Chapter

25 Antibiotics

1. INTRODUCTION

The term **"antibiotic"** was put forward by Vuillemin in 1889, **to designate the active component involved in the process of 'antibiosis' or to the opposition of one living micro-organism to another.** According to another school of thought—'**antibiotics are nothing but the microbial metabolites which in relatively high dilution may inhibit the growth of micro-organisms**'.

Waksman proposed the widely cited definition that—'***an antibiotic or an antibiotic substance is a substance produced by the microorganisms, which has the capacity of inhibiting the growth and even of destroying other micro-organisms***'.

However, the restriction that an **antibiotic** must be a product of a micro-organism is not in keeping with common use.

Later on Benedict and Langlykke coined a more general and acceptable definition of an **antibiotic** which states that—'***a chemical compound derived from or produced by a living organism, which is capable, in small concentrations, of inhibiting the life processes of micro-organisms.***'

Therefore, a substance may be classified as an **antibiotic** provided it meets the following *four* **cardinal requirements** ; namely :

(*a*) that it is a product of metabolism

(*b*) that it is a synthetic product produced as a structural analogue of a naturally occurring antibiotic.

(*c*) that it antagonizes the growth and/or the survival of one or more species of microorganisms

(*d*) that it is effective in low concentrations.

In another latest version **'antibiotics'** may be defined as—**'microbial metabolites or synthetic structural analogues inspired by them which, in small dosage regimens, inhibit the growth and survival of microorganisms without any serious toxicity whatsoever to the parent host.'**

Importantly, selective toxicity happens to be the **'key concept'** amongst the antibiotics. There are several vital and glaring instances whereby the **'clinical utility of natural antibiotics'** has been enormously augmented *via.* critical medicinal chemical manipulation of the **mother structure** that would ultimately give rise to not only broader antimicrobial spectrum but also higher potency, lower toxicity and more convenient way of administration.

It is pertinent to state at this point in time that in the modern era the enormous and wide application of '**antibiotics**' in animal nutrition and disease has eventually resulted in the overwhelming sensitization of a comparatively huge number of the susceptible people across the globe, most of whom have developed serious reactions upon contact with such type of drug substances. In the same vein, the most frequent and wide agricultural usage have also made a significant contribution to the ever increasing '**pool of antibiotic resistant bacteria**' in a community.

2. CLASSIFICATION

In this chapter the **antibiotics** will be discussed explicitely under the following *four* main heads, namely :

(*a*) β-Lactam antibiotics,
(*b*) Aminoglycoside Antibiotics,
(*c*) Chloramphenicol, and
(*d*) Tetracyclines.

These *four* types of antibiotics shall be treated in an elaborated manner in the pages that follows :

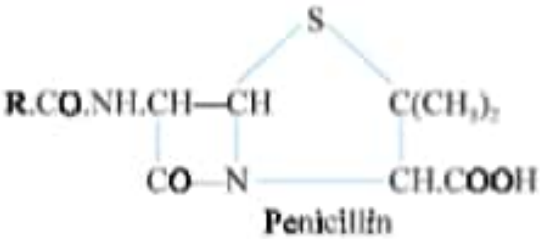
3. β-LACTAM ANTIBIOTICS

The **β-lactam antibiotics** may be further sub-divided into *two* categories, namely :

(*a*) Penicillins, and (*b*) Cephalosporins.

3.1. Penicillins

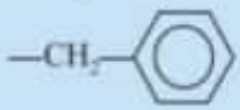
Penicillin is the name assigned to the mixture of natural compounds having the molecular formula $C_9H_{11}O_4N_2SR$, and differing only in the nature of 'R'.



Penicillin

These are mainly produced by various strains of *Penicillium notatum* and *Penicillium chrysogenum*. There are at least **six naturally occurring penicillins,** whose chemical names, other names and the nature of 'R' are given in the following table :

3.1.1. Naturally Occurring Penicillins

S. No.	Chemical Name	Other Names	—R
1	**Pent-2-enylpenicillin**	Penicillin-I or F	$-CH_2.CH=CH.CH_2CH_3$
2	**Benzylpenicillin**	Penicillin-II or G	

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3	***p*-Hydroxybenzyl-penicillin**	Penicillin-III or X	
4	***n*-Heptylpenicillin**	Penicillin-IV or K	$-(CH_2)_6.CH_3$
5	***n*-Amylpenicillin**	Dihydro-F-penicillin	$-(CH_2)_4.CH_3$
6	**Phenoxymethyl-penicillin**	Penicillin-V	$-CH_2-O-C_6H_5$

3.1.2. Structure of the Penicillins

Following are the various salient features which ultimately determine the general structure of all the **penicillins** :

1. The **penicillins** are all strong monobasic acids, *i.e.,* they form salts.

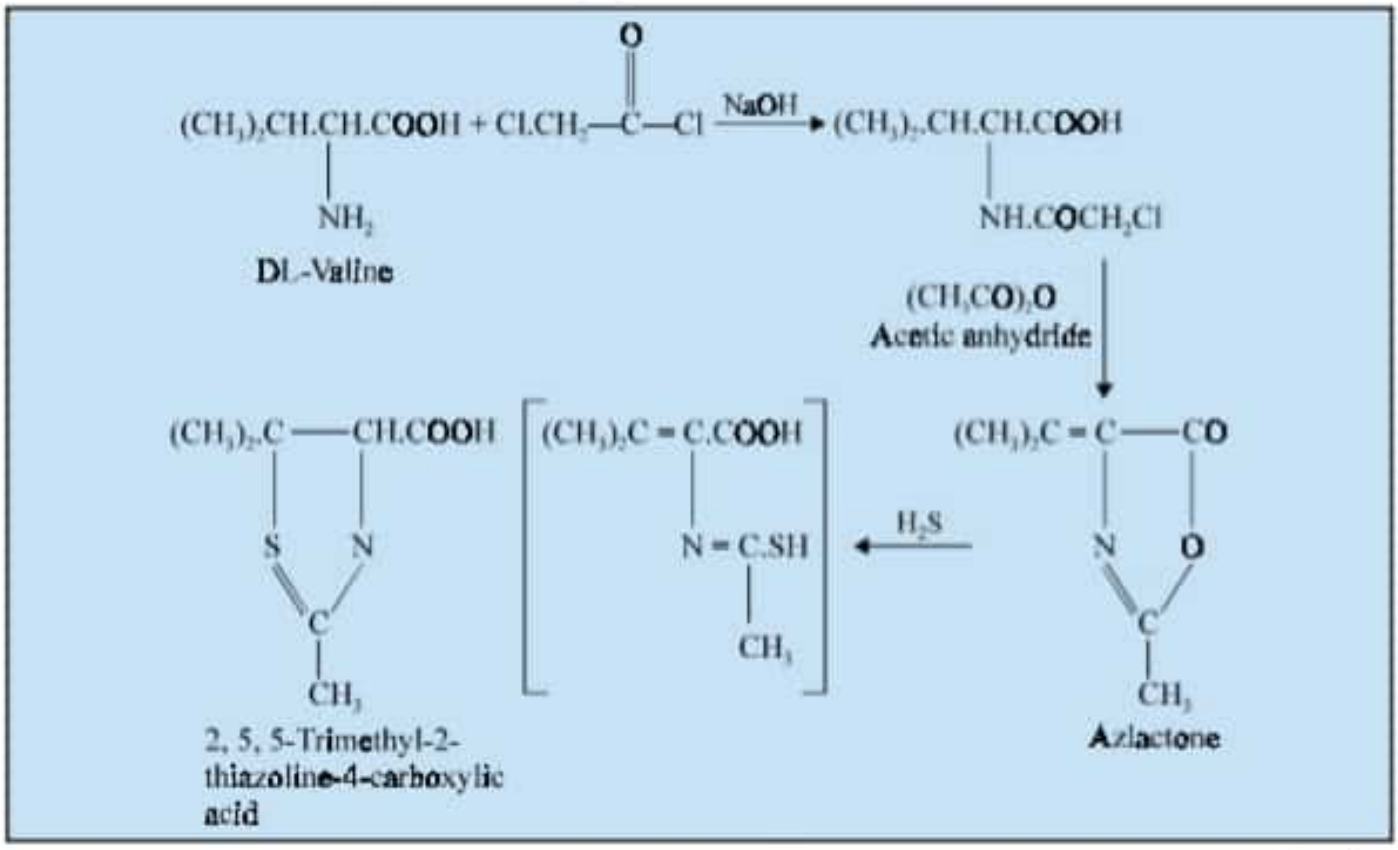
2. The **penicillins** are hydrolysed by hot dilute inorganic acids ; one carbon atom is eliminated as carbon dioxide (CO_2) and two products are obtained in equimolecular proportions, one being an **amine,** *Pencillamine* and the other an **aldehyde, Penniloaldehyde**.

$$C_9H_{11}O_4N_2SR + 2H_2O \xrightarrow{HCl} CO_2 + \underset{\text{Amine}}{C_5H_{11}O_2NS} + \underset{\text{Aldehyde}}{C_3H_4O_2NR}$$

All the **penicillins** give the same amine, but different aldehydes, because it bears the variable component 'R' in it.

3. ***D-Penicillamine* ($C_5H_{11}O_2NS$)**

Penicillamine instantly gives the indigo colour reaction with ferric chloride, a test characteristic of cysteine, thereby suggesting that the amine is probably a substituted cysteine. The structure of **penicillamine** was later on proved to be **D-β: β-dimethylcysteine** by synthesis as described below :



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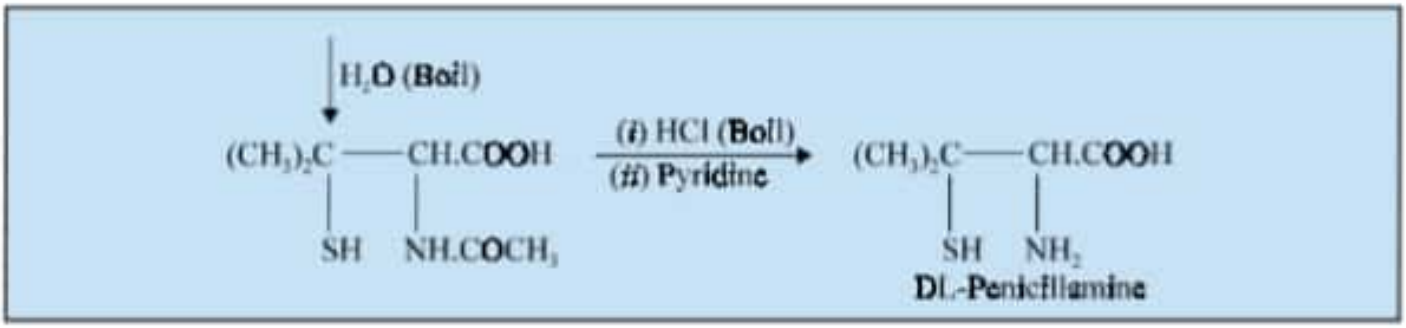
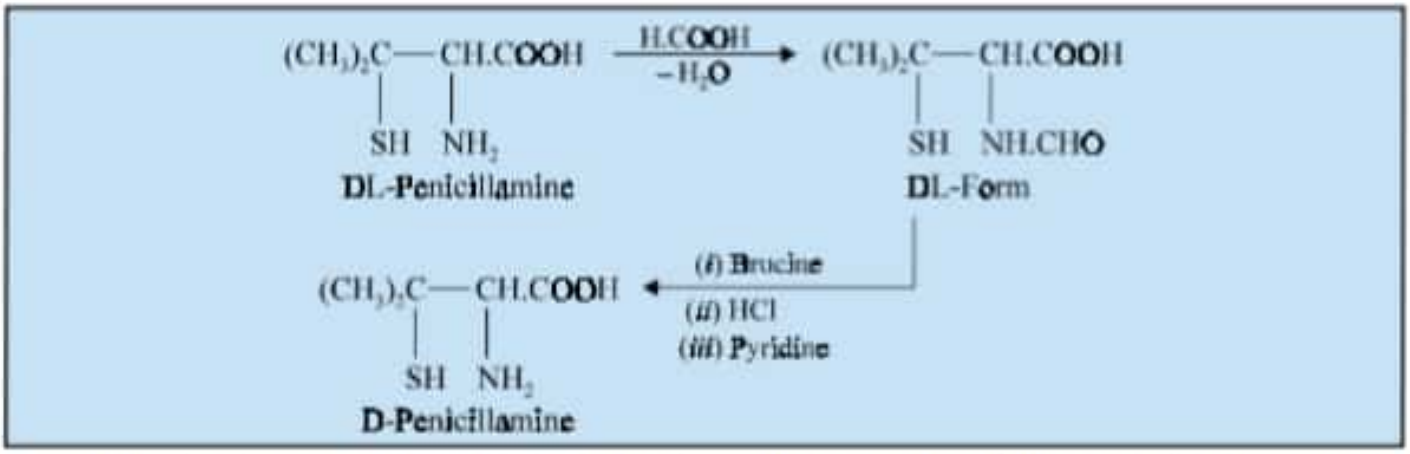


Resolution of the racemic amine

The resulting racemic mixture of **penicillamine** was *first* converted into the formyl derivative ; and *secondly* it was resolved by means of brucine and thirdly, the formyl group was removed by hydrolysis, thus :



D-Penicillamine was found to be identical with the **natural penicillamine**. When treated with diazomethane ($CH_2=N^+=N^-$), **penicillin** is converted into its methyl ester and this, on treatment with an aqueous solution of mercuric chloride, gives the methyl ester of **pencillamine**, thereby proving that the carboxyl group in penicillamine is the carboxyl group present in the **penicillin** moelcule itself.

4. *Penilloaldehyde*

It has been observed that on vigorous hydrolysis, all the **penilloaldehydes** give a substituted acetic acid and an **aminoacetaldehyde**. Hence, the **penilloaldehydes** may be considered as acylated derivatives of **aminoacetaldehyde**. Thus

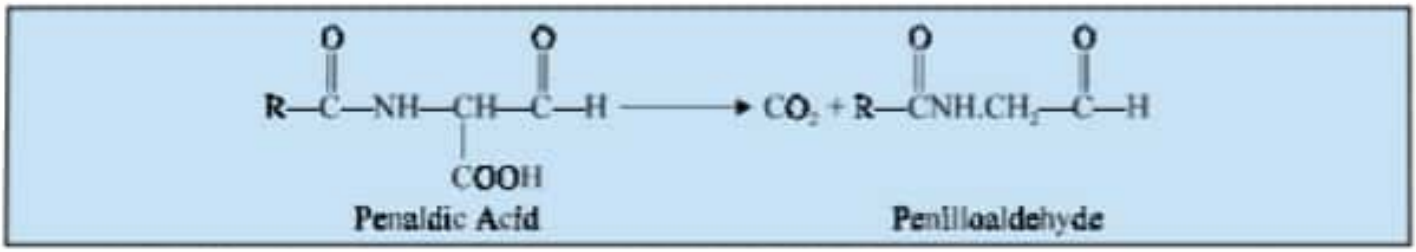
$$R.CO.NH.CH_2.CHO + H_2O \rightarrow R.COOH + H_2N.CH_2.CHO$$

This structure has been confirmed by synthesis :

5. *Carbon Dioxide (CO_2) Molecule*

As stated earlier the acid hydrolysis of penicillin yields three products only *viz.*, **penicillamine**, **penilloaldehyde** and **carbon dioxide**. The liberation of a molecule of **carbon dioxide** gave rise to the belief that it is formed by the ready decarboxylation of an unstable acid. Such an acid is a **β-keto acid**.

Hence, a possible explanation may be put forward that perhaps a **penilloaldehyde-carboxylic acid** (**penaldic acid**) is formed as an intermediate in the hydrolysis of **penicillin**, thus

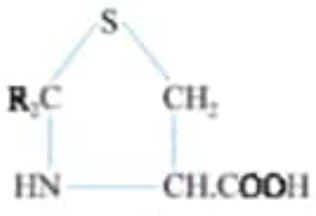


6. *Combination of Penicillamine and Penilloaldehyde in Penicillin*

It has been observed that the hydrolysis of **penicillin** with dilute alkali or with the enzyme (***penicillinase***) yields **penicilloic acid** (a dicarboxylic acid), which readily eliminates a molecule of carbon dioxide to form **penilloic acid**, thereby suggesting that a carboxyl group is present in the β-position with regard to a negative group.

