PHARMACOGNESY III

FROM TEXTBOOKS:
(TREASE AND EVANS PHARMACOGNOSY, 16TH ED.)
PHARMACOGNESY AND PHARMACOBIOTECHNOLOGY, 9TH ED, ROBBERS JE, SPEEDIE MK, TYLER VE.)

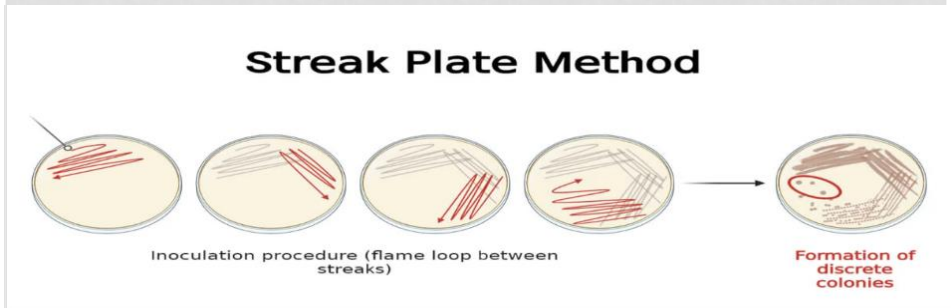
ANTIBIOTIC

- 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.

Actinomycets colony

- 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* .

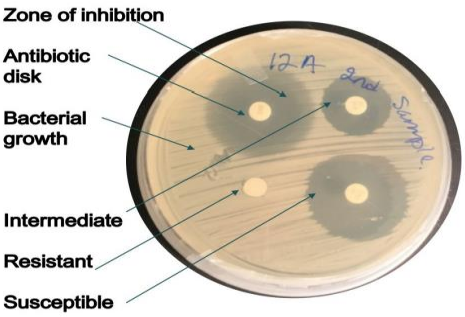
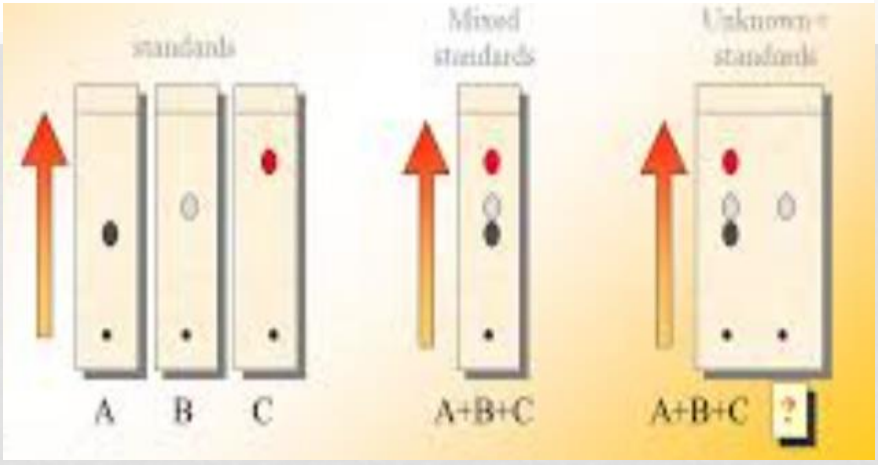


Fig. 1. An agar plate with antibiotic disks showing zone of inhibition (ZOI) and bacterial growth. Based on the comparisons of ZOI on the plate and the standard values, the bacteria tested were determined to be resistant, intermediate, and susceptible to different antibiotics present.

- 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) .

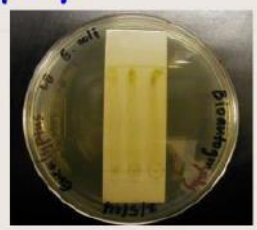
BIO AUTOGRAPHY ASSAY

- 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.



Bioautography

- Bioautography allows you to test the separated compounds on *E. coli* and yeast
- Compounds on the TLC plates are transferred to the agar plates to see if they can inhibit growth.



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: the course of typical penicillin fermentation is carried out in a culture medium containing:

1- Glucose and lactose as sources of carbon nutrition.

2- Corn steep liquor for nitrogen sources.

3- Phosphate buffer.

- During the growth phase, the culture becomes thick owing to the formation of aggregates of fungal cells called (Mycelium), so the 1st 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

1. Sources of nutritional carbon and nitrogen .
2. Ratio of carbon to nitrogen nutrients (C/N) .
3. Mineral composition of medium.
4. Incubation temperature .
5. Initial PH & control of PH during the fermentation period .
6. Rate & method of aeration.
7. 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.

