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CHAPTER 15

Antibiotics

INTRODUCTION

The term antibiotic has its origin in the word antibiosis (i.e. against life). Antibiotics are chemical substances obtained from various species of microorganisms (bacteria, fungi, actinomycetes) that suppress the growth of other microorganisms and eventually may destroy them. The probable points of difference amongst the antibiotics may be physical, chemical, pharmacological properties, antibacterial spectra, and mechanism of action. They have made it possible to cure diseases caused by bacteria, such as pneumonia, tuberculosis, and meningitis, and they save the lives of millions of people around the world.

CLASSIFICATION

Antibiotics are classified on the basis of their mechanism of action and by its chemical nature.

Classification Based on Mechanism of Action

1. *Agents that inhibit the synthesis of bacterial cell walls*: These include the penicillins and cephalosporins that are structurally similar and dissimilar agents, such as cycloserine, vancomycin, bacitracin and the imidazole antifungal agents.
2. *Agents that act directly on the cell membrane of the microorganisms, affecting permeability, and leading to leakage of intracellular compounds*: These include polymyxin, polyene antifungal agents, nystatin, and amphotericin B that bind to cell wall sterols.
3. *Agents that affect the function of 30s and 50s ribosomal subunits to cause reversible inhibition of protein synthesis*: These include tetracyclines, erythromycins, chloramphenicol, and clindamycin.
4. *Agents that bind to the 30s ribosomal subunit and alter protein synthesis*: These include aminoglycosides that leads to cell deaths eventually.
5. *Agents that affect nucleic acid metabolism*: Such as rifampicin, which inhibit DNA dependent RNA polymerase.

Classification Based on Chemical Structure

1. β-Lactam antibiotics
2. Aminoglycoside antibiotics
3. Tetracycline antibiotics
4. Polypeptide antibiotics

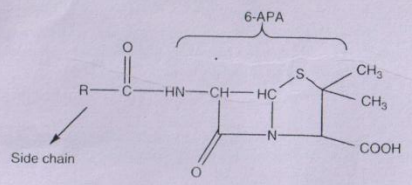
5. Macrolide antibiotics
6. Lincomycins
7. Other antibiotics

1. β-lactam antibiotics

These consists of two major class of agents, that is penicillins and cephalosporins.

a. Penicillins

Penicillin, the most important of the antibiotics, was first extracted from the mould *Penicillium notatum*. Subsequently, a mutant of a related mould, *P. chrysogenum*, was found to give the highest yield of penicillin and is employed for the commercial production of this antibiotic. Penicillin belongs to a group of antibiotics called β-lactam antibiotics . The basic structure of the penicillins consists of a thiazolidine ring fused with a β-lactam ring, which is essential for antibacterial activity. These two rings constitute the fundamental nucleus of all the penicillins, namely, 6-amino penicillanic-acid (6-APA) A variety of semisynthetic penicillins are produced by altering the composition of the side chain attached to 6-APA nucleus. Both the 6-APA nucleus and side chain are essential for the antibacterial activity.



Basic structure of penicillin

Nomenclature

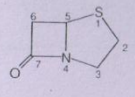
Penicillins are named in the following ways:

a. Chemical abstract

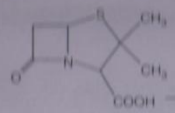
1. The penicillins are described as 4-thia-1-azabicyclo (3.2.0) heptanes.
2. Benzylpenicillin is 6-(2-phenylacetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabiclo(3.2.0)heptane-2-carboxylic acid.

b. Penam

In order to simplify the unsubstituted bicylic ring system of penicillin, it is given the name penam. Accordingly, the penicillins are 6-acylamino-2, 2-dimethyl penam-3-carboxylates.



c. Penicillanic acid derivatives



Penicillanic acid

Production of Penicillins

The strains of *Pseudomonas* species are allowed to grow in a nutrient medium obtained from sugary materials (e.g. glucose, sucrose, lactose, maltose, starch) and proteinaceous substance. From this medium, penicillin may be produced by any of the following three methods:

