Anti-biotics

Antibiotic is a chemical substance produced by M.O (Micro-organism) that has the capacity, in low concentration to inhibit selectively or even to destroy bacteria and other M.O. through an anti-metabolic mechanism.

M.O. producing A.B. called (Actinomycetes)

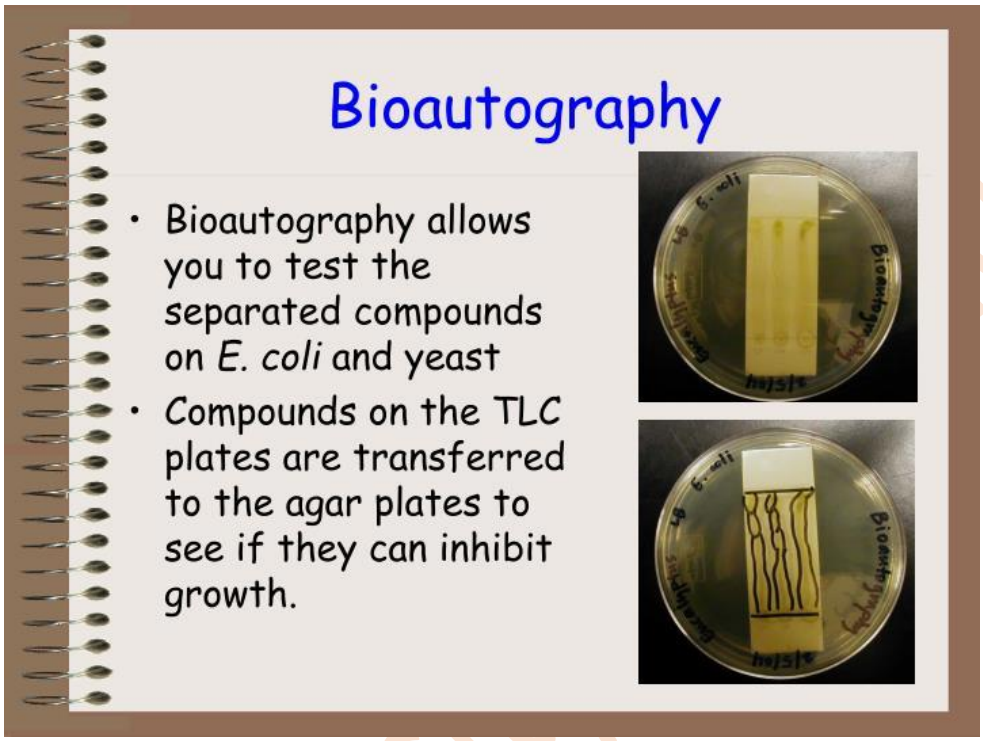
Screening for A.B.:

In searching for new A.B., relatively simple and rapid methods have seen developed for screening M.O., for A.B. producing ability soil sample are commonly employed in the screen because they are a rich source of A.B. producing organisms. A general method for screening first involves treating the soil sample with chemical that inhibit the growth of interfering bacteria and fungi but do not affect actinomycetes, cycloheximide is an as antifungal often employed for this purpose and 1:40 dilution of phenol is used as anti-bacterial agent.

Varying dilution of the treated soil sample are streaked on agar plates containing medium that supports the growth of actinomycetes. After incubation for 3-7 days at25-30C ° the plates are examined for characteristic colonies of actinomycetes, these colonies then transferred on to fresh medium contain pathogenic M.O. for indication of the potential usefulness of the A,B. for example activity against G+ve bacteria can be determined with *Staphylococcus aureus* or *Bacillus subtilis*, activity against G-ve bacteria can be determined with *E. coil* or *Salmonella typhi* and antifungal with *Neurospora crassa* .

The next step in the screening is to determine whether the chemical substance that produced the inhibition is a new A.B. or a known compound , a rapid method that has been developed for this determination is termed **(Bio auto-graphy assay)**

This assay employs paper or thin layer chromatography TLC and biologic assay. Extract containing the newly discovered A.B. is chromatographed along with reference in different solvent systems. Because each A.B. would possess a characteristic mobility on the chromatogram in a given solvent system, a comparison of the mobilities of the unknown A.B. with those of known one in several solvent system would indicate whether the newly discovered A.B. was a known compound.



The detection methods of the A.B. on the developed chromatogram

Chemical methods for detection is impossible and difficult because the A.B. are widely diverse chemically, consequently a biologic method is used to detect the A.B. by placing the developed chromatogram on an agar medium that has been seeded with an appropriate test organism, the anti-biotics diffuse from the chromatogram in to the agar and after incubation , clear zone on the agar owing to inhibition of growth of the test organism indicate the position of the A.B. on the chromatogram.

Commercial production of anti-biotics:

When a new A.B. has been discovered, investigation in to the chemical, physical, biologic properties of the A-B are required, the most important requirement for commercial production of A.B. is:

The organism must excrete the A.B. in to culture medium, however some anti-biotics such as those of the polyene group are retained in the cells of organism and required special extraction procedures for recovery which is very difficult and expensive.

