. It differs from sumatriptan only in the 5-position of the indole ring: the triazole replaces the methylsulfamoyl substituent. Hence, it would be expected that the degradation chemistry of rizatriptan should be similar to that of sumatriptan, particularly with regard to the tertiary amino side chain. For example, Rao et al. reported the *N*-oxide degradant of rizatriptan after its treatment with 3% hydrogen peroxide at 60°C for 2 hours (Scheme 301–302-3) [7]. In a formulation containing aspartame, which is a methyl ester, the quaternary ammonium degradant was formed, with aspartame as the methyl donor onto the tertiary amine moiety [8].



Scheme 301–302-2 Degradation pathways of sumatriptan under acidic, alkaline, and photolytic conditions.



Scheme 301–302-3 Degradation of rizatriptan due to the tertiary amine moiety.

References

1 Sumatriptan. https://en.wikipedia.org/wiki/Sumatriptan (accessed August 18 2024).

2 Xu, X., Bartlett, M.G., J.T., and Stewart, J.T. (2001). Determination of degradation products of sumatriptan succinate using LC-MS and LC-MS-MS. *J. Pharm. Biomed. Anal.* 26: 367–377.

3 Udutha, S., Shankar, G., Borkar, R.M. et al. (2018). Identification and Characterization of stress degradation products of sumatriptan succinate by using LC/Q-TOF-ESI-MS/MS and NMR: toxicity evaluation of degradation products. *J. Mass Spectrom.* 53: 963–975.

4 Li, M. (2012). Section 3.5.10. Oxidation of heterocyclic aromatic rings. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

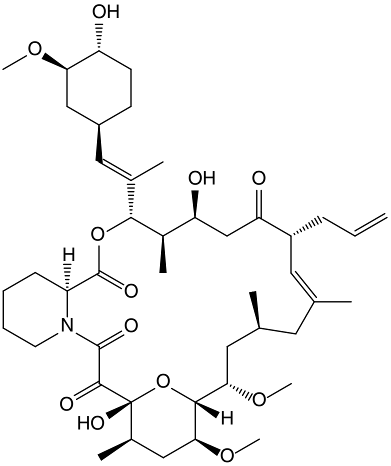
5 Hargreaves, R.J., Lines, C.R., Rapoport, A.M. et al. (2009). Ten years of rizatriptan: from development to clinical science and future directions. *Headache* 49 (Suppl 1): S3–S20.

6 Williamson, D.J., Hill, R.G., Shepheard, S.L., and Hargreaves, R.J. (2001). The anti-migraine 5-HT$_{1B/1D}$ agonist rizatriptan inhibits neurogenic dural vasodilation in anaesthetized guinea-pigs. *Br. J. Pharmacol.* 133: 1029–1034.

7 Rao, B.M., Sangaraju, S., Srinivasu, M.K. et al. (2006). Development and validation of a specific stability indicating high performance liquid chromatographic method for rizatriptan benzoate. *J. Pharm. Biomed. Anal.* 41: 1146–1151.

8 Zecevic, M., Jocic, B., Zivanovic, L., and Protic, A. (2008). Application of multicriteria methodology in the development of improved RP-LC-DAD for determination of rizatriptan and its degradation products. *Chromatographia* 68: 911–918.

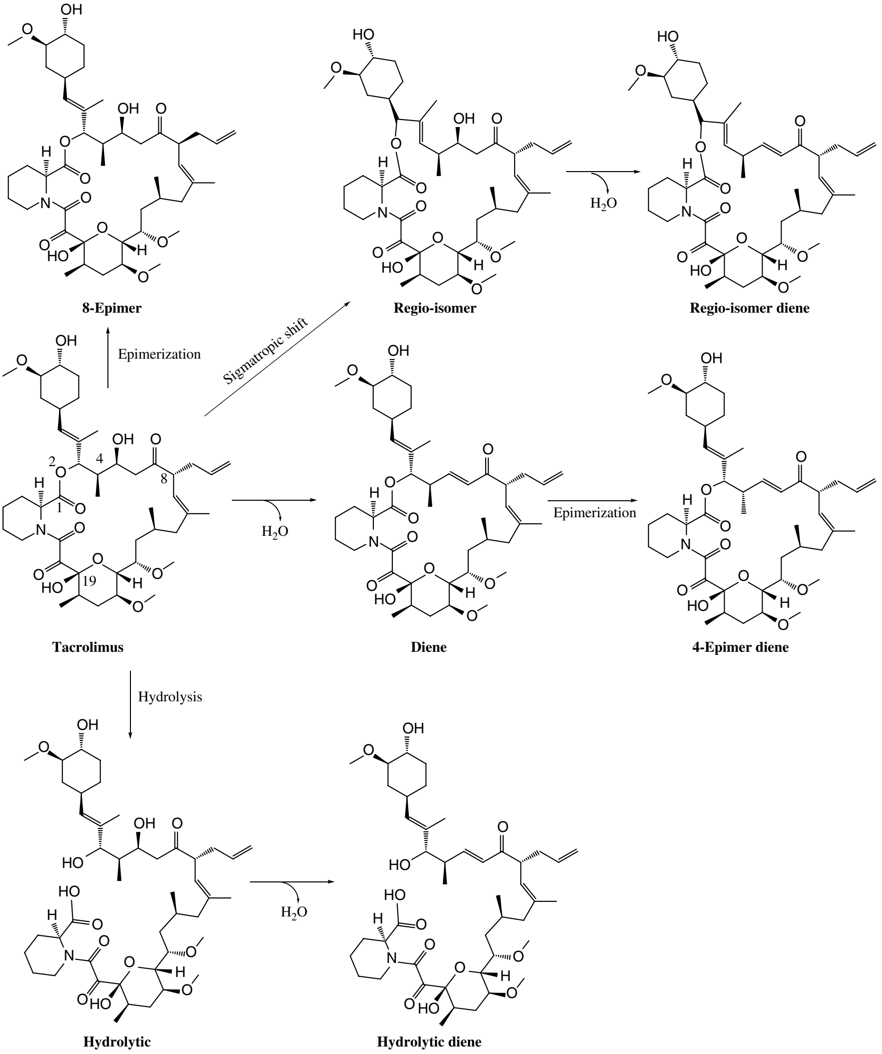
303: Tacrolimus [WHO List of Essential Medicines]



Chemical name: (*1R,9S,12S,13R,14S,17R,18E,21S,23S,24R,25S,27R*)-1,14-Dihydroxy-12-[(*E*)-1-[(*1R,3R,4R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-23,25-dimethoxy-13,19,21,27-tetramethyl-17-prop-2-enyl-11,28-dioxa-4-azatricyclo[22.3.1.04,9]octacos-18-ene-2,3,10,16-tetrone
Trade name: Prograf, Advagraf, Protopic
Formula: $C_{44}H_{69}NO_{12}$
Monoisotopic protonated ion (*m/z*): 804.4893
Molecular weight: 804.03
CAS number: 104987-11-3

Tacrolimus, also known as FK-506 or Fujimycin, is an immunosuppressive drug, which is clinically used to prevent organ rejection for patients undergoing allogenic organ transplant [1]. It is also used as a topic formulation to treat eczema and psoriasis. Tacrolimus is a natural product isolated by Tanaka et al. from the bacterium *Streptomyces tsukubensis* in 1987 [2]. Chemically, it is a macrolide lactone, and the lactone ester linkage can be susceptible to hydrolytic degradation. Nevertheless, it seems that the hydrolysis only occurred meaningfully under strong acidic or alkaline stress conditions [3]. Under the latter stress conditions, dehydration could also take place either as a concurrent degradation pathway or a subsequent one to yield the diene or hydrolytic diene degradants (Scheme 303-1). Such hydrolytic degradation was also reported for similar macrolide lactones, e.g., everolimus and sirolimus [4]. Upon heating, tacrolimus can undergo a sigmatropic shift to produce the regio-isomer [5], which can also dehydrate to yield the corresponding regio-isomer diene. Both the 4-methyl and 8-(3-propenyl) groups can epimerize to afford the corresponding epimeric degradants, 4-epimer diene and 8-epimer [6, 7], which are most likely mediated via the respective keto enolization processes.