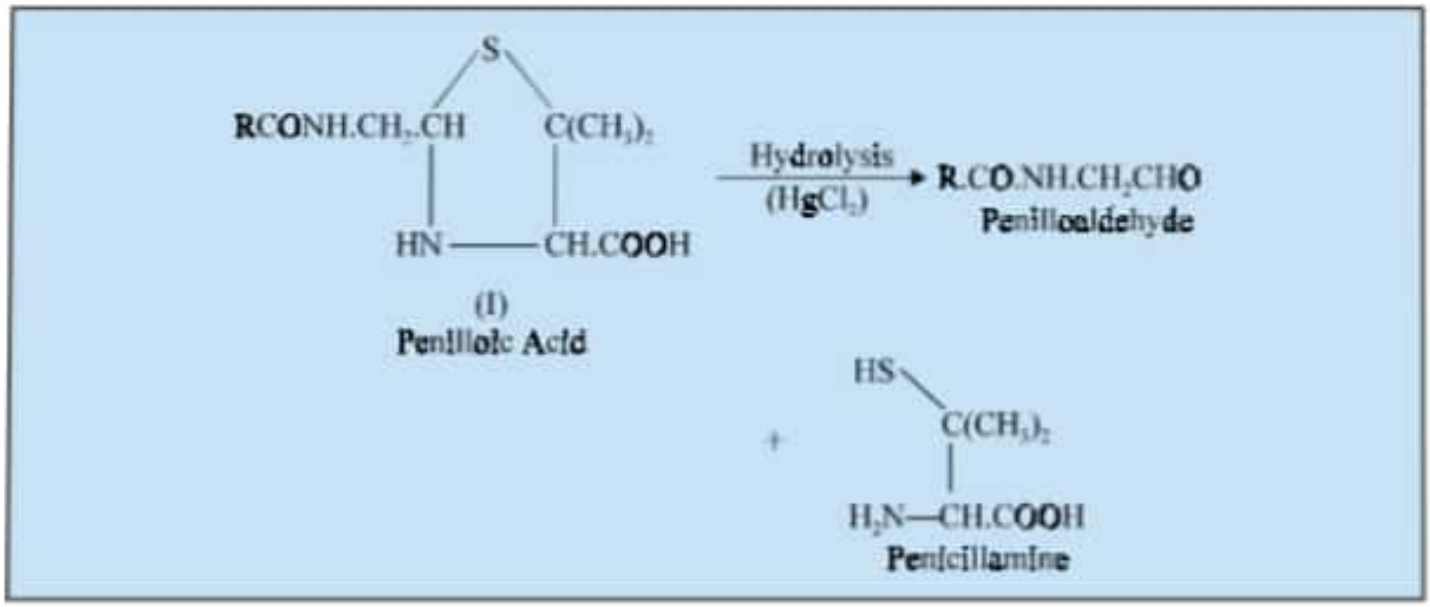
7. *Presence of Thiazolidine Ring*

It has been established experimentally that **penilloic acid** upon hydrolysis with aqueous mercuric chloride yields **penicillamine** and **penilloaldehyde** respectively. This type of hydrolysis is characteristic of compounds containing a **thiazolidine ring**, *i.e.*,

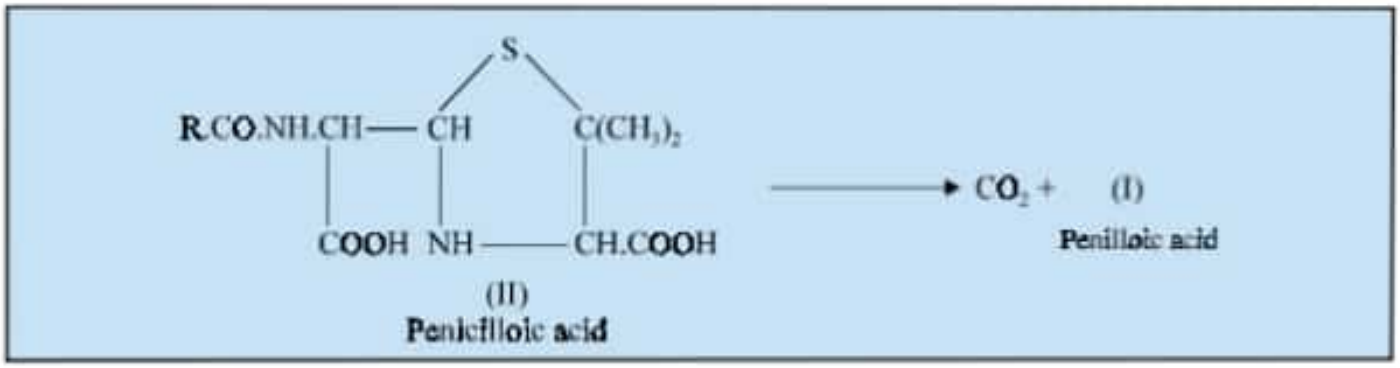


Thiazolidine

Hence, **penilloic acid** could be (I), because this particular structure would give the above required products. Thus

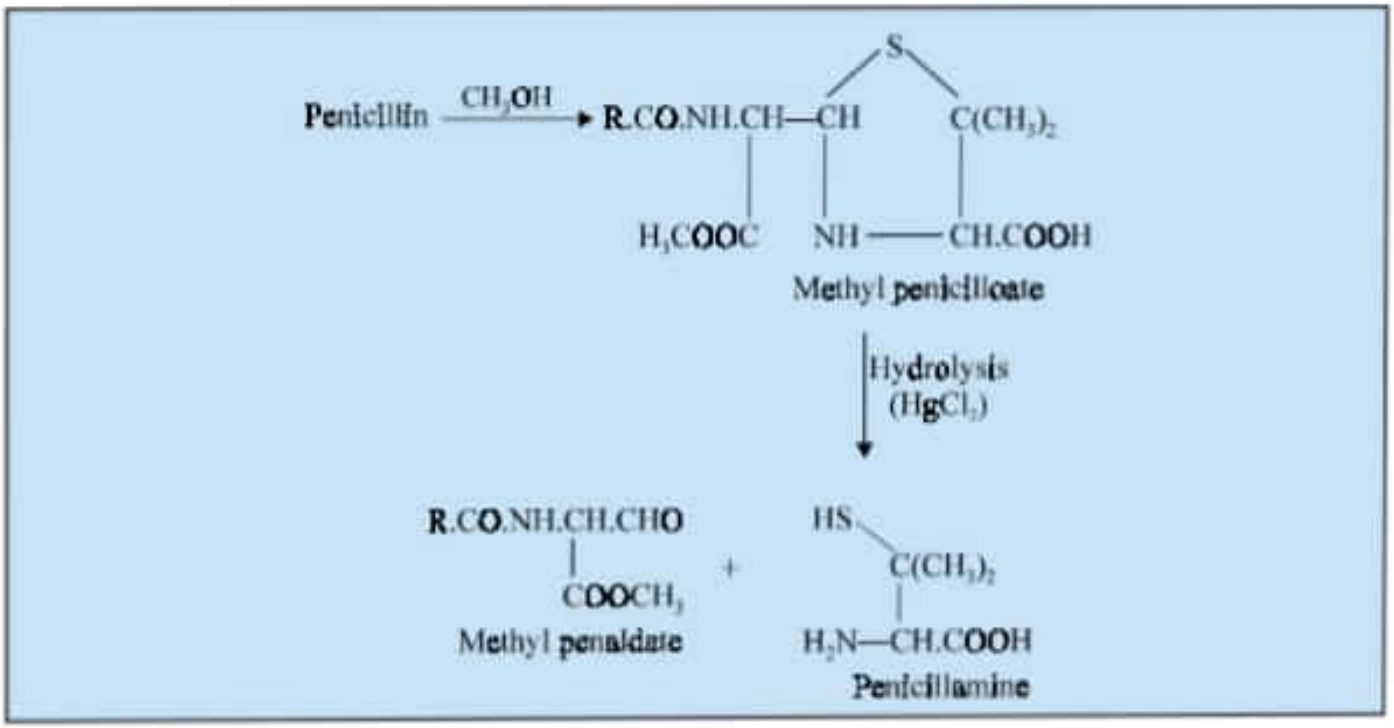


Therefore, if (I) is **penilloic acid**, then **penicilloic acid** would be (II)



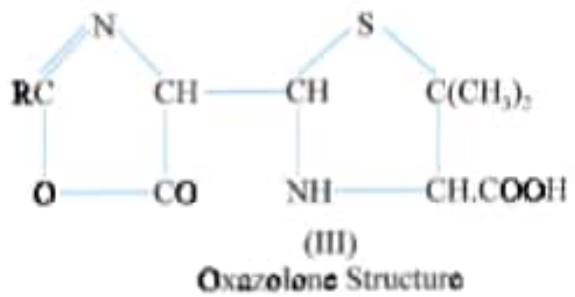
8. ***Evidence for Structure (II)***

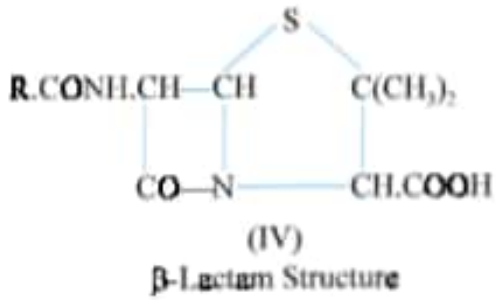
The treatment of **penicillin** with methanol yields the corresponding **ester methyl penicilloate** which, on hydrolysis with aqueous mercuric chloride, gives **methyl penaldate** and **penicillamine**. Thus



9. ***Probable Structures for Penicillin***

Based on the foregoing chemical evidences two probable structures for **penicillin** have been put forward *viz* ; (III) and (IV) :





At this juncture, however, it was not quite possible to decide between the two structures (III) and (IV) on the ground of chemical evidence alone, since **penicillin** is prone to undergo abrupt molecular rearrangement, *e.g.*, on treatment with dilute acid, **penicillin** rearranges to **penillic acid**.

Therefore, it was absolutely necessary to examine the molecule by physical methods (thereby leaving the molecule intact). In fact, an intensive study of the **penicillins** was carried out with respect to their infra-red and X-ray diffraction analysis.

(a) Infra-Red Analysis

The infra-red (IR) spectra of many **penicillins** were examined and therefrom a correlation between various bands and functional groups was established by examining the spectra of synthetic model compounds which contained different portions of structures (III) and (IV) that had been proposed above on the basis of chemical evidence.

This fact may be further illustrated by taking into consideration the methyl ester as well as the **sodium salt of benzyl penicillin** that exhibited the following characteristic peaks of all the **penicillins** in these regions :

Penicillin (as)	Characteristic Peaks (cm^{-1})
Methyl Ester :	3333, 1770, 1748, 1684, 1506
Sodium Salt :	3333, 1770, 1613, 1681, 1515

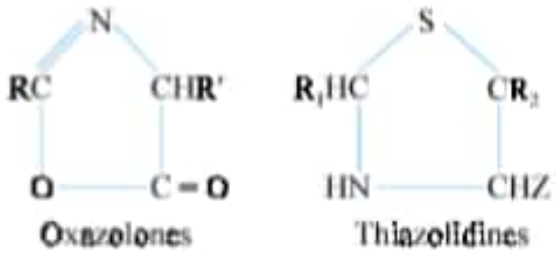
The band at 3333 cm^{-1} in both compounds was due to the NH group (str.)

The 1748 cm^{-1} band of methyl ester and the 1613 cm^{-1} band of the corresponding salt were assigned to the carbonyl group (str.) in the carboxyl group (as ester or salt respectively).

Further, model **oxazolones** were studied that showed two characteristic bands ; one at 1825 cm^{-1} for the carbonyl group and the other at 1675 cm^{-1} for the C = N moiety.

However, the absence of the first band but possible presence of the second in the benzylpenicillin derivatives would not allow an ultimate decision to be reached between (III) and (IV).

On specifically examining a large number of **thiazolidines** in the double bond region down to 1470 cm^{-1}, only the carbonyl bond was revealed to be present (~ 1748 and 1613 cm^{-1}).



Oxazolones Thiazolidines

[Z = – COOCH$_3$; – COO$^{\ominus}$ etc.]

A number of 1°, 2° and 3° amides were studied extensively. It was found that all the three types showed a characteristic band close to 1670 cm^{-1} which may be attributed to the carbonyl group ; but in the case of the primary amides there was an additional band at 1613 cm^{-1} and with the secondary amides the band was found to be close to 1515 cm^{-1}. These findings reveal that the penicillins possess the secondary amide structure (IV), because the secondary amide band at 1670 cm^{-1} was almost equal to 1684 and 1681 cm^{-1}, besides the band at 1515 cm^{-1} was equivalent to 1506 and 1515 cm^{-1}. Thus, in all, four out of five bands have been accounted for duly.

Finally, a large number of **β-lactams** and fused **thiazolidine-β-lactams** were studied intensively. The former category of compounds did not display a band near 1770 cm^{-1}, but all the latter were found

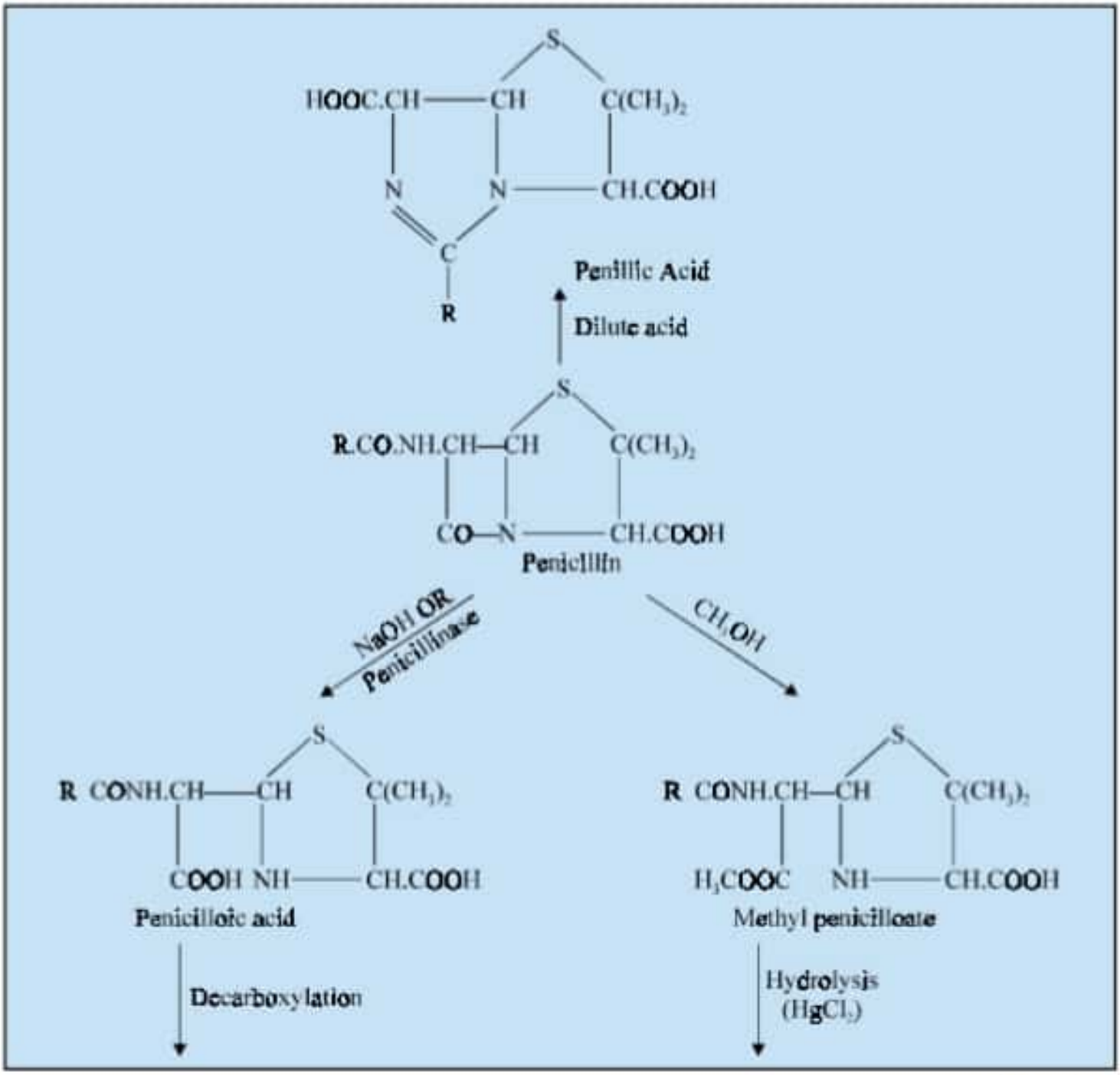
to exhibit a band at 1770 cm^{-1}. This ultimately accounts for the fifth band, and hecne it follows that (IV) is the structure of the **penicillins**.

(b) X-Ray Diffraction Analysis

The **X-ray diffraction analysis** of the sodium, potassium and the rubidium salts of the benzylpenicillin showed the presence of a **β-lactam ring**, thereby further supporting the fact that structure (IV) is the **probable structure of penicillin**.

Based on this structure (IV) and also the various chemical reactions studied so far, one may summarise them as described below :

3.1.3. Chemical Reactions of the Penicillins



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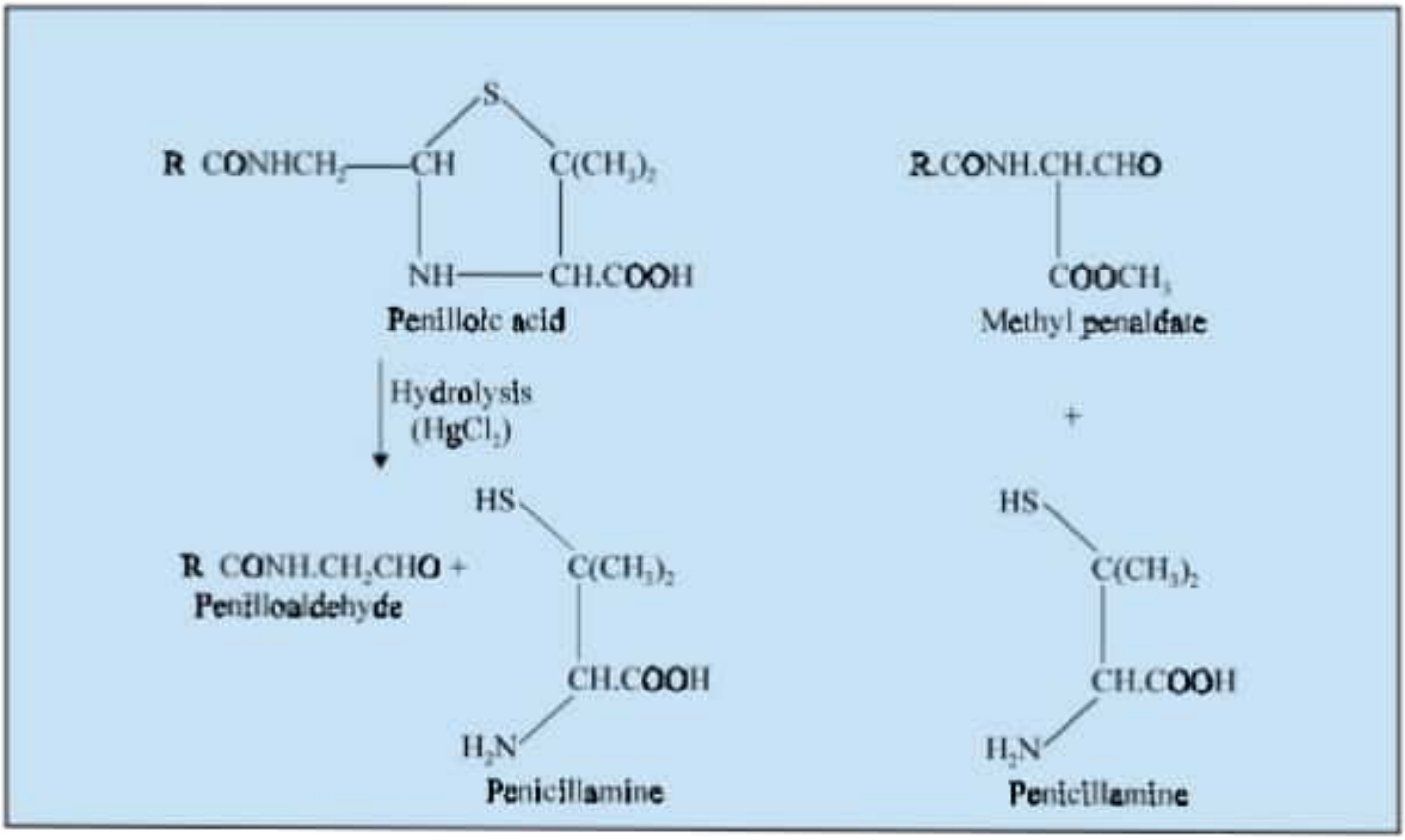


3.1.4. The Penicillin Variants

The advent of latest developments in '**medicinal chemistry**', in fact, put forward the following *five* **penicillin variants**, namely :

(*a*) Natural Penicillins (best streptococcal and narrow spectrum)

(*b*) Penicillinase-resistant Penicilins (antistaphylococcal)

(*c*) Aminopenicillins (improved Gram – ve : *H-influenzae, Enterococcus, Shigella, Salmonella*),

(*d*) Extended-spectrum (antipseudomonal) penicillins, and

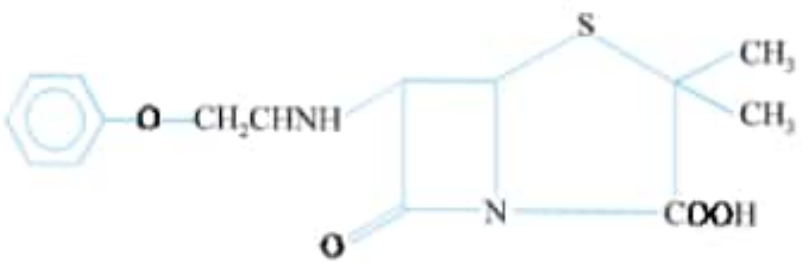
(*e*) β-Lactamase combinations (expand spectrum to staph, β-lactamase producers).