1. *Surface culture method:* An aqueous solution of molasses adjusted to pH 7–8 used as a medium for the microorganism because the molasses under this condition contain sucrose, mineral salts and nitrogenous material almost ideally suited for mould growth. In a few days time the growth of microorganism begins and after 6 or 7 days, the concentration of penicillin reaches up to 0.3–0.4 mg per cc.
2. *Bran method:* For mould growth, moist bran is a good substrate and the resultant penicillin can be extracted as in liquid production, or the penicillin-containing bran can be used directly. It is difficult to sterilize it, as the bran is a bad conductor of heat.
3. *Submerged culture method:* It is an exclusive method for the commercial production of penicillin. In this process, the inoculums are made into large tanks and the medium usually contains corn steep liquor as an adjuvant and lactose as sugar source. Under the conditions of agitation and aeration, the mould grows throughout the bulk of the liquid as globular pellicles consisting mainly of mycelium. Then temperature is maintained at about 24°C and the medium is agitated by a stream of sterile air.

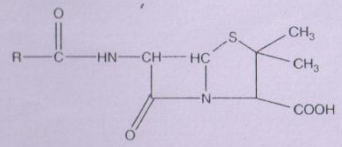
Isolation of Penicillins

The metabolic solution is rapidly cooled and adjusted to a low pH. Solvent extraction then follows. If amyl alcohol is used, the pH is reduced to 2–3, but if butyl alcohol is used, it is adjusted to 6.4 after adding ammonium sulphate. After adding petroleum ether to the cold solvent extract, it is shaken with dilute sodium bicarbonate solution. The solution is adjusted to pH 6–7 and then rapidly freeze evaporated to give the sodium salt. As the final product should be absolutely dry as well as pure, the sodium salt penicillin is dried in a high vacuum.

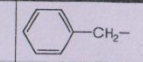
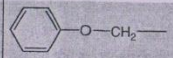
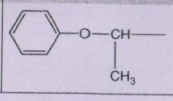
During all these operations, the penicillin solution must be maintained at a temperature just above its freezing point to avoid inactivation. Lastly, since most of the penicillin is administered by injection, the final sample must be nontoxic, sterilized and free from pyrogens for which the concentrated and purified solution of penicillin salt is passed through asbestos pads which absorb microorganisms and pyrogens.

Commercial penicillin consists of a mixture of benzyl, pentenyl and heptyl penicillins. By using certain chemical compounds in the culture medium, it is possible to increase the yield of the desired penicillin. For example, adding 2-phenylethylamine increases the yield of benzyl penicillin.

d. Classification



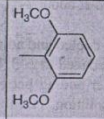
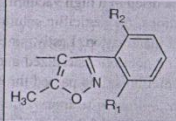
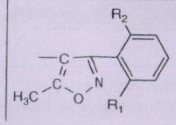
I. Penicillinase-susceptible penicillins

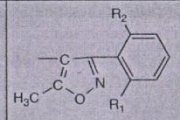
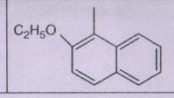
Name	Nature of Substituent (R)
Penicillin G (Benzyl penicillin)	
Penicillin V (Phenoxy methyl penicillin)	
Phenethicillin	

The general impact on antibacterial activity is as follows:

- Excellent gram-positive potency against susceptible *Staphylococci* and *Streptococci*
- Useful against some gram-positive *cocci*
- Good oral absorption, but relatively acid-labile
- Ineffective against gram-negative bacilli
- Susceptible to deactivation by penicillinase

II. Penicillinase-resistant penicillins

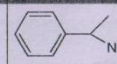
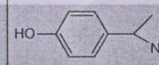
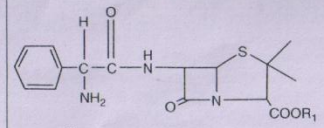
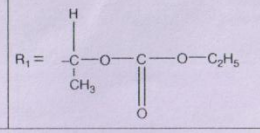
Name	R
(i) Methicillin	
(ii) Oxacillin (R_1=R_2=H)	
(iii) Cloxacillin (R_1=H, R_2=Cl)	
(iv) Dicloxacillin (R_1=, R_2=Cl)	