Phases of anti-biotics production

In the production of A.B. there are two distinct phases in the fermentation process:

The growth phase of the organism which is termed (**Tropho phase**) & anti-biotics production phase **(Idio phase**)

Example: in a course of typical penicillin fermentation carried out in a culture medium containing glucose and lactose as a sources of carbon nutrition, corn steep liquor for nitrogen sources and phosphate buffer.

During the growth phase, the culture becomes thick owing to the formation of aggregates of fungal cells called (Mycelium), so the 1^{st} phase (tropho phase) lasts from the beginning of the culture period to approximately one day later (0-24 hr), during the growth phase glucose rather than lactose is preferentially utilized because it can be used directly as a source of carbon. In the growth phase process, ammonia is liberated by deamination of amino acid of the corn steep liquor, this liberation raise the PH of the medium to 7 which is the optimum PH for penicillin stability and buffer in the medium maintain the PH close to neutrality.

Penicillin production increase rapidly between 24-80 hr, at the start of A.B. production phase glucose has been used up and the fungus then uses lactose for carbon source but lactose cannot be utilized until its hydrolyzed to glucose and galactose so:

The decreased availability of carbon source is thought to be the triggering mechanism for penicillin production.

Factors that are often observed to have qualitative and quantitative importance for A.B. production:

- Sources of nutritional carbon and nitrogen
- Ratio of carbon to nitrogen nutrients (C/N)
- Mineral composition of medium
- Incubation temperature
- Initial PH & control of PH during the fermentation period
- Rate & method of aeration
- Addition & timing of addition of special growth and A.B. promoting substance

For example: some strain of Bacillus yield Bacitracin when C/N ratio is 15 , at lower ratio the yield is less& when the ratio is reduced to 6 another undesired A.B. is produced.

Another example: the use of mercaptothiazole in the culture of streptomyces which give tetracycline will favor chlortetracycline.

Classification of A.B.

According to the general mode of A.B. action:

1. inhibition of protein synthesis example: chloramphenicol, clindamycin, erythromycin, gentamycin, lincomycin, neomycin, tetracycline, streptomycin.

2. alteration in cellular membrane function exp. Amphotericin, nystatin, polymyxin.

3. inhibition of cell wall formation: penicillin, cephalosporin, vancomycin, bacitracin, cycloserine.

4. disruption of deoxy-ribonucleic acid metabolism: exp. Actinomycin, doxorubicin, mitomycin, rifampin, bleomycin, novobiocin.

According to the biosynthetic pathways:

1. A.B. derived from **amino acid** metabolism

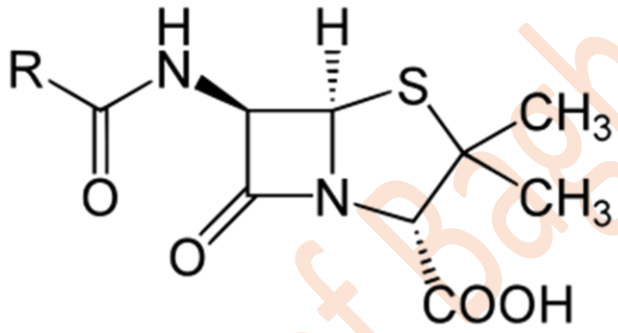
2. A.B. derived from **acetate** metabolism