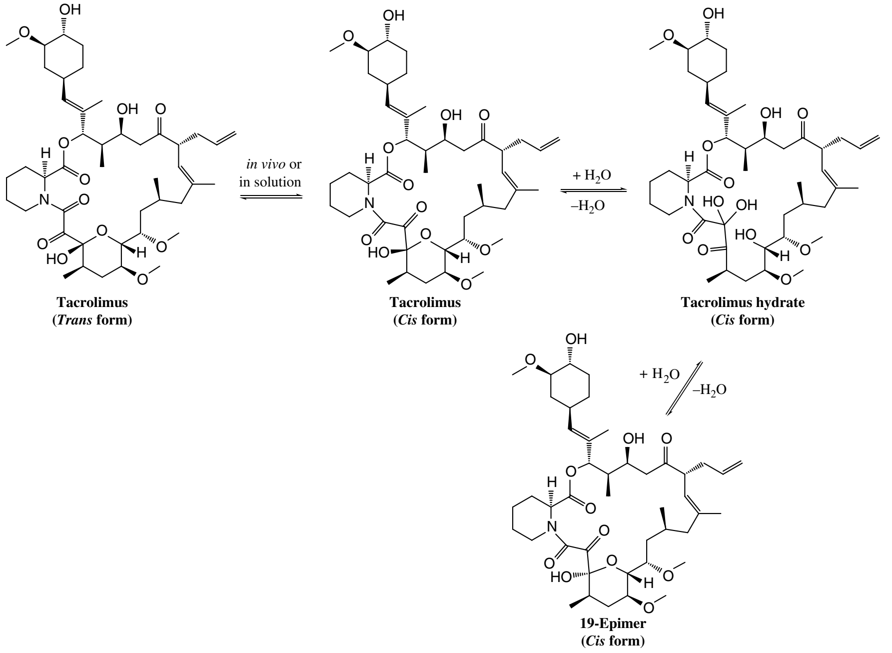
Tacrolimus also contains an amide bond with rotational barrier, and hence, it can exist as both the *cis*- and *trans*-forms. In solid state, tacrolimus exists as the



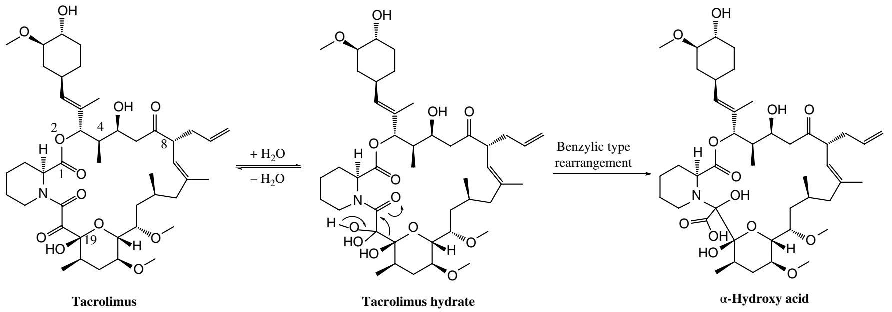
Scheme 303-1 Degradation pathways of tacrolimus. The numbering of the tacrolimus core follows that of Skytte et al. [5]. *Source:* Skytte et al. [5] / with permission of Elsevier.

trans-form and *in vivo* the vast majority of it would quickly convert to the pharmacologically active *cis*-form, based on a study by Namiki et al., indicating that ~92% of tacrolimus was present in its *cis*-form, with its 20-hydrate and 19-epimer at 1% and 7%, respectively (Scheme 303-2) [8]. It is worth noting that both the 20-hydrate and 19-epimer were referred to as the "tautomeric" forms of tacrolimus in the paper by Namiki et al., which does not seem to be accurate.

In a tacrolimus-excipient compatibility study by Peterka et al. [9], a novel degradant was identified as tacrolimus alpha-hydroxy acid, which was attributed to the



Scheme 303-2 *Trans/cis* conversion, formation of tacrolimus hydrate and 19-epimer *in vivo* or in solution.



Scheme 303-3 Formation of tacrolimus α-hydroxy acid via benzylic type rearrangement.

presence of magnesium stearate (Scheme 303-3). A later study revealed that this degradant could also be generated in the forced degradation of tacrolimus with a mixture of 5 mM NaOH and acetonitrile (1:3.5, v/v)[1].

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[1] Wang, J., Tian, Y., Lin, J., Li, M. Unpublished results at the present author's laboratory.

References

1 Tacrolimus. https://en.wikipedia.org/wiki/Tacrolimus (accessed March 13 2024).

2 Tanaka, H., Kuroda, A., Marusawa, H. et al. (1987). Structure of FK506, a novel immunosuppressant isolated from *Streptomyces*. *J. Am. Chem. Soc.* 109: 5031–5033.

3 Prajapati, M., Eiriksson, F.F., and Loftsson, T. (2020). Stability characterization, kinetics and mechanism of tacrolimus degradation in cyclodextrin solutions. *Int. J. Pharm.* 586: 119579.

4 Ilichev, Y.V., Alquier, L., and Maryanoff, C.A. (2007). Degradation of rapamycin and its ring opened isomer: role of base catalysis. *Arkivoc.* 12: 110–131.

5 Skytte, D.M., Jaroszewski, J.W., Johansen, K.T. et al. (2013). Some transformations of tacrolimus, an immunosuppressive drug. *Eur. J. Pharm. Sci.* 48: 514–522.

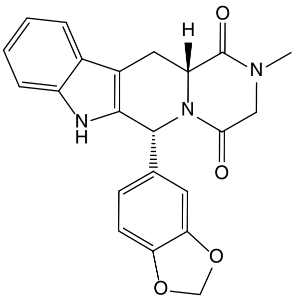
6 Skak, N., Holm, P. (2017). Stabilized tacrolimus composition. US Patent 9,549,918 B2. Filed 17 February 2011. Granted 24 January 2017.

7 Skytte, D.M., Frydenvang, K., Hansen, L. et al. (2010). Synthesis and characterization of an epimer of tacrolimus, an immunosuppressive drug. *J. Nat. Prod.* 73: 776–779.

8 Namiki, Y., Fujiwara, A., Kihara, N. et al. (1995). Determination of the immunosuppressive drug tacrolimus in its dosage forms by high-performance liquid chromatography. *Chromatographia* 40: 253–258.

9 Peterka, T.R., Grahek, R., Hren, J. et al. (2015). Solid state compatibility study and characterization of a novel degradation product of tacrolimus in formulation. *J. Pharm. Biomed. Anal.* 110: 67–75.

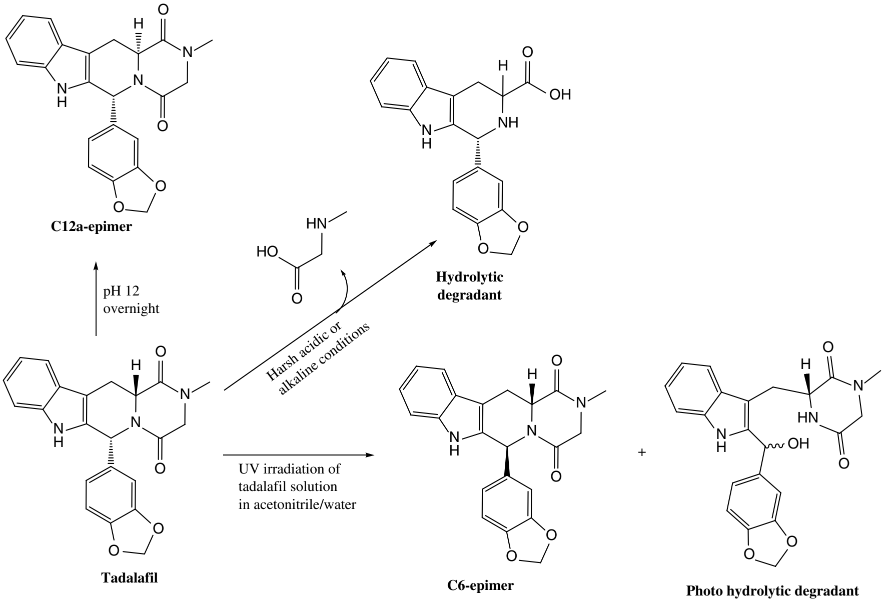
304: Tadalafil



Chemical name: (*2R,8R*)-2-(1,3-Benzodioxol-5-yl)-6-methyl-3,6,17-triazatetracyclo [8.7.0.03,8.011,16]heptadeca-1(10),11,13,15-tetraene-4,7-dione
Trade name: Cialis
Formula: $C_{22}H_{19}N_3O_4$
Monoisotopic protonated ion (*m/z*): 390.1448
Molecular weight: 389.41
CAS number: 171596-29-5

Tadalafil is a highly selective phosphodiesterase 5 (PDE5) inhibitor clinically used to treat erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension [1, 2]. It was developed by Eli Lilly and approved by the US FDA in 2003.

