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SCHOOL OF SCIENCE AND HUMANITIES

DEPARTMENT OF CHEMISTRY

UNIT – I - Alkaloids – SCYA7201

UNIT-1

ALKALOIDS

1.1 INTRODUCTION:

Alkaloids was introduced by W.Meissner in 1819. Alkali like basic-nitrogen containing compounds isolated from plants.

Alkaloids are defined as natural plant compounds with a basic character –containing one nitrogen atom in a heterocyclic ring structure.

Modern definition states that –Basic nitrogeneous plant products mostly optically active and possessing nitrogen heterocycle as their structural units with a pronounced physiological action.

1.2 OCCURRENCE OF ALKALOIDS

Heterogeneous containing nitrogen containing substances in plant families. Distributed widely in higher plants-dicotyledons- Apocynaceae, Papilionaceae, Rubiaceae- Solanaceae. In plants- basic in nature they occur as salts of organic acids- acetic, oxalic, citric, malic, lactic etc.,

Glysoides of sugars- glucose, Rhamnose, galactose

Esters- Eg:Atropine

1.3 FUNCTION OF ALKALOIDS:

(1)Act as reserve substances to supply nitrogen. 2)May be end-products of detoxicfication mechanisms.3)Poisonous substances protect from animals.4)Plant stimulants or regulators for normal metabolism.5) Reservoirs for protein synthesis.

1.4 NOMENCLATURE OF ALKALOIDS

1) According to the plants isolated

Papaverine- Papaver someniferum.

Berberine- Berberis Vulgaris L.

2) According to Physiological Action

Morphine- (Ger- Morphin- God of Dreams)

Narcotine- (Greek Narkoo- to benumb)

Emetine – (Greek- Emetikos- to vomit)

3) Named after the discoverer

Pelletierine- P.J. Pelletier.

Prefixes –epi, iso, neo, pseudo have been used to designate isomeric or slightly modified structures.The Prefix ‘nor’ denotes the structure does not have a methyl group attached to nitrogen atom.

1.5 CLASSIFICATION OF ALKALOID:

1) Taxonomic- according to the Family.

Solanaceous or Papilionaeous family- Tropane, pyridine, steroidal or pyrrolizidine.

Pharmacological- Analgesic alkaloids, Cardioactive alkaloids.

Biosynthetic- Indole alkaloids- Tryptophan and mevalonic acids

Morphine, papverine –Phenylalanine, tyrosine.

2) According to Chemical structure

Phenylethylamine alkaloids-Ephedrine

Pyrrolidine alkaloids-

Pyridine alkaloids- Ricinine, Coniine, Piperine, Pelletierine

Pyridine-Pyrrolidine alkaloids-Nicotine

Tropane alkaloids-atropine, cocaine

Quinoline & Isoquinoline alkaloids- Quinine.

Phenanthrene alkaloids-Reserpine

1.6 PHYSICAL PROPERTIES

1) Colourless crystalline solids-Insoluble in water, soluble in organic solvents like $CHCl_3$, alcohol ether etc.,
2) Bitter in taste- optically active- laevorotatory.
3) Basic in character.
4) Insoluble precipitates with phosphotungstic acid, picric acid.
5) Contains oxygen and nitrogen atoms in their structure. Nitrogen being in their tertiary or secondary state.

1.7 ISOLATION OR PRODUCTION OF ALKALOIDS

1) Alkali Extraction

POWDERED DRUG (Containing alkaloids)

Moisten with alkali Na_2CO_3, K_2CO_3

Free alkaloids

Exhaust with Organic solvent ether, Chloroform, Methylene chloride

Total Extract

Concentrate and Shake with dil.Sulphuric acid

Total extract

Aqueous acid solution | Residual Organic Fraction

Residual AQUEOUS solution

Organic solution of alkaloid base

Crude alkaloid Product

1.8 METHODS FOR DETERMINING STRUCTURE OF ALKALOID

1) Molecular formula determination: Elemental composition and its empricial formula is found by combustion method. The molecular formula is obtained by Rast's method.

   The number of double bond equivalents and the number of rings in the structure can be calculated by the following expression for the molecule $C_aH_bN_cO_d$ = (a-1/2b+ 1/2C+ 1).The presence of unsaturation is ascertained by treating the alkaloid with bromine water, $KMnO_4$.

2) Functional Group Analysis: -Infra-red spectroscopy.

a) Functional nature of Oxygen:

   Oxygen can be present as –OH (alcoholic/phenolic); 2) methoxy (OCH_3);

   3) Acetoxyl($-OCOCH_3$); 4) Carboxylic (-COOH); 5) carbonyl (-CO); 6) carboxylate (-COOK)

1) Hydroxyl group:

   On treatment with acetic acid it forms acetate; it forms benzoate with benzoyl chloride.

$$ROH + (CH_3CO)_2O \longrightarrow ROOCCH_3 + CH_3COOH$$

$$ROH + CH_3COCl \longrightarrow ROOCCH_3 + HCl$$

$$ROH + C_6H_5COCl \longrightarrow ROOCC_6H_5 + HCl$$

The number of hydroxyl groups is determined by acetylation or zerewitinoff's method, involves acetylating the alkaloid and hydrolysing the acetyl derivative with 1N NaOH

$$ROH \xrightarrow{CH_3COCl} ROCOCH_3 \xrightarrow{NaOH} ROH + CH_3COONa$$

The excess alkali is estimated by titrating with standard HCl. The number of acetyl groups can be calculated from the volume of alkali used for hydrolysis.

Hydroxyl groups can be detected by treatment with methyl magnesium iodide and quantitatively estimating the methane content.

$$-OH + MeMgI \longrightarrow -OMgI + CH_4$$

$$NH + MeMgI \longrightarrow NMgI + CH_4$$

2) Phenolic OH:

Soluble in sodium hydroxide;Reprecipiated by CO_2

Coloration with neutral $FeCl_3$.

3) Hydroxylic OH: - oxidation or by dehydration to unsaturated compounds.

4) Carboxylic acids:

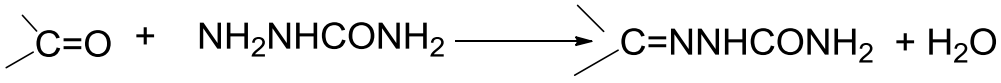
Solubility in aqueous sodium carbonate; ester on treatment with alchol.

The number of carboxylic acids can be determined by titrating against $Ba(OH)_2$ or gravimetrically by silver salt method.

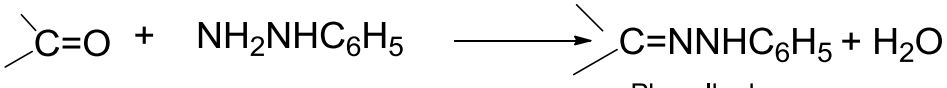
5) Oxo Group:

Carbonyl group:Reaction of the alkaloid with hydroxyl amine, semi carbazide, phenylhydrazine.

Oxime



Semicarbazone

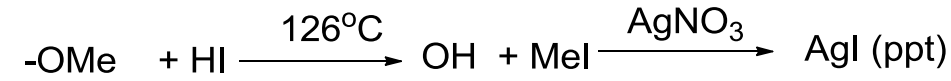


Phenylhydrazone

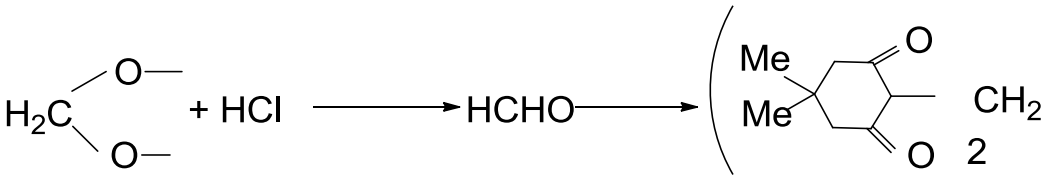
6) Methoxyl group:

a) Zeisel Method

Alkaloid is heated with HI at 126^{o}C and then precipitated as AgI.

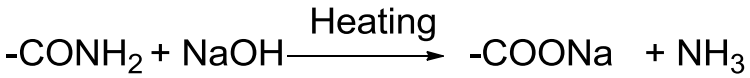


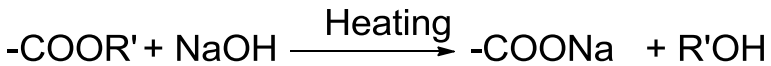
7) Methylenedioxyl group: (-OCH_2OO-)

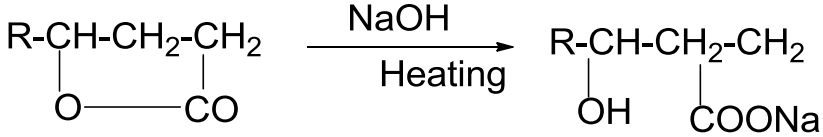


Dimedone

8) Ester,amide, lactum or lactone: These groups can be detected and estimated from the products of alkali or acid hydrolysis.



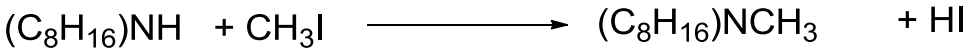




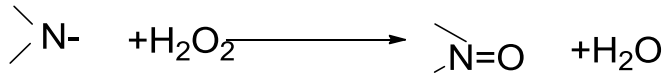
b) Nature of Nitrogen:

It is present as a part of heterocyclic ring as secondary (NH) or tertiary (N).

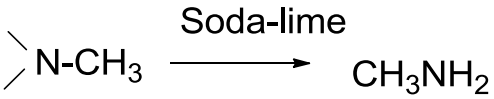
Secondary nitrogen in the alkaloid is detected by reacting it with one molecule of methyl iodide to form N-methyl derivative.



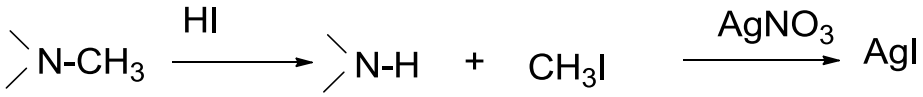
Tertiary nitrogen is treated with 30% H_2O_2 where it is oxidised to amine oxide.



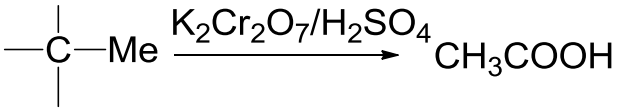
Presence of N-methyl amine is detected by distillation of alkaloid with soda-lime when methyl amine is obtained.



Herzig-Meyer's method: Cleaving N-methyl amine present in the alkaloid with HI at 150-300^{0}C and estimating the methyl iodide formed by conversion to silver iodide with silver nitrate solution.



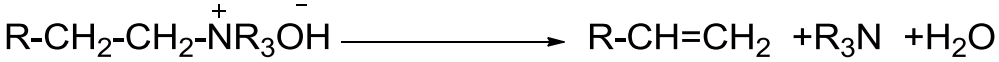
Estimation of C-methyl groups: Kuhn-Roth oxidation in which acetic a cid formed is estimated.



1.9 DEGRADATION OF ALKALOID

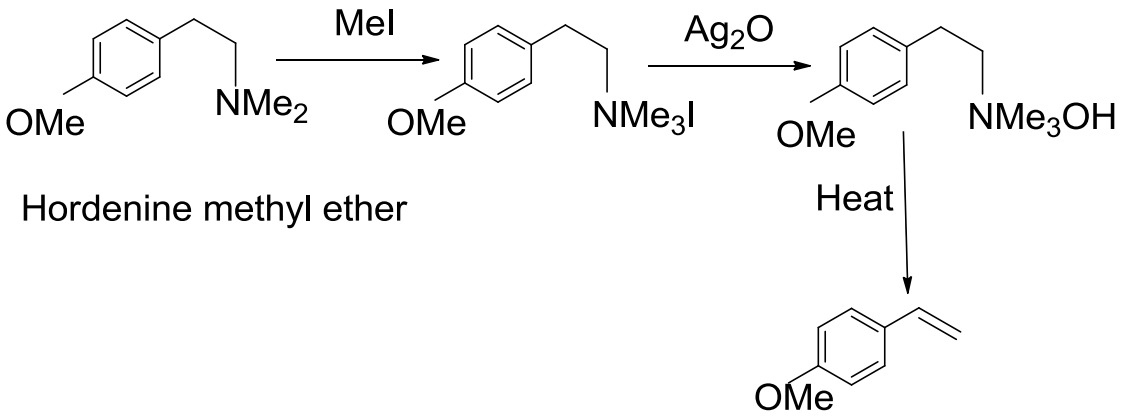
1.9.1 Hofmann Exhaustive methylation method:

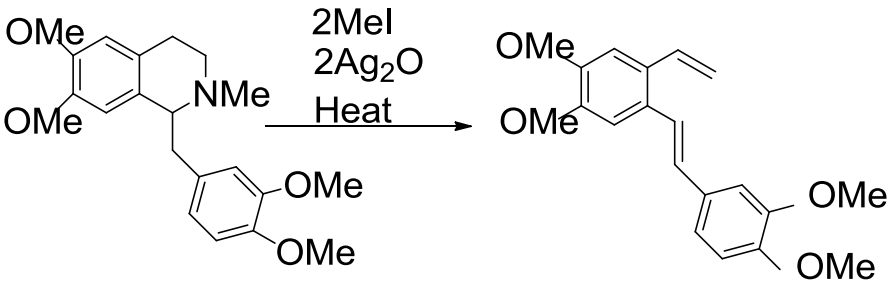
The alkaloid amine is hydrogenated followed by its conversion to quaternary iodide, on excess methyl iodide. The salt is converted to hydroxide by reacting with moist Ag_2O . The hydroxide on heating at 200^{0}c gives an olefin with the elimination of tertiary amine.