Name	R
(v) Floxacillin (R_1=F, R_2=Cl)	
(vi) Nafcillin	

General impact on antibacterial activity is as follows:

- Diminished susceptibility to many penicillinase.
- Active against microrganisms, resistant to early penicillin.
- Oxacillins offer good oral activity.
- Inadequate spectrum against many gram-negative species.

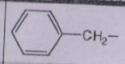
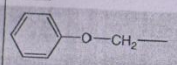
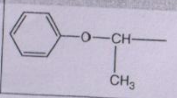
III. Aminopenicillins

Name	R
Ampicillin	
Amoxicillin	
Bacampicillin	 

General impact on antibacterial activity is as follows:

- Extended spectrum of activity against some gram-negative bacteria and retention of gram-positive potency
- Ineffective against *Pseudomonas aeruginosa*

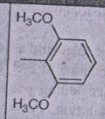
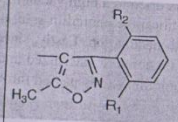
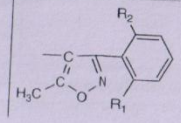
I. Penicillinase-susceptible penicillins

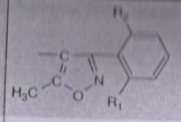
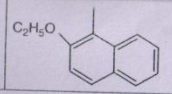
Name	Nature of Substituent (R)
Penicillin G (Benzyl penicillin)	
Penicillin V (Phenoxy methyl penicillin)	
Phenethicillin	

The general impact on antibacterial activity is as follows:

- Excellent gram-positive potency against susceptible *Staphylococci* and *Streptococci*
- Useful against some gram-positive *cocci*
- Good oral absorption, but relatively acid-labile
- Ineffective against gram-negative bacilli
- Susceptible to deactivation by penicillinase

II. Penicillinase-resistant penicillins

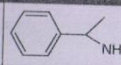
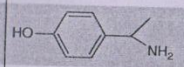
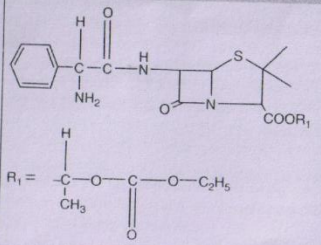
Name	R
(i) Methicillin	
(ii) Oxacillin (R_1=R_2=H)	
(iii) Cloxacillin (R_1=H, R_2=Cl)	
(iv) Dicloxacillin (R_1=, R_2=Cl)	

Name	R
(v) Flocaxillin (R_1=F, R_2=Cl)	
(vi) Nafcillin	

General impact on antibacterial activity is as follows:

- Diminished susceptibility to many penicillinase.
- Active against microorganisms, resistant to early penicillin.
- Oxacillins offer good oral activity.
- Inadequate spectrum against many gram-negative species.

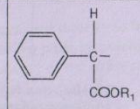
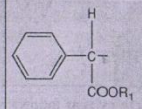
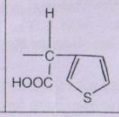
III. Aminopenicillins

Name	R
Ampicillin	
Amoxicillin	
Bacampicillin	

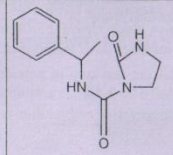
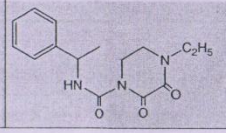
General impact on antibacterial activity is as follows:

- Extended spectrum of activity against some gram-negative bacteria and retention of gram-positive potency
- Ineffective against *Pseudomonas aeruginosa*