In this particular section a few typical examples from each category of **penicillin variants** shall be discussed comprehensively.

3.1.4.1. Natural Penicillins (best streptococcal and narrow spectrum)

The first successful synthesis of **penicillin** was carried out by Sheehan *et al.* (1957, 1959) who synthesized **Penicillin-V** (*i.e.*, **phenoxymethyl-penicillin**).

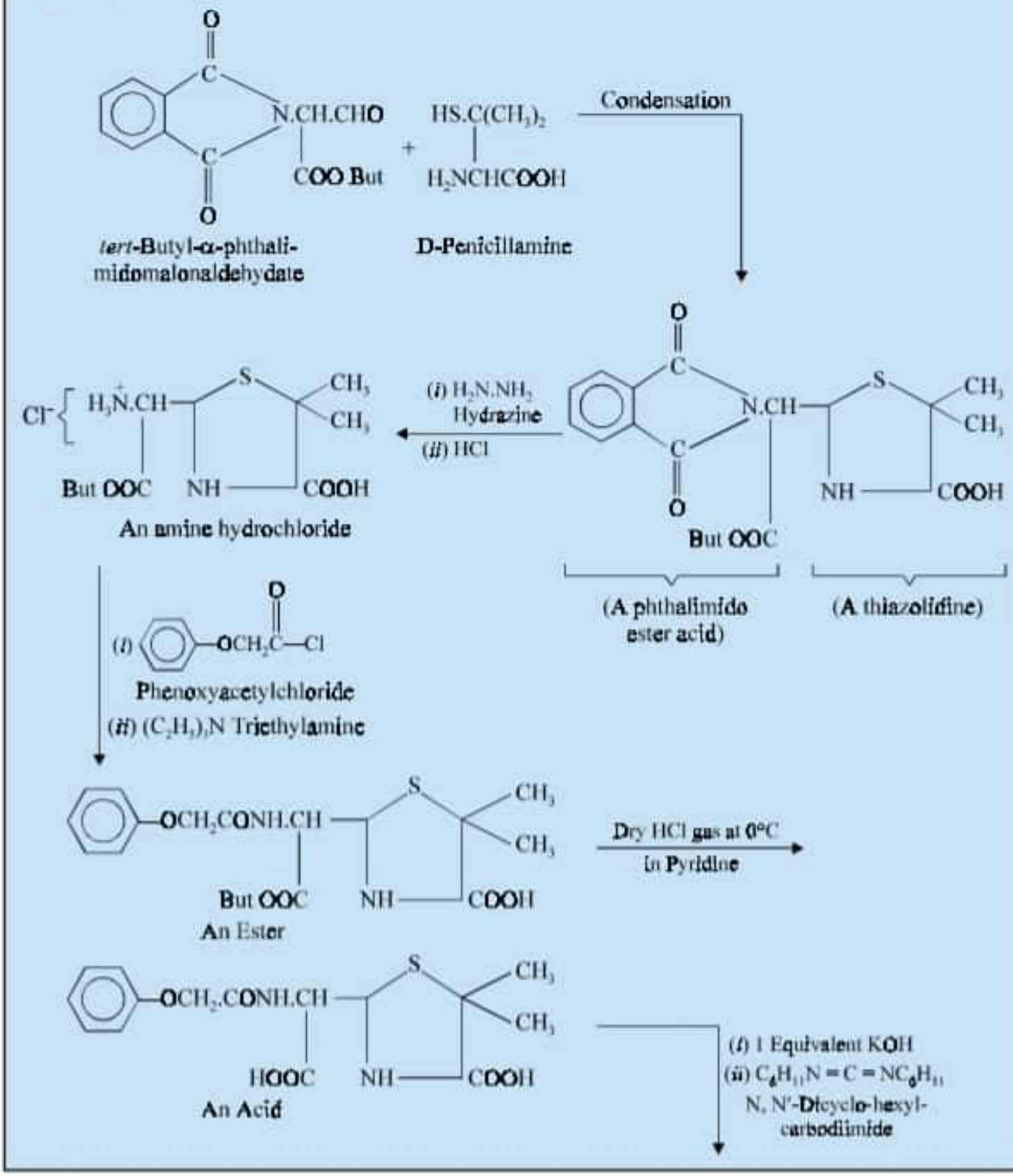
A. *Phenoxymethylpenicillin* INN, BAN *Penicillin*-V USAN,



(6R)-6-2-(-2-Phenoxyacetamido) pencillanic acid ; 4-Thia-1-azabicyclol [3,2,0]-heptane-2-carboxylic acid, 3, 3-dimethyl-7-oxo-6- [(phenoxyacetyl) amino]-, [2S-(2α, 5α, 6β)]- ; Phenoxymethylpenicillin (BP ; Eur. P ; Int. P.,) ; Penicillin-V (USP) ;

V-Cillin$^{(R)}$ (Lilly).

Synthesis



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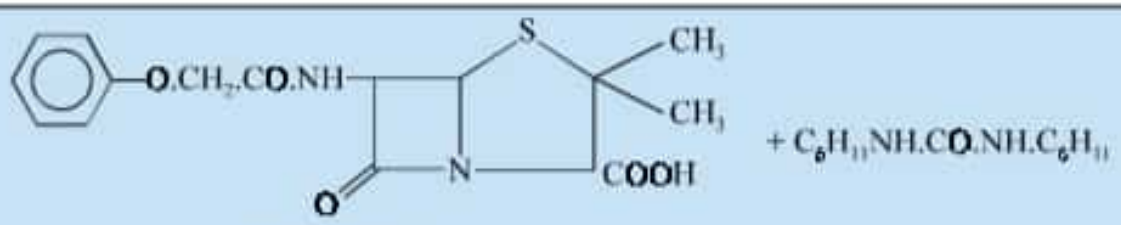


Penicillin-V

Condensation of **D-Penicillamine** and ***tert*-butyl-α-phthalimidomalonaldehydate** yields an intermediate embedded with a phthalimido ester acid and a thiazolidine ring. Treatment of this with hydrazine followed by hydrochloric acid helps the removal of phthaloyl moiety as phthalhydrazide and gives an amine hydrochloride. The resulting product on further treatment with phenoxyacetylchloride in triethylamine introduces the side chain to yield an ester. This product when subjected to a stream of hydrogen chloride gas at 0°C in pyridine helps to remove the blocking *tert*-butyl function thereby yielding **penicilloic acid**. The resulting acid undergoes cyclization to afford **penicillin-V** by stirring for 20 minutes with a solution of N, N′-dicyclohexyl carbodiimide in dioxane.

Penicillin-V is paritcularly effective in the management and control of infections caused by gram-positive bacteria, namely ; streptococcal, staphylococcal, pneumococcal and clostridial infections. It is also the drug of choice in the treatment of a number of gram-positive bacteria *viz.*, gonococcal and meningococcal infections. Besides, it is also used exclusively in the *treatment of pneumonia and other respiratory tract infections caused by Staphylococcus aureus, B. anthracis and Streptomyces pyrogenes. It is now solely used in the treatment of both syphillis and gonorrhea.*

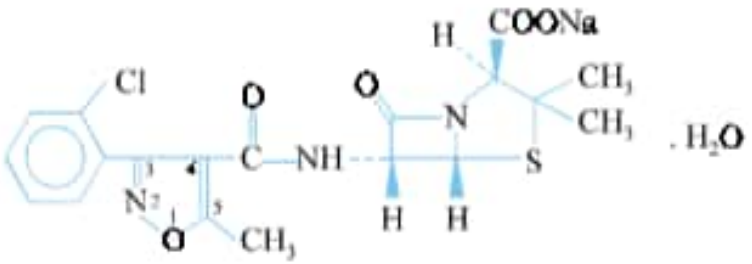
Dose. *Oral, adults and children over 12 years of age or older, usually 125 to 500 mg 3 to 4 times a day.*

Mechanism of Action. The mechanism of action of **penicillin-V** shall be discussed as under : The '**drug**' gets inactivated to a relatively lesser extent by gastric juice in comparison to penicillin G. It is, however, **the *most preferred oral penicillin* for *less serious infections*** due to the fact that serum-levels are found to be 2-5 times higher than matching doses of penicillin G ; besides, there exists relatively less variability in its degree of absorption. The oral bioavailability is about 60% at the most. It gets bound to plasma proteins between 75-80%. The volume of distribution v_d^{ss} stands at 0.73 mL. g^{-1}, which is significantly much higher than that of **penicillin G**. The '**drug**' gets excreted unchanged in the urine between 20-40% . The plasma half-life is nearly 0.5 to 1. hour.

3.1.4.2. Penicillinase-resistant Penicillins (antistaphylococcal)

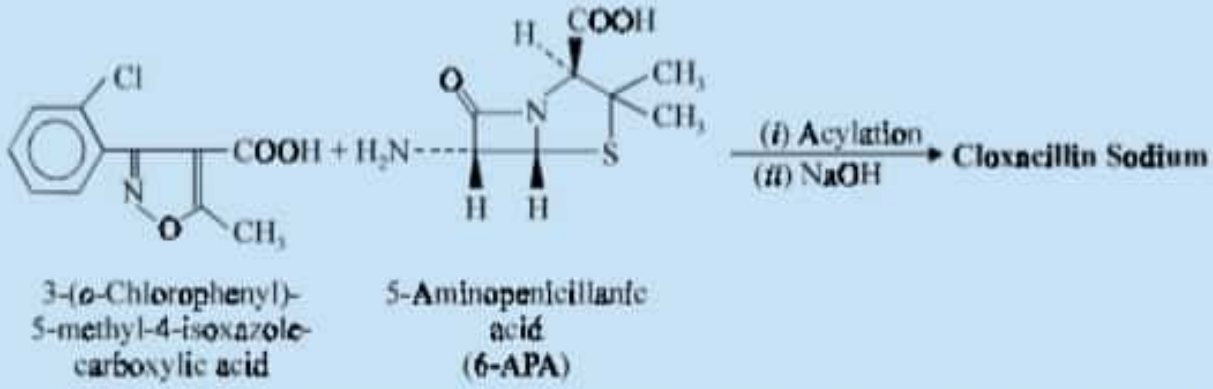
A few typical examples belonging to this class of penicillins are, namely : **cloxacillin**, **methicillin**, **oxacillin** etc., which would be treated individually in the sections that follows :

3.1.4.2.1. Cloxacillin Sodium USAN, INN



[2S-(2α, 5α, 6β)-4-Thia-1-azabicyclo [3, 2, 0] heptane-2-carboxylic acid 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl] carbonyl] amino]- 3, 3-dimethyl-7-oxo-, monosodium salt, monohydrate ; Tegopen(R); Cloxapen(R); USP ;

Synthesis



3-(*o*-Chlorophenyl)-5-methyl-4-isoxazole-carboxylic acid

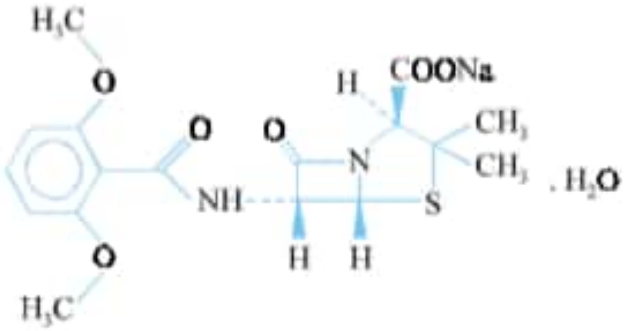
5-Aminopenicillanic acid (6-APA)

6-APA is duly acylated with 3-(*o*-chlorophenyl)-5-methyl-4-isoxazole carboxylic acid and the resulting cloxacillin base is adequately purified by recrystallization. The base thus obtained is converted to the corresponding sodium salt by treating with an equimolar concentration of NaOH.

It is a pencillinase-resistant penicillin (antistaphylococcal) usually administered orally.

Potency : Equivalent of not less than 825 mcg of cloxacillin per mg.

3.1.4.2.2. Methicillin Sodium, USAN, INN



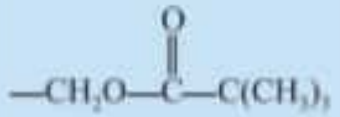
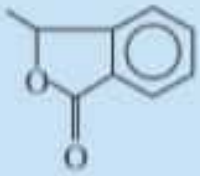
4-Thia-1-azabicyclo [3, 2, 0] heptane-2-carboxylic acid, 6-[(2, 6-dimethoxybenzoyl) amino]-3, 3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2S-(2α, 5α, 6β)]- ; USP ;

Staphcillin(R) ;

The official compound may be prepared by condensing the fermentation-produced 6-APA in an appropriate solvent with 2, 6-dimethoxybenzoyl chloride ; and the resulting methicillin is subsequently precipitated as its corresponding sodium salt by the addition of sodium acetate.

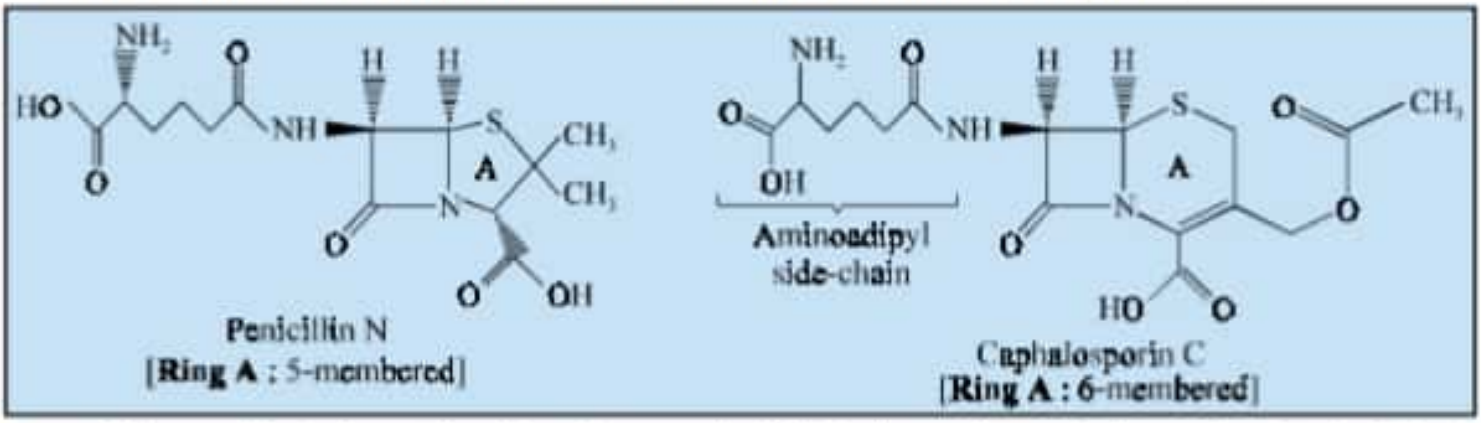
It is usually indicated in the treatment of staphylococcal infections caused by strains resistant to other penicillins. As a precautionary note it is recommended that this '**drug**' should **not** be used in general treatment and therapy so as to avoid the possibility of widespread development of organisms resistant to it.

Mechansim of Action. The '**drug**' is specifically resistant to inactivation by the presence of the enzyme **penicillinase** found in staphylococci. It has been observed to induce penicillinase formation which specifically restrains its usage in the control, management and treatment of **penicillin G-sensitive infections**.