The reaction proceeds by E_2 mechanism in which the β hydroge n and quaternary nitrogen group are present in trans-anti-parallel configuration.

Reactions:

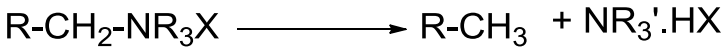




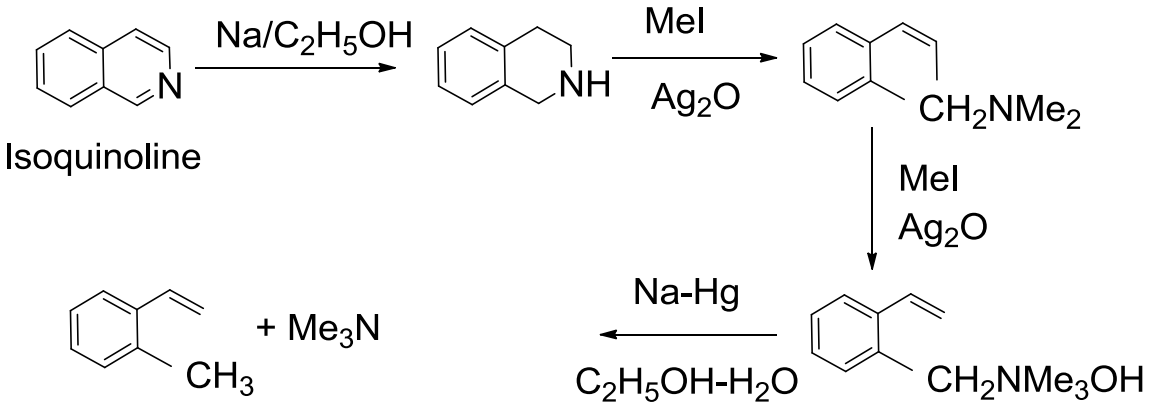
Hofmann's exhaustive methylation fails with unsaturated heterocyclic rings, when there is no β-hydrogen.

1.9.2 Emde's degradation:

The method involves the cleavage of quaternary ammonium salts with sodium amalgam or sodium in liquid ammonia or by catalytic hydrogenation.



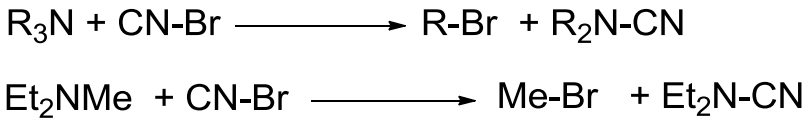
Reactions:



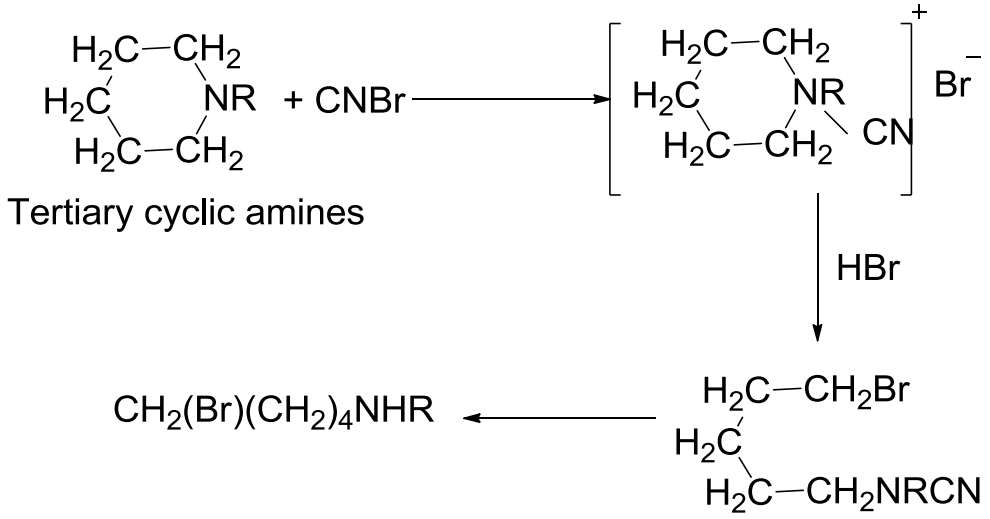
1.9.3 Van Braun's Method:

Tertiary amine containing alkyl substituent is treated with cyanogen bromide resulting in the cleavage of an alkyl-nitrogen bond to give alkyl halide and a substituted cyanamide.

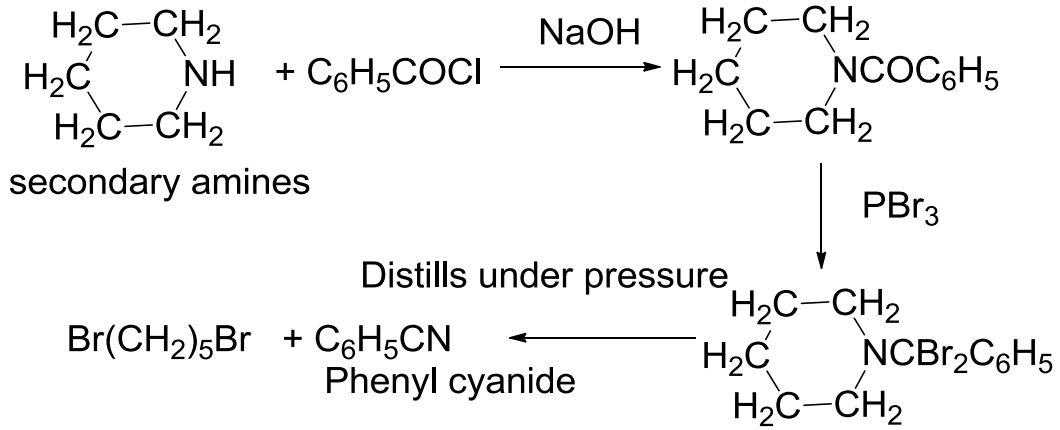
This method can be applied to compounds which does not respond to Hoffman's method. Unsymmetrically substituted amines gives alkyl halide from the smallest alkyl substituent.



For Tertiary cyclic amines:

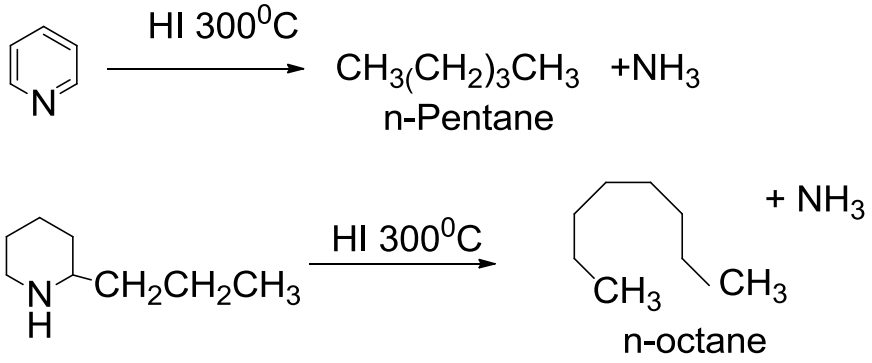


For secondary cyclic amines: The amine is treated with benzoyl chloride in the presence of NaOH which on treatment with Phosphorus followed by distillation under reduced pressure yields α,ω-dibromo derivative.



1.10 REDUCTIVE DEGRADATION:

Ring opening can takes place by heating with HI at 300^0C.



1.11 TROPANE ALKALOIDS-ATROPINE

1.11.1Occurrence: Roots of deadly nightshade, Thorn apple and with l-hyoscyamine which is optically active. Atropine is the racemic form of l-hyoscyamine. It racemizes to atropine when warmed with an ethanolic solution.

1.11.2 Isolation: It is isolated from the roots of belladonna plant. The juice contains hyoscyamine, heated with K_2CO_3 it is racemized to atropine. The atropine is extracted with $CHCl_3$. On evaporation, the residue is treated with sulphuric acid and purified by converting it into an oxalate or a sulphate.

1.11.3 Properties:

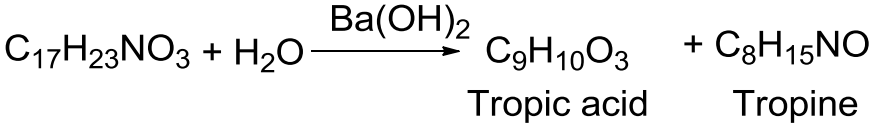
Crystalline compound(m.pt 118^0c), bitter taste. It is a tertiary base with a pKa of 10.

Dilates pupil and it is used to relieve the night sweats and distressing feature of tuberculosis which diminishes the activity of salivary and gastric glands.

1.11.4 Elucidation Of Atropine:

From elemental analysis and molecular weight determination, the molecular formula of atropine is **$C_{17}H_{23}NO_3$.**

Atropine as an ester: When atropine is treated with barium hydroxide solution it undergoes hydrolysis to yield racemic acid, Tropic acid and an optically inactive alcohol, tropine. Thus atropine is the tropine ester of tropic acid or tropine tropate.



Atropine can't be an amide because tropine, the product of hydrolysis is a tertiary base.

a) Structure of Tropic acid:

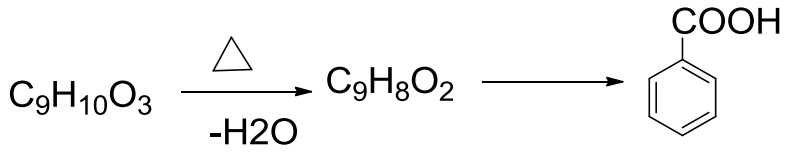
Molecular formula of the compound is $C_9H_{10}O_3$.

Tropic acid consumes one equivalent of alkali and doesn't add bromine it is a saturated monobasic acid.

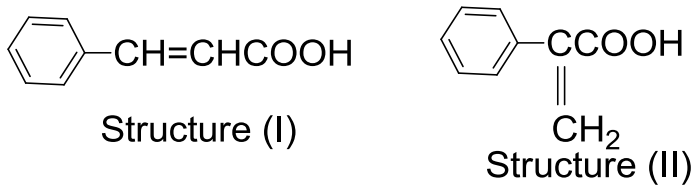
Tropic acid, on acetylation forms monoacetate indicating the presence of one hydroxyl group. The hydroxyl group must be an alcoholic group.

Tropic acid on heated strongly it loses a molecule of water to yield an optically inactive unsaturated acid , atropic acid $C_9H_8O_2$

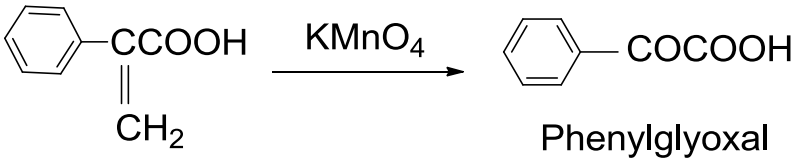
Atropic acid, on oxidation yields benzoic acid. The formation of benzoic acid reveals that atropic acid and tropic acid contain atleast one benzene nucleus with a side chain containing carboxylic acid in their structure.



As atropic acid is an unsaturated acid it mean atropic acid may be either structure (I) or (II) .



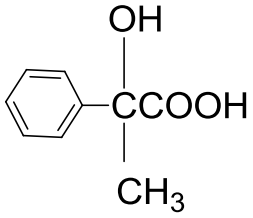
Hence, the structure (II) is atropic acid which is confirmed by oxidation with $KMnO_4$ to form phenylglyoxal.