IV. Antipseudomonal penicillins (Carboxy Penicillins)

Name	R
Carbenicillin (R_1=H)	
Indanyl carbenicillin (R_1=5-indanol)	
Ticarcillin	

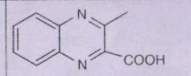
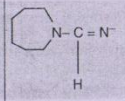
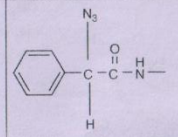
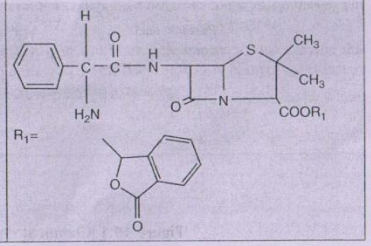
V. Ureidopenicillins

Name	R
Aziocillin	
Piperacillin	

General impact on antibacterial activity is as follows:

- Enhanced spectrum of activity against *P. aeruginosa* and expanded activity against *Klebsiella*.
- Good potency against gram-positive bacteria, but generally not effective against penicillinase producers.
- Good pharmacokinetic profile.
- Good activity *against Escherichia coli, Klebsiella, Shigella, Salmonella,* and many other resistant species.

VI. Miscellaneous penicillins

Name	R
Quinicillin	
Amidinopencillins (Mecillinam)	
Azidocillin	
Talampicillin	

The chemical degradation of penicillins is depicted in Figure 15.1

Inactivation of penicillins by acids, bases, and β-lactamases is as follows:

- The penicillins are very reactive due to the strained amide bond in the fused β-lactum of the nucleus.
- Penicillins undergo a complex series of reactions leading to a variety of inactive degradation products.
- They are extremely susceptible to nucleophilic attack by water or hydroxide ion to form the penicilloic acid. β-Lactamases also cleave the β-lactam ring to give penicilloic acid with a consequent loss of antibacterial activity.
- In strongly acidic solutions (pH < 3), penicillin is protonated at the β-lactam nitrogen, and this is followed by nucleophillic attack of the acyl oxygen atom on the β-lactam carbonyl carbon. The subsequent

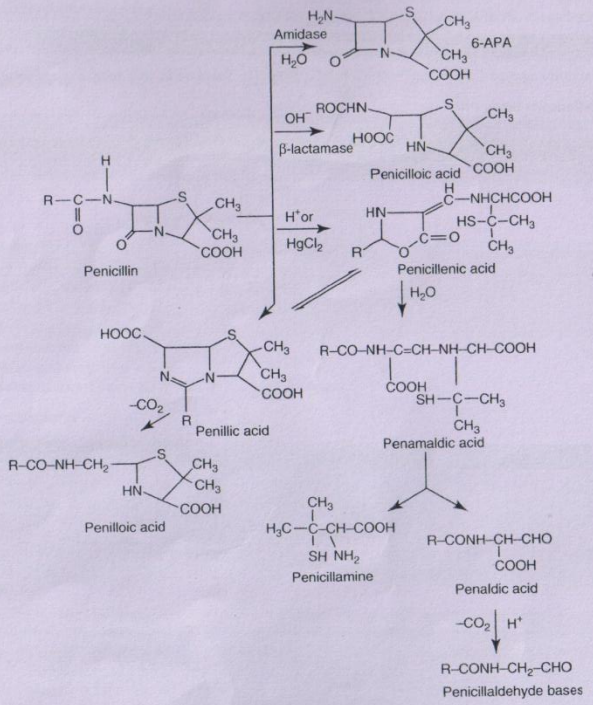


Figure 15.1 Chemical degradation of penicillins.

opening of the β-lactam ring destabilizes the thiazoline ring, which opens to form penicillenic acid that degrades into two major products penicillamine and penilloic acid. A third product, penicillaldehyde is also formed.

