(Medicinal chem) Second yr

Sem III

Antibiotics

Introduction :

→ The term antibiotics has its origin in the word antibiosis (i.e. against life), the latter being used very first time by Vuillemin in 1889 in an attempt to describe the concept of survival of the fittest.

→ Among the many attempts to define the term 'antibiotic', the most appropriate one may be stated as ' An antibiotic is a chemical compound derived from or metabolically produced by micro-organisms and that in high dilution resists the growth or survival of one or more species of micro-organisms.

→ These agents are either bacteriostatic or bacteriocidal.

→ The Sci. Waksman proposed a wide defination as ' an antibiotic is produced by micro-organisms which has capacity of inhibiting the growth & even of destroying other micro-organisms.

Classification of Antibiotics :

- There are various ways by which antibiotics can be classified.
- The probable points of differences regarding chemical & pharmacological properties, antibacterial spectra & mechanism of action serve as the basis of classification :

1) Depending upon spectrum of antibacterial activity :

a) Narrow spectrum antibiotics :

These are the antibiotics that inhibit only certain groups of micro-organisms.

eg.

Nystatin, bacitracin, penicillin etc.

These antibiotics exhibit a high degree of selectivity.

b) Broad spectrum antibiotics :

These antibiotics inhibit both gram +ve & gram -ve bacteria & other intracellular organisms.

eg.

chloramphenicol, tetracyclines, cephalosporins etc.

2) Depending upon the sources from which antibiotics are derived :

These are of three types

a) Natural :

These antibiotics are naturally obtained from the large scale fermentation of micro-organisms.

eg.

bacitracin & polymixin ℟ obtained from some bacilli, streptomycin is obtained from streptomyces griseus.

b) Semisynthetic :

The observation that 6-aminopenicillanic acid (6-APA) can be obtained from cultures of P. chrysogenum led to development of this class.

eg.
During the Commercial production of benzyl penicillin, the phenyl acetic acid is added as side chain precurssor to medium in order to achieve predominance of the product.

c) Synthetic :
This class includes antibiotics which are purely Synthetic origin.
eg chloramphenicol

3) Depending upon the mode of Action :

a) Bacterial cell Wall Synthesis inhibitors :
These are the Drugs which interfers the cell Wall Synthesis in bacteria
eg. Penicillins, Cycloserin, bacitracin, cephalosporins etc.

b) Protein Synthesis inhibitors :
These are the drugs that interfere with protein biosynthesis in micro-organisms.
eg.
tetracyclin, chloramphenicol, lincomycins etc.

c) Bacterial Cell membrane inhibitors :
These are the drugs which inhibits the Synthesis functioning of cytoplasmic membrane of bacteria or fungi.
eg.
amphotericin, polymixins etc.

d) Nucleic acid Metabolism inhibitors :
These are the drugs which inhibits the metabolism of Nucleic acid.
eg.
Actinomycin, rifampicin, griseofulvin etc.

e) Drugs that antagonise the essential metabolic processes in micro-organisms include drugs trimethoprim, Sulphonamides & most of anticancer agents.

4) The general scheme of classification include following diff. categories of antibiotics :

a) β-lactum antibiotics
b) aminoglycoside antibiotics.
c) tetracycline antibiotics
d) peptide antibiotics
e) macrolide antibiotics
f) lincomycins antibiotics
g) unclassified antibiotics.

★ Bacterial Cell Wall Synthesis :

- The bacterial cell wall is about 10-25 µ, thick having porous, permeable structure which controls flow of solute to bacterial cell.
- The cell wall encloses cytoplasmic membrane which is 5-10 mµ thick having semipermeable nature.
- It consist of enzymes that involved in formation of cell wall.
- The cytoplasmic membrane encloses a thick, viscous jelly like transparent colourless material known as proto-plasm ~~is~~ Cytoplasm.
- Protoplasm consists of lipids, polysaccharides, nucleus, ribosomes etc.
- The main function of cytoplasm is to synthesize enzymes, proteins, necessary for functioning bacterial cell.
- chemically the bacterial cell wall composed of amino acids, lipids, carbohydrates, amino sugars etc.

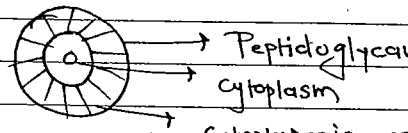


Fig. Bacterial cell wall of grampositive bacteria

- In grampositive bacteria cell wall composed of peptidoglycan. This peptidoglycan is made up of amino sugars & amino acids which is a spongy, gelforming layer consists of alternating sugars. (Amino acids, such as L-glysine, glycine, D & L-alanine amino sugars such as N-acetyl glucosamine, N-acetyl muramic acid etc)

→ The peptidoglycan is composed by glycan chains which are linear strands of alternating units of N-acetyl glucosamine & N-acetyl muramic acid

- These glycan chains then cross linked by peptide chains and compose the peptidoglycan.

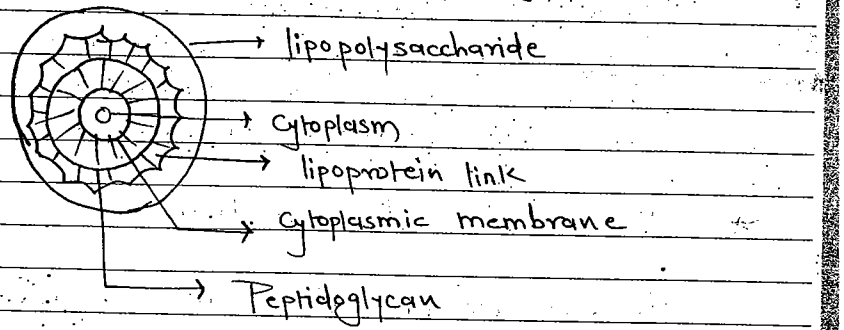


fig. Cell wall of gram negative bacteria

- The cell wall of gram negative bacteria is Complex.
- It Constituents large no. of amino acids. Also lipo-polysaccharides & lipoprotein.
- The bacterial cell wall is a rigid structure and offers protection to intracellular bacterial Components from mechanical damage & osmotic pressure change.
- The cell wall provides protection to the cytoplasmic membrane which is imp. site of most of biological processes of cell.
- Any defect in cell wall causes lysis (breaking) of Cytoplasmic membrane and also inhibits protein synthesis.
- Due to simplified they are highly gram positive bacteria they are highly susceptible to the action of β-lactum antibiotics due to freely permeable nature of cell wall.

- In case of Complex gram negative bacteria with semi-permeable or selective permiable nature of gram negative bacteria less susceptible to antibiotics
- The periplasmic fluid (between outer membrane and cytoplasmic membrane) and outer membrane are protected by other materials.
- Also the presence of β-lactamase enzymes in periplasmic fluid in high Concentration decreases energy of β-lactum antibiotics.

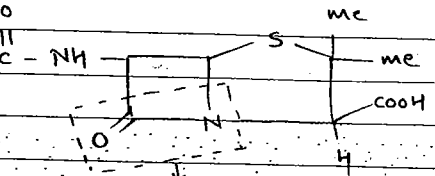
★ Mechanism of Action of Antibiotics :

following are the major routes through which antibiotics exe carries their antimicrobial activity.

a) Inhibition of Cell Wall Synthesis.
b) Inhibition of protein Synthesis.
c) Disorganisation of cytoplasmic membrane
d) Interference in nucleic acid biosynthesis
e) Inhibition of biosynthesis of tetrahydrofolate.

a) Inhibition of bacterial Cell Wall Synthesis.