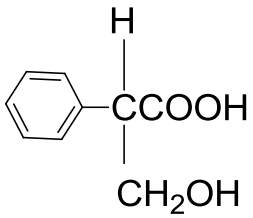
Atropic acid is formed by the dehydration of tropic acid. Hence addition of water to atropic acid gives tropic acid.

Therefore, tropic acid is

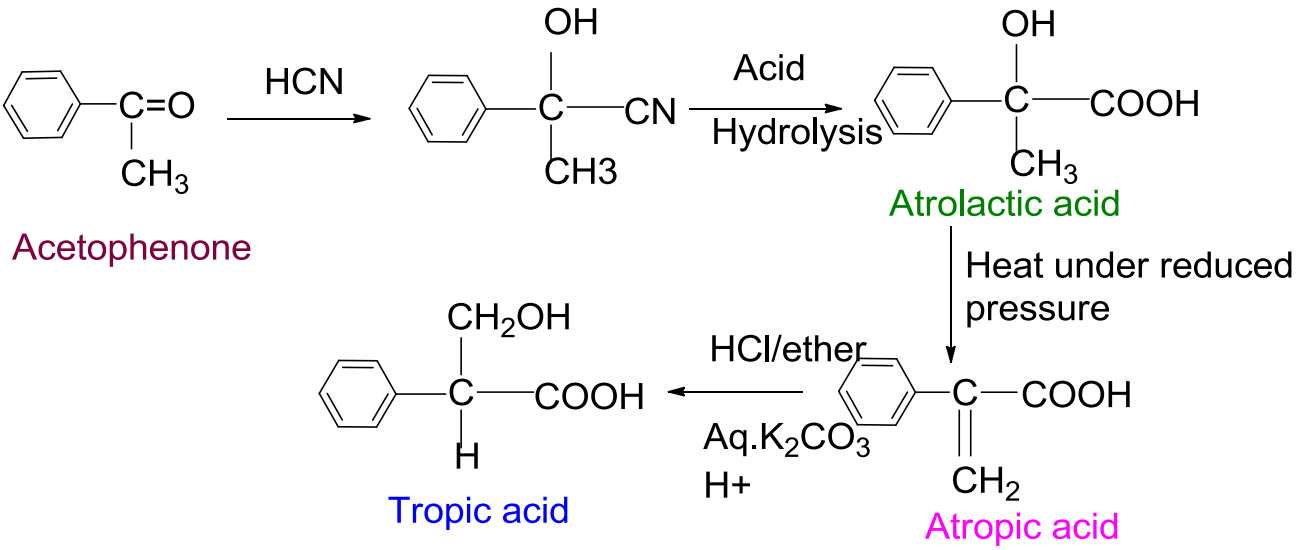
Structure (III)



Structure (IV)



Synthesis of tropic acid from acetophenone



b) Structure of Tropine:

Molecular formula ha been found to be $C_8H_{15}NO$.

Tropine on heating with methyl iodide it yields a crystalline additive product in which nitrogen is in tertiary state.

$$C_8H_{15}NO + CH_3I \longrightarrow C_8H_{15}\overset{+}{O}N\overset{-}{C}H_3I$$

On fusing with alkali, tropine yields methyl amine indicating the formation of N-methyl group. Tropine on heating with HI at 150°c yields one molecule of CH_3I.

Tropine forms monoacetate and monobenzoate indicating the presence of alcoholic hydroxyl group.

$$C_8H_{14}N(OH) \xrightarrow{C_6H_5COCl} C_8H_{14}N(OCOC_6H_5)$$

Monobenzoate

Tropine is oxidised with chromic acid, to yield tropinone, $C_8H_{13}NO$ which gives characteristic reactions of ketone hence the hydroxyl group must be a secondary alcoholic group.

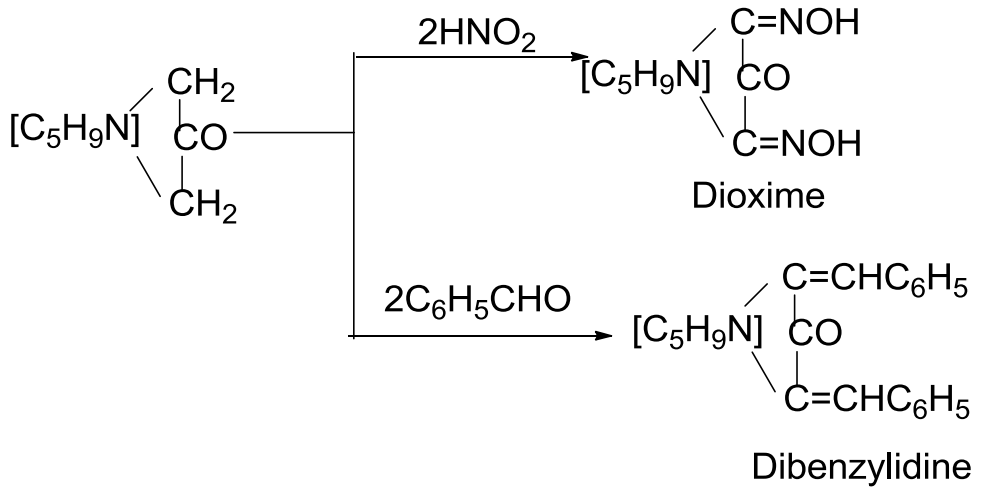
$$C_7H_{13}N(CHOH) \xrightarrow[O]{CrO_3} C_7H_{13}N(CO) + H_2O$$

Tropinone

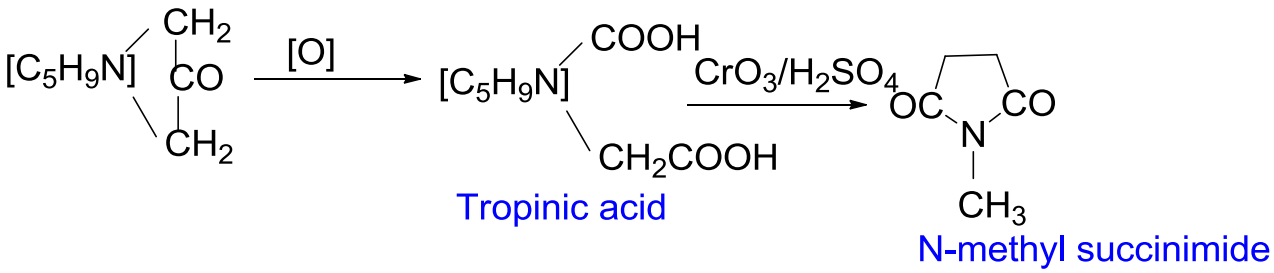
Tropine on treatment with HNO_2 and benzaldehyde it yieldsdioximino and dibenzylidene derivative indicating the presence of $–CH_2\text{-}CO\text{-}CH_2$- group.

Further, Oxidation of tropinone yields a dicarboxylic acid , tropinic acid without a loss of carbon atom hence the $–CH_2-CO-CH_2-$ group or $–CH_2-CH(OH)-CH_2-$ group must be in the ring.

Reactions:

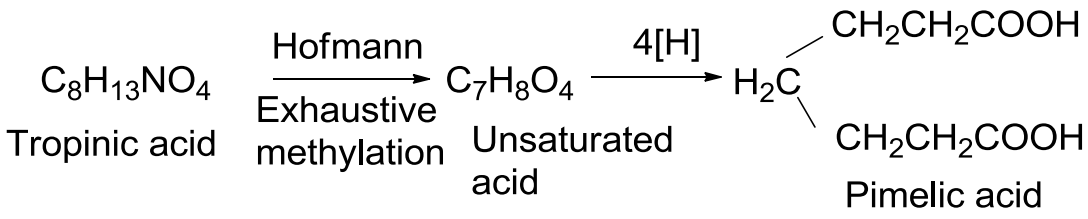


Tropine on oxidation gives tropinic acid on further oxidation gives N-methyl succinimide.



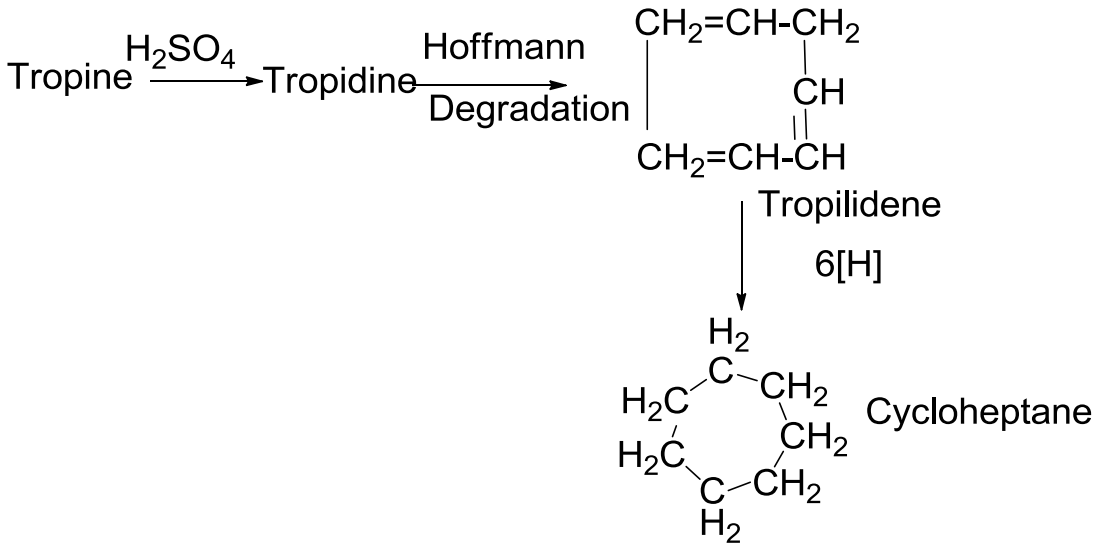
The presence of N-methyl pyrrolidine accounts for 5 C atom as against 8 C atoms in tropine and tropinic acid. Since tropinic acid is a dicarboxylic acid the remaining 3 C atoms is present as –COOH and CH_2COOH.These two groups must attach at α and β positions in pyrrolidine.

Tropinic acid on exhaustive methylation yields an unsaturated dicarboxylic acid which on reduction forms pimelic acid.

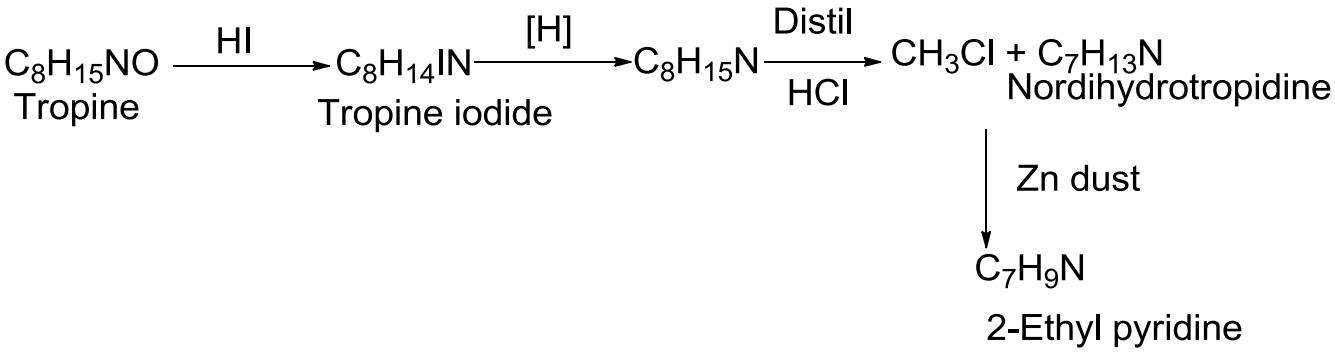


The formation of Pimelic acid indicates the tropinic acid has 7C atoms joined in series with the 8C is present as N-methyl group. It is possible only when –COOH and $–CH_2COOH$ group is in α and α' position in tropinone and tropinic acid.

Dehydration of tropine yields tropidine which on Hoffmann's degradation yields cycloheptatriene or tropilidene which on reduction forms cycloheptane.



Reaction:

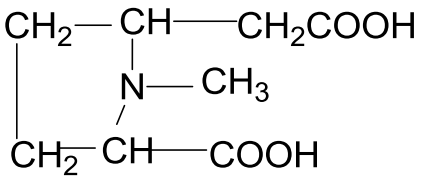


Formation of 2-Ethyl pyridine reveals the presence of pyridine in the tropine molecule.