- Acid-catalyzed degradation in the stomach contributes in a major way to the poor oral absorption of penicillin. Thus, efforts to obtain penicillins with improved pharmacokinetic and microbiologic properties have sought to find acyl functionalities that would minimize sensitivity of the β-lactam ring to acid hydrolysis and at the same time, maintain antibacterial activity.
- Substitution of an electron-withdrawing group for the α-position of the benzyl penicillin has stabilized the penicillin to acid catalyzed hydrolysis. The increased stability imparted by such electron-withdrawing

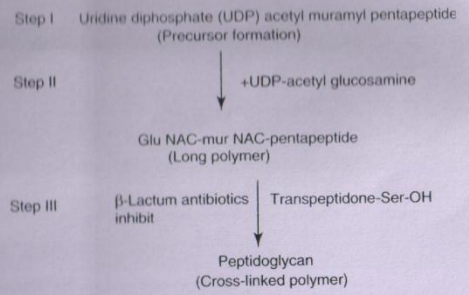


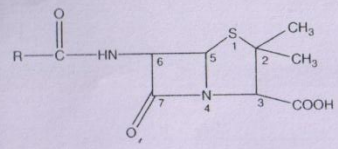
Figure 15.2 Stages involved in the biosynthesis of peptidoglycan.

groups has been attributed to a decrease in the reactivity of the side chain amide carbonyl oxygen atom towards participation in P-lactam ring opening to form the penicillenic acid.

Mode of action: The cell wall of bacteria is essential for the normal growth and development. Peptidoglycan is a heteropolymeric component of the cell wall that provides rigid mechanism for stability by virtue of its highly cross-linked lattice-wise structure. The peptidoglycan is composed of glycan chains, which are linear strands of two alternating amino sugars (*N*-acetyl glucosamine and *N*-acetylmuramic acid) that are cross-linked by peptide chains of an enzyme, transpeptidase. Penicillins inhibit the transpeptidase activity to the synthesis of cell walls. They also block cleavage of terminal D-alanine during the cell wall synthesis. The biosynthesis of peptidoglycan involves three stages (Fig. 15.2).

B-lactam antibiotics inhibit the last step in peptidoglycan synthesis. The transpeptidase enzyme that contains serine is probably acylated by β-lactam antibiotics with the cleavage of -CO-N-bond of the β-lactam ring. This renders the enzyme inoperative and inhibits peptidoglycan synthesis.

SAR of Penicillins



1. **6-Acyl side chain:** Benzyl penicillin has rather serious limitations, that is, narrow spectrum of activitywith acids and alkali degradation occurs and is susceptible to all known β-lactamase. The increased latitude in varying the acyl amino side chain through acylation of 6-APA resulted in more significant improvement in the biological properties of penicillins.
   a. Introduction of α-aryloxyalkyl penicillins in the side chain gives increased acid stability and oralabsorption. Other substitutents on the α-carbon of the side chain, such as amino (ampicillin), chloro, and guanidine display good resistance to inactivation by acids.

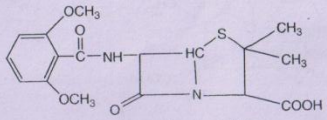
b. Substitution of the α-carbon atom of the side chain with bulky groups confers β-lactamase resistance. Examples: methicillin, nafcillin, oxacillin, etc.
In all these penicillins, an aromatic ring is attached directly to the side chain amide carbonyl, and there is substitution at both positions ortho to the point of attachment. The size of the ring systems plays an important role in determining the ability of the ortho substitutent to confer pen-icillinase resistance.
c. Introduction of an ionized or polar group into the α-position of the side chain in the benzyl carbon atom of penicillin-G confers against the gram-negative bacilli. Amino, hydroxyl, carboxyl,and sulphonyl increase gram-negative activity. Example: ampicillin and carbenicillin.
d. The D-isomer is 2–8 times more active than L-isomer in ampicillin.
e. When acyl side chain is replaced with hydroxymethyl groups, increased gram-negative activity isobserved.
f. *N*-acylated ampicillins (ureidopenicillins) have increased activity against P*seudomonas*.