3. A.B. derived from **CHO** metabolism.

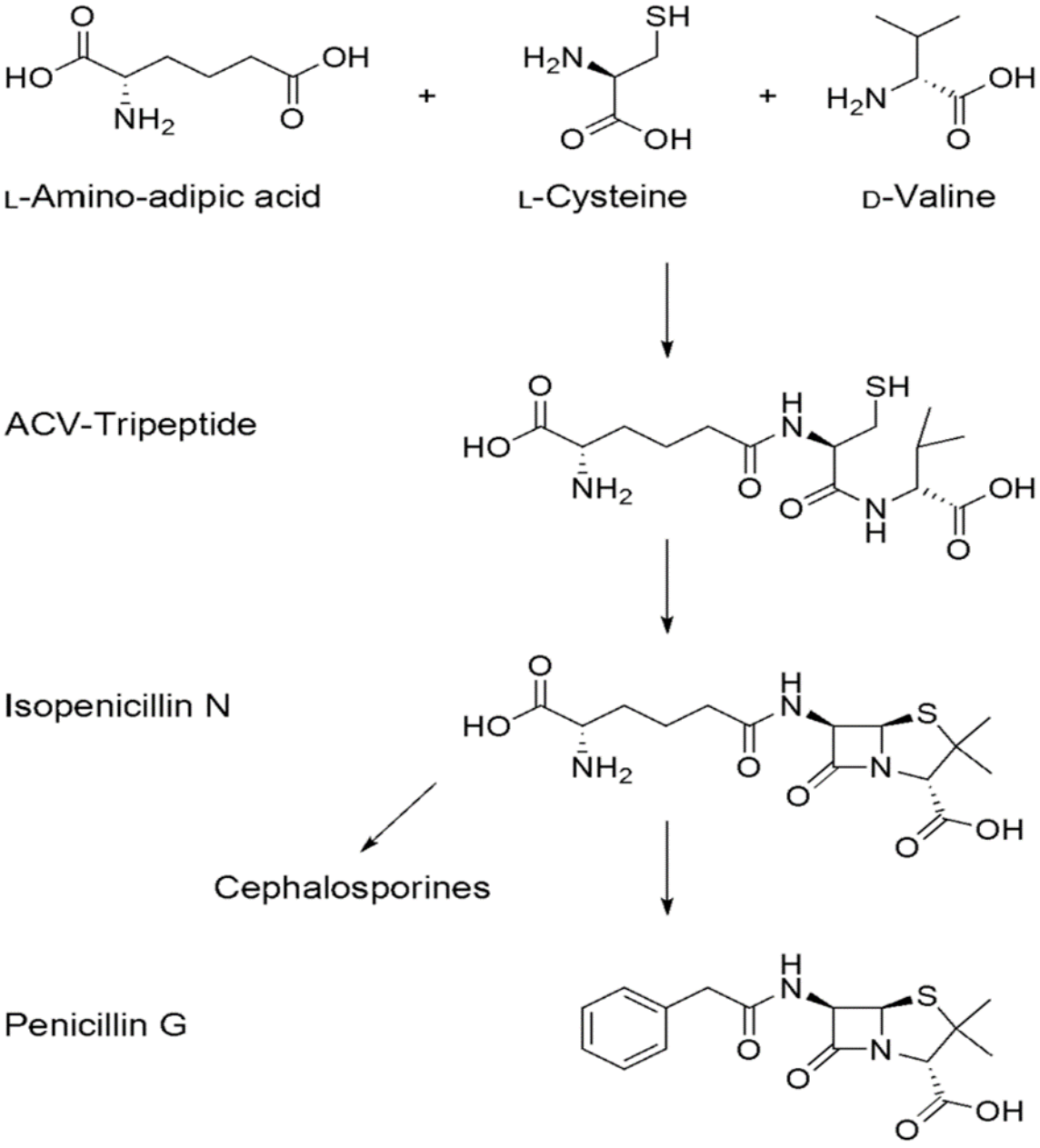
Antibiotics derived from aminoacids metabolisms:

Pencillins: Penicillin is a group of antibiotics which include penicillin G (intravenous use), penicillin V (oral use), procaine penicillin, and benzathine penicillin (intramuscular use). They are derived from *Penicillium* fungi. Penicillin antibiotics were among the first medications to be effective against many bacterial infections caused by staphylococci and streptococci. Penicillins are still widely used today, though many types of bacteria have developed resistance following extensive use. All penicillins are β-lactam antibiotics. About 10% of people report that they are allergic to penicillin.

Basic structure of Penicillin : (5member)thiazolidine+(4member)lactam ring.



Penicillin core structure, where "R" is the variable group.



Penicillin G biosynthesis

There are three main and important steps to the biosynthesis of penicillin G benzylpenicillin:

1. The first step is the condensation of three amino acids—-aminoadipic acid ,L-cysteine, L-valine into a tripeptide .Before condensing into the tripeptide, the amino acid L-valine must undergo epimerization to become D-valine. The condensed tripeptide is named- (L-α-aminoadipyl)-L-cysteine-D-valine (ACV). The condensation reaction and epimerization

are both catalyzed by the enzyme (L-α-aminoadipyl)-L-cysteine-D-valine synthetase (ACVS) .

2. The second step in the biosynthesis of penicillin G is the oxidative conversion of linear ACV into the bicyclic intermediate isopenicillin N by isopenicillin N synthase (IPNS), Isopenicillin N is a very weak intermediate, because it does not show strong antibiotic activity.
3. The final step is a transamidation by isopenicillin N N-acyltransferase, in which the α-aminoadipyl side-chain of isopenicillin N is removed and exchanged for a phenylacetyl side-chain. This reaction is encoded by the gene *penDE*, which is unique in the process of obtaining penicillins.

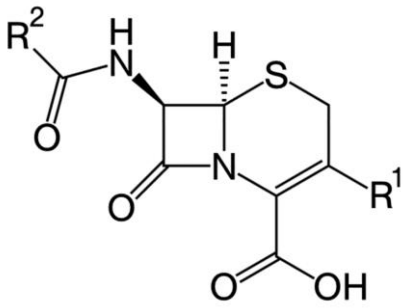
Developments from penicillin:

- The narrow range of treatable diseases or "spectrum of activity" of the penicillins, along with the poor activity of the orally active phenoxymethylpenicillin, led to the search for derivatives of penicillin that could treat a wider range of infections. The isolation of 6-APA, the nucleus of penicillin, allowed for the preparation of semisynthetic penicillins, with various improvements over benzylpenicillin (bioavailability, spectrum, stability, tolerance).

- The first major development was ampicillin in 1961. It offered a broader spectrum of activity than either of the original penicillins. Further development yielded β-lactamase-resistant penicillins, including flucloxacillin, dicloxacillin, and methicillin. These were significant for their activity against β-lactamase-producing bacterial species, but were ineffective against the methicillin-resistant *Staphylococcus aureus* (MRSA) strains that subsequently emerged.