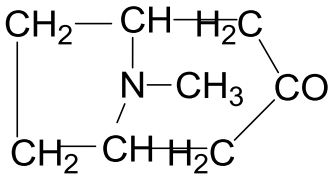
From the above discussion,

- Tropine possesses a seven-membered ring having -CH_2-CH(OH)-CH_2 group.
- Tropine possesses a reduced pyrrole ring in their structure.
- It contains a reduced pyridine ring in their structure.
- It possesses a N-CH_3 group.
- It contains a nitrogen atom common to pyrrolidine and reduced pyridine and it should be in N-CH_3 group.

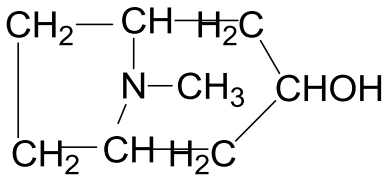
Thus the structure of tropinic acid, tropinone and tropine is as follows:



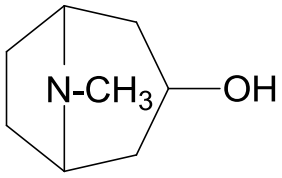
Tropinic acid



Tropinone

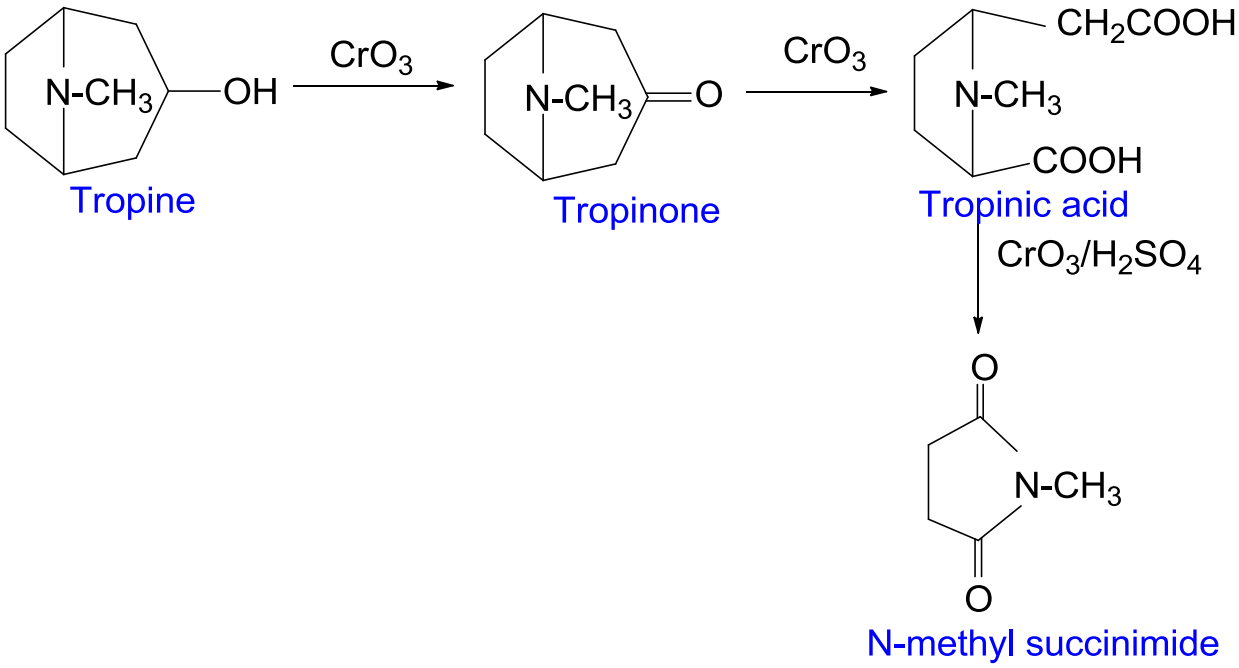


Tropine



REACTIONS:

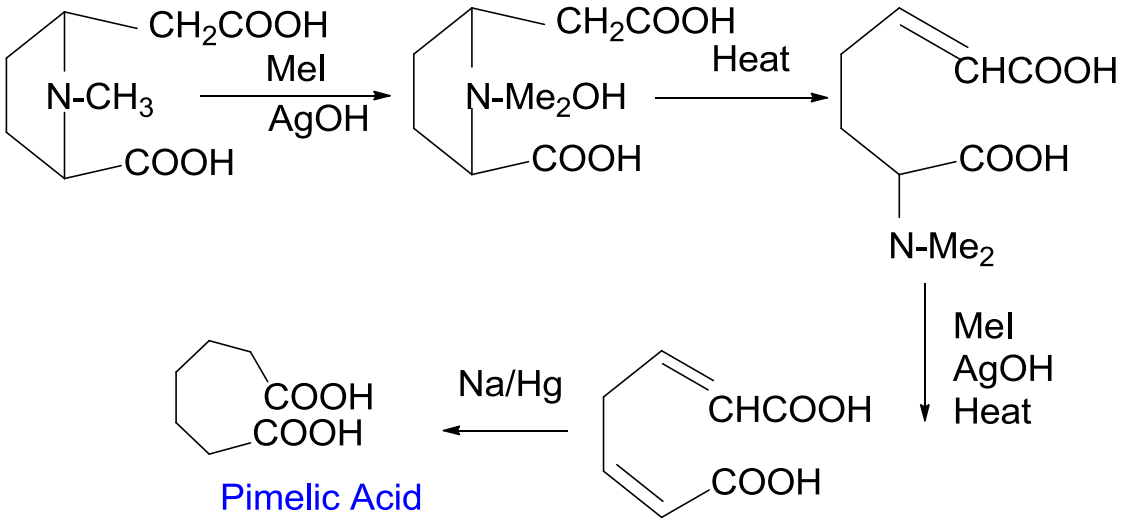
a) Conversion of tropine to N-methyl succinimide.



Tropine Tropinone Tropinic acid

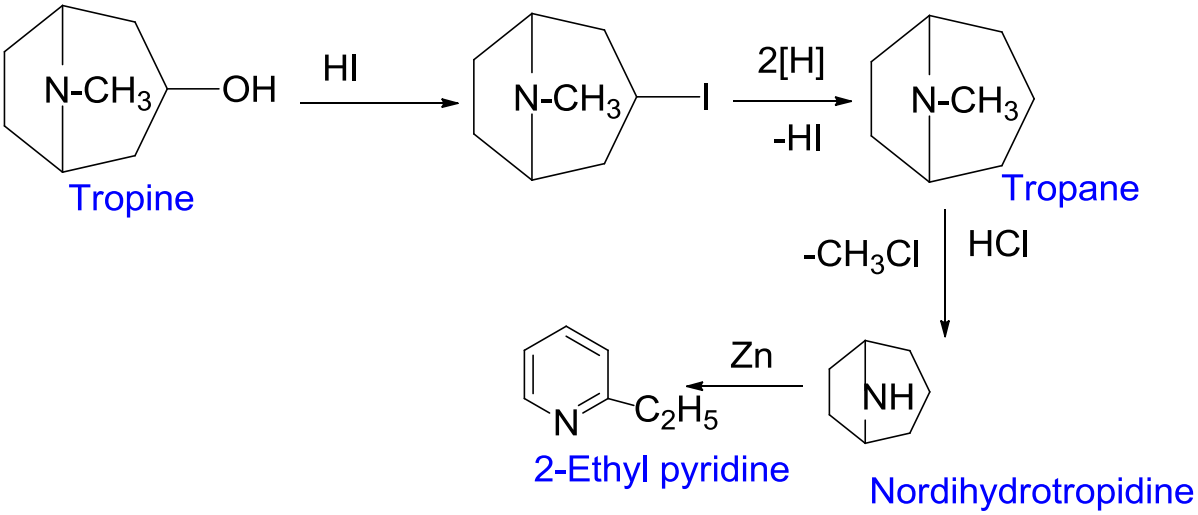
N-methyl succinimide

b) Formation of pimelic acid from tropinic acid.

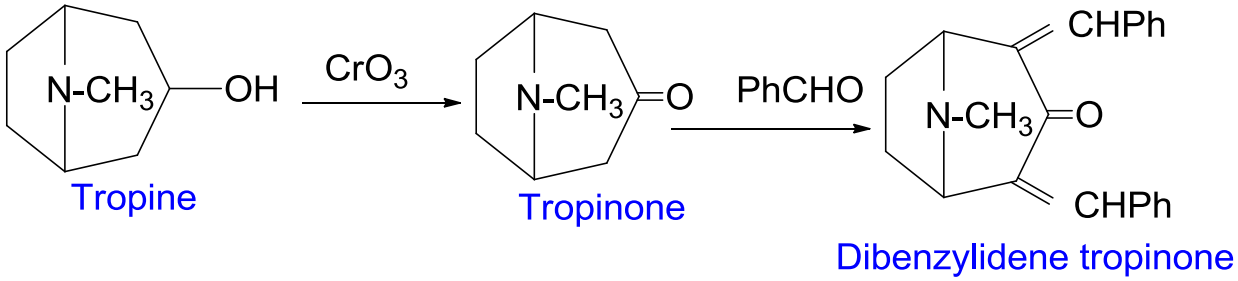


Pimelic Acid

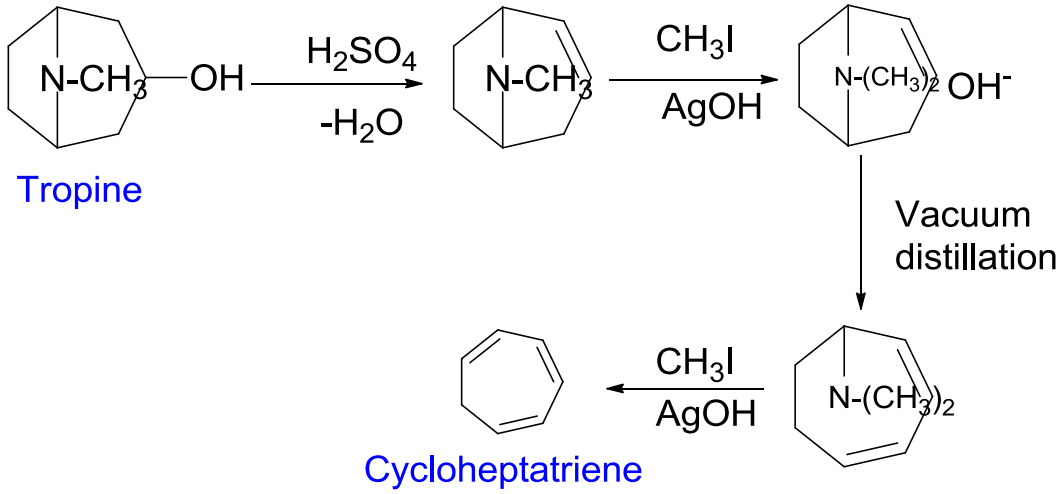
c) Formation of 2-Ethyl pyridine from tropine.



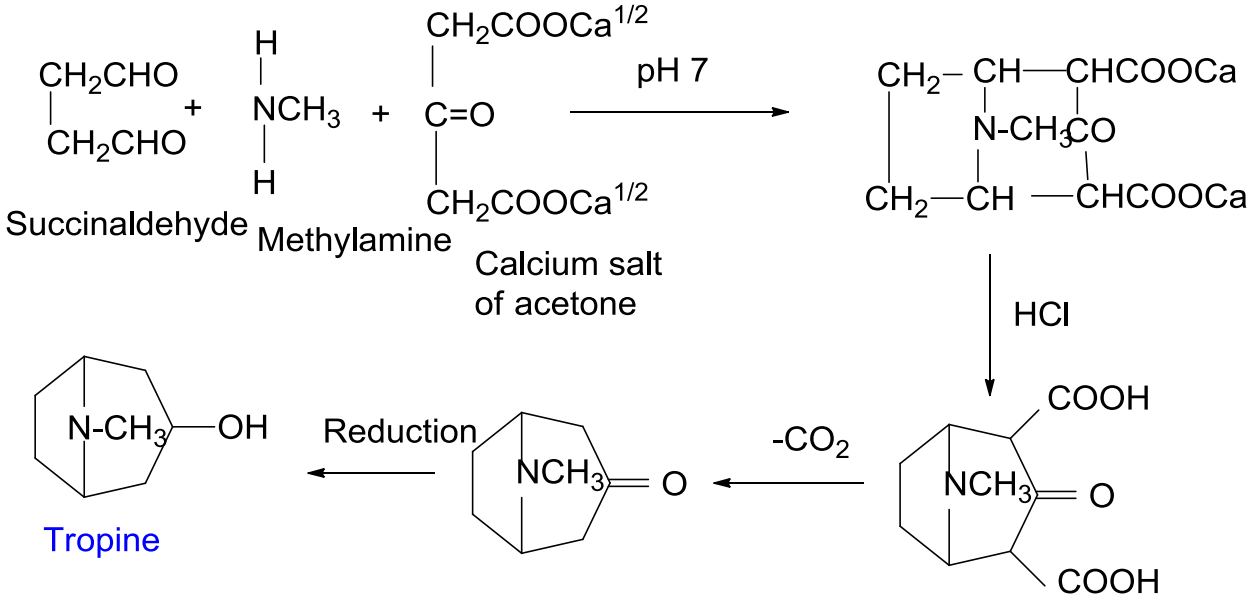
d) Formation of dibenzylidene derivative.