Chemically, tadalafil is an indole derivative with an extended fused ring system that contains a diketopiperazine (DKP) moiety. The 6-membered DKP ring is usually hydrolytically stable but can be hydrolyzed under harsh acidic and alkaline stress conditions (Scheme 304) [3], which typically does not resemble a real degradation scenario. On the other hand, Temussi et al. reported that tadalafil underwent epimerization on the chiral methylene of the DKP ring at pH 12 to produce C12a-epimer [4]. The same authors also studied the photochemical degradation behavior of tadalafil in solution under UV irradiation and observed C6-epimer and two diastereo photo-hydrolytic degradants as the main degradants. In an oxidative forced degradation study of tadalafil in ~4% hydrogen peroxide in methanol at room temperature for 24 hours, it was found to decompose at ~2% [5]. Another stress study also showed that tadalafil had a certain degree of degradation under oxidative condition [6]. Nevertheless, no structural elucidation of the oxidative degradant was performed in either study. It seems likely that the oxidative degradation would occur on the indole ring, as it is known to be susceptible to oxidation by hydrogen peroxide [7].



Scheme 304 Hydrolytic and photochemical degradation pathways of tadalafil.

References

1 Ahmed, N.S. (2019). Tadalafil: 15 years' journey in male erectile dysfunction and beyond. *Drug Dev. Res.* 80: 683–701.

2 Tadalafil. https://en.wikipedia.org/wiki/Tadalafil (accessed March 13 2024).

3 Rezk, M.R., Abdel-Moety, E.M., Wadie, M., and Tantawy, M.A. (2020). Stability assessment of Tamsulosin and Tadalafil co-formulated in capsules by two validated chromatographic methods. *J. Sep. Sci.* 44 (2): 530–538.

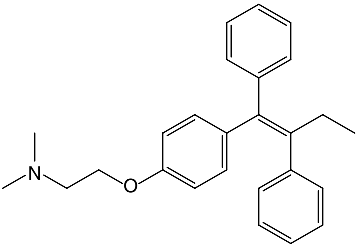
4 Temussi, F., Bassolino, G., Cermola, F. et al. (2010). Investigation on the phototransformation of tadalafil in aqueous media. 6-Epimerization vs. solvent trapping reaction. *Photochem. Photobiol. Sci.* 9: 1139–1144.

5 Pinto, M.A., Nicorena, K.F., Machado, M.M. et al. (2022). Tadalafil and sildenafil illicit association: stability indicating HPLC method, photodegradation kinetic and toxicological studies. *Braz. J. Pharm. Sci.* 58: e19491.

6 Rao, D.V.S., Radhakrishnan, P., and Himabindu, V. (2008). Stress degradation studies on tadalafil and development of a validated stability-indicating LC assay for bulk drug and pharmaceutical dosage form. *Chromatographia* 67: 183–188.

7 Li, M. (2012). Section 3.5.2. Double bonds susceptible to addition by hydroperoxides. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

305: Tamoxifen [WHO List of Essential Medicines]

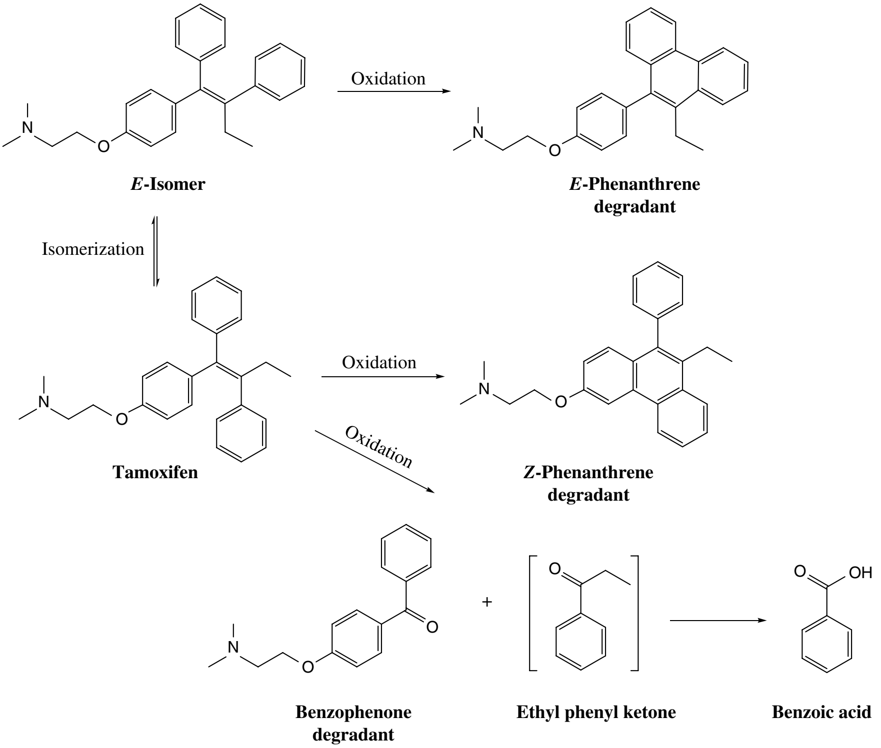


Chemical name: 2-[4-[(*Z*)-1,2-Diphenylbut-1-enyl]phenoxy]-*N*,*N*-dimethylethanamine
Trade name: Nolvadex, Others
Formula: $C_{26}H_{29}NO$
Monoisotopic protonated ion (*m/z*): 372.2322
Molecular weight: 371.52
CAS number: 10540-29-1
Common salt form: Citrate

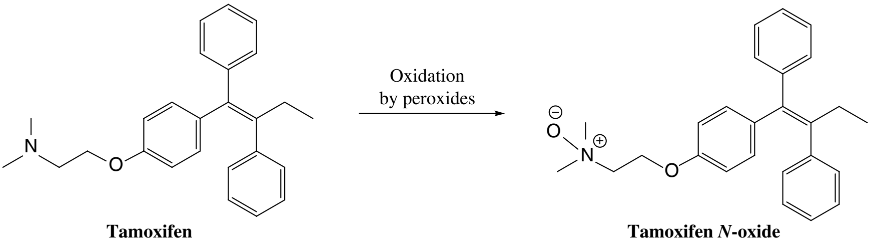
Tamoxifen is a selective estrogen receptor modulator clinically used to treat and prevent breast cancer [1, 2]. It was originally synthesized by Dora Richardson in 1962 (code-named ICI-46,474), as part of a research team headed by Arthur L. Walpole at ICI Pharmaceuticals. The team's original goal was to develop a morning-after contraceptive pill; nevertheless, it turned out to be ineffective as a contraceptive pill. Following on the advice by Walpole, V. Craig Jordan, who studied tamoxifen for his doctoral research at the University of Leeds in the early 1970s, went to Worcester Foundation for Experimental Biology to continue studying tamoxifen and eventually determined that tamoxifen is effective for treating breast cancer. Jordan's work triggered the process that led to the US FDA's approval of tamoxifen as an anti-breast cancer drug in 1977 [3, 4].

Chemically, tamoxifen can be considered a derivative of stilbene, and its central carbon–carbon double bond is susceptible to photochemical *cis–trans* isomerization. Due to conjugation to the three phenyl substituents, tamoxifen can undergo photochemical *cis-trans* isomerization under the irradiation by UV and sunlight (Scheme 305-1) as its UV absorption extends to 320 nm, to produce the *E*-isomer [5, 6]. While tamoxifen (in *Z*-configuration) is an anti-estrogen, its regio *E*-isomer is a full estrogen agonist [7]. In the presence of air, several photo-oxidative degradants were also observed, among which the two phenanthrene degradants were formed via intra-molecular crosslinking, while the other two degradants apparently resulted from the oxidative destruction of the central carbon–carbon double bond [5].

Tamoxifen also contains a tertiary amino moiety that should be susceptible to oxidative degradation to yield the *N*-oxide degradant (Scheme 305-2) [8]. Although it appears that the *N*-oxide degradant has not been specifically reported [9], the analogous α-acetoxytamoxifen *N*-oxide was readily prepared by treating α-acetoxytamoxifen with hydrogen peroxide [10].