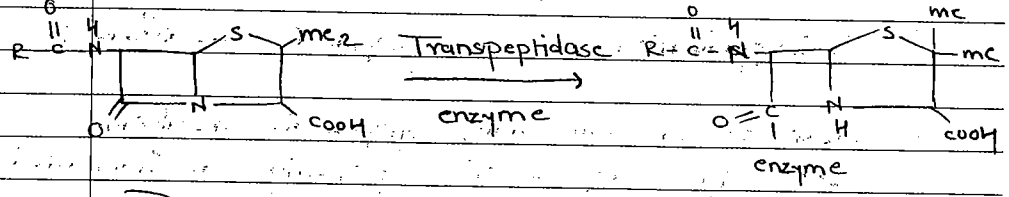
- The Cell Wall is essential for the growth and survival of bacteria.
- The rigid Stability of bacterial cell Wall is provided by a highly cross-linked lattice like structure Composed of peptidoglycan.
- There is a close structural Similarity between penicillins or other β-lactum antibiotics and the D-alanyl-D-alanine end of the polypeptide side chain of peptidoglycan.



β-lactum ring Penicillin

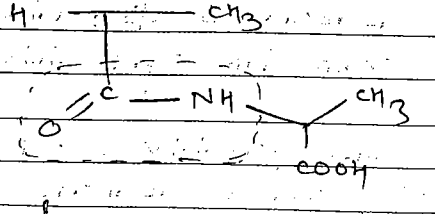
- The labile CO-N bond in β-lactum ring of penicillin lies in the Same position of peptide bond involved in transpeptidation (cross-linking of polypeptides).
- Due to this Similarity, antibiotics binds with trans-peptidase enzyme through Covalent bonding instead of D-alanyl-D-alanin end of polypeptide
- Thus enzymes necessary for transpeptidation reaction are occupied by antibiotic
- This irreversible inactivation of faulty & weak cell Wall Bacteria fail to divide.
- Some antibiotics may change the permeability of cell membrane leaving cell Wall undisturbed.
ex :- Novobiocin, nystatin etc.

Ex : Cephalosporines, Cycloserine, penicillins etc.



Penicillin Penicilloyl transpeptidase

Σ Reaction; Inactivation of transpeptidase enzyme (Complex irreversible nature)



b) Inhibition of bacterial protein Synthesis

- Protein biosynthesis is perhaps one of the important process provides spare parts known as peptides.
- These peptides assembeled as need of organisms in a proper sequence to biosynthesis various enzymes or nucleic acids.
- The imp. events in the protein biosynthesis can be outlined as
  1) Amino acid activation
  2) Formation of aminoacyl - t-RNA
  3) Peptide bond formation
  4) Translocation
- The antibacterial activity results due to attack of drug on one or more of the above events occuring on the ribosomal (r-RNA) Surface.

eg.
Tetracyclines, chloramphenicol, erythromycin, lincomycin, macrolides etc.

c) Disorganisation of the Cytoplasmic membrane:

- Next to the cell Wall, Cytoplasmic membrane Serves the purpose of protecting the vital bacterial cell Constituents from damage.
- The fungal membrane in fungi Contains sterol is eraosterol.
- If this membrane is disorganised due to any reason it results into rapid killing of micro-organisms.
- Antibiotics like polymixins, may damage the integrity of cytoplasm membrane by disorenting the lipophilic groups in membrane which leads to leakage of intracellular Components
- Amphotericin B & nystatin have high affinity for sterols present in fungal membrane shows potent antifungal activity.
- They Combine with membrane sterols & create pores or channels in fungal membrane.

d) Interference in the bacterial nucleic acid Synthesis

- The nucleic acids (DNA/RNA) are ingredients of microbial cell.
- DNA govern both the quality & quantity of RNA Synthesis while RNA is a key instructor that read out message on which Synthesis of various proteins & enzymes, necessary for overall growth of micro-organism depends.
- RNA molecules Synthesized by polymerisation of ribonucleoside triphosphate under the influence of DNA dependent RNA polymerase enzyme. Thus DNA template acts a platform for transcription
- Hence the sequence of peptides in RNA being Synthesized is nothing but copy of sequence in that particular DNA template.
- Antibiotics may affect Nucleic acid biosynthesis in two diff. ways :-
  1) By interacting Specifically with enzyme (DDRP) which catalyses the polymerisation.
  2) By interacting with DNA template & disturbing whole process.

- Rifampicin is an antibiotic acting through first mechanism.
- It doesn't interfere in chain elongation & termination because when it is added to culture medium where RNA being synthesized, partly formed.
- RNA continue the process till completion, but it inhibits initiation because it stops formation of new RNA chains.
- Actinomycin is an example acting through second mechanism.
- It is unselective in action affects both host cells & bacteria.

e) Inhibition of the tetrahydrofolate biosynthesis:

- In both cells & micro-organisms the tetrahydrofolate serves as an essential co-factor in the transfer & reduction of 1-carbon fragment and for production of nucleic acid.
- PABA is an essential metabolite in bacterial cell acts as precursor of folic acid.
- Due to structural similarities with PABA, the Sulphonamides interferes with reaⁿ between ~~pteridin~~ pteridine pyrophosphate intermediate & PABA.
- Trimethoprim selectively attacks dihydrofolate ~~reduction~~ reductase enzyme that catalyses the reduction of dihydrofolate to tetrahydrofolate product.
- Tetrahydrofolate is a co-factor needed for the synthesis of thymidine monophosphate, which is required for the synthesis of DNA & RNA

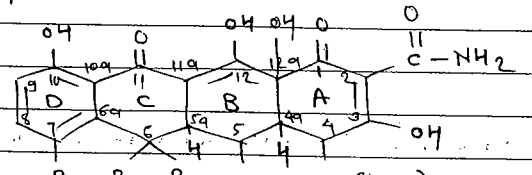
★ Protein Synthesis inhibitors

The drugs that interfer in protein biosynthesis in micro-organisms called as protein synthesis inhibitors.

1) Tetracyclines

- It is found that penicillins are active against gram positive bacteria whereas the streptomycin family are inactive against gram negative bacteria.
- Tetracyclines are obtained from soil actinomycetes.
- They are truely broad spectrum antibiotics which affects the broad range of micro-organisms i.e. gram +ve as well as gram -ve bacteria & some other micro-organisms.
- All tetracyclines are solid bitter in taste & weak water soluble antibiotics.
- The first tetracycline introduced was chlortetracycline in 1947, obtained from S. aureofaciens. After 2 years oxytetracycline was produced in 1949 from mutant strain, Streptomyces rimosus.

Structure :



Tetracycline : $R_1 = H$, $R_2 = OH$, $R_3 = CH_3$, $R_4 = H$

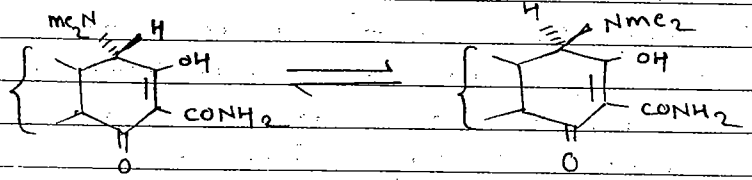
chlortetracycline : $R_1 = H$, $R_2 = OH$, $R_3 = CH_3$, $R_4 = Cl$

oxytetracycline : $R_1 = R_2 = OH$, $R_3 = CH_3$, $R_4 = H$

- These are amphoteric in nature.
- Though these are not Completely absorbed through GIT till Well known for their oral use.
- Basic Nucleus of tetracycline is poly+ polycyclic naphthacenecarboxamide.