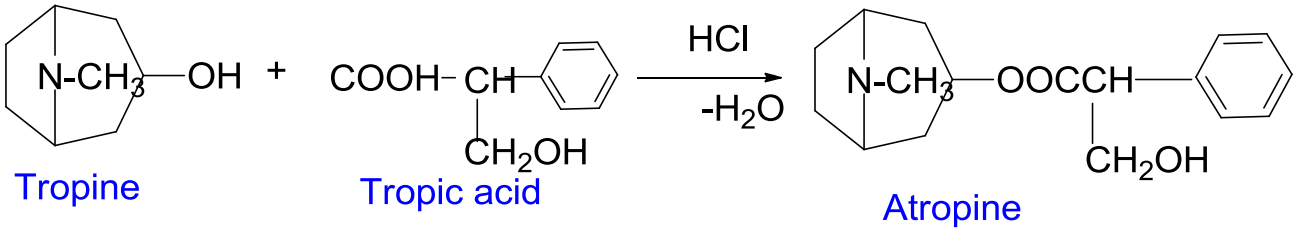
e) Formation of cycloheptatriene from tropine.



Synthesis of Tropine:



Structure of Atropine:



1.12 COCAINE:

1.12 .1 Introduction: Isolated from the leaves of *Erythroxylon cocaL.*Leaves are powderd and digested with carbonate and water. It is extracted with light petroleum. The alkaloids are removed by adding dil.sulphuric acid. The acid solution is evaporated to yield a crystalline solid of cocaine.

1.12.2 Physical Properties:Colourless crystals, sparingly soluble in water, strong tertiary base.

1.12.3 Constitution of Cocaine:

Molecular formula of cocaine from elemental analysis was found to be $C_{17}H_{21}NO_4$.

Nature of nitrogen atom: It is a strong tertiary base (pK 8.7) and adds one molecule of methyl iodide to form methiodide. It also reacts with cyanogen bromide to give methyl bromide indicating the presence of N-CH_3 group.

$$C_{17}H_{21}NO_4 + CH_3I \longrightarrow C_{17}H_{21}NO_4.CH_3I$$

$$C_{17}H_{21}NO_4 + CNBr \longrightarrow C_{16}H_{18}NO_4.CN + CH_3Br$$

Hydrolysis: When cocaine is heated with water, it is hydrolysed to methanol and benzoyl ecgonine.

$$C_{17}H_{21}NO_4 + H_2O \longrightarrow \underset{\text{Benzoyl Ecgonine}}{C_{16}H_{19}NO_4} + CH_3OH$$

Benzoyl ecgonine mustc ontain a –COOH group. Thus cocaine is the methyl ester of benzoyl ecgonine. It is further proved that when benzoyl ecgonine is heated with methanol it forms cocaine.

When benzoyl ecgonine is boiled with $Ba(OH)_2$ it undergoes hydrolysis yields benzoic acid and ecgonine. But Ecgonine shows reactions of alcohol. Hence, benzoyl ecgonine is the benzoyl derivative of ecgonine.

$$C_{16}H_{19}NO_4 + H_2O \xrightarrow{Ba(OH)_2} \underset{\text{Ecgonine}}{C_9H_{15}NO_3} + \underset{\text{Benzoic acid}}{C_6H_5COOH}$$

a) Constitution of Ecgonine:

Molecular formula of the compound is $C_9H_{15}NO_3$.

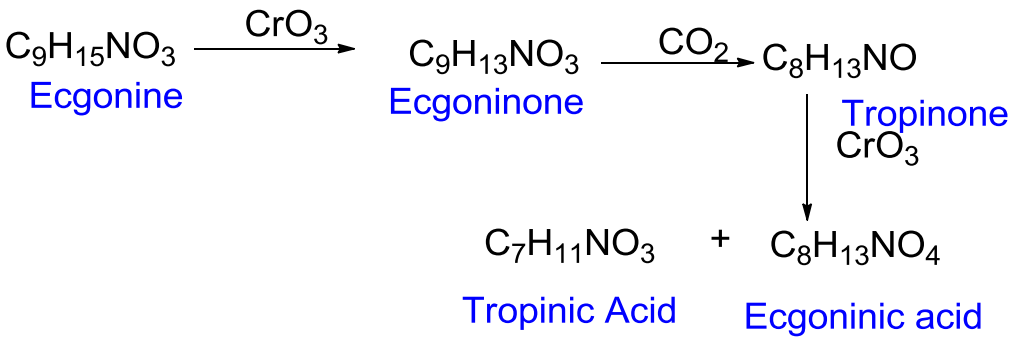
It is a tertiary base as it forms a crystalline solid $C_9H_{15}NO_3.CH_3I$ with methyl iodide.

Ecognine forms an ester and salt with alcohol and alkali it means it contains a carboxyl group.

Presence of –OH group is indicated by its reaction with acid chlorides and anhydrides to form acyl derivative. The acyl derivative can be esterified indicating that ecgonine as both alcohol and an acid.

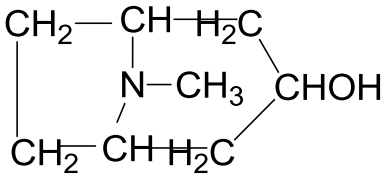
Ecgonine on oxidation yields a ketone, ecgoninone, means ecgonine contains a secondary alcoholic group i.e., –CHOH group.

Ecgonine on oxidation with CrO_3 forms a ketone ecgoninone which soon loses a molecule of CO_2 to form tropinone, further oxidation yields tropinic acid.



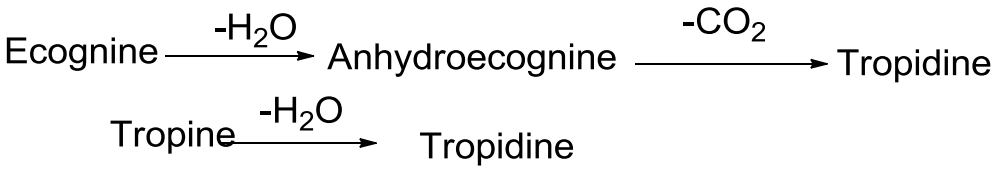
Based on the above reactions,

Ecgonine contains the tropane skeleton.

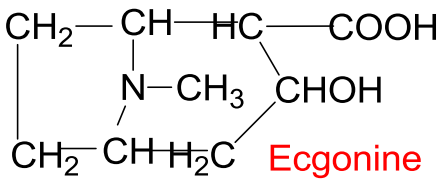


Tropine

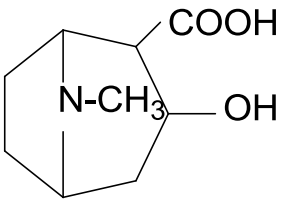
Ecgonine undergoes dehydration to form anhydroecgonine which on decarboxylation forms tropidine.



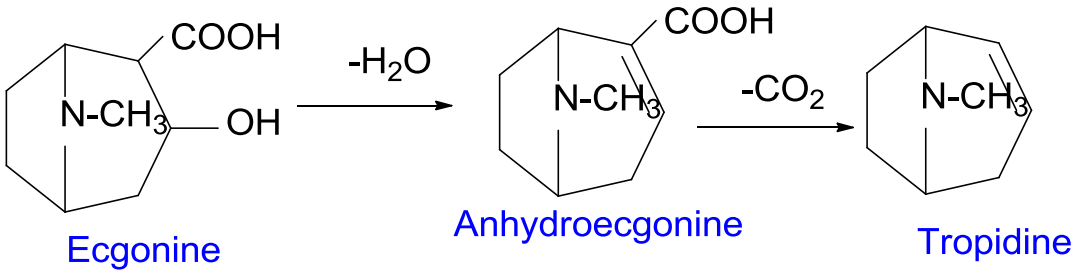
Ecgonine undergoes easy decarboxylation which reveals it is a β-keto acid.



Ecgonine



a) Reactions:

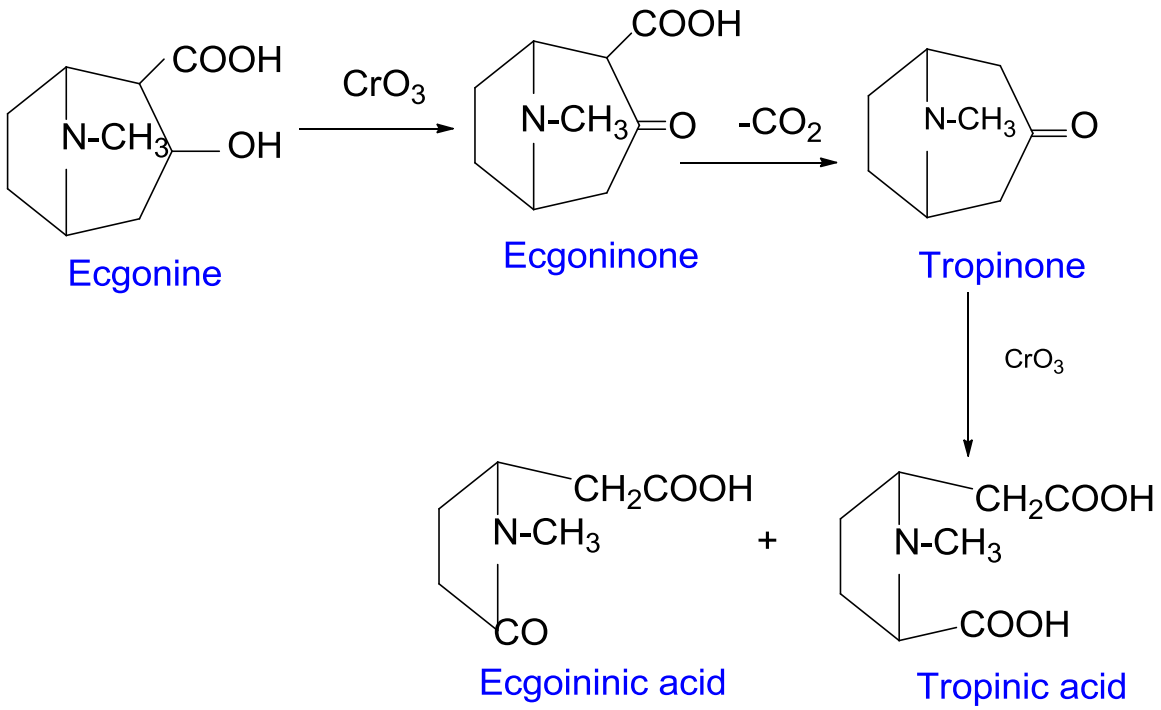


Ecgonine

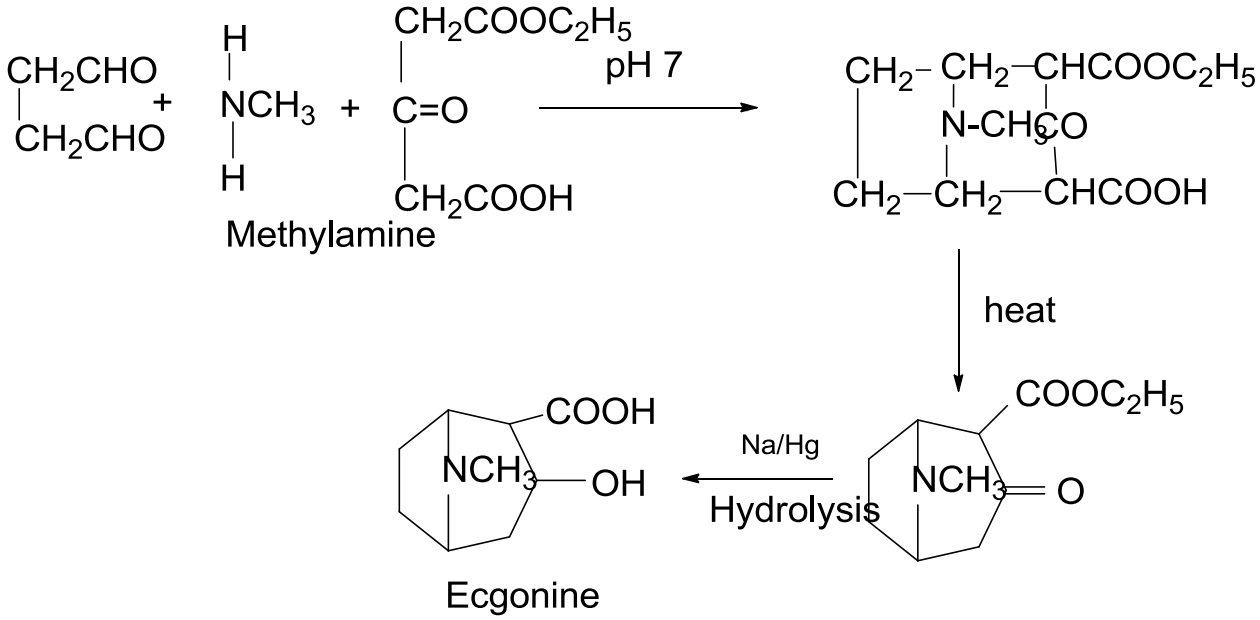
Anhydroecgonine

Tropidine

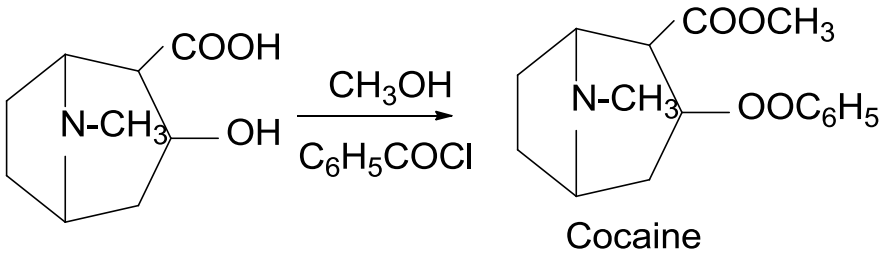
b) Reaction 2:



Synthesis of Ecgonine:



Constitution of Cocaine:



1.13 QUININE

1.13.1 Isolation:

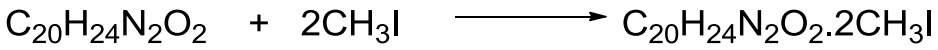
It is obtained from the barks of cinchona bark, contains thirty alkaloids but its antimalarial activity is due to quinine, quinidine, cinchonine and cinchonidine. It is Isolated by crushing the Bark into fine powder . Lime and caustic soda is added and extracted with petroleum ether. IT is then washed with dilute sulphuric acid and allowed to stand for several hours where the mixture of sulphates is recrystallized with quinine sulphate having maximum solubility. It is obtained by precipitation with alkali, washing and drying.

1.13.2 Constitution of Quinine:

Elemental analysis shows the molecular formula of the compound is $C_{20}H_{24}N_2O_2$.

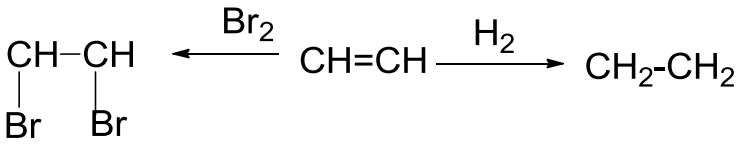
Presence of two tertiary nitrogen atoms:

Quinine adds two molecules of methyl iodide to form a diquaternary salt, it is a ditertiary base.



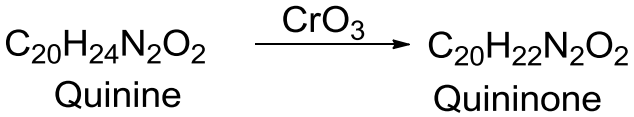
Presence of **olefinic linkage:**

Quinine adds one molecule of bromine and absorbs one molecule of hydrogen in the presence of a catalyst indicating the presence of one ethylenic bond.



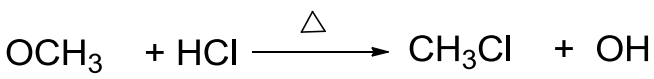
Presence of **secondary alcoholic group:**

Quinine forms monoacetate and monobenzoate indicates that it must contain one –OH group. Quinine on oxidation gives ketone, quininone.



Presence of **methoxyl group:**

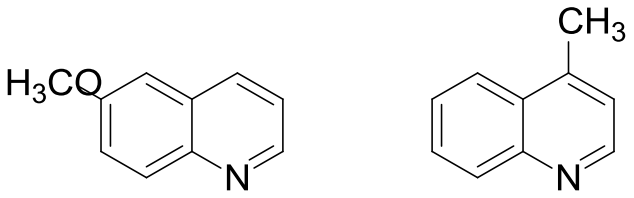
Quinine on heated with HCl, it eliminates as methyl chloride indicating the methoxyl group in quinine.



Presence of a **vinyl group:** On controlled oxidation with $KMnO_4$ it yields a monocarboxylic acid and formic acid which reveals the presence of vinyl group.

Presence of **quinoline group:**

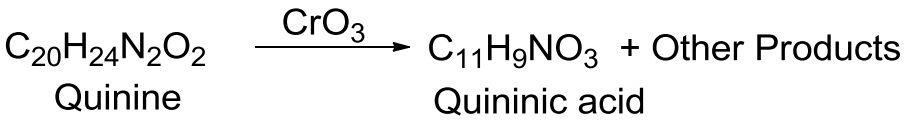
Quinine on fusion with concentrated KOH, it yields a mixture of 6-methoxy quinoline and lepidine, indicating that quinoline nucleus is present in quinine.

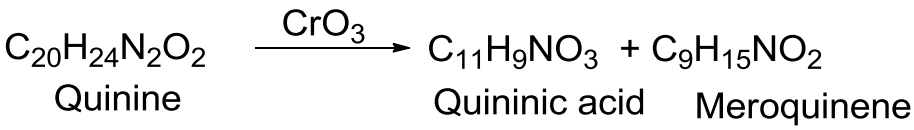


6-methoxy quinoline Lepidine

Presence of **meroquinone:**

Oxidation of quinine with chromic acid produces quininic acid.



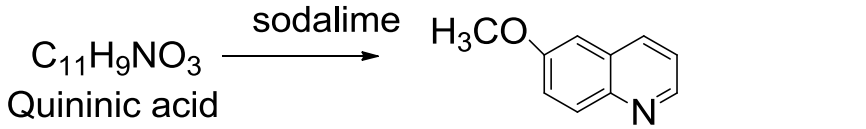


Controlled oxidation of quinine gives quininic acid and meroquinene.

a) Structure of quininic acid:

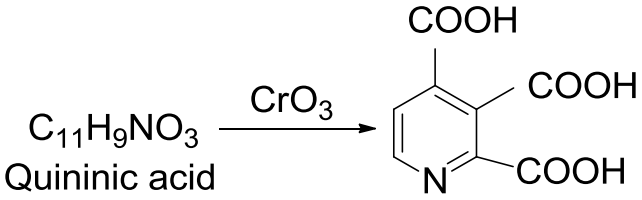
Molecular formula is $C_{11}H_9NO_3$

When quininic acid is heated with sodalime it undergoes decarboxylation yielding methoxyquinoline.



When quininic acid is oxidised with chromic acid it yields pyridine 2,3,4 tricarboxylic acid indicating that the methoxyl group is a substituent in the benzene ring of quinoline and COOH is at position 4.

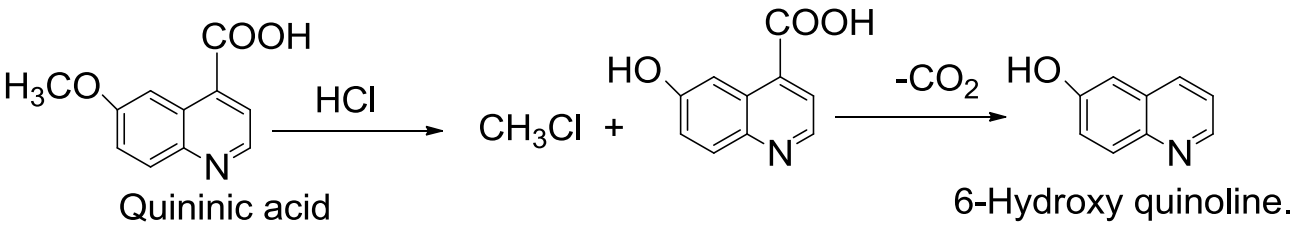
Reaction:



Pyridine 2,3,4-tricarboxylic acids

To ascertain the position of methoxy group , quininc acid is heated with HCl to yield demethylated product which on decarboxylation yields 6-hydroxy quinoline.

Reactions:

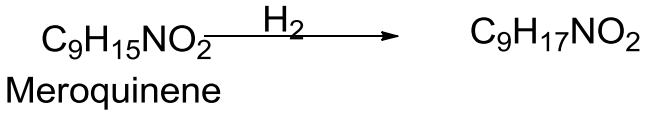


b) Structure of Meroquinene:

Molecular formula of Meroquinene is $C_9H_{15}NO_2$.

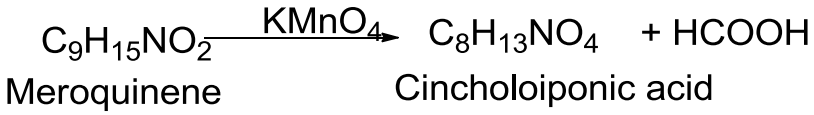
It forms monosodium salt as well as ester it represents the presence of –COOH group.

Merquinene is reduced with hydrogen suggesting the presence of one ethylenic bond, indicates the presence of side chain in the molecule.



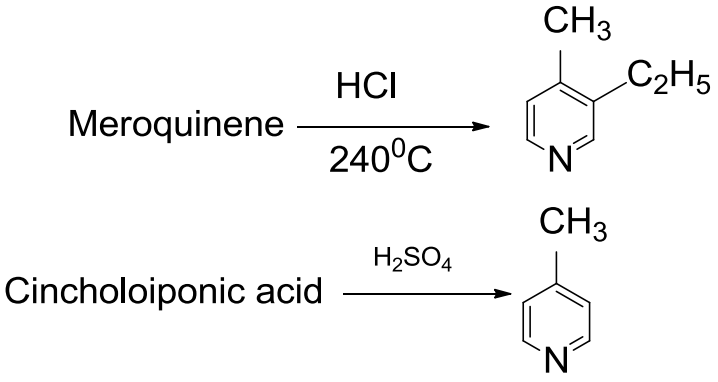
Meroquinene is benzoylated, acetylated and nitosated indicating the presence of secondary amino group .

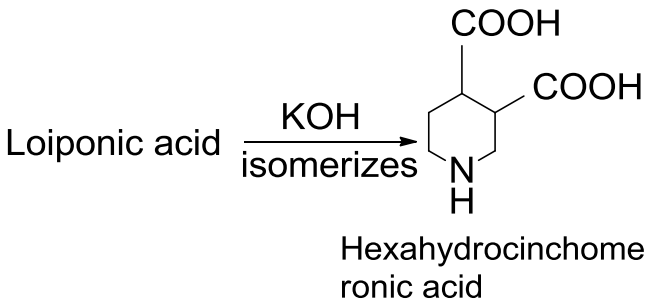
Meroquinene is oxidised with acidified $KMnO_4$, it yields a cincholoiponic acid and formic acid., indicates the presence of $–CH=CH_2$ group in the side chain.



When cincholoiponic acid is oxidised with acid $KMnO_4$ it yields loiponic acid, $C_7H_{11}NO_4$. It is a carboxylic acid with a methylene group indicate that cincholoiponic acid contains $–CH_2COOH$.

The three acids reveals the presence of a piperidine ring as shown by the following reactions.