- Another development of the line of true penicillins was the antipseudomonal penicillins, such as carbenicillin, ticarcillin, and piperacillin, useful for their activity against Gram-negative bacteria. However, the usefulness of the β-lactam ring was such that related antibiotics, the cephalosporins, still retain it at the center of their structures.

Cephalosporin: β-lactam+ dihydrothiazine



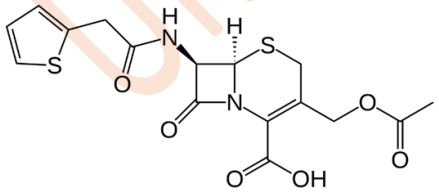
- The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus *Acremonium*, which was previously known as "*Cephalosporium*".

Classification:

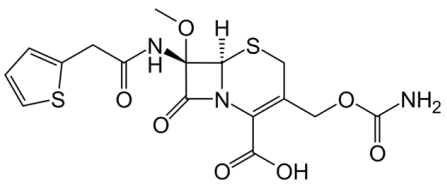
- The cephalosporin nucleus can be modified to gain different properties. Cephalosporins are sometimes grouped into "generations" by their antimicrobial properties. The first cephalosporins were designated first-generation cephalosporins, whereas, later, more extended-spectrum cephalosporins were classified as second-generation cephalosporins.

- Each newer generation has significantly greater Gram-negative antimicrobial properties than the preceding generation, in most cases with decreased activity against Gram-positive organisms. Fourth-generation cephalosporins, however, have true broad-spectrum activity.

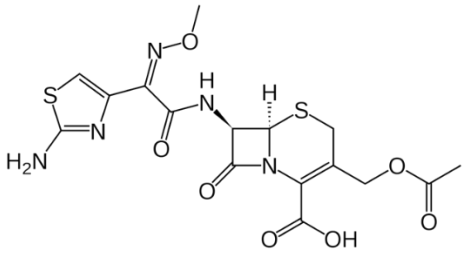
- 1. cephalothin.
- 2. cephoxitin.
- 3. cephotaxime.
- 4. cephepime.



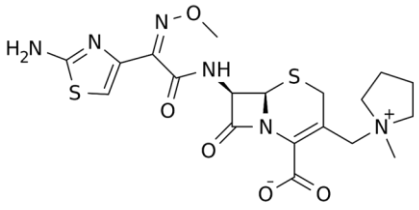
Cephalothin



cephoxitin

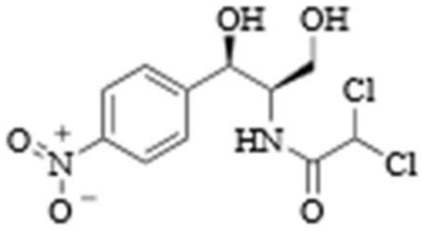


Cephotaxime



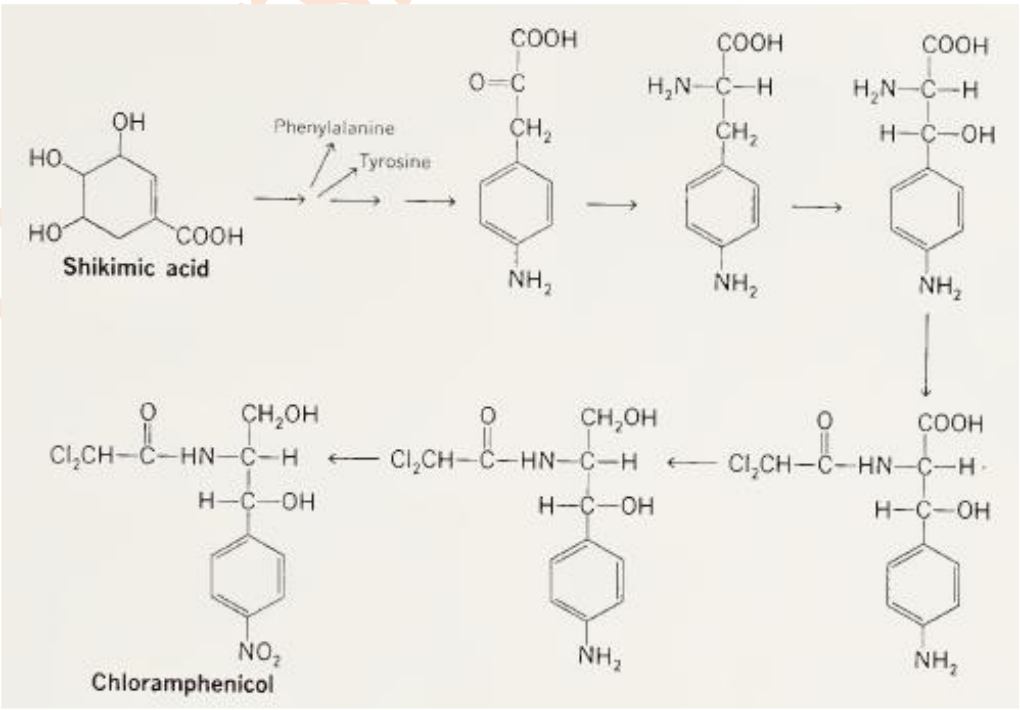
cephepime

Chloramphenicol:



Chloramphenicol is an antibiotic useful for the treatment of a number of bacterial infections. This includes meningitis, plague, cholera, and typhoid fever.