Scheme 305-1 Photochemical degradation pathways of tamoxifen in aqueous methanol solution exposed to air. When the photolysis was carried out in argon atmosphere, only the -isomer was formed as the predominant degradant. *Source:* DellaGreca et al. [5] / with permission of Elsevier.



Scheme 305-2 Probable oxidative degradation of tamoxifen by peroxides.

References

1 Tamoxifen. https://en.wikipedia.org/wiki/Tamoxifen (accessed March 13 2024).

2 Maximov, P.Y., McDaniel, R.E., and Jordan, V.C. (2013). *Tamoxifen: Pioneering Medicine in Breast Cancer*. New York: Springer, Basel.

3 MD Anderson News Release June 12, 2024 (2024). MD Anderson celebrates the life and legacy of V. Craig Jordan, Ph.D. https://www.mdanderson.org/newsroom/md-anderson-celebrates-the-life-and-legacy-of-v-craig-jordan-PhD.h00-159698334.html#:~:text=Pharmacologist%20V.%20Craig%20Jordan,%20Ph.D.,%20a (accessed October 2 2024).

4 Abderrahman, B. (2024). V. Craig Jordan obituary: pharmacologist who revolutionized breast cancer treatments. https://www.nature.com/articles/d41586-024-02448-9#:~:text=The UK–US pharmacologist V. Craig Jordan (accessed October 2 2024).

5 DellaGreca, M., Iesce, M.R., Isidori, M. et al. (2007). Phototransformation products of tamoxifen by sunlight in water. Toxicity of the drug and its derivatives on aquatic organisms. *Chemosphere* 67: 1933–1939.

6 Jalonen, H.G. (1988). Simultaneous determination of tamoxifen citrate and its E isomer impurity in bulk drug and tablets by high-performance liquid chromatography. *J. Pharm. Sci.* 77: 810–813.

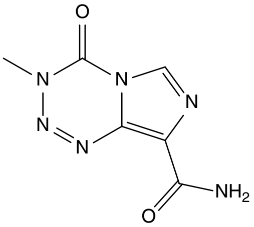
7 Jordan, V.C. and Murphy, C.S. (1990). Endocrine pharmacology of antiestrogens as antitumor agents. *Endocr. Rev.* 11: 578–610.

8 Li, M. (2012). Section 3.5.3.1. Formation of *N*-oxides via nucleophilic attack. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

9 Shete, H. and Patravale, V. (2013). Long chain lipid based tamoxifen NLC. Part I: preformulation studies, formulation development and physicochemical characterization. *Int. J. Pharm.* 454: 573–583.

10 Umemoto, A., Monden, Y., Komaki, K. et al. (1999). Tamoxifen-DNA adducts formed by α-acetoxytamoxifen *N*-oxide. *Chem. Res. Toxicol.* 12: 1083–1089.

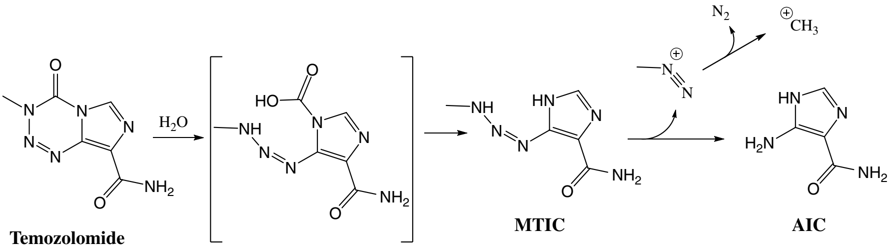
306: Temozolomide



Chemical name: 3,4-Dihydro-3-methyl-4-oxoimidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxamide
Trade name: Temodar, Temodal
Formula: $C_6H_6N_6O_2$
Monoisotopic protonated ion (*m/z*): 195.0625
Molecular weight: 194.15
CAS number: 85622-93-1

Temozolomide is an antitumor medication, which is primarily used for treating brain tumors; it is a first-line treatment for glioblastoma and a second-line treatment for astrocytoma [1]. It was designed as a prodrug of the putative active metabolite of dacarbazine, a prior approved anti-cancer drug, by Malcolm Stevens' group at Aston University in Birmingham, England, in 1987 [2]. The drug candidate, originally code-named CCRG 81045, was further developed by Schering-Plough, with the code name of SCH 52365, and received market approval in 1999 [1, 3].

Upon hydrolytic cleavage, temozolomide releases the active drug, MTIC (a triazene derivative), which further decomposes to AIC and methyldiazonium ion. The diazonium ion, itself a strong methylation agent, along with methyl cation, its ultimate decomposition product, methylates tumor DNA to suppress tumor growth. This activation pathway is also the main degradation pathway of temozolomide (Scheme 306), in which the detectable degradant is AIC [4–6].



Scheme 306 Degradation pathway of temozolomide.

References

1 Temozolomide. https://en.wikipedia.org/wiki/Temozolomide (accessed October 3 2024).

2 Stevens, M.F.G., Hickman, J.A., Langdon, S.P. et al. (1987). Antitumor activity and pharmacokinetics in mice of 8-carbamoyl-3-methylimidazo[5,l-*d*]-l,2,3,5-tetrazin-4(3*H*)-one (CCRG 81045; M & B 39831), a novel drug with potential as an alternative to dacarbazine. *Cancer Res.* 47: 5846–5852.

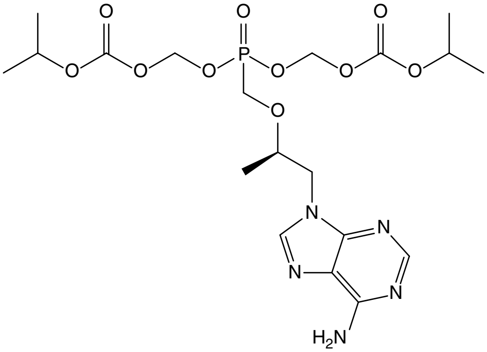
3 Liang, L., Nale, L., James, L. et al. (2007). Dose intense schedules of temozolomide (SCH 52365) achieve better efficacy than continuous low dose schedules in human brain cancer and melanoma models. *Cancer Res.* https://aacrjournals.org/cancerres/article/67/9_Supplement/3195/538778 (accessed October 3 2024.

4 Saravanan, G., Ravikumar, M., Jadhav, M.J. et al. (2007). A stability-indicating LC assay and degradation behavior of temozolomide drug substances. *Chromatographia* 66: 291–294.

5 Lopes, I.C., de Oliveira, S.C.B., and Oliveira-Brett, A.M. (2013). Temozolomide chemical degradation to 5-aminoimidazole-4-carboxamide – electrochemical study. *J. Electroanal. Chem.* 704: 183–189.

6 Jedynak, L., Puchalska, M., Zezula, M. et al. (2013). Stability of sample solution as a crucial point during HPLC determination of chemical purity of temozolomide drug substance. *J. Pharm. Biomed. Anal.* 83: 19–27.

307: Tenofovir Disoproxil [WHO List of Essential Medicines]



Chemical name: Bis{[(isopropoxycarbonyl)oxy]methyl} ({[(2*R*)-1-(6-amino-9*H*-purin-9-yl)-2-propanyl]oxy}methyl)phosphonate