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

1. Inhibition of protein synthesis:

For 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.

B- According to the biosynthetic pathways

a. A.B. derived from amino acid metabolism .

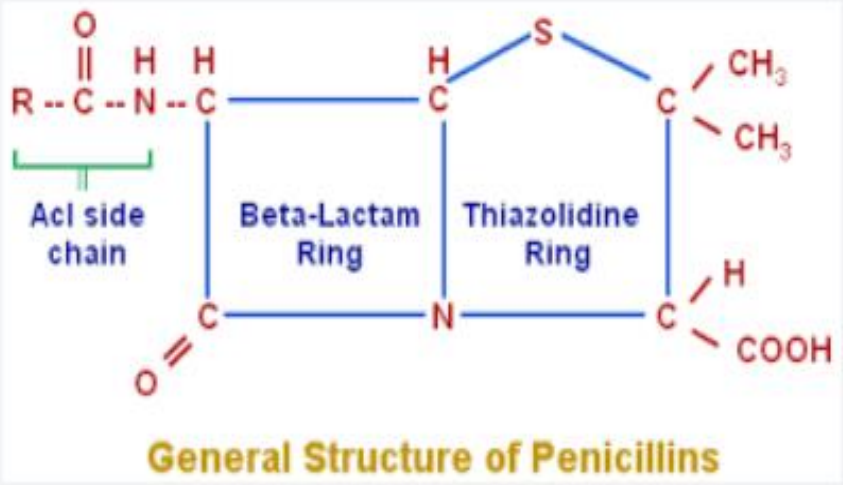
b. A.B. derived from acetate metabolism .

c. 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



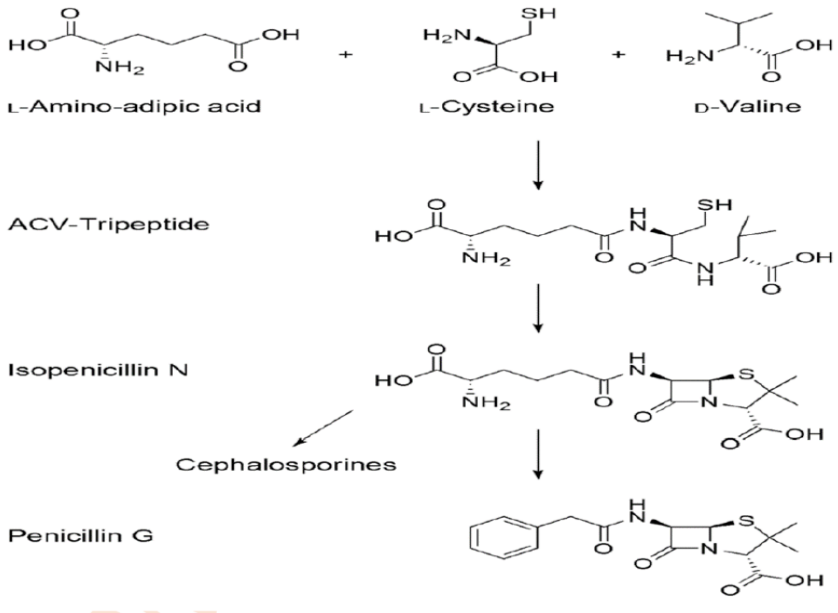
BIOSYNTHESIS OF PENICILLIN- G

- 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, D-valine into a tripeptide . The condensed tripeptide is named - (L-a-aminoadipyl)-L-cysteine-D-valine (ACV). The condensation reaction is catalyzed by the enzyme (L-a-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 a-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.



Penicillin G biosynthesis

DEVELOPMENTS FROM PENICILLIN

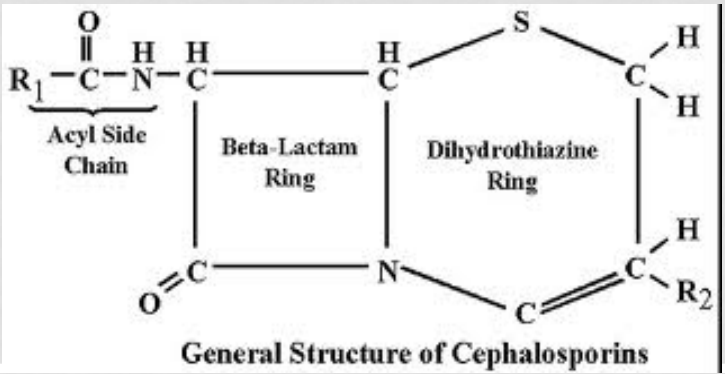
- The narrow range of treatable diseases or "spectrum of activity" of the penicillins, along with the poor activity of the orally active phenoxymethylpenicillin, led to the search for derivatives of penicillin that could treat a wider range of infections.
- The isolation of 6-APA, the nucleus of penicillin, 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.