SAR :

1) The tetracyclic backbone skeleton is essential for activity.
2) The enolised System from C_1 to C_3 must be intact for good activity.
3) The amide function at C_2 must remain unmodified in order to retain activity, should not be removed.
4) Epimerisation of C_{5a} & C_4 causes loss of activity i.e. epitetracyclin are very much less active than Neutral isomer.



Neutral Epimer

5) Conversion of Carboxamide to nitriles causes a 20 fold loss of activity. Though Some Carboxamide methylamine are highly active.
6) Substitution at C_6 decreases clinical stability
ex: oxytetracyclin less stable than

7) Substitution at C_7 position increases potency of drug both $e^{\ominus}$ releasing & $e^{\ominus}$ withdrawing substituents gives analogs with antibacterial activity
eg.
chlortetracyclin has -Cl at C_7 position ($e^{\ominus}$ withdrawing & Minocyclin has $-NMe_2$ at C_7 position (EDG) but both are active enough.

8) cis A/B fusion with α-OH group at 12 a position necessary for retention of activity.

9) Substitution at 5, 6, 7 & 9, product with retention of activity in Some cases with improved activity. In some cases with improved activity. Also removal of these doesn't drastically alters activity.

10) 6-thiatetracyclin, thiacyclin these are more active with Superior activity.

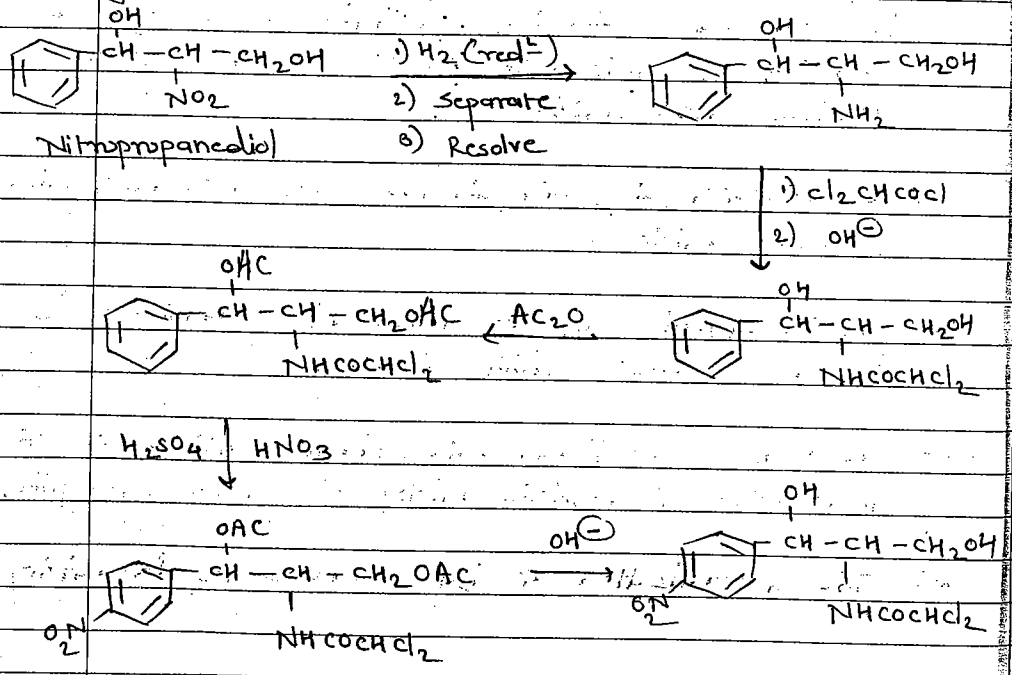
11) Aminoalkylation at Carboxamide using mannich base increases Water Solubility of drug.

12) Removal of 4-dimethylamino function affords loss of about 75% of antibiotic activity of parent tetracyclin.

13) The aromatization at ring C causes loss in activity.

★ Synthesis of chloramphenicol :

- chloramphenicol is synthesized by nitro-propanediol. This is reduced catalytically to the mixture of aminodiols.
- The threeisomer is then seperated by crystallization and resolved as a diasteromeric salt to give the D(-) isomer. on acylation with dichloroacetyl chloride it initially gives the triacetate.
- Its saponification gives a new product. The free hydroxyl groups are then Converted to the acetates by means of acetic anhydride & the resulting product is then nitrated with nitric Sulphuric acid mix.
- Its Saponification removes the acetate protecting groups & affords chloramphenicol.



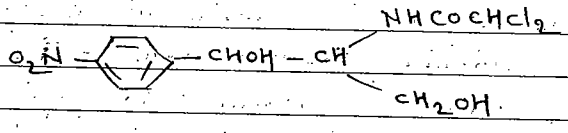
chloramphenicol.

→ The chloramphenicol is obtained From culture of streptomyces Venezuleae.

→ It is a drug of choice, in the treatment of typhoid fever.

SAR of chloramphenicol :

In an attempt to establish a relationship between the structure of chloramphenicol & its biological activity Several Compounds have been synthesized & following observations made :



1) Replacement of $-NO_2$ group with no. of other substituents like $-CN$, $-CONH_2$, $-SO_2$, $-NHR$, $-NH_2$, $-OH$, $-NMe_2$, $-NHCH_2R$, Br, I, Cl, F, C_6H_5 or various heterocyclic groups resulted in some reduction or Complete loss of antibacterial activity.

2) Shifting of $-NO_2$ group From para position reduces antibacterial activity

3) When phenyl group replaced by other aromatic or alicyclic groups like napthalene, pyridyl, quinolyl, thienyl, furyl, cyclohexyl etc. derivative obtained are less potent than chloramphenicol..

4) Pri. alcoholic group required since replacement with -H or -R or ester leads to Comp. which are much less potent.

5) ~~The dichloroacetyl group is the~~

5) Of the four stereoisomers of chloramphenicol the anti-bacterial activity resides in only D-threo Compound. other isomers are inactive Compound.

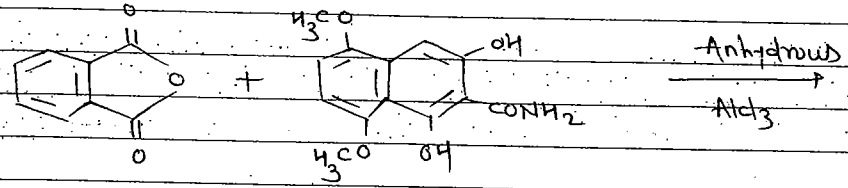
Mode of Action :

- Chloramphinicol is a broad-Spectrum antibiotic shows action through inhibiting protein biosynthesis of bacterial cell.
- It binds to the 50S subunit of ribosomes & appear to act by inhibiting the movement of ribosomes along mRNA probably by inhibiting the peptidyl transferase reaction by which the peptide chain is extended.
- Most probably, by acting as a peptide analogue, it prevents formation of peptide bonds.
- At high doses, chloramphenicol may inhibit the mammalian mitochondrial protein synthesis.