Name	R	Official Status	Brand Name(s)	Dose
Pivampicillin Hydrochloride		—	Pondocillin® (Burgers, U.K.) ;	500 mg 3 to 4 times daily
Talampicillin Napsylate		—	Talpen® (Beecham Research, U.K.)	250 to 500 mg 3 times daily

3.2. Cephalosporins

After the spectacular world-wide recognition and tremendous success of the **penicillins**, the best known family of **β-lactams** are termed as the **cephalosporins**, wherein the **β-lactam ring** is strategically fused to a **6-membered dihydrothiazine ring system** as shown below : Caphalosporin C



Giuseppe Brotzu's epoch making discovery, in 1945, in the species **cephalosporium** fungi obtained from *C. acremonium* showed a remarkable inhibition in the growth of a rather wide spectrum of both Gram-positive and Gram-negative organisms. Abraham and Newton (1961) at Oxford for the first time not only isolated successfully but also characterized **cephalosporin C**.* However, the confirmation of its structure was ascertained by X-ray crystallography.**

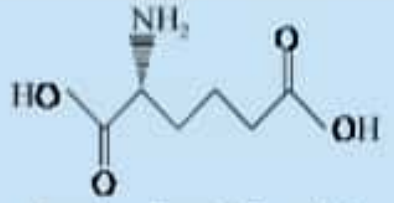
Inspite of the glaring evidence that **cephalosporin C** was resistant to *S. aureus* β-lactamase, besides its prevailing antibacterial activity was inferior in comparison to **penicillin N** and other penicillin structural analogues.

It has been observed critically that the **natural products** usually exhibit a relatively **lower level of antibacterial activity**. Therefore, the articulate and judicial '*cleavage*' of the amide bond of the **aminoadipyl side-chain** present in **cephalosporin C** provides **7-amino-cephalosporanic acid (7-ACA),** which is most ideally suitable for the synthesis of a wide range of semisynthetic cephalosporins *via* acylation of the C(7)-amino functional moiety*** as depicted under :

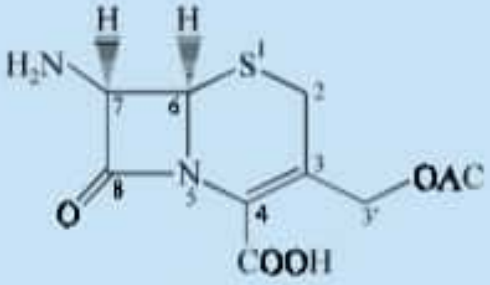
*EP Abraham and GGF Newton, *Biochem J.*, **79**, 377, (1961)

DC Hodgkin and EN Masien, *Biochem. J.*, **79, 393, (1961).

***Fechtig B *et al.*, *Helv. Chim Acta.* **51,** 1108, 1968.



Aminoadipyl Side-chain
(Present in Cephalosporin C)



7-ACA

3.2.1. Classification

The '**cephalosporins**' may be classified under the following *four* categories : Aminoadipyl

(*a*) First generation (staph, some enteric Gram-negative, bacilli)

(*b*) Second generation (more active *Vs* Gram-negative, some active *Vs H. influenzae* and anaerobes)

(*c*) Third generation (best Gram-negative spectrum, β-lactamase resistant, poor *Vs* staph.)

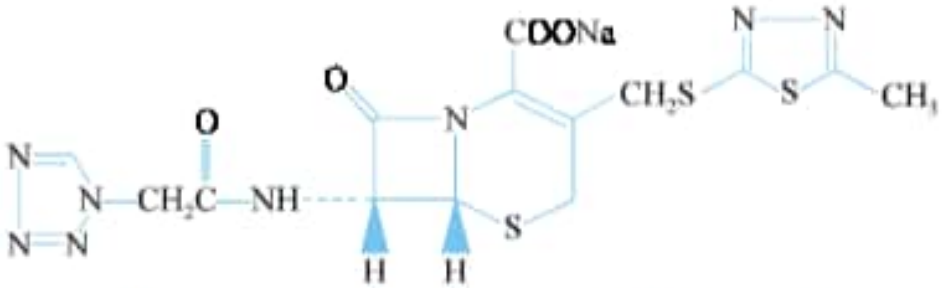
(*d*) Fourth generation.

A few typical examples of '**cephalosporins**' belonging to each of the above *four* generation shall now be discussed more explicitely in the sections that follows :

3.2.1.1. First generation cephalosporins

The following *three* drugs belonging to this class of compounds shall be treated in an elaborated manner, namely : **cefazolin**, **cephalexin**, and **cephradine**.

3.2.1.1.1. Cefazolin Sodium USAN



5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, 3-[[(5-methyl-(6R-*trans*)-1,3,4-thiadiazol-2-yl) thio] methyl]-8-oxo-7-[[(1H-tetrazol-1 yl) acetyl]-amino]-, monosodium salt, USP ;

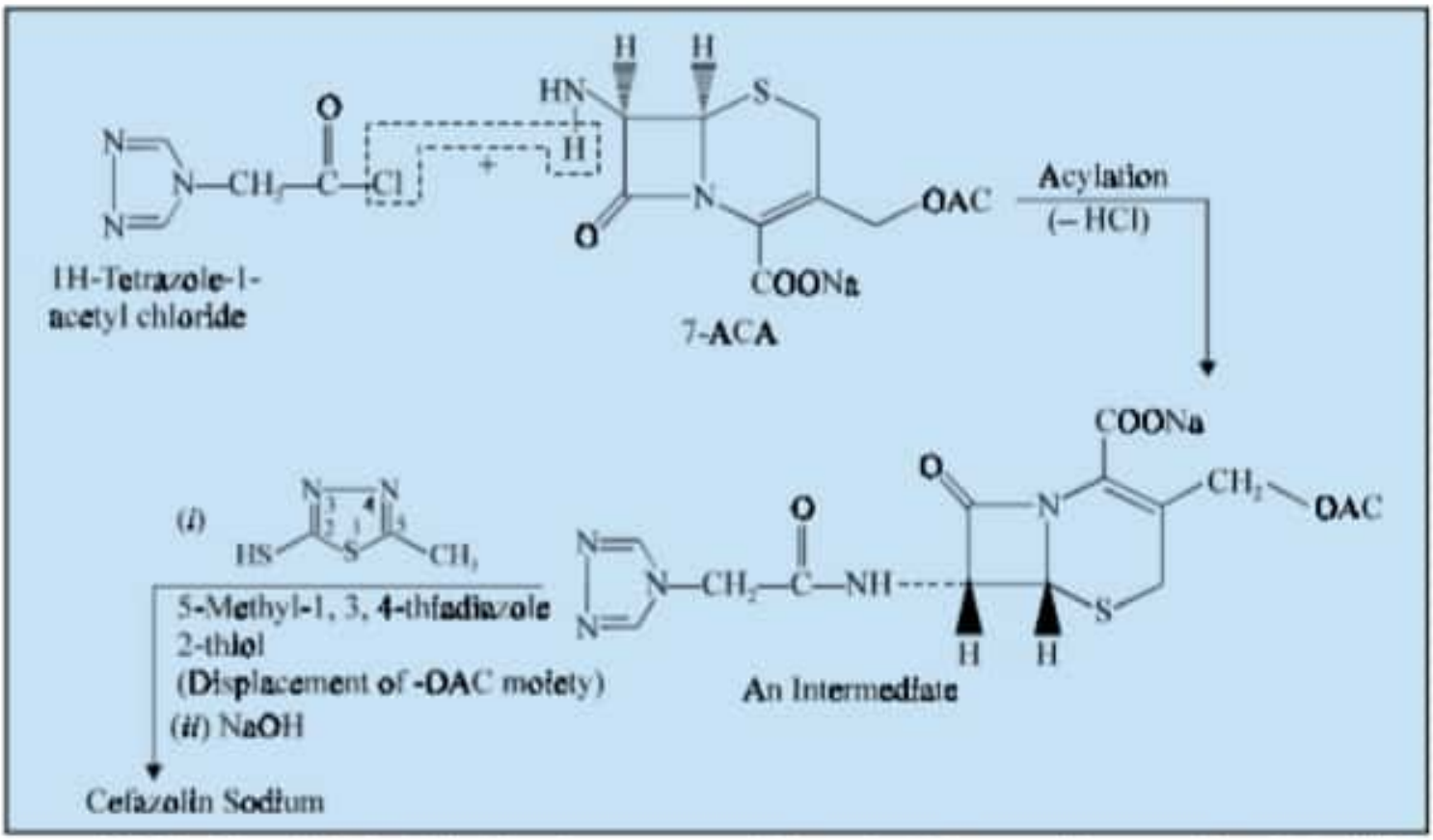
Ancef$^{(R)}$; Kefzol$^{(R)}$;

Synthesis

The acylation of the sodium salt of **7-aminocephalosporanic acid** (*i.e.*, **7-ACA**) with 1H-tetrazole-1-acetyl chloride gives rise to the formation of an intermediate with the elimination of a mole of HCl. The resulting product on being treated with 5-methyl-1, 3, 4-thiadiazole-2-thiol affords the displacement of the acetoxy moiety which upon treatment with an equimolar concentration of NaOH yields the official compound.

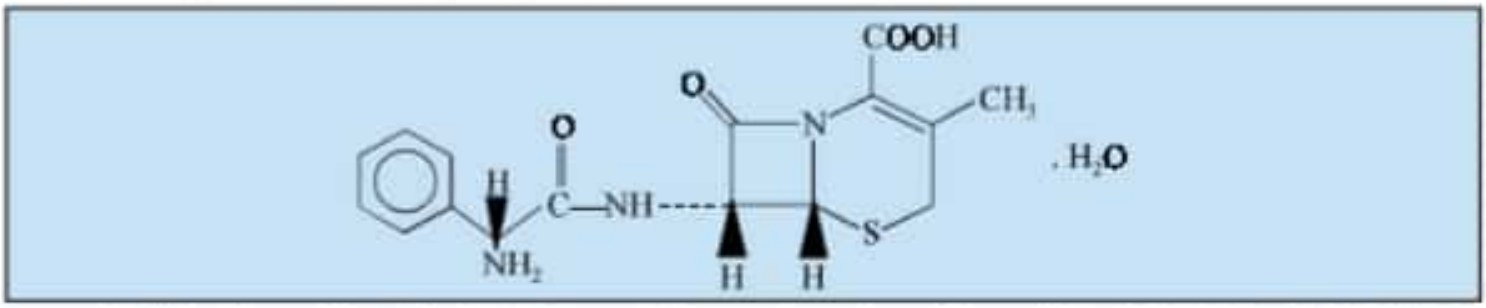
It is a **first-generation cephalosporins** given IM or IV. The '**drug**' may be employed to treat infections of the skin, bone, soft tissues, respiratory tract, urinary tract, and endocarditis and septicemia caused by susceptible organisms. It has been observed that amongst UTIs, cystitis responds much predominantly and better in comparison to *pyelonephritis*.* It is regarded to be the preferred cephalosporin for most surgical prophylaxis due to its inherent long half-life.

*Inflammation of kidney and renal pelvis.



Mechanism of Action. The '**drug**' possesses activity against Gram-positive organism, but exhibits a relatively narrow spectrum against Gram-negative strains due in part to their susceptibility to the β-lactamases. However, the Gram-negative activity essentially confined to *E. coli, Klebsiella* and *Pr mirabilis.* It has also been observed that certain *Gram-negative organisms* and *penicillinase-producing staphylococci* which are resistant to both **penicillin G** and **ampicillin** are evidently sensitive to **cefazolin**.

3.2.1.1.2. Cephalexin USAN, INN, BAN



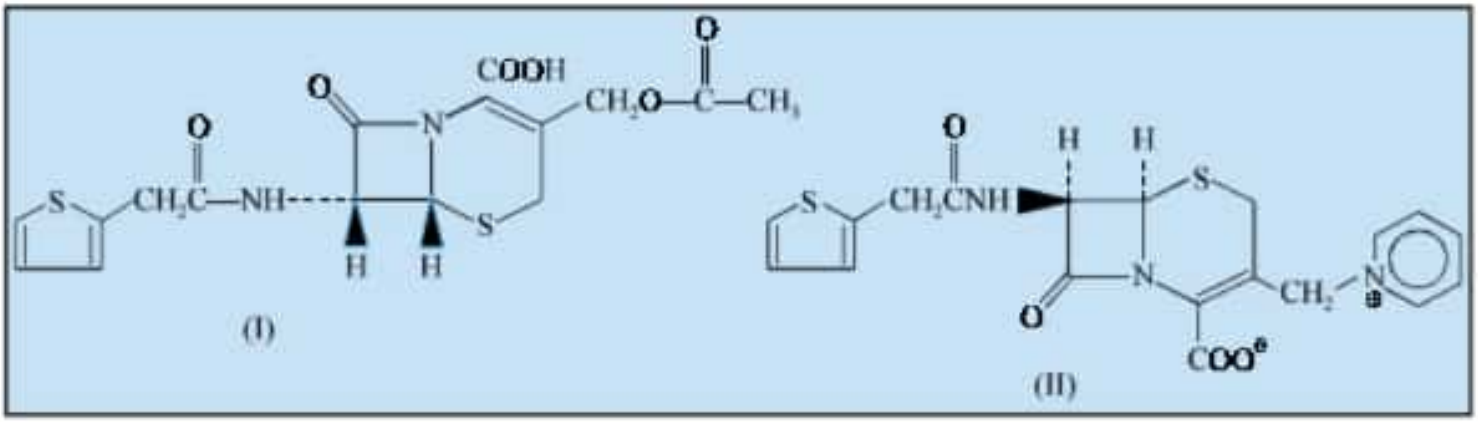
5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R-(6α,7β (R*)]]-7-[(aminophenylacetyl) amino]-3-methyl-8-oxo-, monohydrate ;

Keflex$^{(R)}$;

It is approved and recommended for use against respiratory infections caused by pneumococcus together with β-hemolytic streptococci ; otitis media by *H. influenzae, Branhamella catarrhalis,* pneumococcus, staphylococci ; skin and soft tissue infections by staphylococci and streptococci ; bone and joint infections by *Pr mirabilis* and staphylococci ; and above all the UTIs produced by *E. coli, Klebsiella* and *Pr mirabilis.*

Mechanism of Action. The '**drug**' has been specifically designed as an **orally active semisynthetic cephalosporin**. Importantly, there are *two* vital reasons that are solely responsible for the oral inactivation of cephalosporins, namely :

(*a*) **β-Lactam ring's instability to acid hydrolysis**, such as : **cephalothin** (I) and **cephaloridine** (II) :

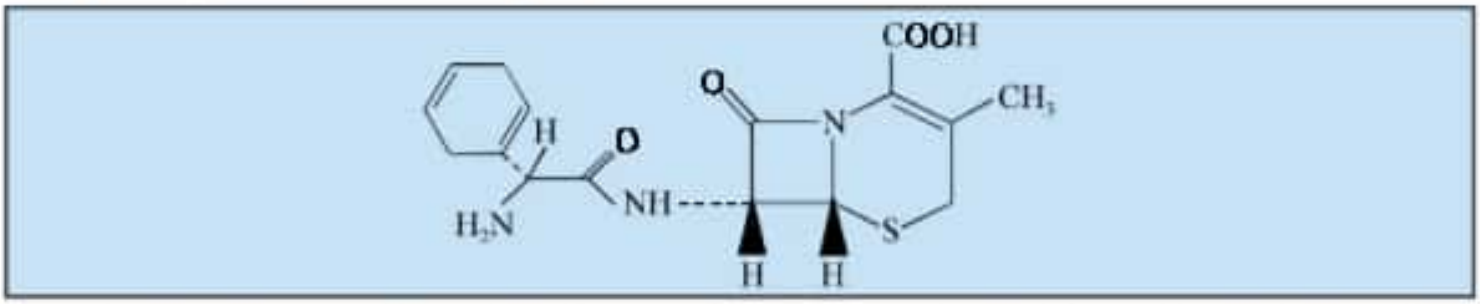


(*b*) **Solvolysis or microbial transformation of the 3-methylacetoxy moiety**, for instance : **cephalothin** and **cephaloglycin.**

The presence of α-amino moiety of **cephalexin** makes the drug '**acid stable**', whereas the reduction of the 3-acetoxymethyl to the methyl function helps in a big way to circumvent profusely the reaction taking place at the specific desired site.

Note. The '*drug*' is significantly much less potent than cephalothin and cephaloridine ; and, hence, is grossly inferior to both of them for the treatment of systemic infections of a vary serious nature.

3.2.1.1.3. Ceptradine USAN BAN, INN



5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R-[6α, 7β-(R*)]]-[(amino-1,4-cyclohexadien-1-ylacetyl] amino]-3-methyl-8-oxo-; USP ;

Anspor(R) ; Velosef(R) ;

A is recognized as a short-acting first-generation **cephalosporin** administered IM or IV. Recommended usually for the treatment of UTIs and respiratory tract infections.

Mechanism of Action. The '**drug**' is minimally protein bound and gets excreted almost 100% after oral administration *via* the kidneys. It is, however, found to be fairly stable in an acidic media (*e.g.*, gastric juice).

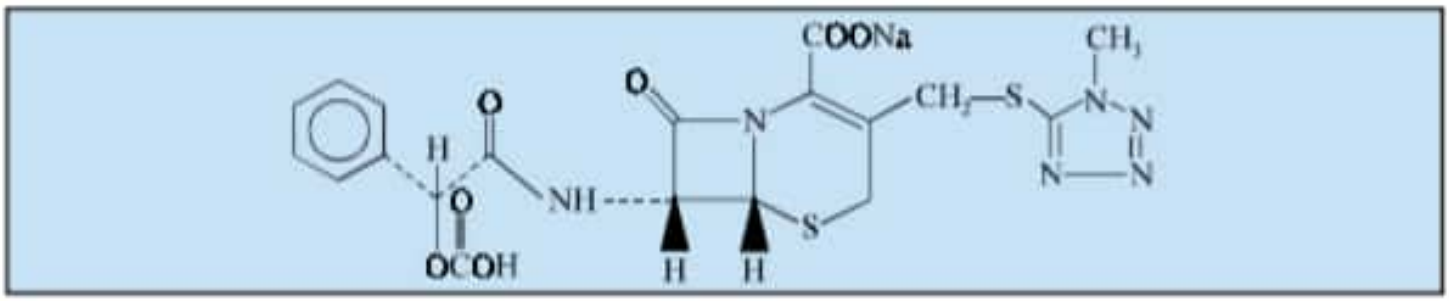
SAR of Cephradine. The '**drug**' has a close resemblance to '**cephalexin molecule**' chemically ; and the former may be viewed as a partially hydrogenated derivative of the latter. Therefore, perhaps cephradine possesses quite similar antibacterial as well as pharmacokinetic characteristics.