- Its use is only recommended when safer antibiotics cannot be used. Monitoring both blood levels of the medication and blood cell levels every two days is recommended during treatment. It is available intravenously, by mouth, and as an eye ointment. Common side effects include bone marrow suppression, nausea, and diarrhea. The bone marrow suppression may result in death.
- Biosynthesis of Chloramphenicol:



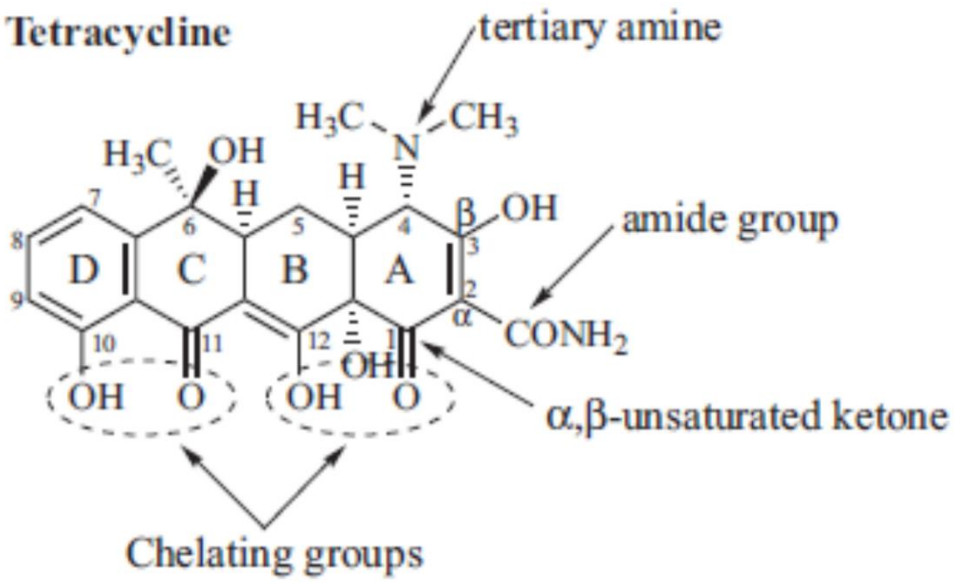
Chemically, chloramphenicol proved to be fairly simple. The most unusual feature was the presence of a nitro group on a normal biologic metabolite. The molecular skeleton of the antibiotic suggested a biosynthetic origin via phenylpropanoid metabolism. Experimental studies with radioactive precursors have confirmed a shikimic acid-phenylpropanoid pathway for the biosynthesis of chloramphenicol, but the pathway apparently branches from normal phenylpropanoid metabolism prior to the formation of phenylalanine or tyrosine. p-Aminophenylpyruvic acid has been suggested as an early metabolite in the biosynthetic pathway, and subsequent steps involving transamination, hydroxylation, acylation, reduction of the carboxyl group, and terminal oxidation of the amino group are suspected.

Polypeptide antibiotics:

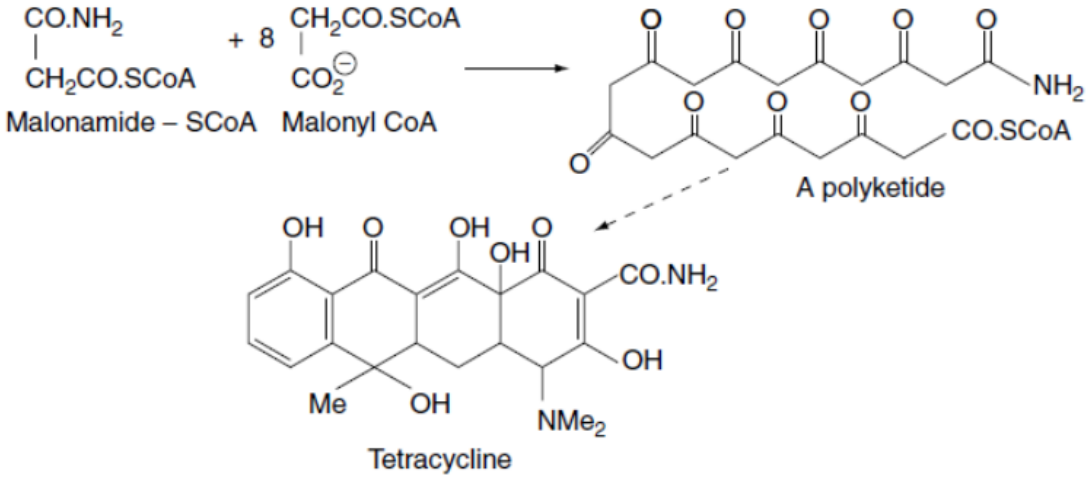
- **Polypeptide antibiotics** are a chemically diverse class of anti-infective and antitumor antibiotics containing non-protein polypeptide chains. Examples of this class include actinomycin, bacitracin, colistin, and polymyxin B. Actinomycin-D has found use in cancer chemotherapy. Most other polypeptide antibiotics are too toxic for systemic administration, but can safely be administered topically to the skin as an antiseptic for shallow cuts and abrasions.