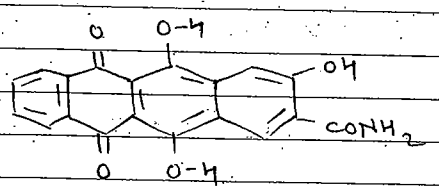
Uses :

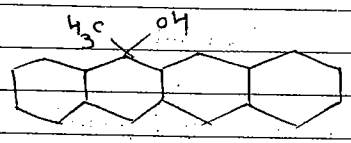
- It is a drug of choice for typhoid fever.
- It is also used in intraocular infections, H. influezae meningitis, anaerobic infections caused by Bact. fragilis & others.
- It is highly effective topically. eg. in external ear infections.
- It has been extensively used in urinary tract infections.

★ Synthesis of chlortetracycline :



phthalic anhydride 1,3-dihydroxy-5,8-methoxy naphthalene-2-carboxamide



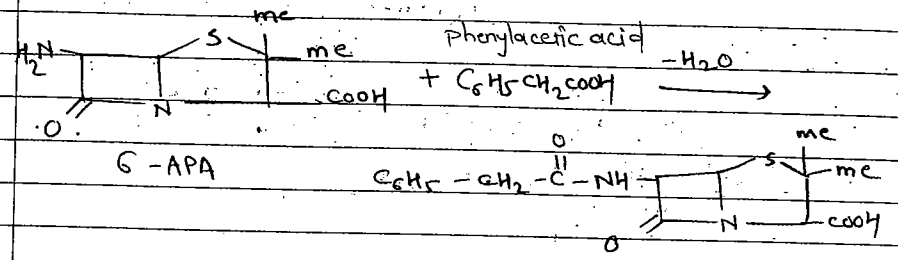


★ Cell Wall Synthesis Inhibitors :

★ Synthesis of Penicillin G :

Penicillin G, it is also called as benzyl penicillin

1) Synthesis from 6-aminopenicillanic acid (6 APA)



Penicillin G

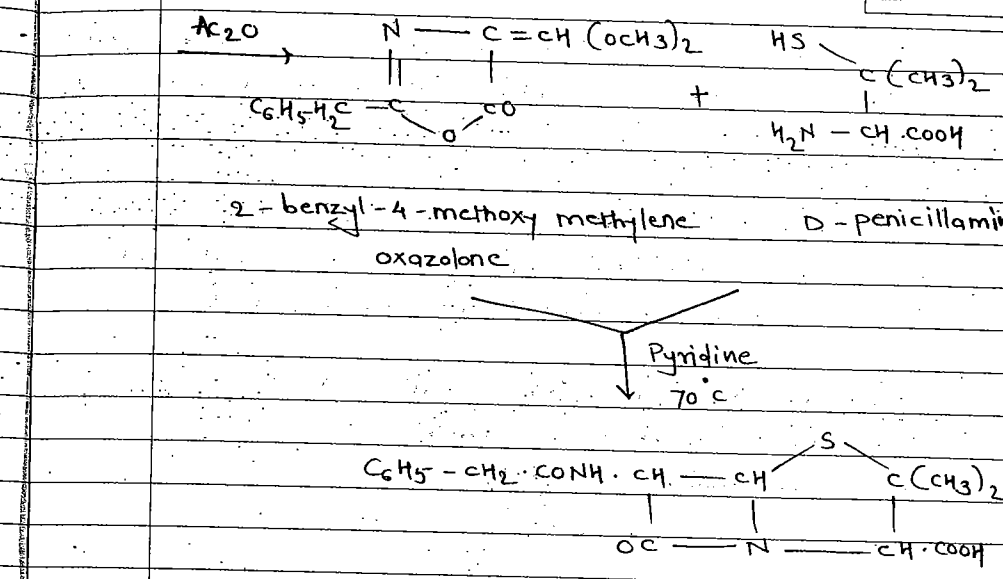
2) Penicillin-G is obtained in small quantities by condensing D-penicillamine with 2-benzyl-4-methoxy methylene oxazolone, in pyridine at 70°c. The benzyl penicillin has isolated as the crystalline triethyl amine salt. The starting material has prepared by methyl phenaceturate in the following way:

$C_6H_5CH_2\cdot CO\cdot NH\cdot CH_2\cdot COOCH_3 \xrightarrow{HCOOCH_3} C_6H_5-CH_2\cdot CONH\cdot CH\cdot CHO$ (CHO on CH, $COOCH_3$ on CH)

Methyl phenaceturate

$\downarrow CH_3OH$, HCl

$C_6H_5-CH_2\cdot CONH\cdot CH(COOCH_3)\cdot CH(OCH_3)_2 \xleftarrow{OH^-} C_6H_5CH_2\cdot CONH\cdot CH(COOH)\cdot CH(OCH_3)_2$



2-benzyl-4-methoxy methylene oxazolone

D-penicillamine

Penicillin G.

SAR of Penicillin G :

1) Modification of -COOH group :

Various carboxylic derivatives of penicillin have been studied by converting -COOH to $-CH_2OH$ or -CHO on reduction. Also isocyanates & benzyl carbamates have been studied but none of these derivative shows any useful antibacterial activity.

2) The EWG at α-position increases stability of benzyl penicillin

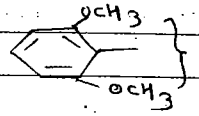
e.g. α-aminobenzyl, α-halobenzyl, α-phenoxy methyl penicillins, gives more stable than benzyl penicillin.

3) Sulphoxides & sulphone derivatives of benzyl penicillin were prepared by oxidation of sulphur atom leads to decrease the antibacterial activity.

4) Breaking of both rings or breaking thiazolidane ring with conservation of β-lactum ring, the comp. without significant antibacterial activity.

5) The substitution at ortho position on phenyl ring gives comp. with increased in activity.

eg. methacillin



The increase in steric hinderance at α-position in benzyl penicillin increases β-lactamase resistance.

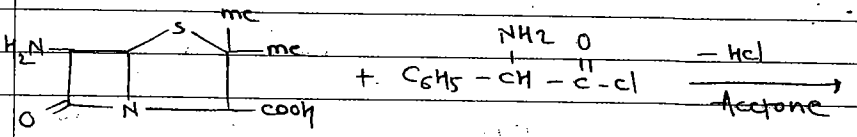
6) Incorporation of acidic substituent -COOH group at α-carbon of benzyl penicillin gives carbenicillin with increased clinical activity.

★ Synthesis of Ampicillin :

- Ampicillin is a semisynthetic penicillin. It is active against all organisms
- Ampicillin is not degraded by gastric acid. It's oral absorption is incomplete. Food interferes with absorption of ampicillin.
- Mostly used for acute infections of urinary tract. Respiratory tract infections including bronchitis, otitis media, Sinusitis etc. are generally treated with ampicillin

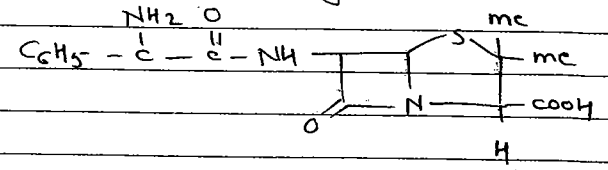
Synthesis :

(1) Prepared by acylation of 6-amino penicillanic acid (6-APA) with α-amino phenyl acetyl chloride



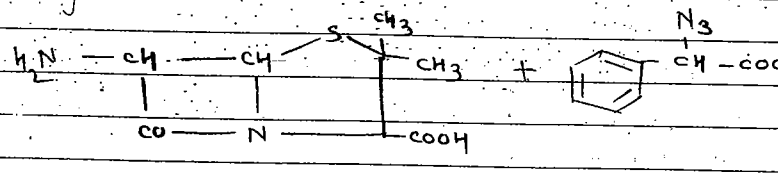
6-APA

α-amino phenyl acetyl chloride



Ampicillin

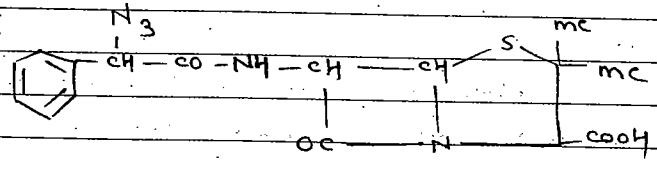
(2) Prepared by acylation of 6-APA with acid chloride from D-2-azidophenylacetic acid followed by a catalytic reduction.