Note. The secondary pharmaceutical products (*i.e.*, dosage forms) contain usually a non-stoichiometric hydrate essentially containing upto 16% water ; and, therefore, all such products must indicate explicitely by the labeling on the package itself.

3.2.1.2. Second Generation Cephalosporins

In this particular class of compounds the following typical examples shall be treated individually in delails *e.g.*, **cefamandole**, **cefoxitin**, and **cefuroxime**.

3.2.1.2.1. Cefamandole Nafate USAN, INN



5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R- [6α, 7β(R*)]]-7-[[(formyloxy) phenylacetyl] amino]-3-[[(1-methyl 1H-tetrazol-5-yl)-thio]methyl]-8-oxo-, monosodium salt ; Mandol$^{(R)}$;

It is a short-acting **second-generation cephalosporins** normally administered IM or IV. It exhibits a broader spectrum of activity with an increased activity against *Haemophilus influenzae*, besides the *enterobacteriaceae* produced as a result from a overwhelmingly enhanced stability to the **β-lactamases**.

SAR of Cefamandole. The various salient features are :

(*i*) A formate ester of **cefamandole**, a semisynthetic **cephalosporin** which essentially inducts D-mandelic acid as the '**acyl portion**' ; and a sulphur-containing heterocycle (*e.g.*, 5-thio-1,2,3,4-tetrazole) instead of the acetoxy moiety positioned on the C-3 methylene C-atom.

(*ii*) Esterification of the α-hydroxyl function of the D-mandeloyl moiety eventually circumvents the instability function of this '***drug***' particularly in solid-state dosage forms.* This important salient feature caters for the satisfactory concentrations of the **parent antibiotic** *in vivo via* spontaneous hydrolysis of the prevailing ester between a neutral to alkaline pH range.

(*iii*) D-Mandeloyl functional group present in this '**drug**' seems to afford noticeable resistance to a few **β-lactamases**, by virtue of the fact that **certain β-lactamase-producing Gram-negative organisms** (specifically **Enterobacteriaceae**) which display obvious resistance of **cefazolin** and other **first generation cephalosporins** (see Section 3.2.1.1.) are found to be sensitive to **cefamandole**,

(*iv*) Besides, the '**drug**' is also active against a few **ampicillin-resistant strains** of *Neisseria* and *Haemophillus* species ; and

(*v*) Permeability and intrinsic acitivity along with viable resistance to the **β-lactamases** are the glaring factors that establishes the ensuing sensitivity of individual bacterial strains to this '**drug**'**.

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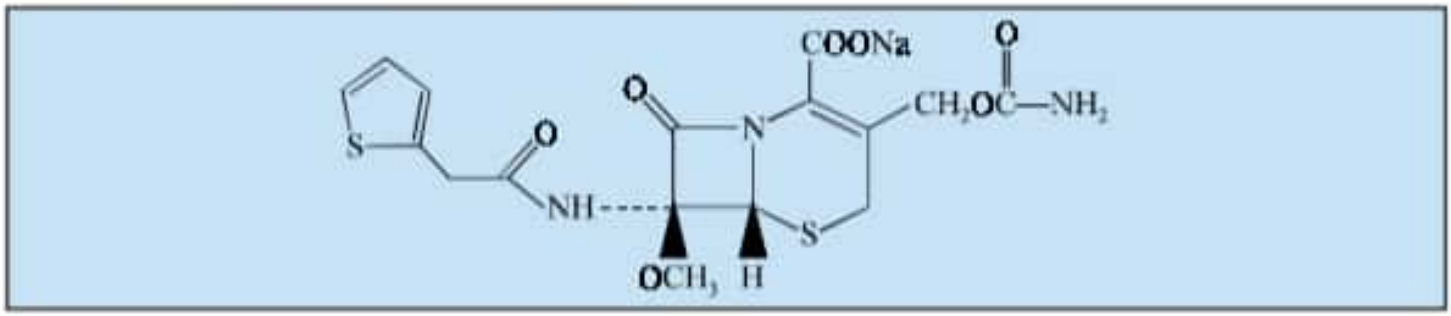
*Indelicato JM *et al. J Pharm Sci,* **65** : 1175, 1976.

Ott JL *et al. Antimicrob Agents Chemother,* **15 : 14, 1979.

3.2.1.2.2. Cefoxitin Sodium USAN, INN



(6R-*cis*)-5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, 3-[[(aminocarbonyl) oxy] methyl]-7-methoxy-8-oxo-7-[(2-thienylacetyl) amino]-, sodium salt ; USP ;

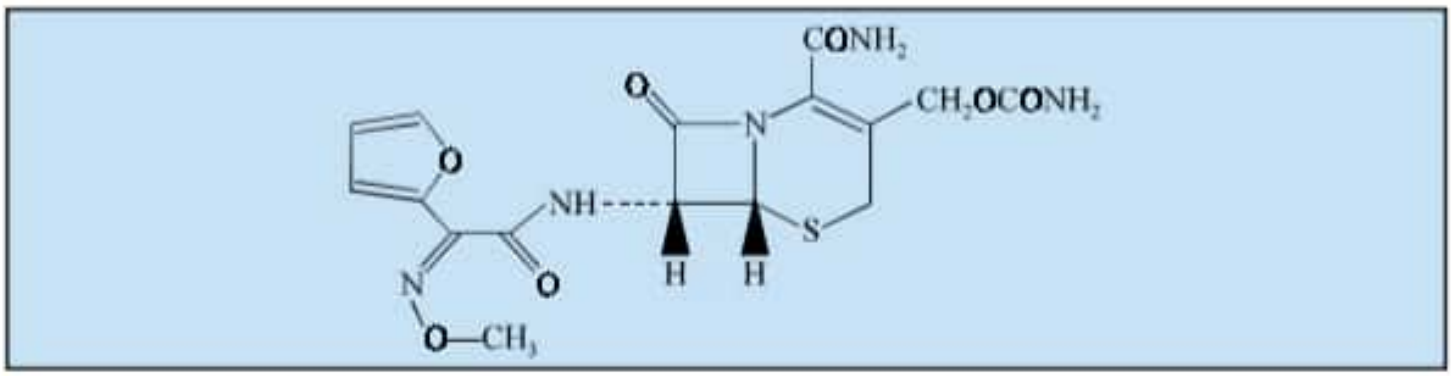
Mefoxin(R) ;

It is invariably used as an '**alternative drug**' for the treatment of intra-abdominal infections, colorectal surgery or appendectomy and ruptured viscus because it is active against most enteric anaerobes including the organism *Bacteroides fragilis.* It is also indicated in the management and treatment of bone and joint infections produced by *S. aureus,* gynecological and intra-abdominal infections caused by *Bacteroides* species together with other common enteric anaerobes and Gram-negative bacilli ; lower respiratory tract infections produced by *Bacteroides* species, *E.coli, H. influenzae, Klebsiella* spp. *S. aureus* or *Streptococcus* spp. (except enterococci) ; septicemia caused by *Bacteroides* spp., *E. coli, Klebsiella* spp., *S. aureus* or *Strep pneumoniae* ; skin infections produced by *Bacteroides* spp., *E. coli,* Klebsiella spp., *S. aureus* or *epidermidis* or *Streptococcus* spp. (except enterococci) or UTIs by *E. coli, Klebsiella* spp. or indole positive *Proteus,* and for preoperative prophylaxis.

Mechanism of Action. The '**drug**' is found to be resistant to certain **β-lactamases** which are responsible for the hydrolysis of **cephalosporins**. It has been duly observed that cefoxitin helps to antagonize the action of **cefamandole** (see Section 3.2.1.2.1) against *E. cloacae* and also that of carbenicillin against *P. aeruginosa.* As the half-life is comparatively of shorter duration ; therefore, the drug must be administered 3 to 4 times per day.

Note. Solutions of its sodium salt stable for 24 hours at an ambient temperature and lasts upto 1 week when refrigerated (0-10°C). However, 7α-methoxyl solutions helps to stabilize the β-lactam to alkaline hydrolysis to a certain extent.

3.2.1.2.3. Cefuroxime Sodium USAN, BAN, INN



5-Thia-1-azabicyclo [4, 2, 0] oct-2-ene-2-carboxylic acid, (6R, 7R)-7-[2-(2-furyl) glyoxylamido]-3-(hydroxymethyl)-8-oxo-, 7-(Z)-mono (*o*-methyloxime) carbamate (ester) ;

Kefurox(R) ; Zinacef(R) ;

It is approved for the treatment of meningitis caused by *Streptococcus pneumoniae, N. meningitidis* and *S. aureus*. It exhibits an excellent activity against all gonococci, hence is also employed to treat gonorrhea. It also finds its usage in the treatment of lower respiratory tract infections invariably caused by *H. influenzae* and *parainfluenzae, Klebsiella spp., E. coli, Strep pneumoniae,* and pyrogens and *Staph aureus*. It is also recommended for use against UTIs produced by *E. coli* and *Klebsiella*, which designates a more limited approval in comparison to other second generation cephalosporins. It is also indicated in bone infections, septicemias, and above all the surgical prophylaxis.

Mechanism of Action. The '**drug**' exerts its activity against *H. influenzae*, besides its inherent ability to penetrate directly into the **cerebrospinal fluid (CSF)** which renders it specifically beneficial for the treatment of *meningitis* caused by that susceptible organism. **Cefuroxime** gets distributed evenly throughout the entire body segments. It has been observed that almost 85% of the '**drug**' gets eliminated in the urine. Its half-life range between 1.3—1.7 hour but may get extended upto even 24 hours in the specific instance of renal failure.

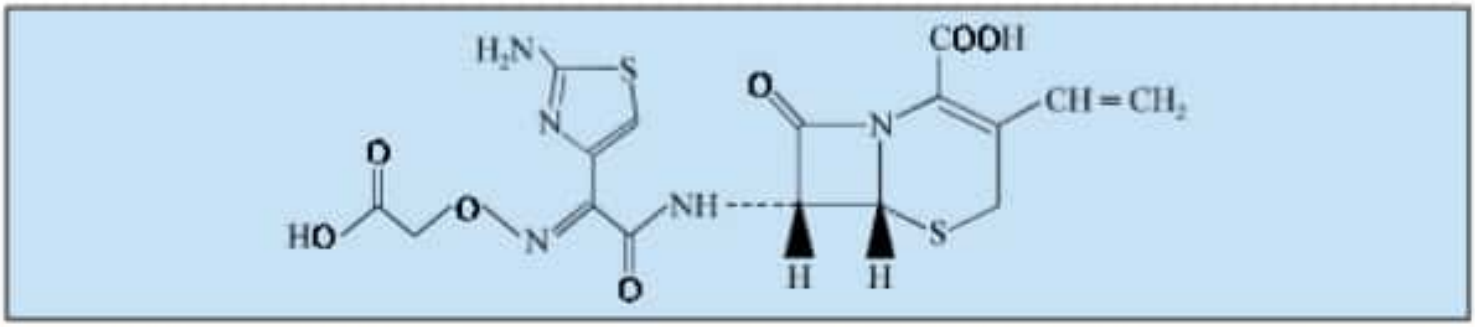
Note. The sodium salt of cefuroxime is poorly absorbed by the oral route. However, its corresponding 'axetil ester' is also available for the oral administration of otitis media, pneumonia and UTIs.

SAR of Cefuroxime. The presence of a *syn*-alkoxiamino substituent in the drug molecule is closely associated with the prevalent β-**lactamase activity** in these cephalosporins. Perhaps its inclusion into the '**second generation cephalosporins**' is duly justified due to the fact that its antibacterial spectrum bears a close similarity to that of **cefamandole** (see Section 3.2.1.2.1).

3.2.1.3. Third Generation Cephalosporins

Though there are several drugs that are approved and marketed belonging to the '**third generation cephalosporins**', but only *three* such compounds shall be discussed in this particular section, namely : **cefixime**, **ceftazidime**, and **ceftibuten**.

3.2.1.3.1. Cefixime USAN, INN



5-Thia-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R-[6α, 7β(Z)]]-7-[[(2-amino-4-thiazolyl) [(carboxymethyoxy) imino] acetyl] amino]-3-ethenyl-8-oxo- ; USP ;

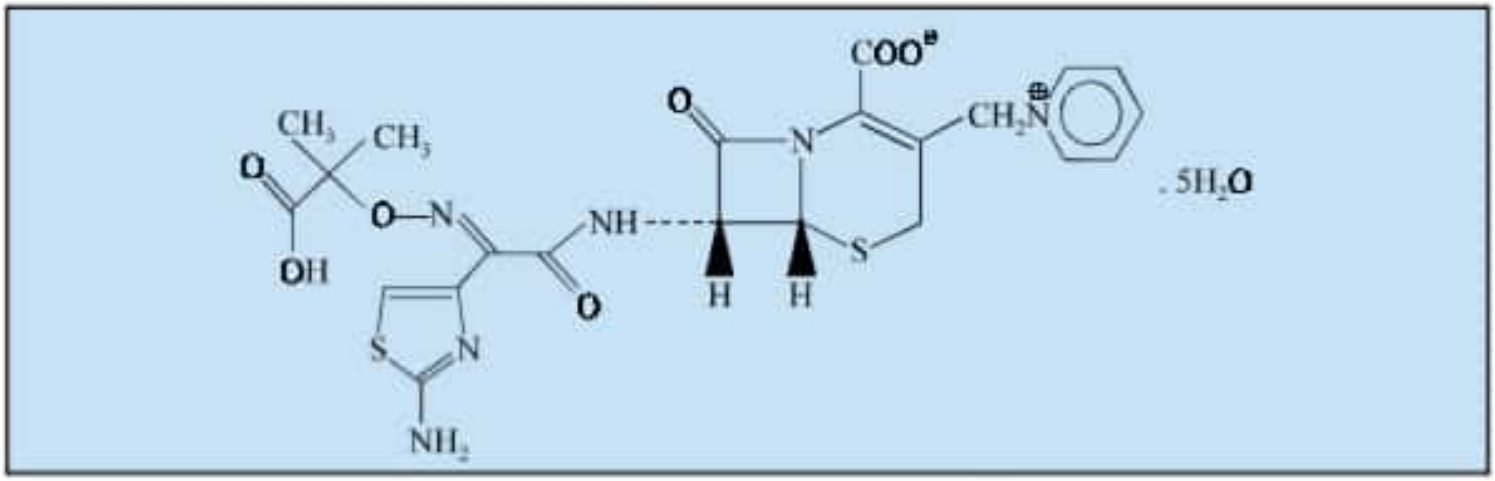
Suprax(R) ;

It is a well-known orally active **third generation cephalosporin** having superb and excellent therapeutic profile against a plethora of *E. coli, Klebsiella, H. influenzae, Branhametla catarrhalis, N. gonorrhoeae* and *meningitidis,* besides including β-*lactamase producing strains*. It is found to be active against certain common **streptococci** spp. whereas **staphylococci** are genuinely resistant. It is invariably recommended for the respiratory infections*, otitis media and uncomplicated UTIs; however, its actual therapeutic role is yet to be understood exhaustively.

*Infections due to acute bronchitis, pharyngitis, and tonsititis.

Mechanism of Action. The '**drug**' gets absorbed gradually and rather incompletely from the GI tract and exhibits a bioavailability ranging between 40-50%. Importantly, the apparent appreciable good oral absorption of this drug substance is due to its facilitated and augmented transport across the **intestinal brush-border membranes** that essentially implicate the ensuing carrier system for the **'dipeptides'**.* However, this result was not quite expected by virtue of the fact that the prevailing '**drug**' predominantly is devoid of the **ionizable α-amino moiety** either present in the '**dipeptides**' or the '**β-lactams**' previously known to be transported by the aforesaid carrier system.**

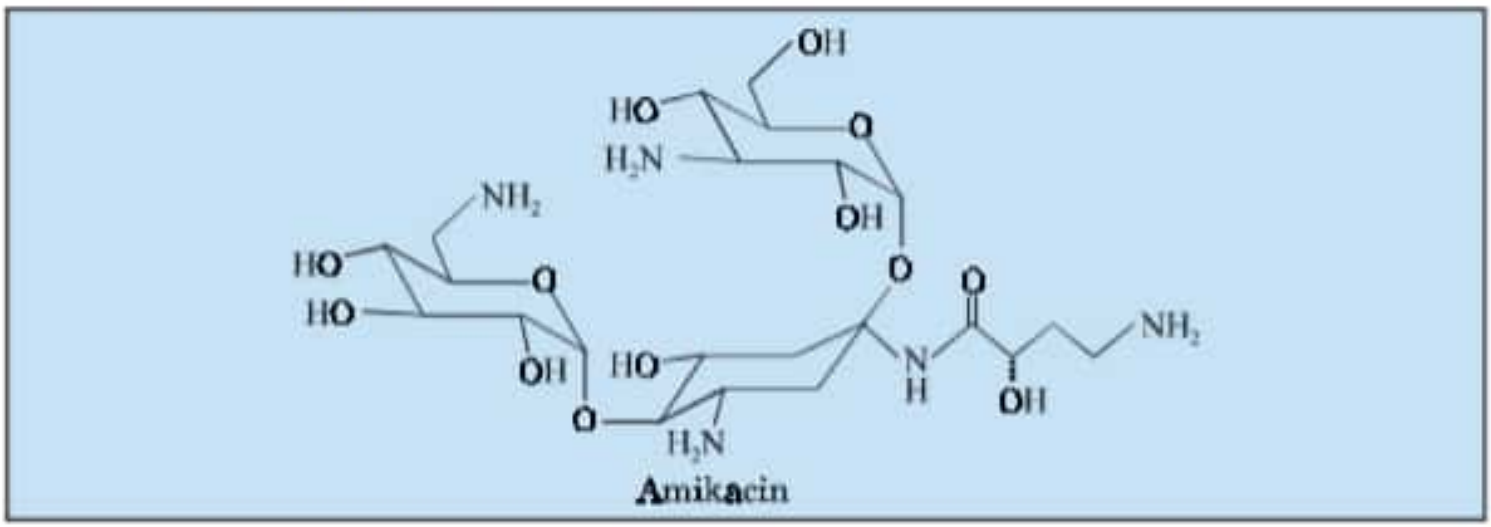
3.2.1.3.2. Ceftazidime Sodium USAN, INN



Pyridinium, [6R (6α, 7β(Z))]-1-[[7-[[(2-amino)-4-thiazolyl)-I(1-carboxy-1-methylethoxy) imino] acetyl] amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo [4,2,0] oct-2-ene-3yl]-, hydroxide, inner salt ; USP ;

Fortaz(R) ; Tazicef(R) ; Tazidime(R) ;

The '**drug**' displays its special interest due to its inherent high activity against the *Pseudomonas* and *Enterobacteriaceae* but fails to do so for *enterococci*. It is well recognized widely as an '**alternative drug**' specifically for the management and treatment of hospital-acquired Gram-negative infections. However, a combination with **amikacin** in the treatment of infections in immunocompromised patients when *Ps aeruginosa* happens to be a causative organism.