Trade name: Viread

Formula: $C_{19}H_{30}N_5O_{10}P$

Monoisotopic protonated ion (*m/z*): 520.1803

Molecular weight: 519.45

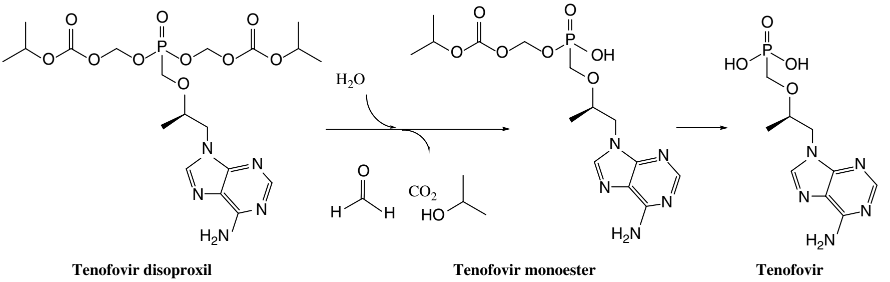
CAS number: 201341-05-1

Common salt form: Fumarate

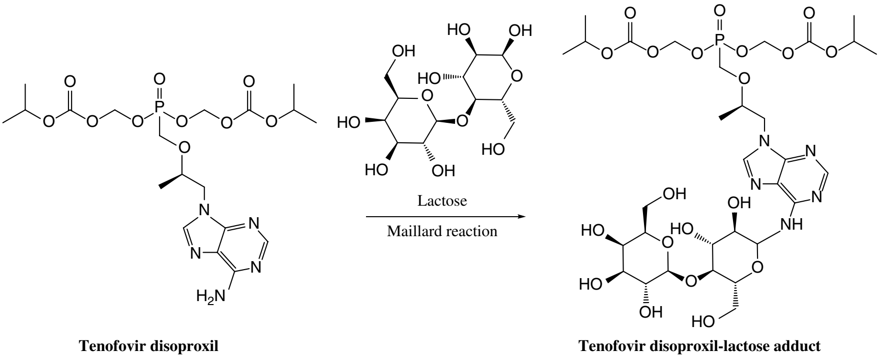
Tenofovir disoproxil, developed by Gilead Sciences, is an antiviral medication used to treat chronic hepatitis B and to prevent and treat HIV/AIDS [1]. It is an ester prodrug of tenofovir, which is a nucleotide analog that inhibits the reverse transcriptase of retroviruses. The active metabolite, tenofovir, is released upon metabolic activation via hydrolytic enzymes.

The carbonate moieties of tenofovir disoproxil are susceptible to hydrolytic degradation, particularly under alkaline conditions, leading to the formation of tenofovir monoester and tenofovir, respectively (Scheme 307-1) [2].

On the other hand, the amino group of tenofovir disoproxil would be expected to undergo the Maillard reaction with excipients containing reducing sugars such as lactose (Scheme 307-2) [3, 4].



Scheme 307-1 Hydrolytic degradation pathway of tenofovir disoproxil.



Scheme 307-2 Maillard degradation of tenofovir disoproxil in the presence of lactose, a commonly used excipient containing reducing sugar.

References

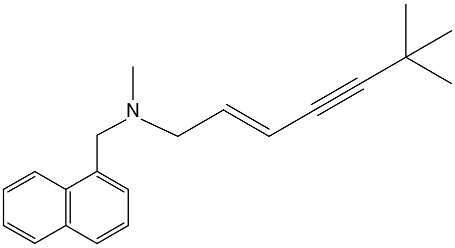
1 Tenofovir disoproxil. https://en.wikipedia.org/wiki/Tenofovir_disoproxil (accessed October 3 2024).

2 Ashenafi, D., Varalaxmi Chintam, V., van Veghel, D. et al. (2010). Development of a validated liquid chromatographic method for the determination of related substances and assay of tenofovir disoproxil fumarate. *J. Sep. Sci.* 33: 1708–1716.

3 Lai, X., Chen, X., Li, M. et al. (2022). Purification and mass spectrometry study of Maillard reaction impurities in five acyclic nucleoside antiviral drugs. *J. Pharm. Biomed. Anal.* 212: 114637.

4 Kurmi, M., Sahu, A., Ladumor, M.K. et al. (2019). Stability behaviour of antiretroviral drugs and their combinations. 9: identification of incompatible excipients. *J. Pharm. Biomed. Anal.* 166: 174–182.

308: Terbinafine [WHO List of Essential Medicines]

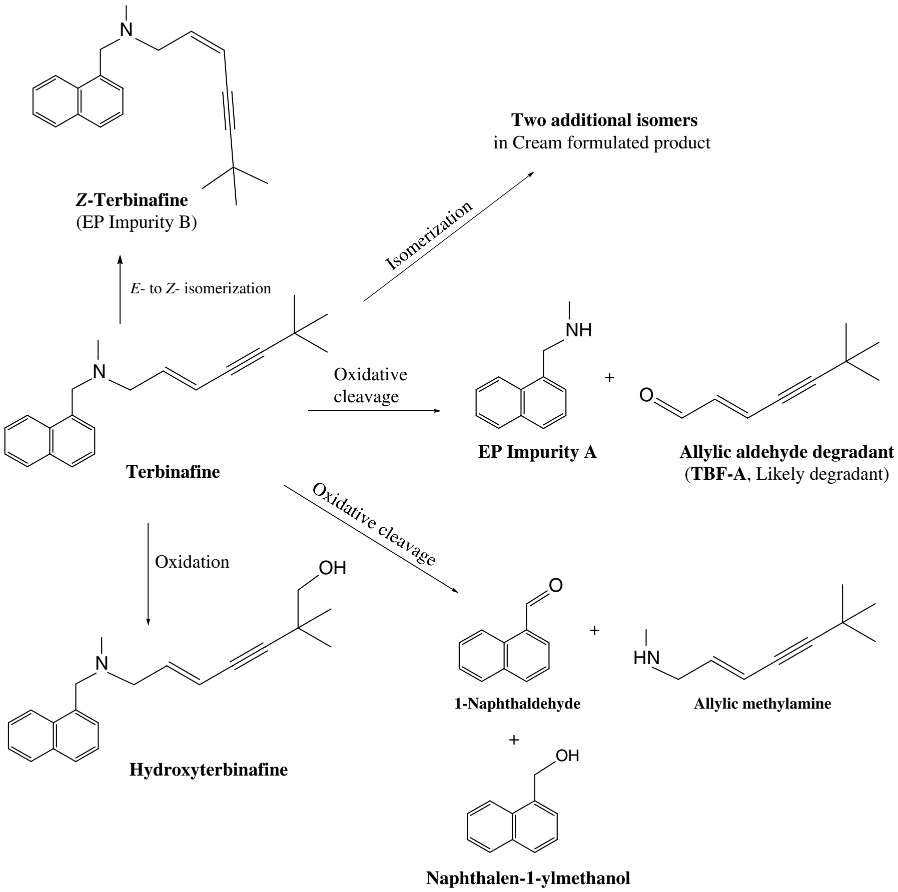


Chemical name: (*2E*)-*N*,6,6-Trimethyl-*N*-(1-naphthylmethyl)-2-hepten-4-yn-1-amine
Trade name: Lamisil, Terbin, others
Formula: $C_{21}H_{25}N$
Monoisotopic protonated ion (*m/z*): 292.2060
Molecular weight: 291.44
CAS number: 91161-71-6
Common salt form: HCl salt

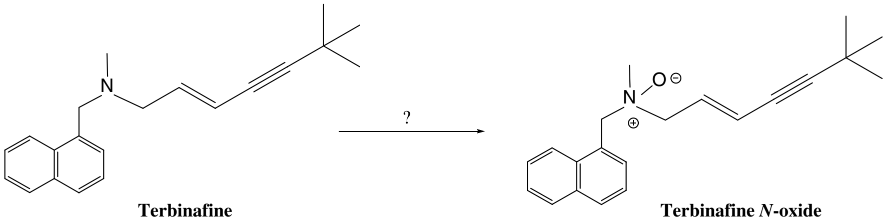
Terbinafine is an antifungal medication in the family of allylamines that inhibit the fungal enzyme squalene epoxidase [1, 2]. It was developed in 1979 based on naftifine, the first allylamine antifungal drug [3] and approved by the US FDA in 1996.

Despite its relatively long history, there seems to be no in-depth study with regard to its degradation chemistry. A photodegradation study of terbinafine performed by Kryczyk-Poprawa et al. appeared to have examined the majority of the photodegradants, and yet, the structural elucidation of the degradants relied only on LC–MS analysis. Upon irradiation by UVA light, the major photodegradation pathways appeared to be the two oxidative cleavage routes (Scheme 308-1). Another significant photooxidative degradation led to the formation of hydroxyterbinafine. Photoisomerization leading to the formation of *Z*-terbinafine was also observed. For the irradiation on the cream formulated terbinafine, two additional photoisomers were also detected [4]. In an accelerated stability study of formulated terbinafine by Matysova et al., EP Impurity A was detected as the only degradant [5]. The formation of EP Impurity A should be accompanied by the simultaneous occurrence of the allylic aldehyde degradant (TBF-A) or a structurally closely related degradant. It is interesting to note that the allylic aldehyde degradant was identified as a reactive metabolite of terbinafine, which is implicated in the probable idiosyncratic liver toxicity of terbinafine [6].

Terbinafine contains a tertiary amino moiety, and as such, terbinafine *N*-oxide would be expected to be a likely degradant (Scheme 308-2) [7]. Nevertheless, it seems that the *N*-oxide degradant has not been reported so far, despite the fact that it is commercially available as a reference material.