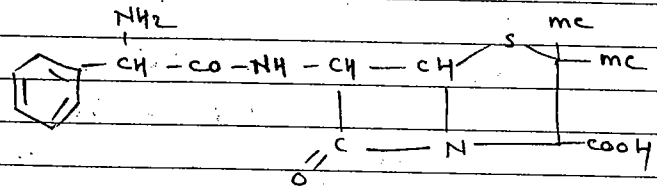
6-APA

D-2-azidophenyl acetic acetyl acid chloride

Acylation



Catalytic red^{n} H_2



Ampicillin

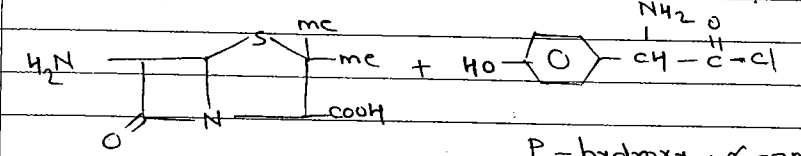
★ Amoxycillin :

– Amoxycillin is semisynthetic penicillin has an amino & hydroxy substitution in side chain. It is active against all organisms.

– It is a close et congener of ampicillin, except that in amoxycillin oral absorption is better.

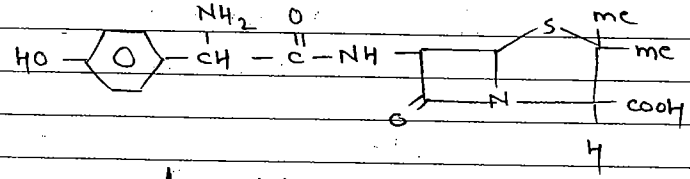
Synthesis :

(1) Prepared by acylation 6-APA with P-hydroxy, α-amino glycine hydrochloride



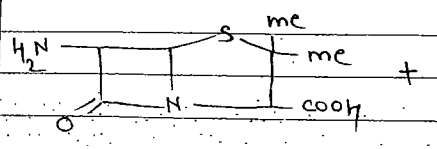
6-APA

P-hydroxy, α-amino glycine hydrochloride

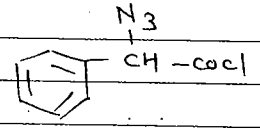


Amoxycillin

(2)

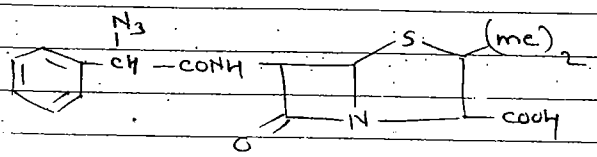


6-APA

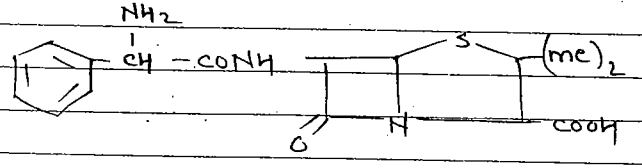


D-2-azidophenyl acetigl ~~acid~~ chloride

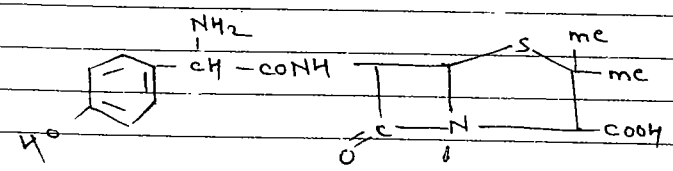
↓ Acylation



↓ catalytic red^{n} H_2

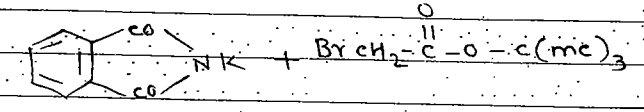


Acylation ↓ 4-hydroxyphenyl acetic acid



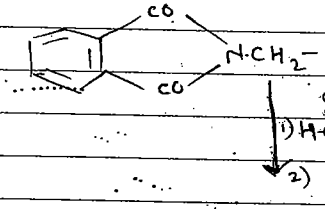
Amoxycillin

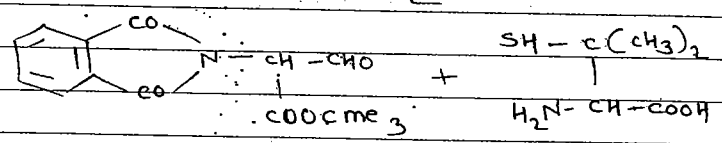
Synthesis of Penicillin V :