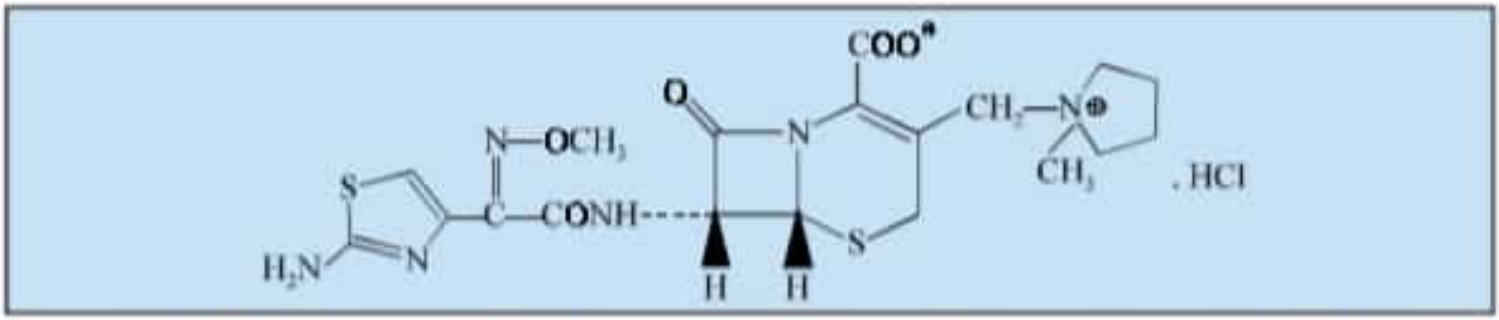


Amikacin

*Tsuji A *et al. J. Pharm. Pharmacol.*, **39**, 272, 1987.

Westphal JP *et al. Clin. Pharmacol. Ther.* **57, 257, 1995.

3.2.1.4.1. Cefepime Hydrochloride USAN, INN



Pyrrolidinium, [6R-(6α, 7β(Z)]]-1-[[7-(2-amino-4-thiazolyl) (methoxy-imino) acetyl] amino-2-carboxy-8-oxo-5-thia-1-azabicyclo [4,2,0] oct-2-ene-3yl]-methyl]-1-methyl-, hydroxide, inner salt hydrochloride ;

Maxipime(R) ; Axepin(R) ;

It is profoundly recognized as an altogether new approved **fourth-generation cephalosporin** which essentially possesses an extended Gram-negative spectrum against Gram-negative aerobic bacilli usually covered by **cefotaxime** and **ceftazidime** including certain strains that are found to be resistant to these **third-generation cephalosporins**.

It is, however, pertinent to state here that **cefepime** definitely exhibits an improved antibacterial profile against *Streptococcus pneumoniae* and *Staphylococcus aureus* in comparison to the **third-generation cephalosporins**. Interestingly, its specific activity against *P. aeruginosa* is found to be variable just like other antibiotics ; and the profile of activity resides between that of **ceftazidime** and **ceftoxime**.

The '**drug**' gets excreted mostly in the urine having a half-life of 2.1 hours. It is found to be bound almost minimally to the plasma proteins.

Note. Cefepime HCl may be administered IVor IM for the treatment of UTIs, pneumonias and skin infections.

4. AMINOGLYCOSIDE ANTIBIOTICS

The aminoglycoside antibiotics constitute an important category of antibacterial agents in the therapeutic armamentarium, *e.g.*, streptomycins, neomycins, paramomycins, kanamycins, gentamycins and the corresponding derivatives of these antibiotics.

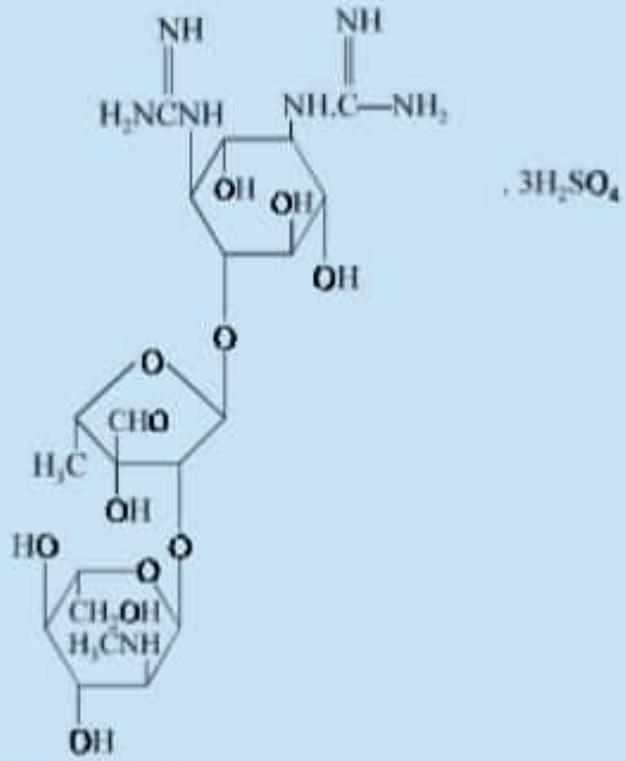
These are a bunch of closely related chemically basic carbohydrates that are mostly water-soluble. Their respective hydrochlorides and sulphates are crystalline in nature. They are found to be effective in inhibiting the growth of gram-positive as well as gram-negative bacteria. They are also effective to a great extent against mycobacteria.

In general, *they are prepared biosynthetically exclusively from an admixture of carbohydrate components of the fermentation media.*

They usually act by causing interference with the **'reading'** of the genetic code.

A few typical examples cited earlier shall be discussed below :

A. *Streptomycin* INN, *Streptomycin Sulphate* BAN, *Streptomycin Sulfate* USAN,



Streptomycin

BP ; USP ; Eur. P ; Int. P ; Ind. P ; Isoject Streptomycin Injection(R) (Pfizer) ;

Streptomycin Sulphate(R) (Glaxo, U.K.).

Streptomycin is chiefly employed in the *treatment of tuberculosis in conjunction with other drugs such as* **isoniazid and rifampicin**.

Streptomycin and **penicillin** exert a synergistic action against bacteria and are usually employed together in the treatment of subacute bacterial endocarditis caused by *Streptococcus faecalis*

It exerts **bacteriostatic action** in low concentrations and **bactericidal** in high concentrations against a plethora of Gram-negative and Gram-positive organisms. The only infection wherein this '*drug*' alone is the '**drug of choice**' are **tularemia*** and **bubonic plague**.** A combination with a *tetracycline* it may be employed in the treatment of **brucellosis***** and infections produced by *Pseudomonas mallei*. It is also an alternative drug of choice in the treatment of chancroid, rat-bite fever and tuberculosis.

Dose. *For non-tuberculosis infections, usual, 1g per day up to 5 to 10 days.*

Mechansim of Action. The '**drug**' exerts its maximum effectiveness against the organism *Mycobacterium tuberculosis*. Interestingly, the antibiotic is not a cure itself but has proved to be an excellent and valuable adjunct to other modalities of therapeutic treatment for tuberculosis. It acquires a rapid development with respect to certain strains of microorganisms. The combined administration of *streptomycin* and **penicillin** has been suggested to combat infections which may be due to organisms that are sensitive to both these antibiotics. The **'drug'** is neither absorbed nor destroyed appreciably in the GI tract.

---

* An acute plaguelike infectious disease caused by *Francisella tularensis*.

**It is caused by *Yersinia pestis* usually found in infected rats, ground squirrels and gets transmitted to humans by the bite of rat flies.

***A widespread infectious febrile disease affectiing humans and cattle (also called **Malta Fever**).

SAR of Streptomycin. The '**drug**' serves as a **triacidic base** due to the presence of *two* characteristic chemical entities, namely : (*a*) two strongly basic guanido moieties ; and (*b*) rather weakly basic methylamino function. Furthermore, hydroxy-streptomycin differs from streptomycin in essentially having a strategically positioned OH moiety in place of one of the H-atoms of the streptose methyl function. Besides, streptomycin B(*i.e.*, mannisido streptomycin) possesses a **mannose residue** attached to a glycosidic linkage *via* a OH moiety at C-4 of the N-methyl-L-glucosamine functional group. The designated stereochemical structure of the '**drug**' has been reconfirmed *via* the total synthesis.*

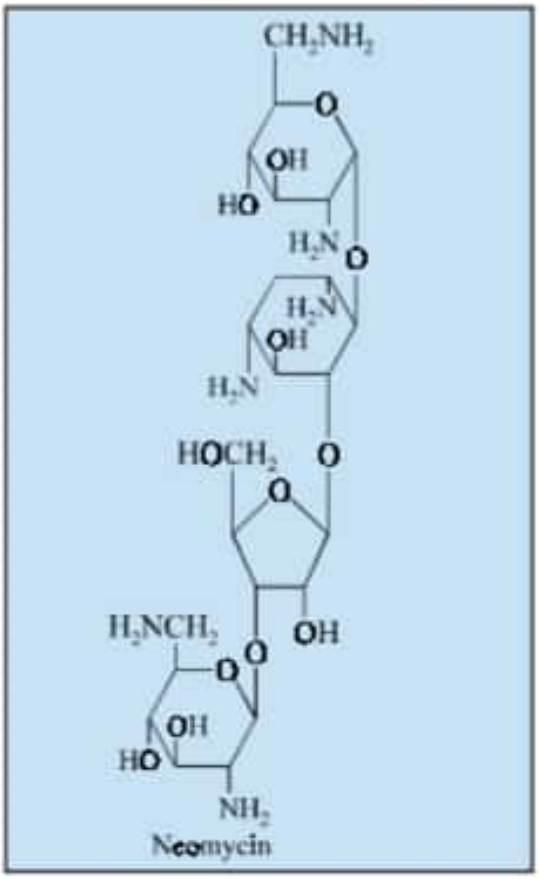
B. *Neomycin* INN, *Neomycin Sulphate* BAN, *Neomycin Sulphate* USAN,

Fradiomycin Sulphate; BP ; USP ; Eur. P ; Int. P ; Ind. P ;

Neobiotic(R) (Pfizer) ; Mycifradin(R) (Upjohn).

Neomycin *is mostly used in a wide variety of local infection such as burns, ulcers, wounds, impetigo, infected dermatoses, furunculosis, conjunctivitis, etc. It is also employed as an adjuvant in topical steroid preparations to control secondary infections in the case of inflammatory disorders.*

The '**drug**' is employed to produce intestinal antisepsis prior to large bowel surgery, for the treatment of gastroenteritis produced by toxigenic *E. coli*, and also to afford suppression of ammonia producing bowel flora in the management of hepatic coma. As it causes a rapid overgrowth of nonsusceptible organism, including staphylococci, oral therapy must not be prolonged in any case for more than 3 days. It displays broad-spectrum activity against a good number of pathogenic organisms. Besides, it demostrates a low incidence of toxic and hypersensitivity reactions.



Neomycin

Dose: *Topical, to the skin, as 5% solution, aerosol or ointment 2 to 3 times a day.*

*Umezawa S *et. al. J. Antibiotic* (Tokyo) **27,** 997, 1974.

has also been indicated for preoperative antisepsis of the bowel. However, this '*drug*' could not be useful in tuberculosis perhaps due to the fact that it develops resistance to mycoorganism rather rapidly.

Dose : (Base equivalent)-Oral, adult, for intestinal infection, 1g after every 8 hours for 5 to 7 days ; For preparative preparations, 1g every hour for 4 doses followed by 1g every 6 hours for 36 to 72 hours.

Mechanism of Action. Based on both clinical experience and experimental demonstration* it has been duly observed that the '*drug*' develops cross-resistance overwhelmingly in the **tubercle bacilli** specifically along with some other medicinal entities, such as : vincomycin, dihydrostreptomycin and antitubercular drug substances.

SAR of Kanamycin. Kanamycins A, B and C *i.e.*, the three closely related analogues of kanamycin have been duly established by the aid of chromatography. Kanamycin A is the '*drug*' available for therapeutic usage. It has been proved that the vital point of difference amongst the kanamycins resides solely in the **sugar residuces** strategically linked to the *glycosidic oxygen* at the C-4 position of the central deoxystreptamine. Interestingly, the kanamycins do **not** essentially possess the **D-ribose residue** as is present in *neomycins* and *paromomycins*. In all the three structural variants of kanamycin the presence of **kanosamine entity** is found to be attached glycosidically at the C-6 position of deoxystreptamine *i.e.*, 3-D-glucosamine. They also differ in the substituted D-glucoses which are observed to be attached glycosidically at the C-4 position of the inherent deoxystreptamine ring.

CAUTION : (1) ***The 'drug' causes either retarded or impaired loss of hearing.***

(2) ***Kanamycin and penicillin salts must not be combined in the same solution perhaps due to the possible inactivation of either agents significantly.***

5. CHLORAMPHENICOL

Chloramphenicol (**chloromycetin**) is a levorotatory broadspectrum antibiotic originally produced from several streptomycetes, namely : *S. venezualae, S. omiyamensis* and *S. phacochromogenes var.* chloromyceticus. It has been reported to be the drug of choice for the treatment of typhus and typhoid fever.

However, **chloramphenicol** is of paramount interest owing to the following *three* reasons :

(*a*) It is a naturally occurring aromatic nitro compound of which there is only one previously recorded example of *hiptagin*, obtain from the root bark of *Hiptage madablota* Gaertn is noteworthy.

(*b*) It is capable of exerting its effect against viral diseases as well as those due to bacterial invasion and opens up the whole field of the chemotherapy of *virus* and *rickettsial* infections in man including typhus, undulant fever, *Salmonella septicaemia*, whooping cough, gastroenteritis, *lymphogranuloma inguinale*, typhoid and paratyphoid. So far, chloramphenicol-fast strains have not been isolated.

(*c*) It is amenable to synthesis on an industrial scale.

5.1. Structure of Chloramphenicol

The structure of **chloramphenicol** has been established on the basis of the following vital chemical evidences. They are :

*Morikubo Y, *J Antibiot* [A] : **12,** 90, 1959.

(*i*) The molecular formula of chloramphenicol is $C_{11}H_{12}O_5N_2Cl_2$.

(*ii*) Its absorption spectrum is similar to that of nitrobenzene.

(*iii*) The presence of a nitro group was revealed by the reduction of chloramphenicol with tin (Sn) and hydrochloric acid, followed by diazotization and then coupling to yield an orange precipitate with β-naphthol (Rebstock *et al.* 1949).

(*iv*) When reduced catalytically (with palladium, Pd) it gives a prodcut which has an absorption spectrum very similar to that of *para*-toluidine and the resulting solution gives a positive test for ionic chlorine.

(*v*) Hydrolysis of **chloramphenicol** with either acid or alkali produces dichloroacetic acid together with an optically active base $C_9H_{12}O_4N_2$. Thus :

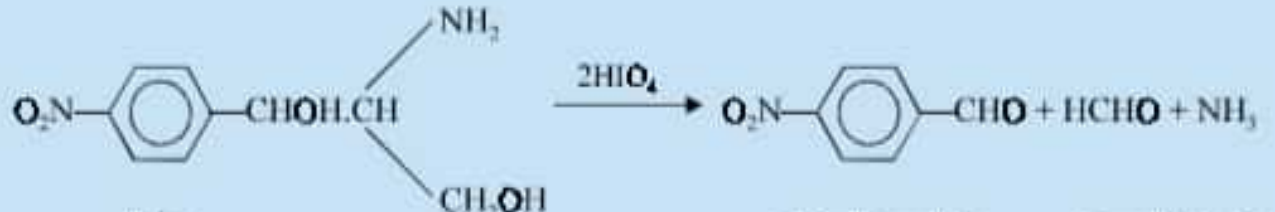
$$\underset{\text{Chloramphenicol}}{C_{11}H_{12}O_5N_2Cl_2} \xrightarrow{H_2O} \underset{\text{Dichloro-acetic acid}}{Cl_2CH.COOH} + \underset{\text{(Base)}}{C_9H_{12}O_4N_2}$$

(*vi*) The resulting base was shown to contain a primary amino group, and on being treated with methyl dichloroacetate, the base regenerated **chloramphenicol** (Rebstock *et al.* 1949).

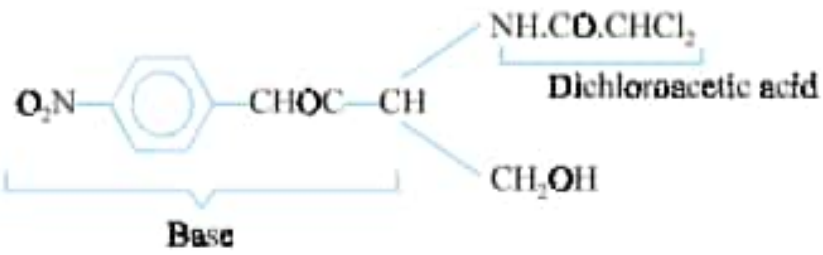
(*vii*) **Chloramphenicol** is converted into a diacetyl derivative on treatment with acetic anhydride in pyridine ; whereas the base obtained from **chloramphenicol** yields a triacetyl derivative on similar treatment thereby suggesting that **chloramphenicol** probably contains two-OH groups.