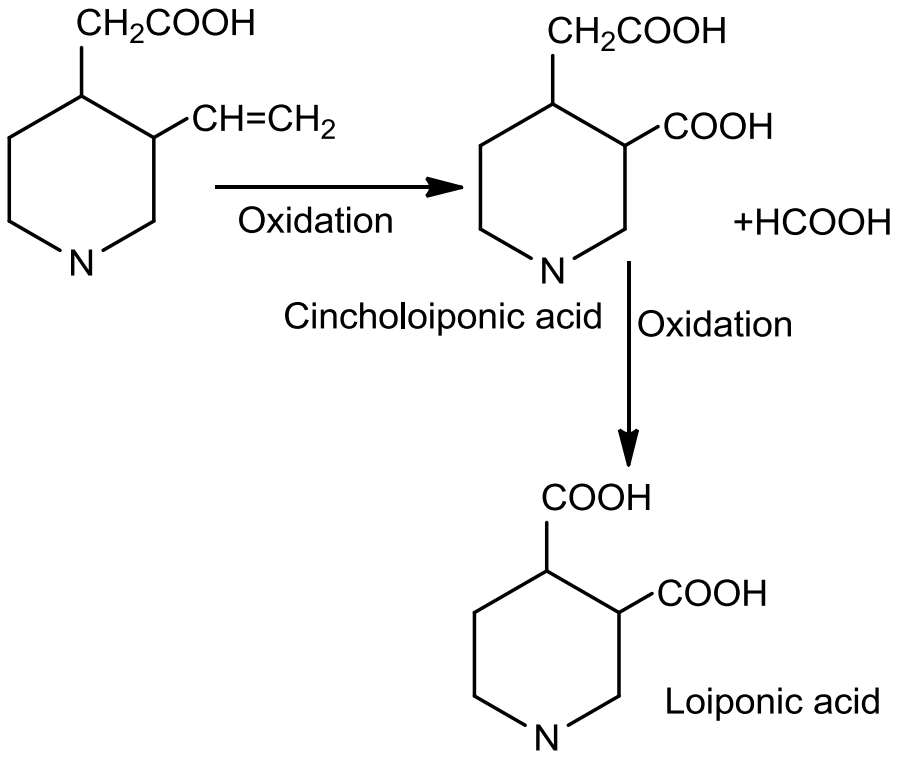


Thus the skeleton of meroquinine will be,

The position of remaining carbon can be at any one of the three positions,

1.As N- methyl group- since all the three acids are secondary bases it is been ruled out.

Since meroquinine contains a side chain of $–CH=CH_2$, the possibility of one carbon in the side chain will leads to the presence of allyl group. This allyl group would result in propyl group not an ethyl group. Carbon can be attached to COOH at 4 position. The reactions of meroquinine can be explained by this structure.

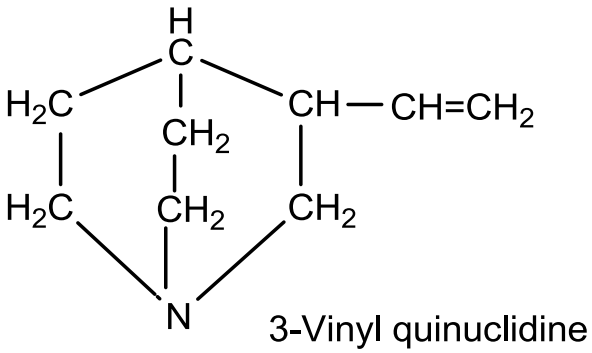


c) Point of linking between quininic acid and meroquinine:

In quinine molecule, the carboxylic groups are not present. Quininic acid and meroquinine contains free carboxylic acid group indicating that the two fragments are linked through this –COOH group.

Quinine is a di tertiary base while meroquinine is a secondary base indicating that the tertiary nitrogen is converted to –NH and at the same time –COOH group is produced. It is possible only when the nitrogen is in a condensed ring system.

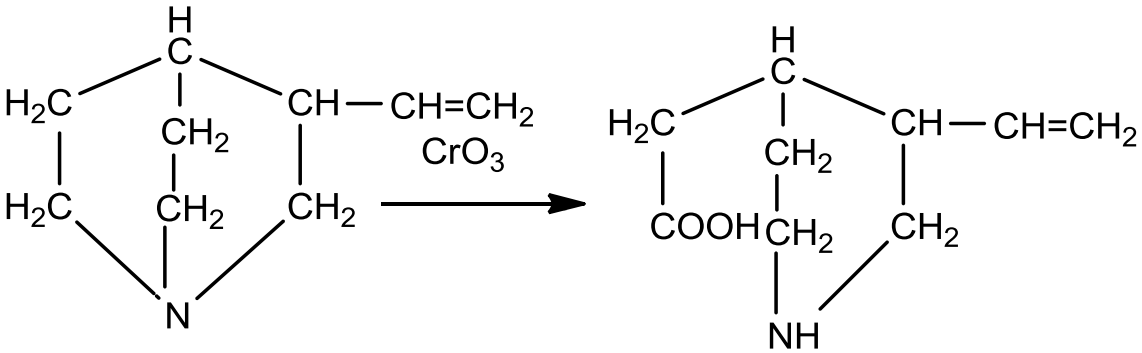
A possible structure could be:



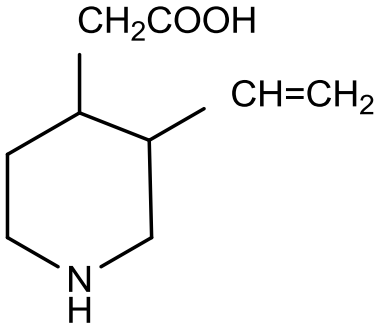
3-Vinyl quinuclidine

When 3-Vinyl quinuclidine is oxidised with CrO_3 one C-N bond is cleaved thus producing an secondary nitrogen and a COOH group.

Reaction:



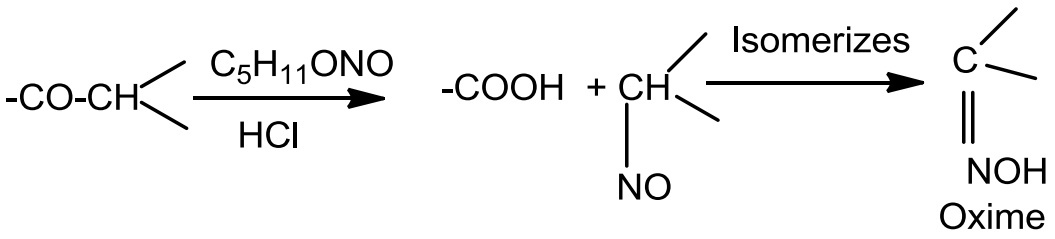
Structure of Meroquinine:



In quinine molecule, the quinoline fraction is joined at position 4 to the quinuclidine at position-8.

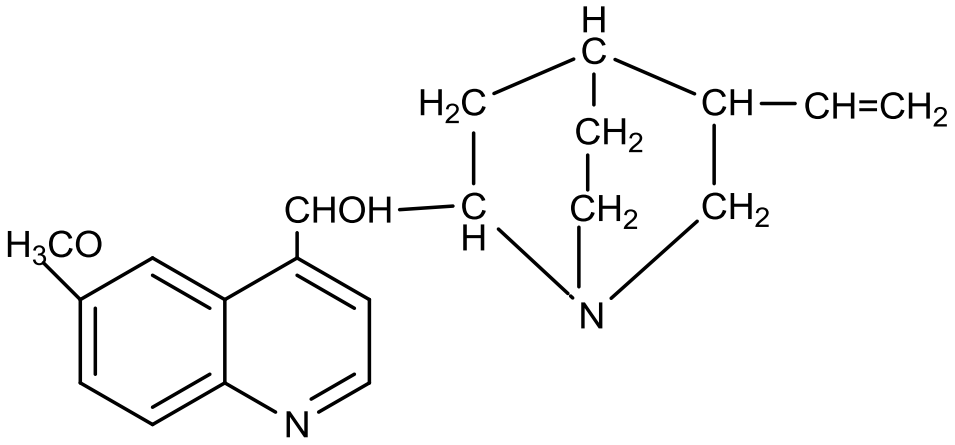
d) Position of secondary alcoholic group:

Quinine is oxidised to quninone, on treating with amyl nitrite and HCl yields quininic acid and an oxime. The formation of acid and an oxime reveals the presence of –COCH- (ie a methylene group is adjacent to carbonyl group).

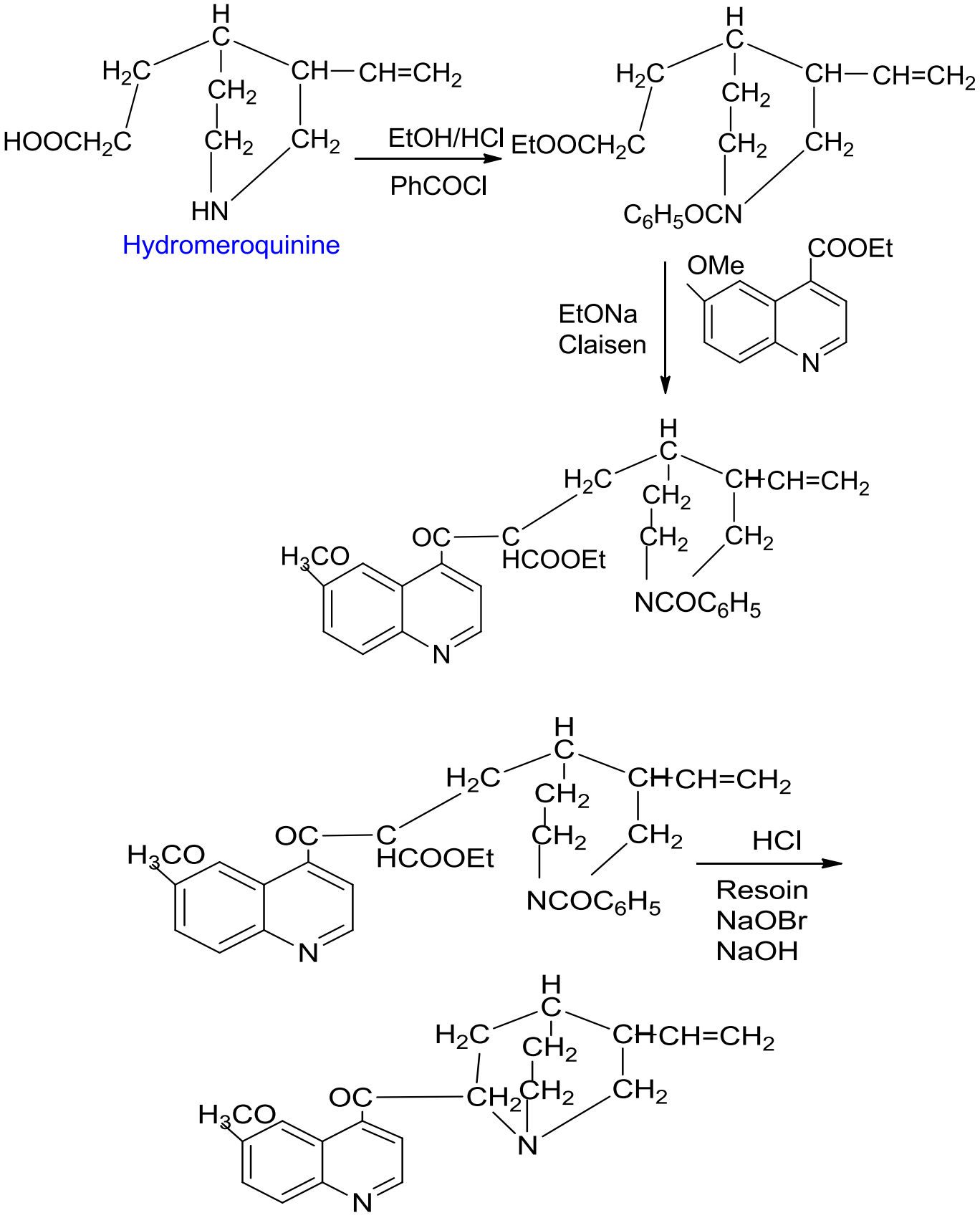


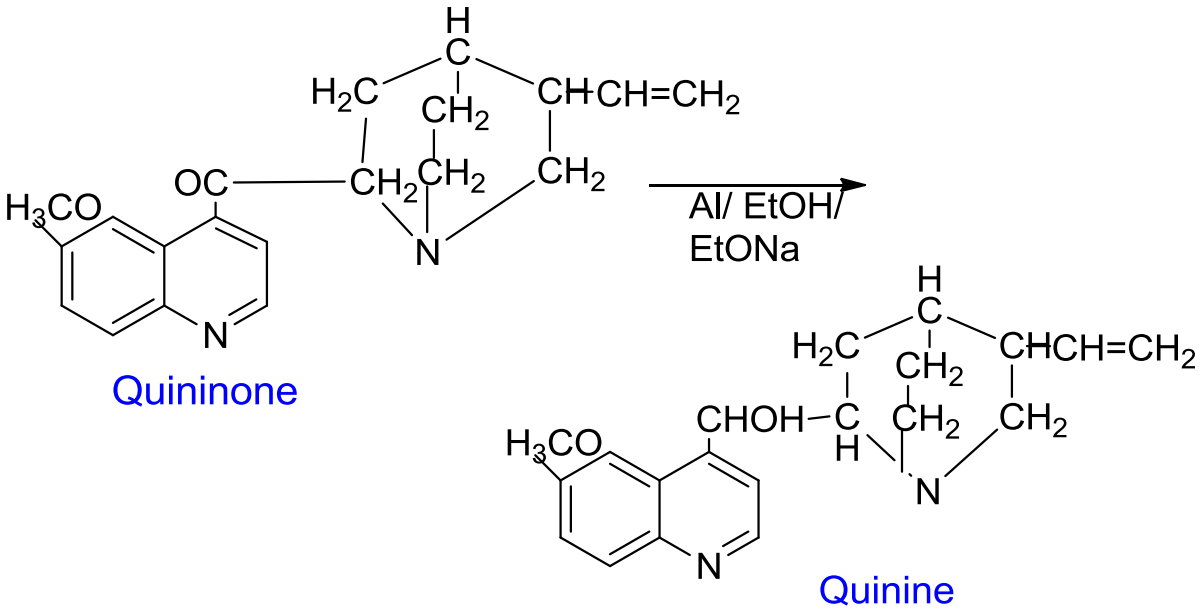
The above reaction is ascertained by its hydrolysis to meroquinine and hydroxyl amine. It follows that both quinoline and quinuclidine units are linked by –CHOH groups.