Potassium phthalimide t-butyl bromoacetate

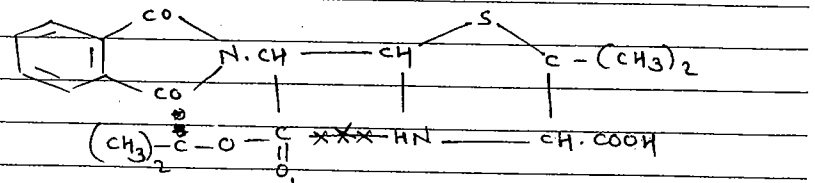
↓

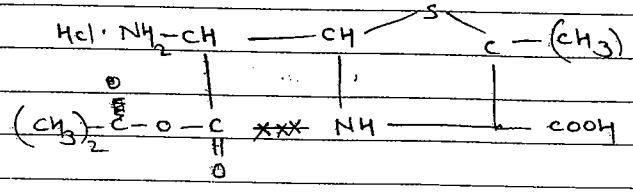


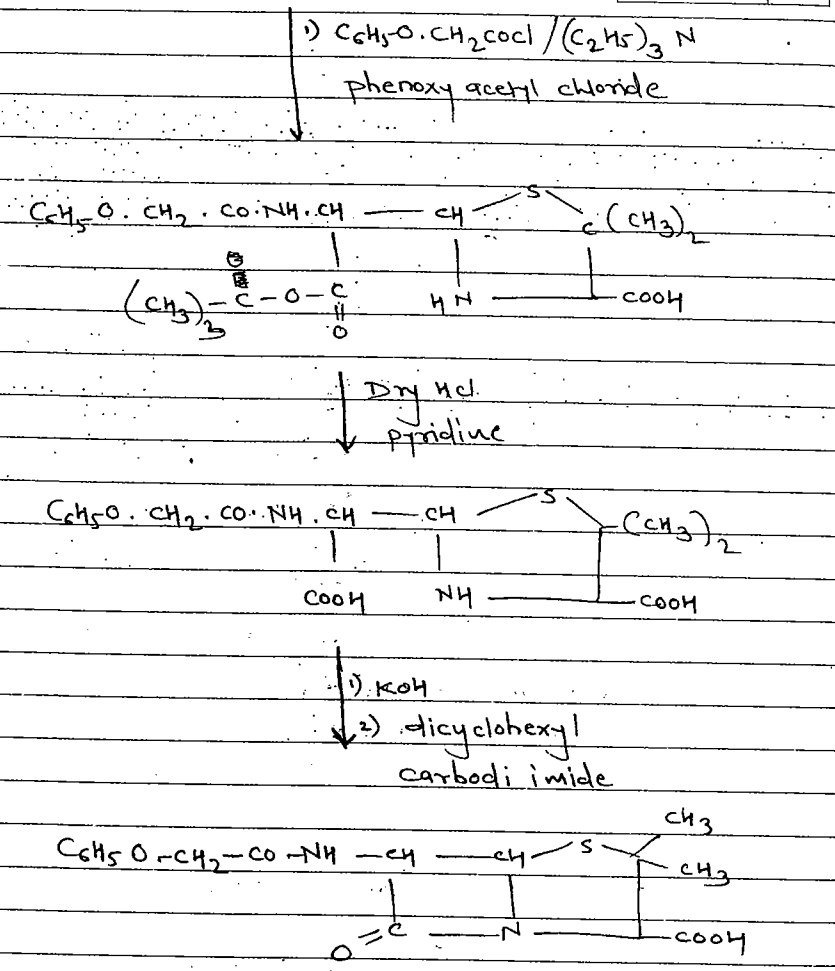


t-butyl phthalimido malonaldehydate D-penicillamine

↓ condensation



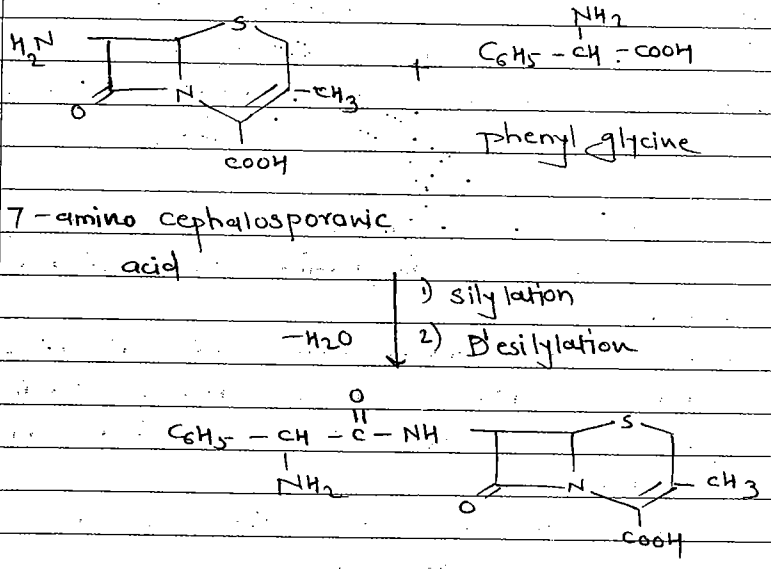




Penicillin V (phenoxy methyl penicillin)

Synthesis of Cephalexin :

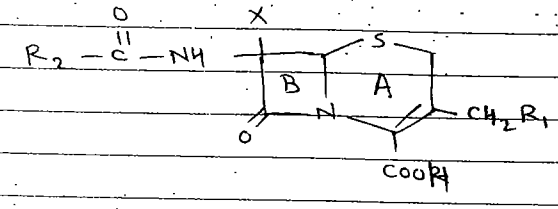
- Cephalexin is one of β-lactum antibiotic having similar antibacterial activity to that of ampicillin.
- It is first generation Cephalosporin antibiotic
- It is used to treat infection of respiratory and urinary tracts of skin.
- It is synthesized from 7-amino cephalosporanic acid.



Cephalexin

★ Structure & Activity of Cephalosporines :

Cephalosporines antibiotics obtained from fungus Cephalosporium



A → β-lactum ring

B → Dihydro-thiazine ring

When X = H ⟶ Cephalosporine

X = $-OCH_3$ ⟶ cephalomycin

When R_1 = $OCOCH_3$, R_2 = ph·CH(NH2)— ⟶ cephaloglycin

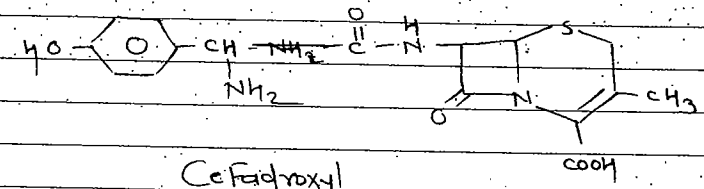
- The low potency of naturally occuring cephalosporines leads to derive Semisynthetic analog which are more potent.
- These Semi-synthetic analog which are more potent obtained by attaching a side chain to 7-amino Cephalosporanic acid just like penicillins made from 6-APA.
- Hence SAR of Cephalosporines appears parallel to SAR of penicillins.

1) Cephalosporins are broad spectrum antibiotics shows antibacterial effectiveness as ampicillins.

2) Cephalosporins are less Sensitive than β-lactamase resistant penicillins towards enzyme hydrolysis. This is mainly due to fused Cepham ring.

3) Drug design of Cephalosporins follows the prominant footprints marked in penicillins evolution.

eg: Cephahydroxyl is designed from Sucess of amoxicillin



Cefadroxyl

4) Presence of phenyl glycine moiety at 7-amino Cephalosporanic acid increases oral activity.
ex. Cephaloglycin.

5) Allylic acetoxy group at C3 is not necessary for antibacterial activity.
eg. Cephalexin doesn't Contain it but still active one

6) Like aziocillin, acylation of amino group of 2-phenyl glycine moiety leads to Cephalosporins to show antipseudomonal activity.
eg. Cefoperazone

7) Introduction of Sulfonic acid moiety in acyl side chain increases antipseudomonal activity.
eg. CefSuldowin

8) When $-X = -OCH_3$ Cephamycin family with increased clinical use. The steric & electronic properties of $-OCH_3$ protect drug from enzyme attack.
eg. Cefoxitin

9) Several cephalosporins penetrate cerebrospinal fluid & hence used in treatment of meningitis eg. Moxalactam.

10) Cephalosporins still alternatives for penicillins.

★ Mode of Action of Cephalosporins :

- The Cephalosporins are belived to act in a manner analogous to that of penicillins by binding to the penicillin binding proteins followed by cell lysis.
- The full details of the manner in which bacterial cells are killed are obscure as yet.
- Cephalosporins are bacteriocidal in clinical terms

★ Demi-Synthetic penicillins :

- These are obtained from acylation of 6-APA & which in turn prepared by biochemical methods such as fermentation or enzymatic splitting of natural penicillins.
- The Semisynthetic penicillins possess following propeties :-

1) It possesses improved acid stability.
2) They are active against gram +ve bacteria and some extent against penicillin-ase producing staphylococci.
3) They are active against gram -ve bacteria but also have broad spectrum of activity into gram -ve bacteria.