(*viii*) When the **chloramphenicol** base is treated with periodic acid (HIO_4) two molecules of the latter are consumed with the formation of one molecule each of ammonia, formaldehyde and *para*-nitrobenzaldehyde respectively.

However, these products may be accounted for provided the base is assumed to be 2-amino-1-nitrophenyl propane-1, 3-diol (Rebstock *et al.* 1949). Thus :

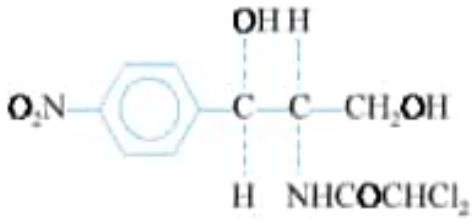


Hence, **chloramphenicol** may be written as :



D-(–)-*Threo*-2-dichloroacetamide-1-*p*-nitrophenylpropane-1, 3-diol.

A. *Chloramphenicol* INN, BAN, USAN,

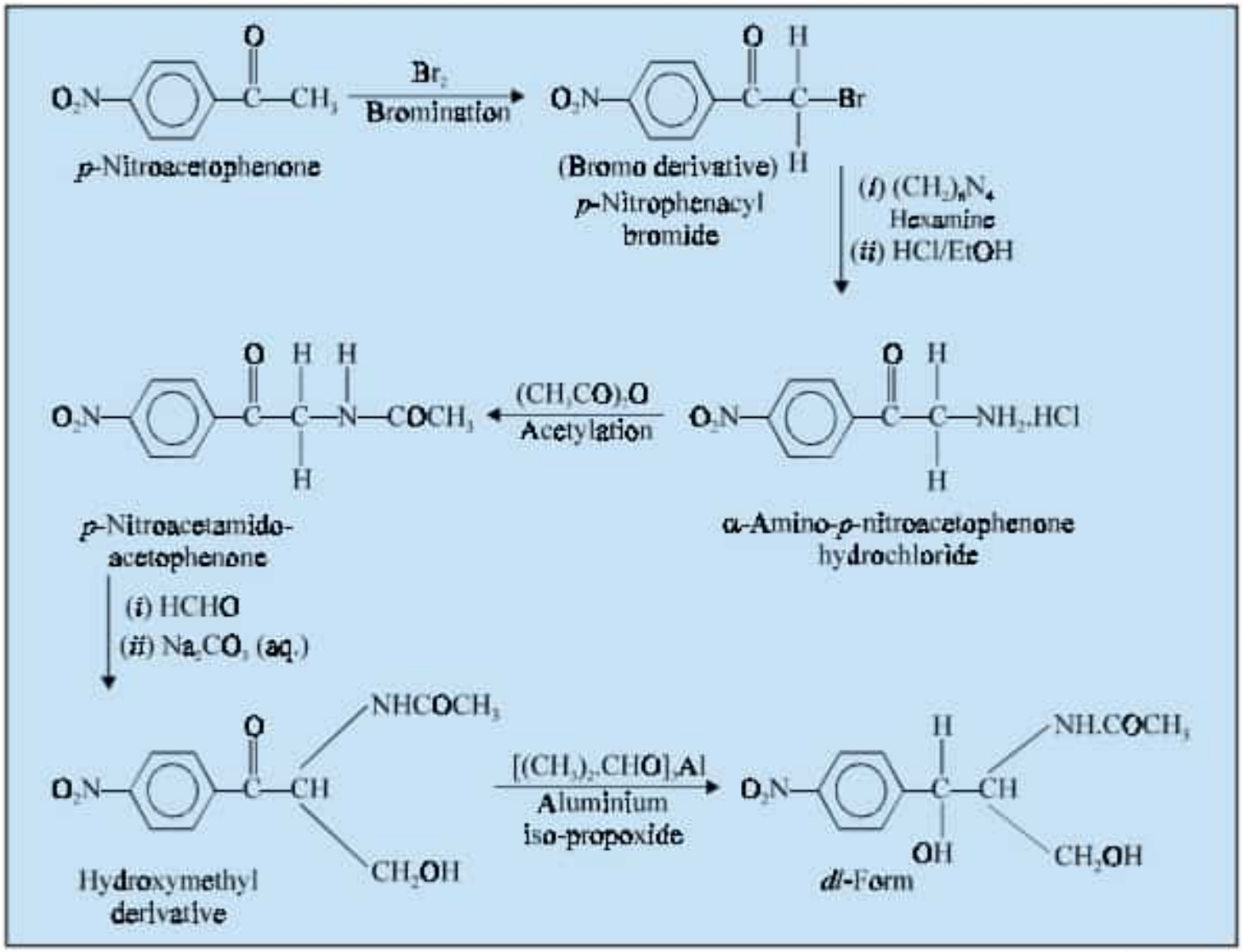


D-*threo*-(–)-2, 2-Dichloro-N-[β-hydroxy-α-(hydroxy methyl)-*p*-nitrophenyl] acetamide ; Acetamide, 2,z-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) etheryl]-, [R-(R*, R*)]-; BP ; USP ; Eur. P ; Int. P ; Ind. P ;

Chloromycetin(R) (Parke-Davis).

5.2. Synthesis of Chloramphenicol

Chloramphenicol has been successfully synthesized by different methods and the present global demand of this drug is adequately met exclusively by chemical synthesis. The synthesis put forward by Long *et al.* (1949) is discussed below :



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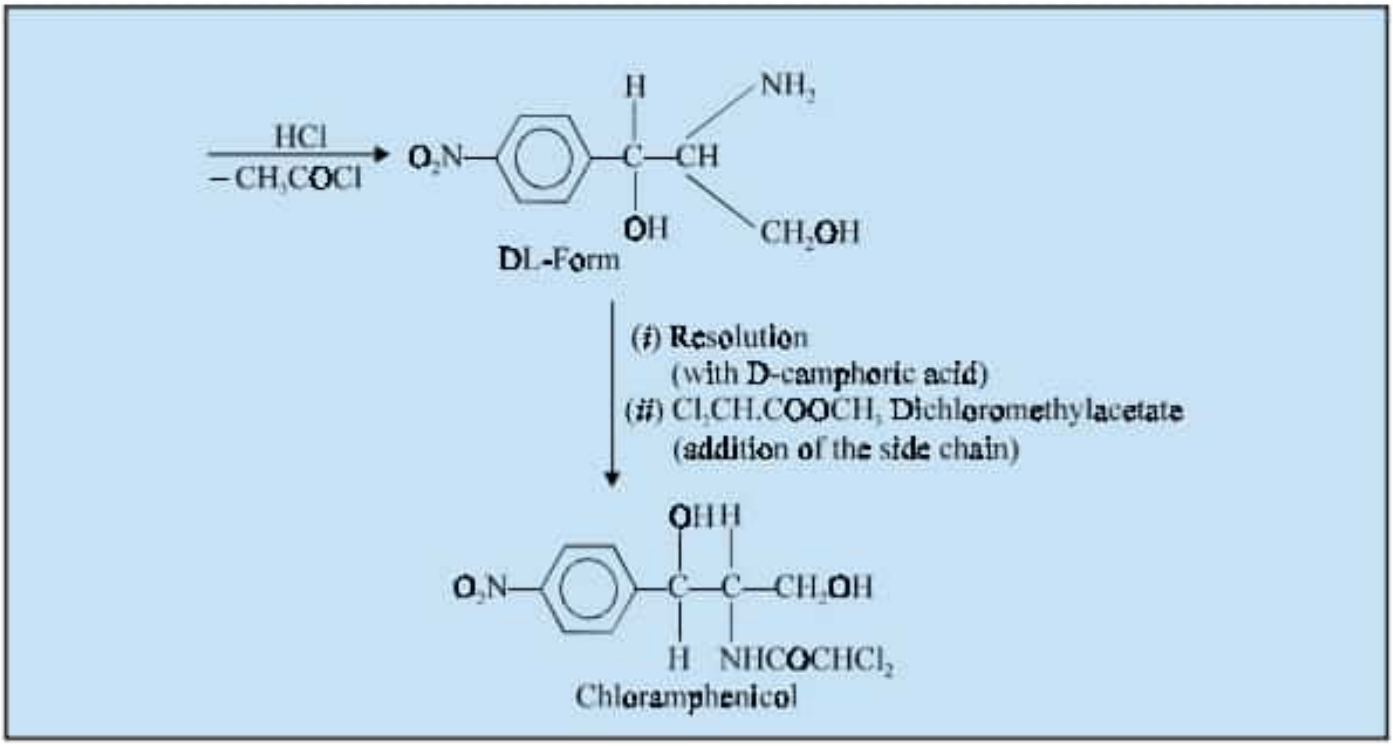


para-Nitroacetophenone on bromination gives the corresponding bromo derivative which on treatment with hexamine followed by acidic ethanol yields α-amino-*p*-nitroacetophenone hydrochloride. This on acetylation gives the acetamido derivative which on treatment with formaldehyde followed by aqueous sodium carbonate affords the corresponding hydroxy methyl analogue. Reduction of the keto moiety is effected by treatment with aluminium iso-propoxide to give the product in DL-form which on reaction with HCl removes the acetyl function to yield the **chloramphenicol base** in its DL-form. The resulting product is first subjected to resolution with α-camphoric acid and secondly with dichloromethyl acetate to afford the addition of the side chain to yield **chloramphenicol**.

Typhoid fever and similar salmonellal infections are usually considered the prime indications for the use of **chloramphenicol**. It is also employed *in acute infections due to Heamophilus influenzae, including meningitis attributed to ampicillin-resistant strains.* It also find its enormous applications in *topical infections of eye and skin.* It has also been used *to eradicate vibrios from patients with cholera.* It is employed *for rickettsial infections like typhus and Rocky Mountain spotted fever.*

Chloramphenicol is particularly recommended for the management and treatment of serious infections produced by the strains of both Gram-positive and Gram-negative organism that have developed eventually resistance to either **ampicillin** or **penicillin G,** for instance : *H. influenzae, Salmonella typhi, S. pneumoniae, B. fragilis,* and *N. meningitidis.*

It is used topically extensively for the superficial *conjunctival infections* and *blepharitis* essentially caused by *E. coli, H. influenzae, Moraxella lacunata, Streptococcus hemolyticus,* and *S. aureus.* However, it is still the drug of choice for the **typhoid fever.**

Dose. *Usual, adult, 500 mg every 6 hours.*

Mechanism of Action. The '**drug**' has specifically the ability to penetrate right into the **central nervous system (CNS)** ; therefore, it is still an important alternative therapy for meningitis. The major course for the metabolism of **chloramphenicol** essentially involves the formation of the 3-*o*-glucuronide.

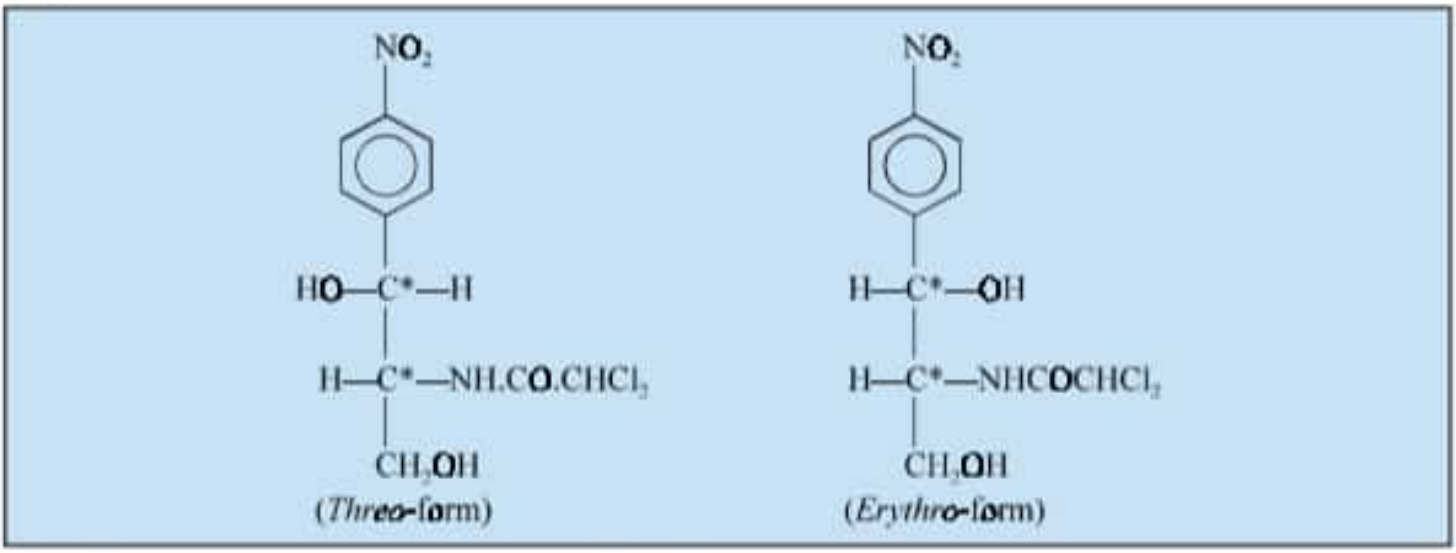
Howerver, the minor reactions necessarily include : (*i*) reduction of the inherent *para*-nitro moiety to the corresponding '*amine*' function ; (*ii*) hydrolysis of the amide moiety ; (*iii*) hydrolysis of α-chloroacetamido group ; and (*iv*) reduction to yield α-hydroxyacetyl analogue.*

Chloramphenicol gets absorbed very fast from the GI tract, having a bioavailability of almost 90%. It has been observed that about 60% of the drug in blood is bound to serum albumin. It is biotransformed in the liver within a range of 85-95%. The volume of distribution v_d^{ss} stands at 0.7 mL. g^{-1}. The plasma half-life varies between 1.5–5 hours, except over 24 hours in neonates 1-2 days old, and 10 hours in infants 10-16 days old. The '*drug*' may cross the placental barrier and in turn intoxicate the fetus ; therefore, it must be avoided as far as possible in pregnant women.

Note. The '*prodrug*' of chloramphenicol viz., chloramphenicol palmitate (USP), which is a tasteless product, is solely intended for pediatric usage profusely, because the parent drug has a distinct bitter taste.

5.3. Structure Activity Relationship

Chloramphenicol possesses *two chiral (asymmetric) carbon atoms* in the **'acylamino-propanediol chain'** as shown below :



Thus there are *two* possible pairs of enantiomorphs.

It has been observed that the biological activity resides almost exclusively in the **'D-Threo-isomer'** whereas the **L-*Threo***, and **D- and L-*Erythro* isomers** are virtually inactive.

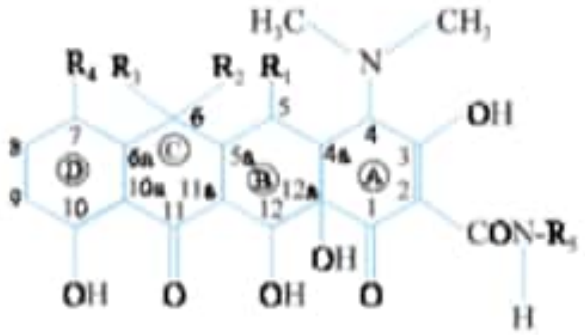
A large number of structural analogues of **chloramphenicol** have been prepared on the basis of the following themes : removal of the chlorine atom, transference of chlorine atom to the aromatic nucleus, transference of the nitro moiety to the *ortho*- or *meta*-position, esterification of the hydroxyl function(s), replacement of the phenyl ring with furyl, naphthyl and xenyl rings respectively, addition of alkyl or alkoxy substituents to the aryl ring and lastly replacement of the inherent nitro group by a halogen atom. It is, however, pertinent to mention here that none of these structurally modified analogues showed an activity approaching to that of **chloramphenicol** towards *Shigella paradysenteriae*

*Glazko A : *Antimicrob Agents Chemother.*, 655, 1966.

6. THE TETRACYCLINES

The epoch-making discovery of **chlortetracycline** (**aureomycin**) in 1947 by Duggar paved the way for a number of structural analogues used as broad-spectrum antibiotics that belong to the tetracycline family. **The tetracyclines** which are found to be effective therapeutically are listed in the following table.

6.1. Salient Features of the Tetracyclines



Name of Compound	Official Status	Brand Name(s)	R_1	R_2	R_3	R_4	R_5
Tetracycline	BPC ; (1973); USP ;	Tetracyn® (Pfizer) ; SK-Tetracycline® (SK & F)	H	OH	CH_3	H	H
Oxytetracycline	USP ;	Terramycin® (Pfizer)	OH	OH	CH_3	H	H
Chlortetracycline HCl	BP, USP ; Eur. P.; Int. P.; Ind. P.;	Aureomycin® (Lederle)	H	OH	CH_3	Cl	H
Demeclocycline HCl	BP, USP ; Eur. P.;	Ledermycin® (Lederle, UK)	H	OH	H	Cl	H
Methacycline HCl	BP (1973); USP ;	Rondomycin® (Wallace)	OH	=	CH_2	H	H
Doxycycline	USP ;	Vibramycin® (Pfizer)	OH	H	CH_3	H	H
Rolitetracycline	USP ;	Syntetrin® (Bristol)	H	OH	CH_3	H—CH_2—N	

6.2. Nomenclature

Based on the above conventional numbering of various carbon atoms and subsequent labelling of the **four** aromatic rings present in the **tetracycline** nucleus, oxytetracycline is chemically designated as :

"4-Dimethylamino-1, 4, 4*a*, 5, 5*a*, 6, 11, 12*a*-octahydro-3, 6, 10, 12, 12*a*-penta-hydroxy-6-methyl-1, 11-dioxo-2-naphthacenecarboxamide".