2. Many esters of the carboxyl group attached to C-3 have been prepared as prodrugs to increase lipophilicity and acid stability. Example: Acetoxymethyl ester derivatives are used for preparing prodrugs.
3. Introduction of C-6 α-methoxy group confers greater stability against β-lactamase without signifi-cant loss of potency.
4. The sulphur of the thiazolidine ring can be replaced with O, CH_2, and CH-β-CH_3 with increased broad-spectrum antibacterial activity. The geminal dimethyl group at C-2 position is a characteristic of the penicillin.
5. In general, derivatization of the C-3 carboxylic acid functionality is not tolerated unless the free penicillin carboxylic acid can be generated *in vivo*. Doubly activated penicillin esters, undergo rapid cleavage *in vivo* to generate active penicillin. Example: pivampicillin and becampicillin.
6. The N-4 atoms at the ring junction are vital for antibacterial activity; the nitrogen atom contributes to the reactivity of the β-lactam carbonyl centre.

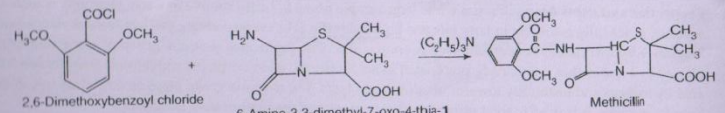
SYNTHESIS AND DRUG PROFILE

I. Penicillinase resistant penicillins

i. Methicillin



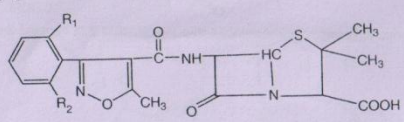
2,6-Dimethoxyphenyl penicillin



2,6-Dimethoxybenzoyl chloride + 6-Amino-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid → Methicillin

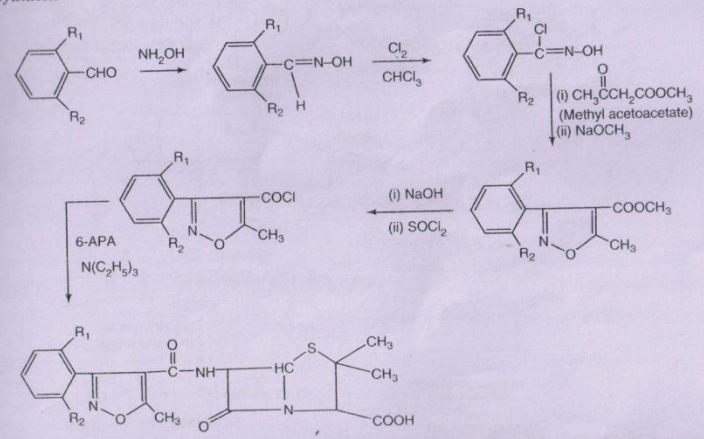
Properties and uses: Methicillin sodium is a white crystalline solid, odourless, soluble in water, slightly soluble in chloroform, but insoluble in ether. It is particularly resistant to inactivation by the penicillinase found in *Staphylococci* and somewhat more resistant than penicillin G to penicillinase from *Bacillus cereus*. Methicillin sodium has been introduced for use in the treatment of *Staphylococci* infections caused by the strains resistant to other penicillins. It is given by IM or by slow IV infusion every 4–6 h.

ii. Oxacillins (Isoxazolyl penicillins)



Properties and uses: Oxacillin sodium monohydrate is a white powder, soluble in water and methanol, insoluble in methylene chloride. The use of oxacillin and other isoxazolyl penicillins should be restricted to the treatment of infections caused by *Staphylococci* that are resistant to penicillin G, although their spectrum of activity is similar to that of penicillin G.

Synthesis

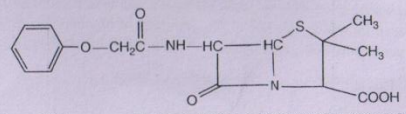


Name	R_1	R_2
Oxacillin	–H	–H
Cloxacillin	–H	–Cl
Dicloxacillin	–Cl	–Cl
Floxacillin	–F	–Cl

Assay: It is assayed by adopting liquid chromatography technique.