Scheme 308-1 Photodegradation pathway of terbinafine based on the results of Kryczyk-Poprawa et al. [4] with modification. *Source:* Kryczyk-Poprawa et al. [4] /John Wiley & Sons.



Scheme 308-2 Likely *N*-oxidation of terbinafine.

References

1 Terbinafine. https://en.wikipedia.org/wiki/Terbinafine (accessed August 27 2024).

2 Ryder, N.S. (1992). Terbinafine: mode of action and properties of the squalene epoxidase inhibition. *Br. J. Dermatol.* 126 (Suppl 39): 2–7.

3 Zhang, A.Y., Camp, W.L., and Elewski, B.E. (2007). Advances in topical and systemic antifungals. *Dermatol. Clin.* 25: 165–183.

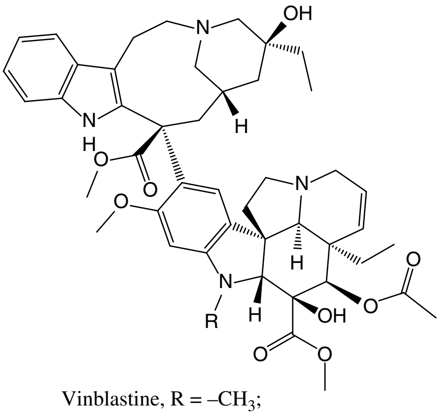
4 Kryczyk-Poprawa, A., Zmudzki, P., Koczurkiewicz, P. et al. (2019). *Photochem. Photobiol.* 95: 911–923.

5 Matysova, L., Solich, P., Mareka, P. et al. (2006). Separation and determination of terbinafine and its four impurities of similar structure using simple RP-HPLC method. *Talanta* 68: 713–720.

6 Iverson, S.L. and Uetrecht, J.P. (2001). Identification of a reactive metabolite of terbinafine: insights into terbinafine-induced hepatotoxicity. *Chem. Res. Toxicol.* 14: 175–181.

7 Li, M. (2012). Section 3.5.3.1. Formation of *N*-oxides via nucleophilic attack. In: *Organic Chemistry of Drug Degradation*. Cambridge: RSC Publishing.

309–310: Vinblastine, Vincristine



Vinblastine, R = $–CH_3$;
Vincristine, R = Formyl.

309: Vinblastine [WHO List of Essential Medicines]

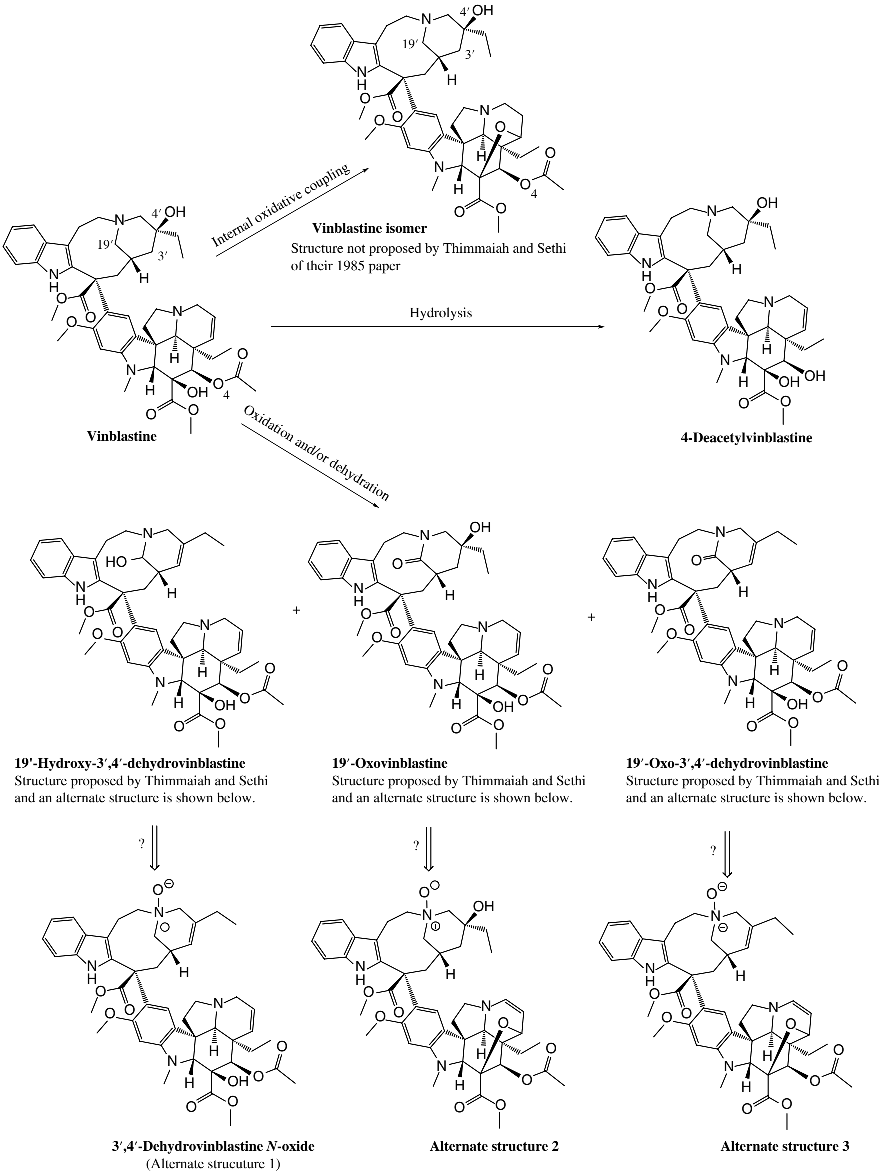
Chemical name: Dimethyl (2β,3β,4β,5α,12β,19α)-15-[(*5S,9S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino [5,4-b]indol-9-yl]-3-hydroxy-16-methoxy-1-methyl-6,7-didehydroaspidospermidine-3,4-dicarboxylate
Trade name: Velban
Formula: $C_{46}H_{58}N_4O_9$
Monoisotopic protonated ion (*m/z*): 811.4277
Molecular weight: 810.99
CAS number: 865-21-4
Common salt form: Sulfate

310: Vincristine [WHO List of Essential Medicines]

Chemical name: (*3aR,3a1R,4R,5S,5aR,10bR*)-Methyl 4-acetoxy-3a-ethyl-9-((*5S,7S, 9S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-2,4,5,6,7,8,9,10-octahydro-1*H*-3,7-methano[1]azacycloundecino[5,4-b]indol-9-yl)-6-formyl-5-hydroxy-8-methoxy-3a,3a1,4,5,5a,6,11,12-octahydro-1*H*-indolizino[8,1-cd]carbazole-5-carboxylate
Trade name: Oncovin
Formula: $C_{46}H_{56}N_4O_{10}$
Monoisotopic protonated ion (*m/z*): 825.4069
Molecular weight: 824.97
CAS number: 57-22-7
Common salt form: Sulfate

Vinblastine and vincristine are structurally highly related *Vinca* alkaloids, which are isolated from the Madagascar periwinkle plant *Vinca rosea (Catharanthus roseus)*

[1, 2]. The plant has been used as a folk remedy for centuries. Both drugs have the same pharmacological mode of action by interfering with the microtubule assembly, thus inhibiting cell division. Their indications include the treatment of cancers such as Hodgkin's lymphoma, lung cancer, bladder cancer, and leukemia. Vinblastine was first isolated by Robert Noble and Charles Thomas Beer at the University of Western Ontario in 1958 [1] and approved by the US FDA in 1965. On



Scheme 309–310 Degradation pathways of vinblastine. The structure for the isomeric degradant and alternate structures for the three other degradants are proposed by the present author based on degradants of similar *Vinca* steroids [6, 7].

the other hand, vincristine, also known as leurocristine, was isolated in 1961 and approved by the US FDA in 1963 [3].

Vinblastine and vincristine each contain three ester functional groups, and thus are expected to be susceptible to hydrolytic degradation, in addition to other types of degradation. In a pH 8.8 buffer solution that was heated at 37°C for 72 hours, five degradants were observed [4], among which 4-deacetylvinblastine is a hydrolytic degradant and its structure was further confirmed via the synthesis of the impurity according to a previously published procedure [5]. For the remaining four degradants, three resulted from oxidation and or dehydration, and their structures were proposed solely based on mass spectrometric analysis by the original authors (Scheme 309–310), while the structure of the isomeric degradant was not proposed [4]. Based on the degradation patterns of similar *Vinca* steroids [6, 7], the structure for the isomeric degradant and alternate structures for the three other degradants are proposed by the present author.