CEPHALOSPORINES

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



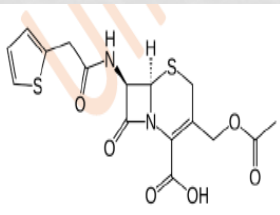
General Structure of Cephalosporins

CLASSIFICATION

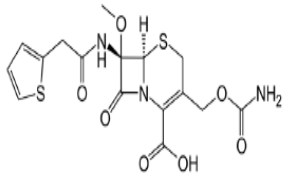
- The cephalosporin nucleus can be modified to gain different properties.
- Cephalosporins are sometimes grouped into "generations" by their antimicrobial properties. The first cephalosporins were designated first-generation cephalosporins, whereas, later, more extended-spectrum cephalosporins were classified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

EXAMPLES FOR DIFFERENT CEPHALOSPORINE GENERATIONS

1. Cephalothin.
2. Cephoxitin.
3. Cephotaxime.
4. Cephepime.



Cephalothin

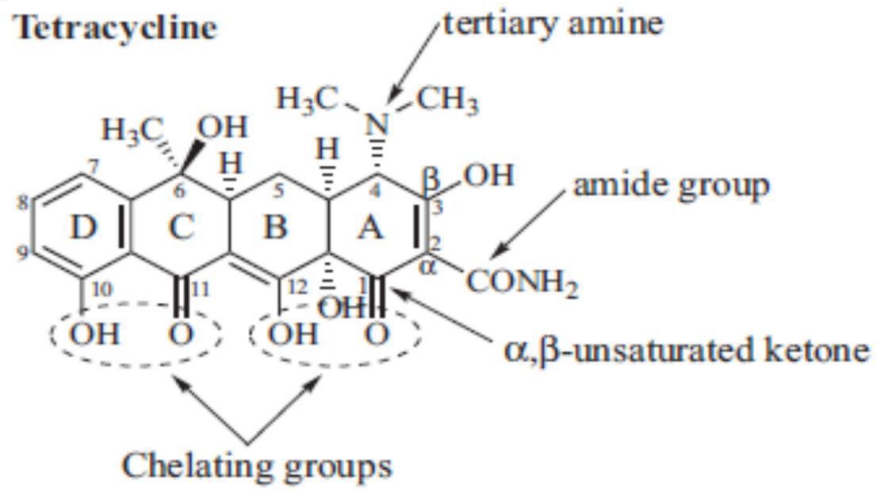


cephoxitin

ANTIBIOTICS DERIVED FROM ACETATE METABOLISM

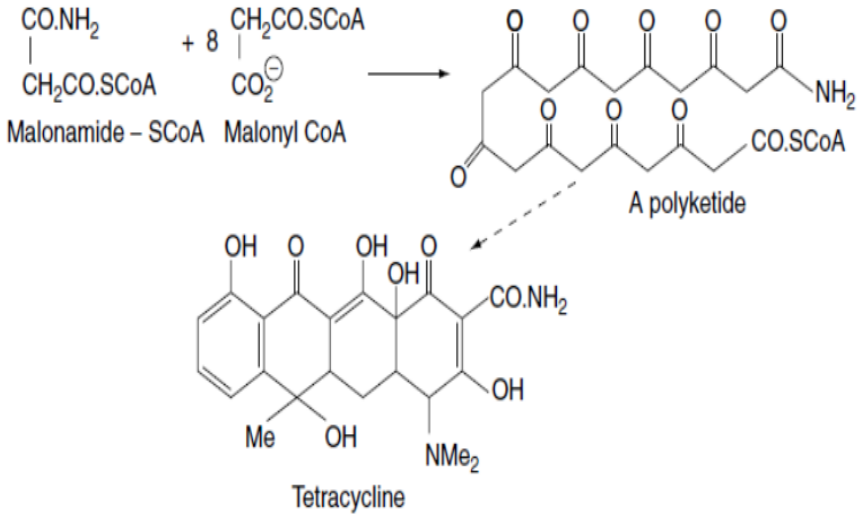
1- Tetracycline:

- 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.