★ Mode of Action of Penicillin :

→ The mode of action of penicillins is a selective & irreversible inhibition enzymes processing the developing peptidoglycan ring.
→ There is a close structural similarity between penicillins & the D-alanyl-D-alanine end of the polypeptide side chain of peptidoglycan.
→ The enzyme carries out transpeptidation mistakenly attack to penicillin results into faulty cell wall.
→ The enzyme penicillin comp. having irreversible nature inhibits the enzymes processing the developing of peptidoglycan layer.
→ Thus penicillin shows the mechanism of action through inhibition of bacterial cell wall synthesis.

★ Mode of action of penicillin & cephalosporins :

- All β-lactum antibiotics interfees with synthesis of bacterial cell wall. The strength of bacterial cell wall provided by substance called peptidoglycan which is formed by bacteria itself.
- It is made up to two types of sugars as N-acetyl muramic acid (NAMA) & N-acetyl glucosamine (NAG)
- They are linked together in the cytoplasm to form a disaccharide.
- Antibiotics inhibit the transpeptidase (enzyme) so that cross linking reaction does not takes place. Transpeptidase enzyme produced by bacteria
- In the final step cell wall swell & finally bursts which is known as bacteriocidal action.

Coagulants And Anticoagulants

★ Mechanism of blood Coagulation :

— Blood Coagulation is nothing but the process of blood clotting. Normally the blood is kept in the fluid state in the body, by the presence of inhibitors of the activation of prothrombin (one of the coagulating factor.)

— The mechanism of blood clotting formation involves two reactions :

1) Prothrombin + Ca^{++} + Thromboplastin ⟶ Thrombin
2) Thrombin + fibrinogen ⟶ fibrin

— The first step involves the reaction between prothrombin and thromboplastin in presence of Ca^{++} ions.

— Prothrombin is formed in the liver which reacts with thromboplastin.

— The thromboplastin is released from injuries tissue cells & from blood platelets.

— After injury when liquid blood come in contact with a foreign substance, the reaction bet^n these prothrombin & thromboplastin occurs in presence Ca^{++} ion to form Thrombin

— This thrombin formed in first step reacts with fibrinogen to give monomer of fibrin.

— fibrinogen is a protein formed in the liver converted to fibrin which constituents a blood clot polymer of fibrin is formed by polymerisation, leads to network formation of fibrin & hence blood clot.

Coagulants :-

- The agents which are used to control bleeding called as coagulants.

Haemorrhagic condition is brought about by vascular defects, platelet defect or plasma coagulation disorder, & low prothrombin concentration which gets caused by anticoagulant therapy. coagulants find uses in the treatment of severse haemorrhage.

- In finds use in the treatment of the haemorrhagic disorders which occurs due to an overdose of heparin. it can be administered by intravenous injection. it is uses in excess, it is having anticoagulant effect.

- It has to protected from light. it finds use topically for its haemostatic or clotting effect on blood. it may be applied as powder.

- generally coagulants include vit. k & its analogues protein & amino acid, plasma coagulants etc.

- The chapter on vitamins, vit k & analogues have been described protamine sulphate & dried thrombin have been the other official drugs.

Vit-k

vit-k, fat-soluble vitamin is not a single entity but occurs naturally in the form of at least two distinct subs vit K_1 & vit k_2. both are derivatives of napthoquinones.

- vitamin k_1 (phytonadione) is the only naturally occuring form of this vitamin. many other closely related compounds possess vit-k activity.

1) prothrombinoplastin (Globulin) → Thromboplastin

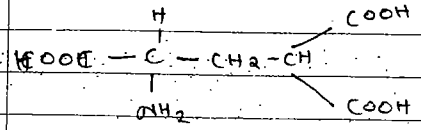
Prothrombin —(Ca++, Vit-k)→ Thrombin (proteolytic enzyme)

2) Fibrinogen (dimer protein synthesised in liver) —(Thrombin, Ca++)→ Fibrin (monomer)

Fibrin (monomer) —(Ca++ & transamidase)→ insoluble firm clot (polymer)

The above mentioned blood clotting factors remain biologically inactive in the absence of vit-k it is clear from the above figure that in the clotting Ca++ ions play an imp role, within the prothrombin etc is necessary to produce strong binding sites for Ca++ ions.

- vit k hydroquinone is a coenzyme in the conversion of glutamyl residue of the prothrombin precursors protein to γ-carboxyglutamyl residues which participate in the



γ - carboxyglutamic acid

Vit-k hydroquinone is produced by the reduction of vit k. Thus reaction needs the presence of NADPH2.

It is also significant that γ- carboxyglutamic acid has been used as residue in all vit k dependant

Uses of Vitamin-K →

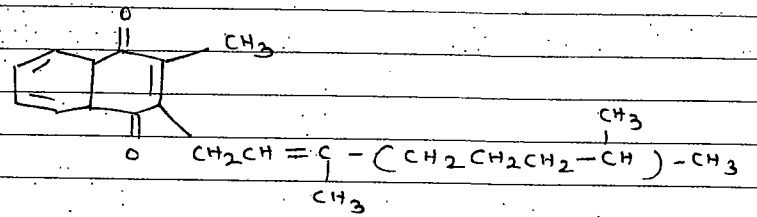
1) Vitamin k may be used in the treatment of destructive jaundice. however vitamin supplement will be ineffective if prothrombin synthesis gets hampered due to excessive liver damage.

2) Vitamin k & its synthetic analogeous can be used in the treatment of oral anticoagulant toxicity.
even injection of green leafy vegetables will minimises its intensity & Frequency of such toxic symptoms.

3) Administration of vitamin k to mother prior to delivery can assure an adequate supply & safeguard against haemorrhage in infant.

Vitamin k & its synthetic analogs

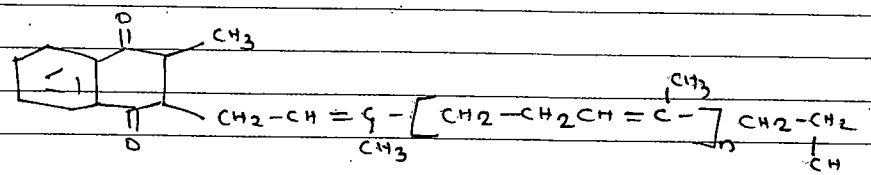
Naturally occuring vitamins



2- methyl - 3 - phytyl - 1, 4 - naphthaquinone.

or

vitamin k1 (Phytonadione)

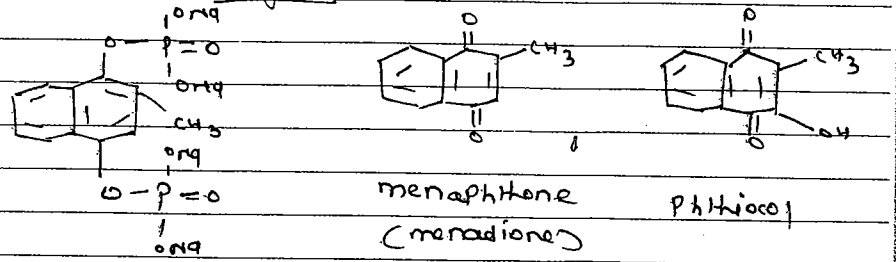


n = 4 : vit k2 (30 carbon atoms)

n = 5 : vit k2 (35 carbon atoms)

3- Famesyl geranyl- geranyl-2-methyl

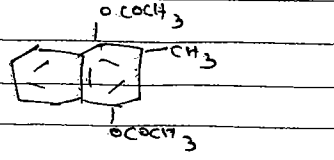
II] Synthetic analogs



menaphthone (menadione)

phthiocol

menadiol sodium phosphate



Acetomenaphthone

Q. Explain Heparin as an anticoagulant.

* Anticoagulants :→

"Anticoagulants are the drugs which prolong the coagulation time of blood."