Vincristine, also known as leurocristine, is structurally highly similar to vinblastine, and the only difference lies in the substituent on the nitrogen of the lower tryptophan moiety. Hence, its degradation chemistry would be expected to be highly similar to that of vinblastine. In a study by Sethi and Thimmaiah [8], degradants due to hydrolysis at the 4-position were observed; on the other hand, the structures of certain degradants proposed by them appear to be unlikely to occur under in vitro degradation conditions used in the study. Vendrig et al. studied the degradation kinetics of vincristine in aqueous solutions and found that it was most stable at pH 4.8, under which condition the half-life of vincristine was found to be 136 hours [9].

References

1 Vinblastine. https://en.wikipedia.org/wiki/Vinblastine (accessed March 15 2024).

2 Vincristine. https://en.wikipedia.org/wiki/Vincristine (accessed March 15 2024).

3 Neuss, N., Gorman, M., Boaz, H.E., and Cone, N.J. (1962). Vinca alkaloids. XI. Structures of leurocristine (LCR) and vincaleukoblastine (VLB). *J. Am. Chem. Soc.* 84: 1509–1510.

4 Thimmaiah, K.N. and Sethi, V.S. (1985). Chemical characterization of the degradation products of vinblastine dihydrogen sulfate. *Cancer Res.* 45: 5382–5385.

5 Hargrove, W.W. Novel acyl derivatives of desacetylvinkaleukoblastine and process for their preparation. U.S. Patent 3,392,173. Filed 9 March 1964. Granted 9 July 1968.

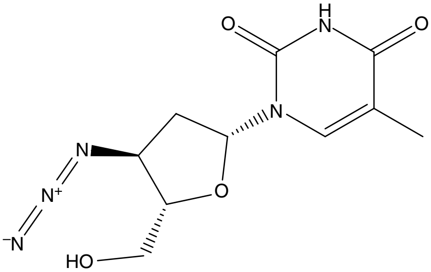
6 Sima Sariaslani, F., Duffel, M.W., and Rosazza, J.P. (1985). One-electron oxidation of vindoline and 16-*O*-acetylvindoline catalyzed by peroxidase. *J. Med. Chem.* 28: 629–633.

7 Thimmaiah, K.N., Lloyd, W.D., and Sethi, V.S. (1990). Chemical characterization of the in vitro degradation products of vindesine sulfate. *Microchem. J.* 42: 115–120.

8 Sethi, V.S. and Thimmaiah, K.N. (1985). Structural studies on the degradation products of vincristine dihydrogen sulfate. *Cancer Res.* 45: 5386–5389.

9 Vendrig, D.E.M.M., Beijnen, J.H., van der Houwen, O.A.G.J., and Holthuis, J.J.M. (1989). Degradation kinetics of vincristine sulphate and vindesine sulphate in aqueous solutions. *Int. J. Pharm.* 50: 189–196.

311: Zidovudine [WHO List of Essential Medicines]

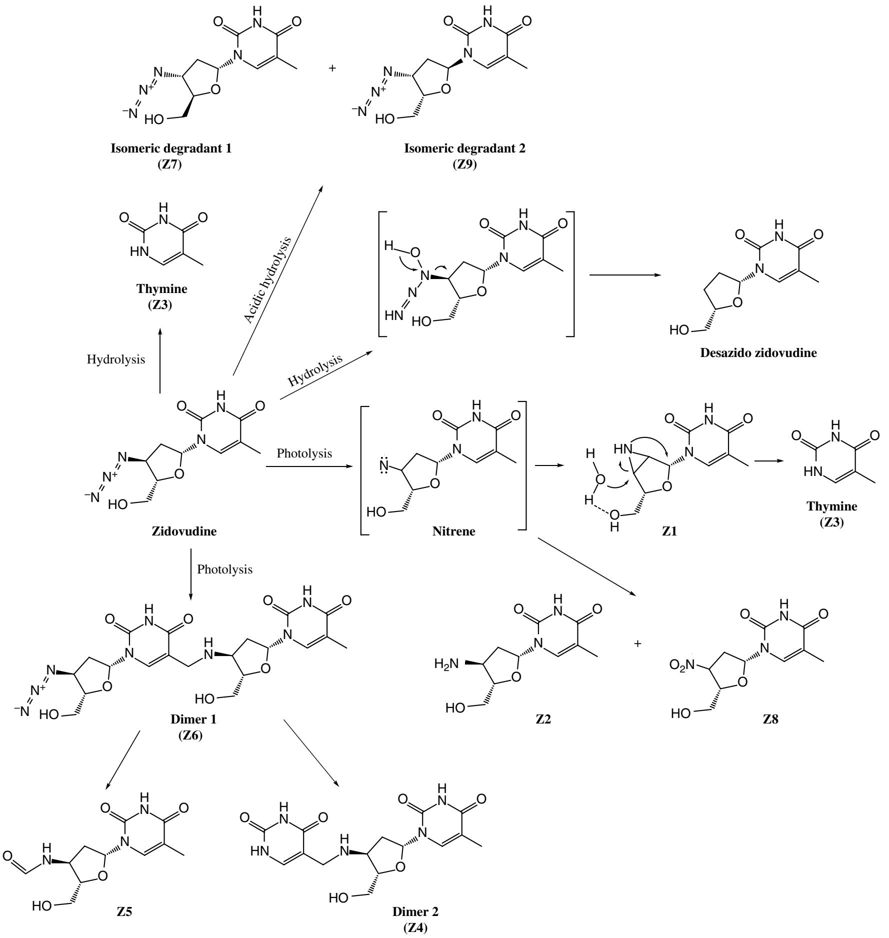


Chemicalname:1-[(*2R,4S,5S*)-4-Azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione
Trade name: Retrovir, others
Formula: $C_{10}H_{13}N_5O_4$
Monoisotopic protonated ion (*m/z*): 268.1040
Molecular weight: 267.25
CAS number: 30516-87-1

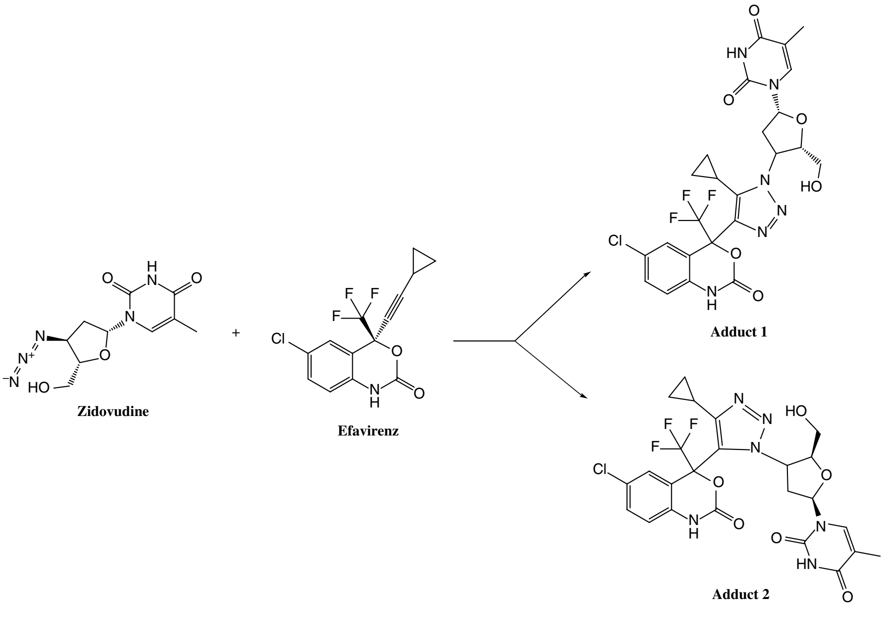
Zidovudine, also known as azidothymidine or AZT, is a nucleoside analog inhibitor of the HIV reverse-transcriptase, which is the first antiviral drug approved to treat HIV/AIDS [1]. It was originally synthesized by Jerome Horwitz's group at Wayne State University in 1964 in an effort to develop a treatment for cancers caused by retroviruses [2]. In 1984, just a few years after AIDS was identified in the United States, Marty St. Clare, a virologist at then Burroughs-Wellcome (eventually became part of GSK), started to screen compounds for anti-AIDS treatment and found zidovudine was effective in inhibiting HIV approximately 5 months into the screening program [3].