Antibiotics derived from acetate metabolism:

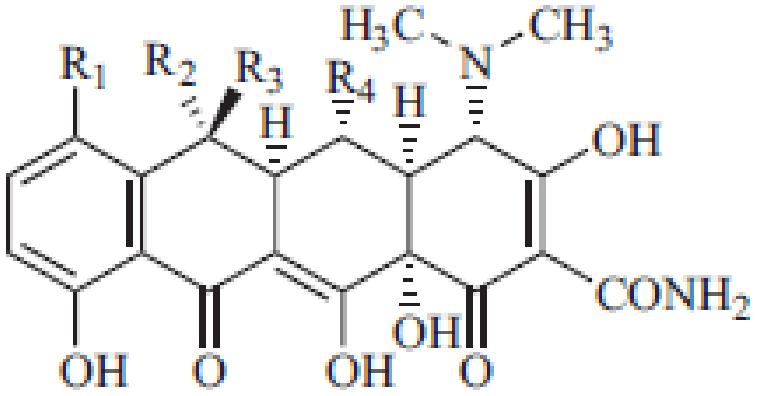
- **Tetracycline:**
- Tetracycline is an antibiotic used to treat a number of bacterial infections. It is commonly used to treat acne and rosacea. Historically it was important in reducing the number of deaths from cholera.
- It is broad-spectrum and of the polyketide class. It is produced by the *Streptomyces* genus of Actinobacteria. It is a protein synthesis inhibitor.



Although tetracycline has numerous functional groups, including a tertiary amine, hydroxyls, an amide, a phenolic hydroxy and keto groups, it is still possible to see that tetracycline is a member of the polyketide class of natural products by looking at the lower portion of the molecule. C10, C11, C12 and C1 are oxygenated, indicating that the precursor of this compound was a poly-b-keto ester. C10 and C11 and C12 and C1 form part of a chelating system that is essential for antibiotic activity and may readily chelate metal ions such as calcium, magnesium, iron or aluminum and become inactive. This is one of the reasons why oral formulations of the tetracycline antibiotics are never given with foodstuffs that are high in these ions e.g. Calcium in milk or with antacids which are high in cations such as Mg. This group of antibiotics has been long known and they have a very broad spectrum of activity against Gram-positive and Gram-negative bacteria, spirochetes, mycoplasmae, rickettsiae and chlamydiae. Tetracycline comes from mutants of Streptomyces aureofaciens, and the related analogue oxytetracycline from S. rimosus. These antibiotics are widely used as topical formulations for the treatment of acne, and as oral/injection preparations.



Biosynthesis of Tetracycline.



Tetracycline, $R_1 = H, R_2 = CH_3, R_3 = OH, R_4 = H$
Oxytetracycline, $R_1 = H, R_2 = CH_3, R_3 = OH, R_4 = OH$
Doxycycline, $R_1 = H, R_2 = H, R_3 = CH_3, R_4 = OH$
Minocycline, $R_1 = N(CH_3)_2, R_2 = H, R_3 = H, R_4 = H$

Minocycline and doxycycline are produced Semi synthetically from natural tetracyclines. Minocycline has a very broad spectrum of activity and has been recommended for the treatment of respiratory and urinary tract infections and as a prophylaxis for meningitis caused by Neisseria meningitides.

Doxycycline (Vibramycin) has use in treating chest infections caused by Mycoplasma and Chlamydia and has also been used prophylactically against malaria in regions where there is a high incidence of drug resistance.

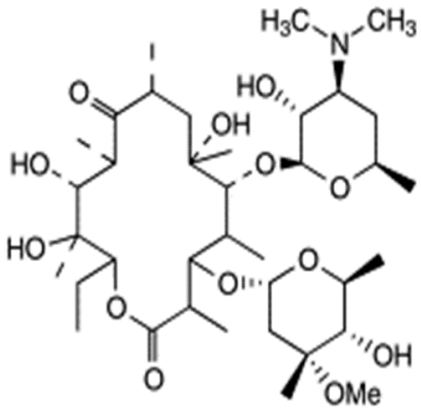
MACROLIDE ANTIBIOTICS

The macrolide antibiotics are characterized by a macrolactone ring which is glycosidically linked to one or more sugars. Biosynthetic studies have established that the macrolactone ring is formed by a condensation of acetate and/or propionate units, apparently via malonyl-CoA and 2-methylmalonyl-CoA. Methyl substituents on the lactone ring appear to be residual from incorporation of propionate units rather than the result of terminal biologic methylation.