Some other members of the **tetracycline** family may conveniently be named as follows :

Methacycline : 6-Methylene-5-oxytetracycline ;

Doxycycline : α-6-Deoxy-5-oxytetracycline ;

Rolitetracycline : N-(Pyrrolidinomethyl)-tetracycline.

6.3. General Characteristics of the Tetracyclines

Following are the **general characteristic features** of all the members of the **tetracycline** family :

(*a*) The **tetracyclines** are obtained by fermentation procedures from **streptomyces species** or by the chemical transformations of the **natural products**.

(*b*) The important members of this family are essentially derivatives of an **octahydron-aphthacene**, *i.e.*, a hydrocarbon made up of a system of four-fused rings.

(*c*) The **antibiotic spectra** and the chemical properties of these compounds are quite similar but not identical.

(*d*) The **tetracyclines** are amphoteric compounds, *i.e.*, forming salts with either acids or bases. In neutural solutions these substances exist mainly as **Zwitter ions**.

(*e*) The acid salts of the **tetracyclines** that are formed through protonation of the dimethylamino group of C-4, usually exist as crystalline compounds which are found to be very much soluble in water. However, these **amphoteric antibiotics** will crystallize out of aqueous solutions of their salts unless they are duly stabilized by an excess of acid.

(*f*) The corresponding hydrochloride salts are used most commonly for oral administration and are usually encapsulated owing to their bitter taste.

(*g*) The water soluble salts are obtained either from bases such as sodium/potassium hydroxides or formed with divalent/polyvalent metals, *e.g.*, Ca^{++}. The former ones are not stable in aqueous solutions, while the latter ones, *e.g.*, calcium salt give tasteless products that may be employed to prepare suspensions for liquid oral dosage forms.

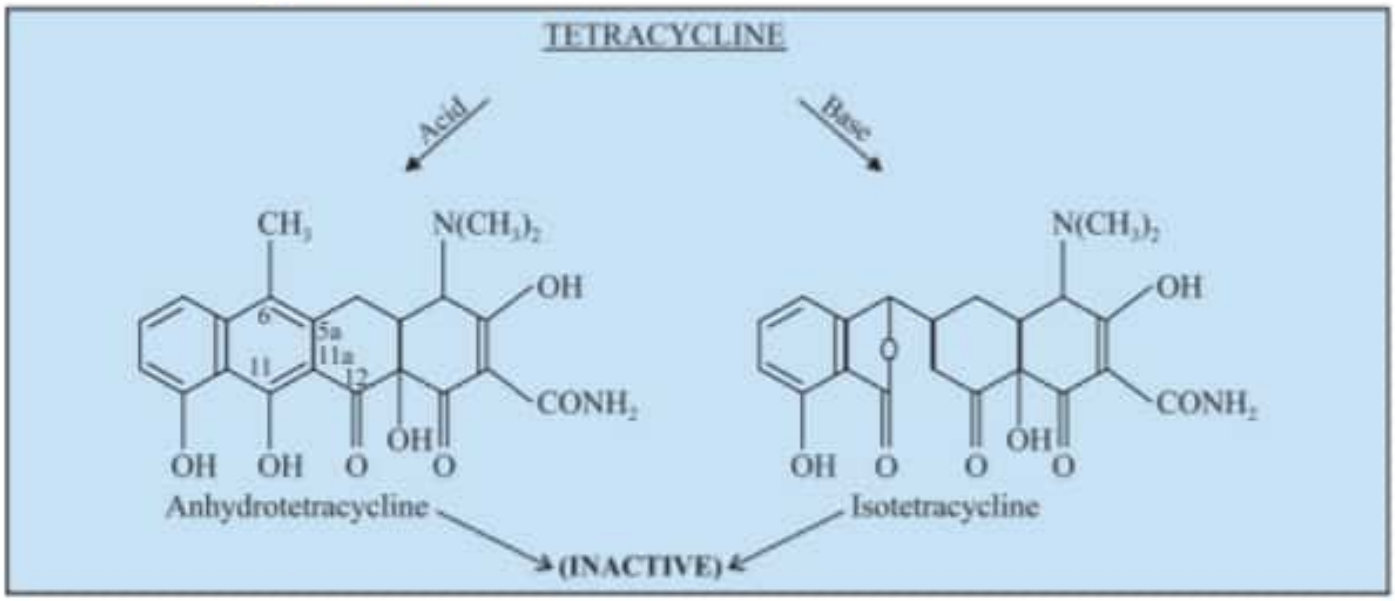
(*h*) The unusual structural features present in the **tetracyclines** afford three acidity constants (pKa values) in aqueous solutions of the acid salts. The thermodynamic pKa values has been extensively studied by Lesson *et al.* and discussed in the chapter on 'Physical-chemical factors and biological activities'.

(*i*) An interesting property of the **tetracyclines** is their ability to undergo epimerizaton at C-4 in solutions having intermediate pH range. These isomers are called **epitetracyclines**.

The **four *epi*-tetracyclines** have been isolated and characterized. They exhibit much less, activity than the corresponding **'natural' isomers ;** thus accounting for an apparent decrease in the therapeutic value of aged solution.

epi (less active) ⇌ **Natural** (more active)

(*j*) It has been observed that the strong acids and bases attack the tetracyclines having a hydroxy moiety at C-6, thereby causing a considerable loss in activity through modification of the C-ring as shown below :



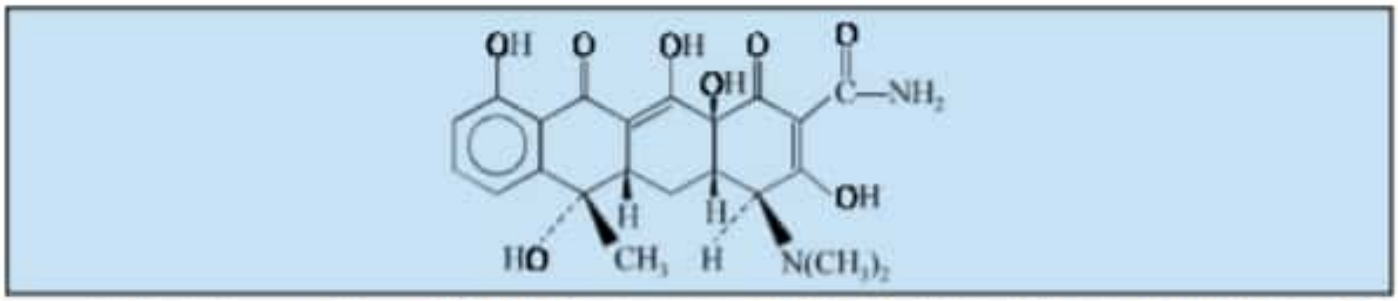
Strong acids produce a dehydration through a reduction involving the OH group at C-6 and the H atom at C-5*a*. The double bond thus generated between positions C-5*a* and C-6 induces a shift in the position of the double bond between the carbon atoms C-11 and C-11*a* thereby forming the relatively more energetically favoured resonant system of the naphthalene group found in the **inactive anhydrotetracyclines**.

The strong bases on the other hand promote a reaction between the hydroxyl group at C-6 and the carbonyl moiety at C-11, thereby causing the bond between C-11 and C-11*a* atoms to cleave and eventually form the lactone ring found in the **inactive isotetracyclines**.

(*k*) The **tetracyclines** form stable chelate complexes with many metals, *e.g.*, Ca^{++}, Mg^{++}, Fe^{++}, etc.

A few typical examples of the tetracyclines shall be dealt with in the sections that follows :

6.3.1. Tetracycline USAN, BAN, INN



2-Naphthacenecarboxamide [4S-(4α, 4*a*α, 5*a*α, 6β, 12*a*α)]-4-(dimethylamino)-1, 4, 4*a*, 5, 5*a*, 6, 11, 12*a*-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-; USP;

Achromycin(R); Cyclopar(R); Panmycin(R); Tetracyn(R);

The '**drug**' is the durg of choice in the treatment of chloera, relapsing fever, granuloma inguinale and infections produced by rickettsia, *Borrelia, Mycobacterium fortuitum* and *marinum*, and *Chlamydia psittaci* and *trachomatis* (except pneumonia and inclusion conjunctivitis).

It may be employed as an '**alternative drug**' in the following *two* situations, namely :

(*a*) For silver nitrate in the prevention of neonatal ocular prophylaxis of chlamydial and gonococcal cojunctivitis, and

(*b*) For treatment of actinomycosis, anthrax, chancroid, mellioidosis, plague, rat-bite fevers, syphilis and yaws.

It has also been reported to be beneficial in the treatment of *toxoplasmosis.*

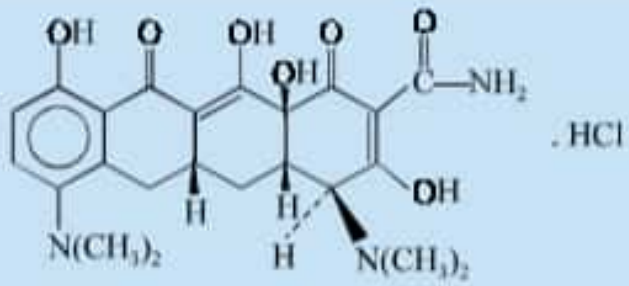
Mechanism of Action. The mechanisms of action of its combination with other agents have been established adequately, such as :

Tetracycline + $MgCl_2 . 6H_2O$—**Panmycin (R)**—Enhances the rate and peak of plasma concentration.

Tetracyclines + Aluminium/Calcium gluconates—Observed enhanced plasma levels in experimental animals.

From the above two cited examples one may evidently conclude that the **tetracyclines** may form stable complexes with bivalent metal ions (*e.g.*, Mg^{2+}, Ca^{2+};) that would appreciably minimize the absorption from the GI-tract. In reality, these '**adjuvants**' seen to compete with the tetracyclines for substances present in the GI-tract which might otherwise be free to complex with these antibiotics, and thus ultimately retard their absorption significantly. Of course, there is no concrete evidence which may suggest that the metal ions (Mg^{2+}, Ca^{2+}) *per se* serve as '**buffers**', a theoretical explanation quite often put forward in the literature.

6.3.2. Minocycline Hydrochloride USAN, BAN, INN



2-Napthhacenecarboxamide, [4S-(4α,4*a*α, 5*a*α, 12*a*α)]-4,7-*bis* (dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,10,12,12*a*-tetrahydroxy-1,11-dioxo-, monohydrochloride ; USP ; Minocin(R) ; Vectrin(R) ;

The '**drug**' is found to be 2-4 folds as potent as **tetracycline** ; however, it essentially shares an equally low potency against *Enterococcus fecalis.* Besides, it is observed to be 8 times more potent against *Streptococcus viridans,* and 2-4 times against Gram-positive organisms in comparison to tetracyclines. It is the drug of choice for the treatment of infections caused by *Mycobacterium marinum.* It remarkably differs from the other structural analogues of **tetracyclines** wherein the observed bacterial resistance to the drug stands at a *low ebb and incidence* ; it is particularly true for *Staphylococci,* in that the prevailing cross-resistance is only upto 4%.

Minocycline has been indicated for the management and treatment of *chronic bronchitis* and othe **upper respiratory tract infections (URTs)**. Though it essentially possesses comparatively low renal clearance, which is partially compensated for by means of its high serum and tissue levels, it has

been duly recommended for the treatment of **urinary tract infections (UTIs)**. The '**drug**' has been equally useful in the virtual erradication of *N. meningitidis* in specific asymptomatic carriers.

Mechanism of Action. The '**drug**' is usually absorbed by the oral route upto 90-100%. However, its absorption is predominantly diminished to a small extent milk and food intake ; and appreciably by the presence of **'iron preparations'** and '**nonsystemic antacids**'. It is protein-bound in plasma between a range of 70-75%. The volume of distribution v_d^{**} stands at 0.14 – 0.7 mL. g^{-1}. The plasma half-life ranges between 11–17 hours. It gets excreted unchanged in urine upto 10%; however, its biological half-life is usually prolonged chiefly in the incidence of renal failure.

6.4. Structure Activity Relationship (SAR)

The **structure activity relationship** amongst the various members of the **tetracycline** family has ben studied extensively.

The high level of antimicrobial activity of tetracycline established earlier reveal that the substitutions on the C-5 and C-7 were not an essential requirement.

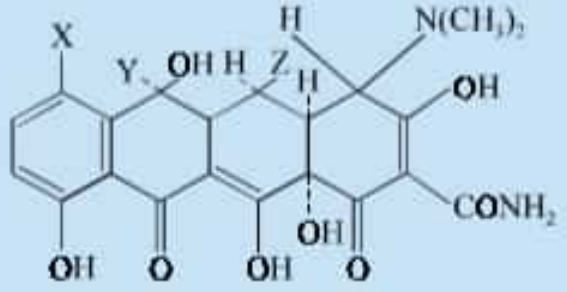
The activity of 6-dimethyltetracycline (**demecycline**) and **demeclocycline** has established that the methyl function at C-6 may be replaced by hydrogen.

The activity of **deoxycycline** and **6-deoxy-6-demethyltetracycline** (**minocycline**) shows that the presence of hydroxy moiety at C-6 is not essential either.

The **6-deoxy-6-methylenetetracyclines** and their corresponding **mercaptan adducts** possess typical characteristics tetracycline activity and illustrate further the level of modification feasible at C-6 with the possible retention of biologic activity.

It is, however, interesting to observe that the subsequent removal of the 4-dimethylamino function affords a loss of about 75% of the antibiotic effect of the **parent tetracyclines**.

The X-ray diffraction studies reveal that the following stereochemical formula represents the orientations, as observed in the **natural tetracyclines** :



Tetracycline : X, Z = H ; Y = CH_3 ;

Chlortetracycline : X = Cl ; Y = CH_3; Z = H ;

Oxytetracycline : X = H ; Y = CH_3 ; Z = OH ;

Demeclocycline : X = Cl ; Y = Z = H ;

* Tally FT *et al. J Antimicrob. Chemother.* **35,** 449, 1995.

X-ray diffraction studies further reveal that the 4-dimethylamino function is placed in a *trans*-orientation rather than the *cis*-form as inferred earlier by chemical investigations. It further establishes the presence of a conjugated system existing in the structures of **tetracycline** from C-10 through C-12.

6.5. Newer Tetracyclines

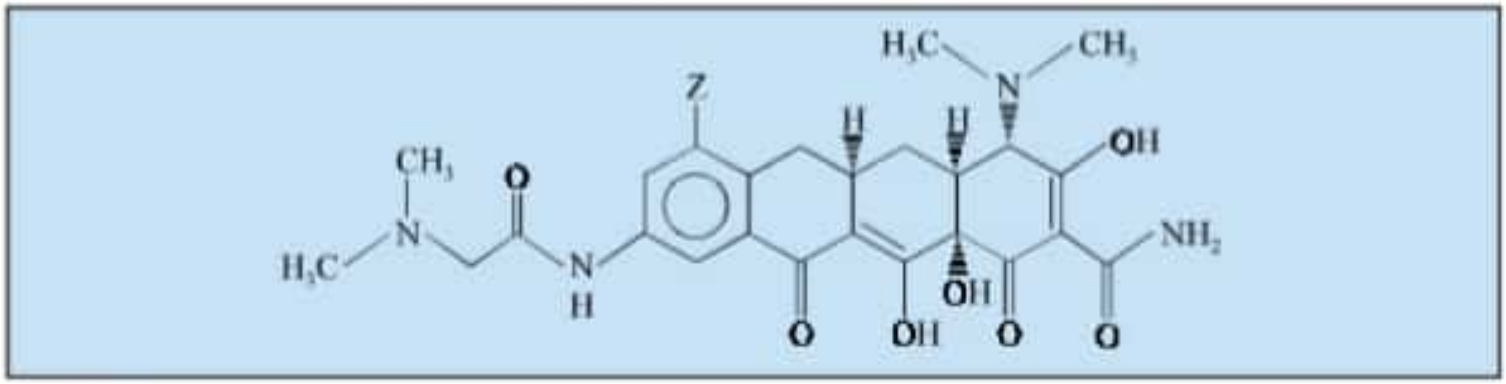
Since 1992, several newer breeds of '**tetracyclines**' have emerged that were exclusively based on the recent researches focussed on the following aspect, namely :

(*a*) superb broad spectrum antimicrobial profile of the **'tetracyclines'**, and

(*b*) recent astronomically broad emergence of bacterial **genes** and **plasmids** encoding tetracycline resistance.

Therefore, keeping in view of the stringent limitations imposed on the '**tetracyclines'** as a class has caused the researchers at the Lederle Laboratories to augment extensive and intensive studies to rediscover **SARs of tetracyclines** with strategical substitutions in the **aromatic ring 'D'** in a meaningful and sincere effort to lay hand on to certain newer breeds of tetracyclines that might give rise to such drug substances which are specifically effective against the resistant strains.*

The concerted efforts ultimately gave birth to a **few newer** tetracyclines as illustrated below :*



Examples :

(*a*) 9-(Dimethylglycylamino) minocycline : [DMG-MINO] ; $Z = N(CH_3)_2$;

(*b*) 9-(Dimethylglycylamino)-6-demethyl-6-deoxytetracycline [DMG-DMDOT] ; Z = H ;

Salient Features. The salient features of the '**glycylcyclines'** are as stated under :

(*i*) retain essentially both potency and broad spectrum profile as displayed by the '**parent tetracyclines'** against specifically the **tetracycline-sensitive microbial strains**, and

(*ii*) exhibit predominantly maximum activity against bacterial strains which show tetracycline resistance either through the ribosomal protecting determinants or afford mediation by efflux.

The future prospects of a possible '**second generation tetracyclines'** are almost written on the wall provided the meaningful and fruitful clinical trials of the ongoing **glycylcyclines** do emerge both favourable *pharmacokinetic* and *toxicological* profiles for such '**medicinal compounds'** in the near future.

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* Tally FT *et al. J Antimicrob. Chemother*, **35,** 449, 1995.