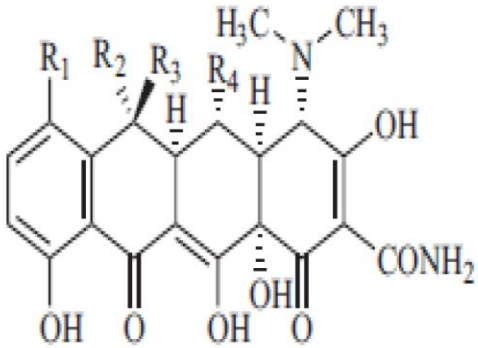


- Although tetracycline has numerous functional groups, including a tertiary amine, hydroxyls, an amide, a phenolic hydroxy and keto groups, it is still possible to see that tetracycline is a member of the polyketide class of natural products by looking at the lower portion of the molecule.
- C10, C11, 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.

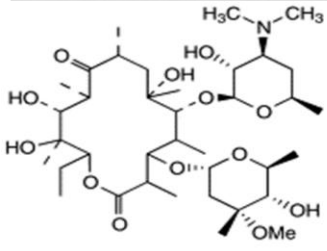
MACROLIDE ANTIBIOTICS

- 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.

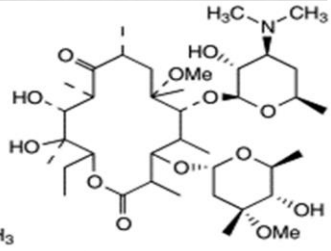
ERYTHROMYCIN A

- 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.

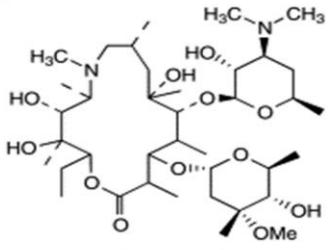
- The therapeutic antibiotic is marketed as a mixture containing predominantly erythromycin A with small amounts of erythromycins B and C.



Erythromycin A



Clarithromycin



Azithromycin

ANTIBIOTIC DERIVED FROM CARBOHYDRATE METABOLISM

- **Gentamicin:**

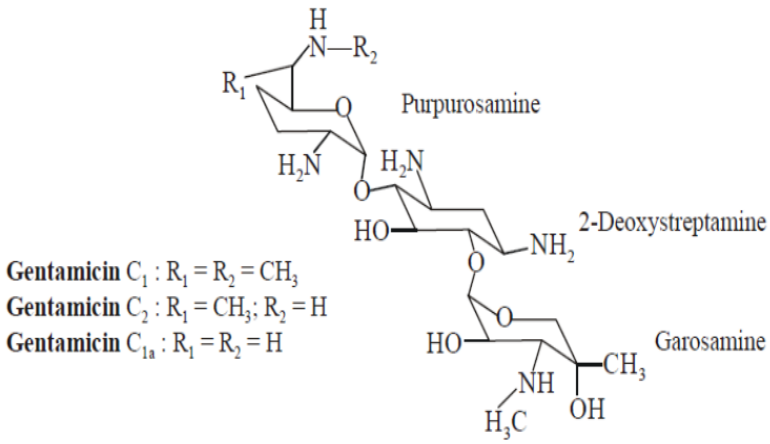
Is produced by *Micromonospora purpurea*, an actinomycete. The antibiotic mixture used in medicine consists primarily of gentamicin primarily of gentamicin C1, C1A and C2 with gentamicinC1 being the major component (approximately 60%).

- These antibiotic substances contain two aminosugar residues and a 2-deoxystreptamine unit.
- Gentamicin is inhibitory to pathogenic species of such enterobacteria as Enterobacter, Escherichia, and Klebsiella and to Proteus and Serratia species in lower concentrations (usual MIC, 1 to 2 µg per ml) than other aminoglycoside antibiotics exclusive of tobramycin.

- Gentamicin is available in formulations (0.1 and 0.3%) for topical use, but its principal use is parenteral for treatment of serious gram-negative infections caused by sensitive organisms.
- Resistance to gentamicin occurs, but cross-resistance with other aminoglycoside antibiotics is often absent in clinical situations; the lack of cross-resistance is presumably related to R-factor-induced inactivation involving specific chemical sites which are not found in the gentamicin molecule (e.g., inactivation by 15 adenylation or esterification of 3-hydroxyl function of a glucosamine moiety).

- **Gentamicin is available as the sulfate salt, and the usual adult dose is the equivalent of 1 mg of gentamicin per kg of body weight, intramuscularly or by intravenous infusion, three times a day.**

Chemical Structure



Gentamicin C_1 : $R_1 = R_2 = CH_3$
Gentamicin C_2 : $R_1 = CH_3$; $R_2 = H$
Gentamicin C_{1a} : $R_1 = R_2 = H$

Gentamicin


THANK
You