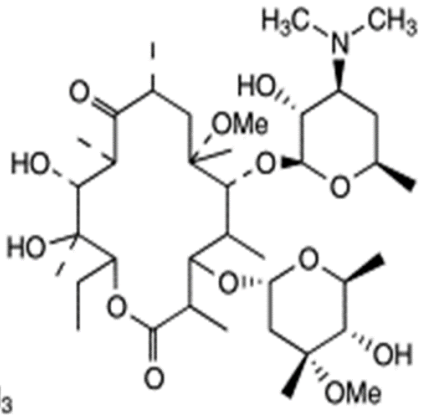
ERYTHROMYCIN A

Erythromycin A is a complex polyketide from Saccharopolyspora erythrea (Actinomycetes), which is a filamentous bacterium, originally classified in the genus Streptomyces. This compound is a member of the natural product class of macrolide antibiotics; these can contain 12 or more carbons in the main ring system. As can be seen from, erythromycin A has the best features of natural products, being highly chiral and having many different functional groups, including a sugar, an amino sugar, lactone, ketone and hydroxyl groups.

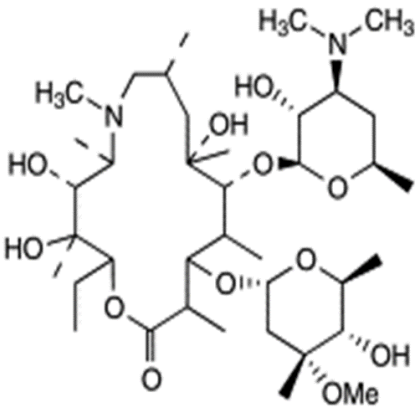
The therapeutic antibiotic is marketed as a mixture containing predominantly erythromycin A with small amounts of erythromycins B and C.



Erythromycin A



Clarithromycin



Azithromycin

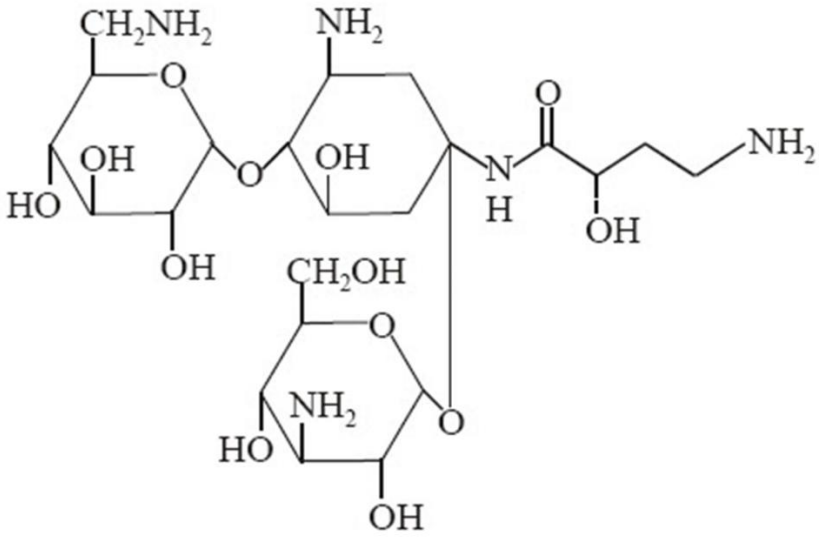
GRISEOFULVIN

Another polyketide antibiotic is griseofulvin (Grisovin) from the fungus (mould) Penicillium griseofulvum. This compound was originally isolated by researchers at the London School of Hygiene and Tropical Medicine.

Griseofulvin is a spiro compound; that is, it has two rings that are fused at one carbon. Initially, the compound was used to treat fungal infections in animals and plants, but it is now recommended for the systemic treatment of fungal dermatophytic infections of the skin, hair, nails and feet caused by fungi belonging to the genera Trichophyton, Epidermophyton and Microsporum. Its main use is in veterinary practice for the treatment of ringworm in animals; it is marketed as Fulcin and Grisovin.

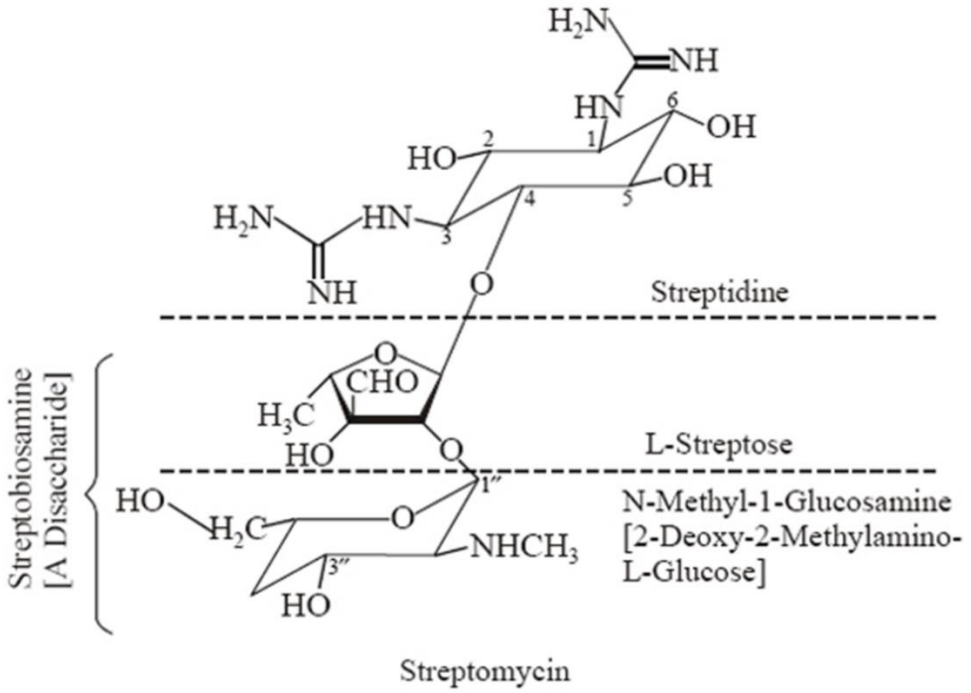
Antibiotic derived from carbohydrate metabolism:

- 1-**Amikacin** :is an aminoglycoside antibiotic used to treat different types of bacterial infections. Amikacin works by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth.

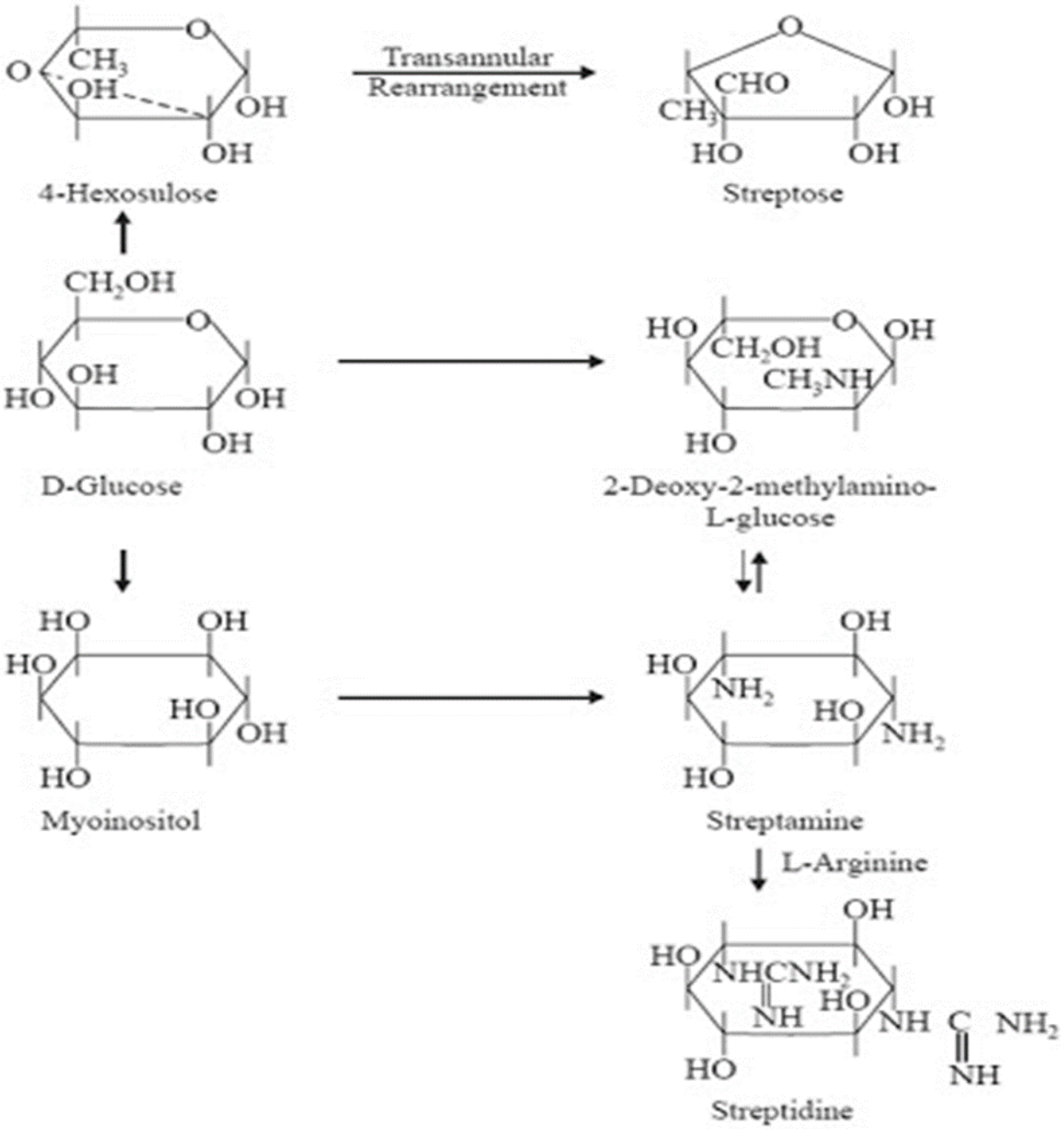


Amikacin

2- **Streptomycin:** is the first of a class of drugs called aminoglycosides to be discovered, and it was the first effective treatment for tuberculosis. It is derived from the actinobacterium *Streptomyces griseus*. Streptomycin is a bactericidal antibiotic. Adverse effects of this medicine are ototoxicity, nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis.



Streptomycin



Biosynthetic Pathway of Components of Streptomycin