A number of drugs are available which decrease the clotting tendency of blood & thereby serve to prevent thrombo-sis & thromboembolic conditions.

Action:→

Anticoagulants show their actions as:

i) They may act directly by inhibiting factors used for coagulation.

ii) They may act by stimulating normal natural anticoagulant.

iii) They may act indirectly by their effect on formation of factors of coagulation.

The various types of products include the physiological anticoagulants such as heparin which acts by prolonging the clotting time with lesser effect on prothrombin time. The synthetic agents like coumarin & phenindione etc derivatives affect the prothrombin time. These agents used in situations which result from intravascular clotting as in thrombosis or phelbothrombosis. They do not touch thrombi or emboli already formed or present but used to retard further intravascular clotting.

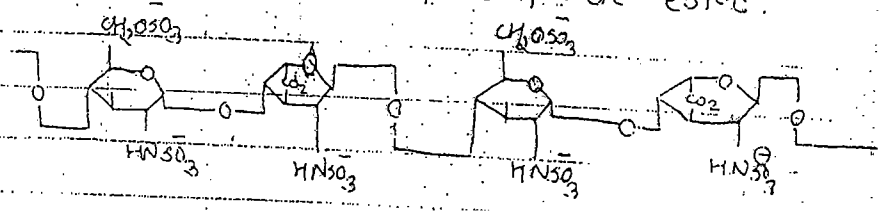
Mucosa = Thick liquid

* Heparin :→

Heparin was discovered in 1916 by McLean. It is naturally occuring substance found in association with lipoproteins in the mast cells of liver & lungs. These mast cells are abundant in liver hence the name heparin. commercial heparin is obtained from lung & intestinal mucosa of pigs & cattles.

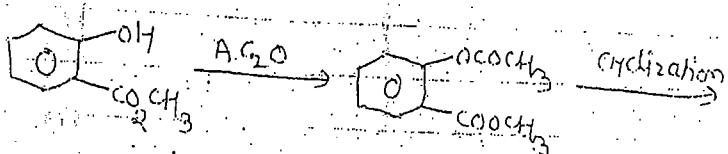
It is generally believed that heparin is the naturally occuring anticoagulant responsible for fluidity of blood.

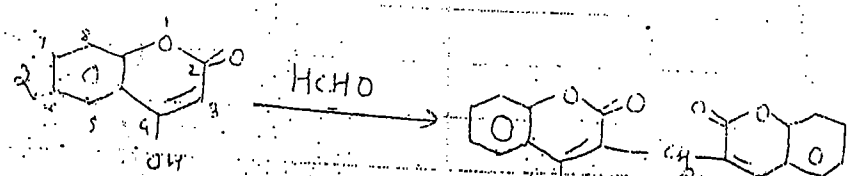
Chemically heparin is a mucopolysaccharide composed of unknown no. of sulfated D-glucosamine & D-glucuronic acid units through an oxygen bridge. It is complex of mucoitin polysulphuric ester.



* Synthesis of dicoumarol :→

The two molecules of the 4-hydroxy coumarin condensed in presence of formaldehyde to give dicoumarol.

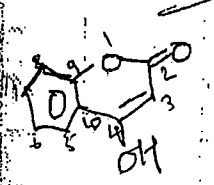




4-hydroxy coumarin

Dicoumarol

Structure Activity Relationship of coumarin derivatives :→



Although clear cut relationship cannot be established for structure & anticoagulant activity but some imp. conclusions have been made as.

i) The intact 4-hydroxy coumarin nucleus and a hydrogen or a hydrocarbon substituent at position 3 is essential for anticoagulant activity

ii) When coumarin nucleus has a hydroxyl or benzamido group at 3-position, anticoagulant action is completely lost

iii) Substituent like methyl, ethyl, phenyl, methoxy in position 3 makes the derivative with slight activity

iv) A hydroxyl group in 4-position in coumarin nucleus shows potent anticoagulant activity but if it is replaced by methyl or phenyl group activity becomes weak.

v) A 2nd coumarin ring attached by methyl bridge to position-3 increases the potency e.g. dicoumarol.

vi) Introduction of methoxy group at 7 & 8 position of benzene ring enhances activity

vii) similarly an acetyl group at 8-position increases activity.

viii) Among the dicoumarol or bis coumarin, the carbonyl group at 2' or 3' position show optimal activity.

ix) A methylthioether ($-SCH_3$) or on methylene bridge of dicoumarol is twice as active as ethyl bis dicoumacetate.

x) Phenyl substitution on methylene bridge decreases activity

Action:→

Heparin is naturally occuring anti-coagulant prevents blood coagulation in both vivo & vitro. It probably acts on all stages of coagulation. It binds to antithrombin & activates it. It also inactivates other coagulating factors. The formation of fibrin monomer & its polymerization are prevented.

Heparin act by prolonging the intravascular clotting time with lesser effect on prothrombin time. Heparin has been shown to inhibit aldosterone secretion.

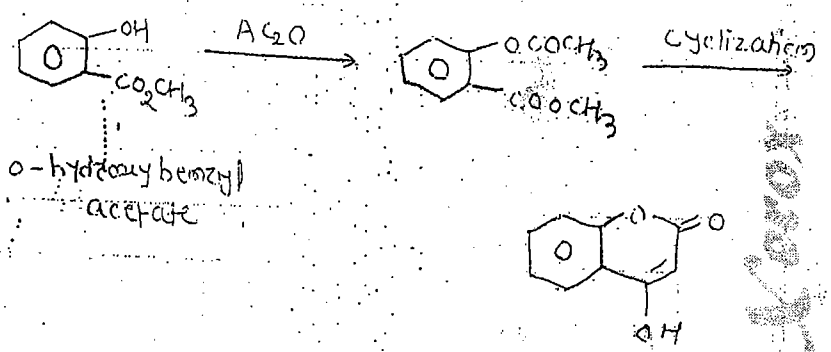
It is ineffective when given orally & therefore is administered parenterally in its sodium salt. Its action is rapid but of short duration.

Q. Vit K as an anticoagulant drug.

Coumarin derivatives:→

These are the anticoagulants acts as Vit. K antagonists. These are the synthetic drugs used to depress intravascular coagulation of blood. They have the advantages over heparin in that they can be administered by mouth & have longer duration of action & are cheap.

Q. Synthesis of 4-hydroxy coumarin:→

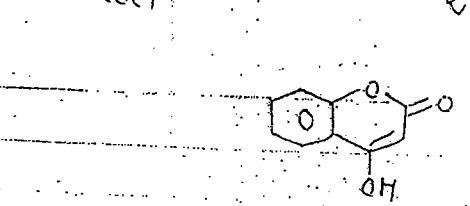
I] 

o-hydroxy benzyl acetate

4-hydroxy coumarin

o-hydroxy benzyl acetate on acetylation followed by cyclization gives 4-hydroxy coumarin.

II] A second method of synthesis consists of the condensation of o-hydroxy benzoyl chloride with malonic esters.



4-hydroxy coumarin.