Zidovudine is susceptible to hydrolytic and photochemical degradation, due to the presence of the glycosidic linkage and the azido group, respectively. In a forced degradation study in 2 M HCl solution at 80°C for 72 hours, two degradants (thymine and desazido degradant) were detected, both of which were rationalized as hydrolytic degradants (Scheme 311-1) [4]. On the other hand, Dunge et al. performed forced degradation under conditions of hydrolysis, oxidation, and photolysis; thymine was found to be the major degradant under all these conditions [5]. In a later study, the same group also reported two minor acidic degradants (Z7 and Z9) being isomeric to zidovudine [6]. Furthermore, many photochemical degradants were observed in both solution and solid-state photolysis, among which Z1, Z2, Z3, and Z8 were presumably mediated via the nitrene intermediate, and Z4–Z6 resulting from the photo-dimerization pathway.

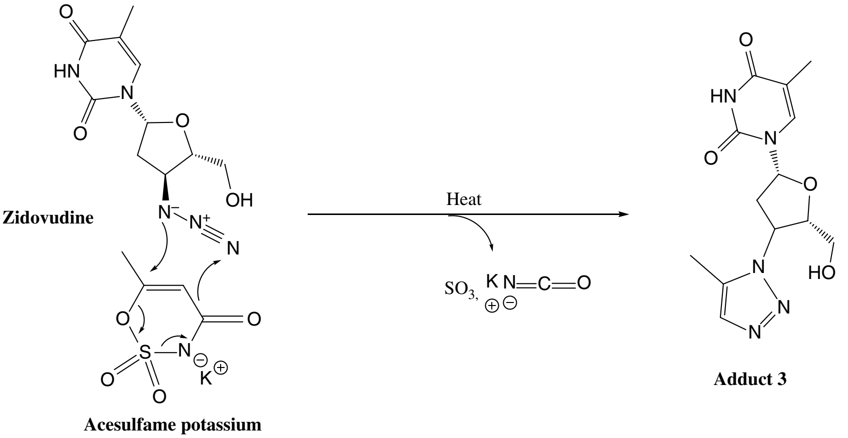
As zidovudine is also commonly used in combination with other antiviral drugs, degradation due to drug–drug interaction is also possible. Kurmi et al. reported two degradants resulting from a 3 + 2 cycloaddition reaction between zidovudine and efavirenz (Scheme 311-2) [7]. Another degradant due to a similar 3 + 2 cycloaddition mechanism was found between zidovudine and acesulfame potassium, an artificial sweetener (Scheme 311-3) [8].



Scheme 311-1 Hydrolytic and photochemical degradation pathways of zidovudine.



Scheme 311-2
3 + 2 cycloaddition process. *Source:*



Scheme 311-3 Degradation of zidovudine due to chemical interaction with acesulfame potassium via a 3 + 2 cycloaddition process. *Source:* Adapted from Aparna et al. [8].

References

1 Zidovudine. https://en.wikipedia.org/wiki/Zidovudine (accessed March 3 2024).

2 Florez, C.V. *Zidovudine or Azidothymidine (AZT)*. Embryo Project Encyclopedia (2020-06-30) ISSN: 1940-5030. https://hdl.handle.net/10776/13154.

3 GSK. The 40 year fight against HIV. https://www.gsk.com/en-gb/behind-the-science-magazine/the-40-year-fight-against-hiv/. (accessed 4 July 2025).

4 Devrukhakar, P.S., Shankar, M.S., Shankar, G., and Srinivas, R. (2017). A stability-indicating LC–MS/MS method for zidovudine: identification, characterization and toxicity prediction of two major acid degradation products. *J. Pharm. Anal.* 7: 231–236.

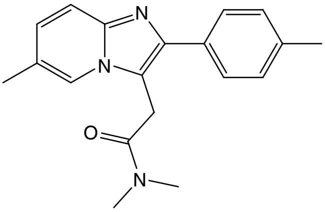
5 Dunge, A., Chakraborti, A.K., and Singh, S. (2004). Mechanistic explanation to the variable degradation behaviour of stavudine and zidovudine under hydrolytic, oxidative and photolytic conditions. *J. Pharm. Biomed. Anal.* 35: 965–970.

6 Kurmi, M., Sahu, A., Tiwari, S.K., and Singh, S. (2017). Stability behaviour of antiretroviral drugs and their combinations. 6: evidence of formation of potentially toxic degradation products of zidovudine under hydrolytic and photolytic conditions. *RSC Adv.* 7: 18803.

7 Kurmi, M., Sahu, A., Balhara, A. et al. (2020). Stability behaviour of antiretroviral drugs and their combinations. 11: characterization of interaction products of zidovudine and efavirenz, and evaluation of their anti HIV-1 activity, and physiochemical and ADMET properties. *J. Pharm. Biomed. Anal.* 178: 112911.

8 Aparna, P., Rao, S.V., Thomas, K.M. et al. (2010). Identification, isolation, and characterization of potential degradation products in a triple combination lamivudine, zidovudine, and nevirapine tablet for oral suspension. *Pharmazie* 65: 331–335.

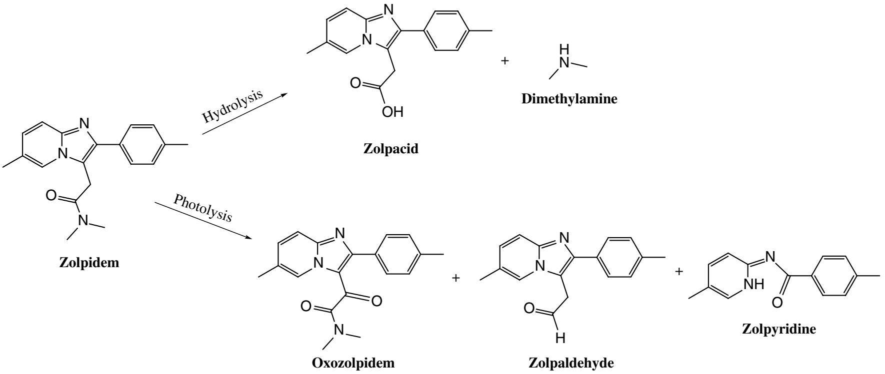
312: Zolpidem



Chemical name: *N,N*-Dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-a]pyridin-3-yl]acetamide
Trade name: Ambien
Formula: $C_{19}H_{21}N_3O$
Monoisotopic protonated ion (*m/z*): 308.1757
Molecular weight: 307.40
CAS number: 82626-48-0
Common salt form: Tartrate

Zolpidem is a $GABA_A$ receptor agonist, which is clinically used for the short-term treatment of insomnia. In the early 1970s, Jean-Pierre Kaplan, a scientist at the French pharmaceutical company Synthélabo (which eventually became part of Sanofi), started to conduct research on GABA, or gamma-aminobutyric acid, along with fellow scientist George Pascal, which led to the discovery of zolpidem [1]. It was first approved in Europe in 1988 and then in the United States in 1992 [2].

Chemically, zolpidem is a dimethylamide derivative and hence, it can be hydrolyzed to form zolpacid and dimethylamine (Scheme 312). It was also reported to be susceptible to photochemical degradation to yield oxozolpidem, zolpaldehyde, and zolpyridine as the photochemical degradants [3, 4].



Scheme 312 Hydrolytic and photochemical degradation pathways of zolpidem.

References

1 The museum of lost things. https://www.museumoflost.com/ambien/#:~:text=Jean-Pierre%20Kaplan%20was%20a%20Paris%20born%20biochemist%2C%20who, developing%20a%20revolutionary%20new%20type%20of%20sleeping%20tablet (accessed October 12 2024).

2 Zolpidem. https://en.wikipedia.org/wiki/Zolpidem (accessed March 15 2024).

3 Malesevic, M., Zivanovic, L., Protic, A., and Jovic, Z. (2011). Multiobjective optimization approach in evaluation of chromatographic behaviour of zolpidem tartrate and its degradation products. *Chromatographia* 74: 197–208.

4 Malesevic, M., Zivanovic, L., Protic, A. et al. (2014). Stress degradation studies on zolpidem tartrate using LC–DAD and LC–MS methods. *Acta. Chromatographica* 26: 81–96.

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