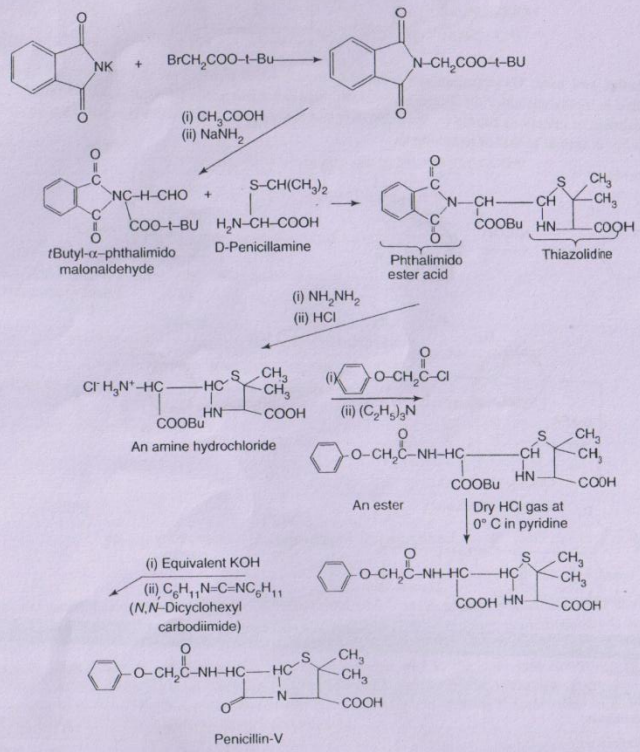
II. Penicillinase Susceptible Penicillins

i. Penicillin-V



3, 3-Dimethyl-7-oxo-6[(phenoxy acetylamino]-4-thia-1-azabiclo(3.2.0) heptane-2-carboxylic acid

Synthesis



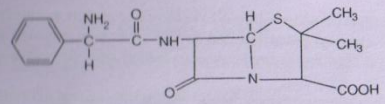
Properties and uses: Penicillin V is a white, odourless, crystalline powder with slightly bitter ta soluble in water. It is more resistant to inactivation by gastric juice than penicillin G and better absorbe the gastro intestinal (GI) tract. Equivalent oral doses provide two or five times greater plasma concen than penicillin G. Penicillin V is given to treat 'trench mouth'. It is useful in the treatment of streptoc pharyngitis, pneumonia, arthritis, meningitis, and endocarditis caused by *S. pyrogenes*.

Dose: Dose of penicillin V by oral route is 125–500 mg six times daily for 10 days. For prophylaxis rheumatic fever, the dose is 125–250 mg twice daily.

Assay: It is assayed by adopting liquid chromatography technique.

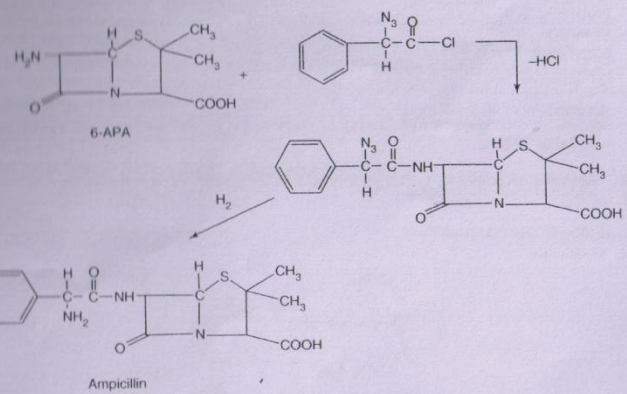
III. Amino penicillins

i. Ampicillin (Amcil, Omnipen)



6[D-α-Aminophenylacetamido] penicillanic acid

Synthesis



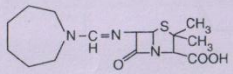
Properties and uses: Ampicillin is a white hygroscopic powder, freely soluble in water, sparingly soluble in acetone, practically insoluble in fatty oils and liquid paraffin. The corresponding product from acylation with 2-azido-4-hydroxyphenyl acetyl chloride is amoxicillin. The protonated α-amino group of ampicillin has a pKa of 7. 3 and is thus extensively protonated in acidic media, which explains ampicillin's stability towards acid hydrolysis and instability towards alkaline hydrolysis. The α-amino group plays an important role in the broader activity. It is used to treat urinary tract infections and respiratory tract infections.

and more than 2 kg, that is, 300 mg/kg in 3–4 divided doses, IV route is preferred for infants and children. Single dose more than 500 mg should not be given via IM injection.

Parenteral: For mild or uncomplicated infections: The adult dose as sodium is 100–125 mg/kg daily, the usual dose, if given via IV injection/infusion is 2 g every 6 or 8 h or 4 g every 12 h, if given via IM injection the dose is 2 g every 8 or 12 h. Prophylaxis of infection during surgery, for adults: as sodium the dose is 2 g just before the procedure or when the umbilical cord is clamped in caesarean section, followed by at least two doses of 2 g at intervals of 4 or 6 h within 24 h of procedure.

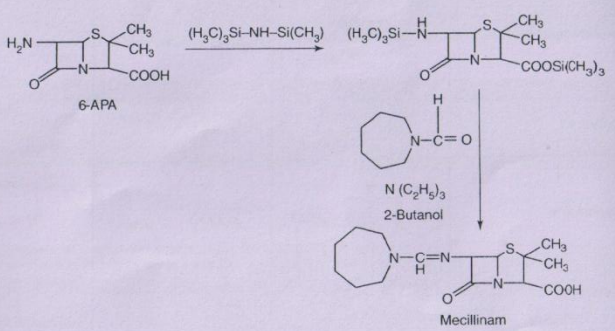
VI. Miscellaneous penicillins

i. Mecillinam (Amdinocillin)



6-β-(Hexahydro-1H-azepin-1-yl)-methylene aminopenicillonic acid

Synthesis



Mecillinam

Properties and uses: Mecillinam is particularly active against enterobacteria including some ampicillin resistant strains and to treat urinary tract infections. It is structurally different from other penicillins, in that, it is not an acylx derivative, but rather alkylidene amino-(amidino) derivative of 6-APA, due to this difference, it has significant gram-negative antibacterial activity as compared to gram-positive antibacterial activity.

Cephalosporins

The cephalosporins were isolated from the fungus *Cephalosporium acremonium* in 1945 by Pro Tzu, Newton, and Abraham (1953). The main product being cephalosporin-C, the molecular modification of cephalosporin-c gave origin to semisynthetic substances. They are β-lactam antibiotics with same fundamental structural requirements as penicillins, the main difference between the two is that cephalosporins

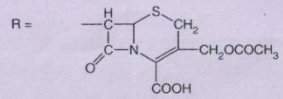
contain dihydrometathiazine ring, while penicillin contains a tetrahydrothiazole (thiazolidine) ring. The cephalosporins are much more acid stable than the corresponding penicillins and also have a mechanism of action similar to that of penicillins; they mainly inhibit the cross-linking of the peptidoglycan units in bacterial cell walls by inhibiting transpeptidase enzyme. However, they bind in the target proteins other than penicillins binding proteins.

Cephalosporins can be divided into three classes:

1. *Cephalosporin N*: It has a penicillin-like structure being a derivative of 6-aminopenicillanic acid.
2. *Cephalosporin P*: An acidic antibiotic, which is steroidal in nature.
3. *Cephalosporin-C*: It is a true cephalosporin and it is a derivative of 7 amino-cephalosporanic acid.

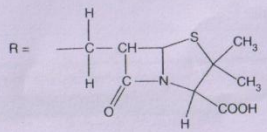
Generalized formula for cephalosporins

In cephalosporin C



Cepahlosporin C contains a side-chain derived from D-α-aminoadipic acid, which is attached to 7-amino-cephalosporanic acid.

In cephalosporin N



Pencillanic acid

A compound structurally similar to cephalosporin P is called *fusidic acid.*