Thus the **structure of quinine is,**



Synthesis of quinine:





1.14 RESPERPINE

Main constuituent of Rauwolfia species. They are hypertensive and sedative reagents.

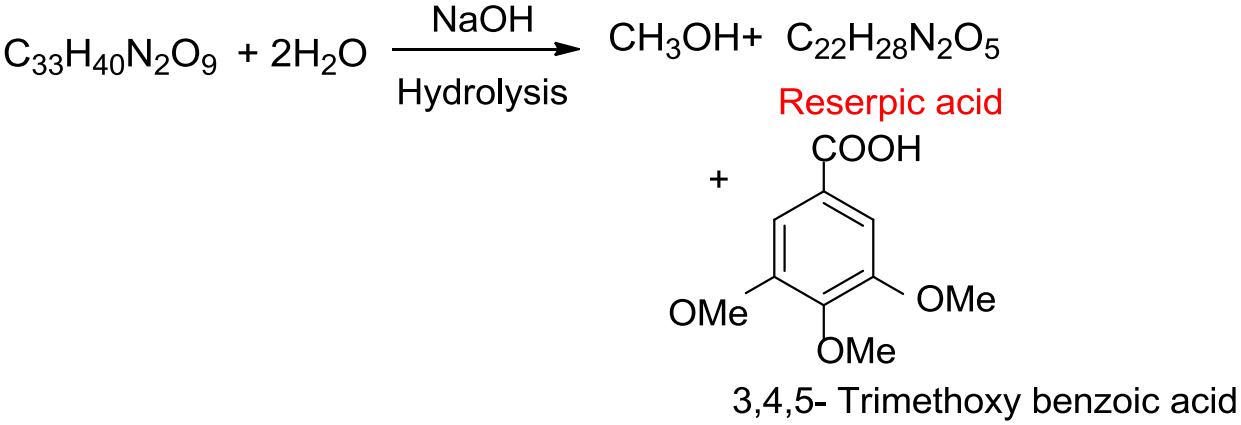
1.14.1 Constitution of Reserpine

Molecular formula is $C_{33}H_{40}N_2O_9$.

Presence of five methoxy groups: Resperpine on heating with HI,yields 5 molecules of CH_3I indicating the presence of 5 methoxy groups in resperpine.

Nature of N atom: It is a weak base indicating both the nitrogen is present in the ring. It doesn't have a hydroxyl group but forms monoacetyl derivative indicating one of the nitrogen as NH group. Other nitrogen is an 3^0 N.

Hydrolysis:When reserpine is hydrolysed with alkali solution it yields a mixture of methyl alcohol, 3,4,5 trimethoxy benzoic acid and reserpic acid ($C_{22}H_{28}N_2O_5$).



As reserpine doesnot contain –COOH or –OH groups, introduction of two-COOH groups and two alcoholic OH groups in its hydrolysis products revelas that reserpine is a

diester. The ester linkage is confirmed by its reduction with $LiAlH_4$ to reserpic alcohol, $C_{22}H_{30}N_2O_4$ and 3,4,5 trimethoxy benzyl alcohol.

a) Structure of Reserpic Acid:

Molecular Formula was found to be $C_{22}H_{28}N_2O_5$

Presence of one carboxyl group: Reserpic acid forms monosodium salt with NaOH indicates the presence of one carboxyl group.

Presence of one –OH group: Reserpic acid contains one alcoholic –OH group, is a secondary alcoholic group because reserpic acid o oxidation yields a ketone.

Presence of two methoxy groups: By zeisel method, it is shown that reserpic acid contains two methoxy groups.

Nature of two nitrogen atoms: In reserpic acid, two nitrogen atoms are present in heterocyclic ring, one as secondary amino and the other as tertiary nitrogen atom.

Thus reserpic acid contains two methoxy group, one COOH group and one alcoholic OH group.

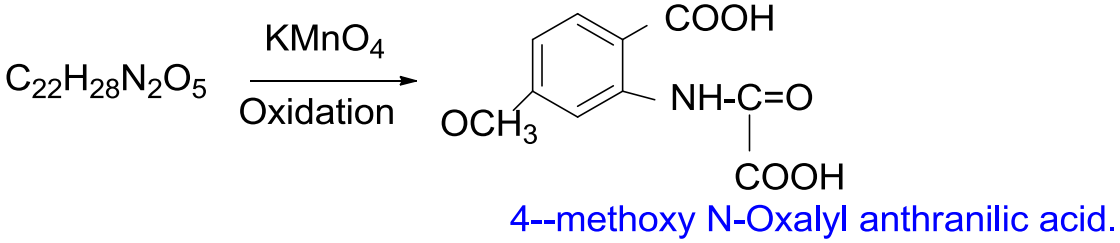
$$C_{19}H_{29}N_2 \quad \left\{ \begin{array}{l} 2\ OCH_3 \\ 1\ -COOH \\ 1\text{-}OH \end{array} \right\}$$

Reduction of Reserpic acid: On reduction with $LiAlH_4$, it yields reserpic alcohol which has two methoxy, one –OH and one $–CH_2OH$ groups.

$$C_{15}H_{20}N_2 \quad \left\{ \begin{array}{l} 2\ OCH_3 \\ 1\ -CH_2OH \\ 1\text{-}OH \end{array} \right\}$$

Oxidation of reserpic acid:

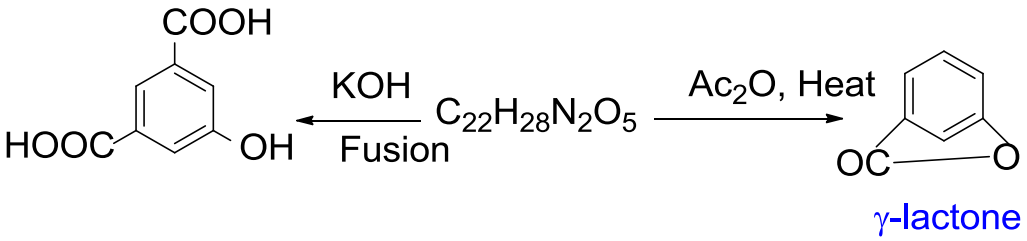
On oxidation with $KMnO_4$ it yields 4-methoxy N-oxalyl anthranilic acid as one of the oxidation products, confirming the presence of one indole nucleus in reserpic acid. Moreover, it reveals that one of the methoxy group is in m-position to NH group.



4--methoxy N-Oxalyl anthranilic acid.

Fusion with KOH:

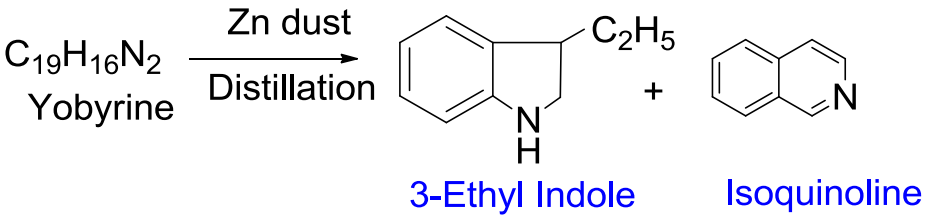
Reserpic acid is fused with potash, to yield 5- hydroxyphthalic acid in which the hydroxyl group and –COOH group must be in m-position to each other. Reserpic acid on heating with acetic anhydride yields a γ-lactone.



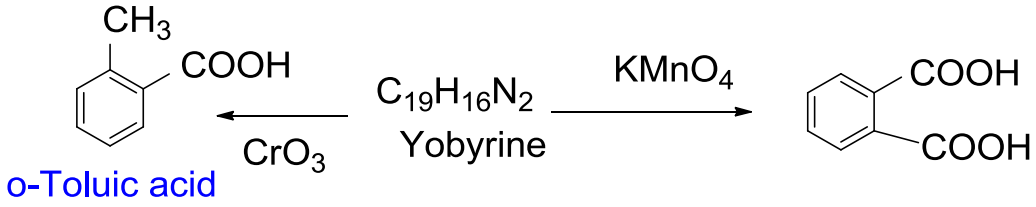
Dehydrogenation: when methyl reserpate is dehydrogenated with Se it yields a hydrocarbon with $C_{19}H_{16}N_2$. this hydrocarbon is obtained by dehydogenation of yohimbine with Se hence called as Yobyrine.

b) Structure of Yobyrine:

When distilled with Zn dust, yobyrine yields 3-ethyl indole and isoquinoline.

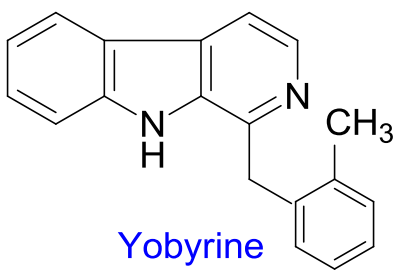


When yobyrine is oxidised with $KMnO_4$, it yields phthalic acid. On oxidation with CrO_3 it yields o-toluic acid.

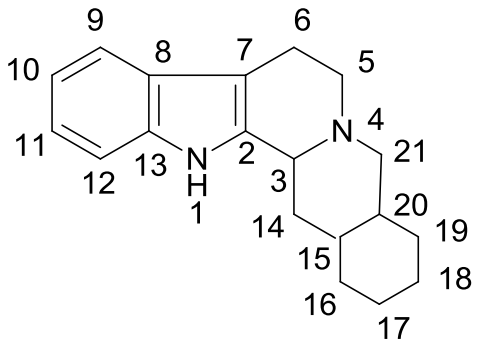


Yobyrine gives condensation products with aldeydes indicating the presence of pyridine ring with a $–CH_2$ substituent adjacent to nitrogen atom.

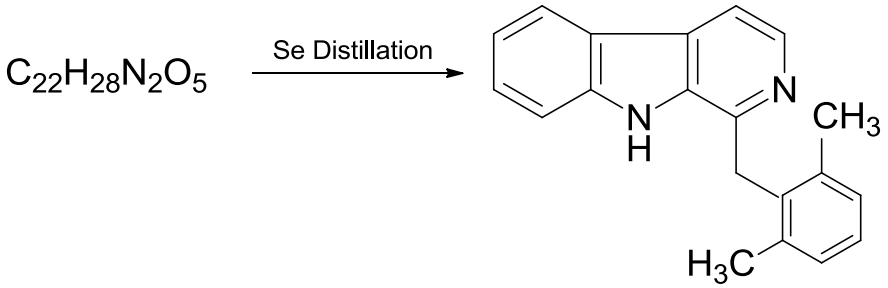
Thus the structure of yobyrine is



As yobyrine is formed from reserpic acid, it follows it possess the following skeletal structure.

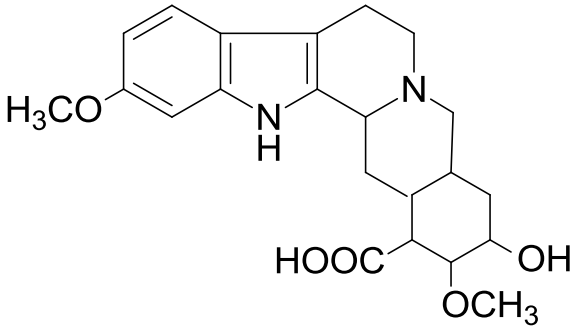


Reserpic acid on dehydrogeation yields 11-hydroxy 16-methyl yobyrine indicating that –COOH group is present at C_{16}. further, one of the methoxy group is present in the m-position to the NH group of the indole.



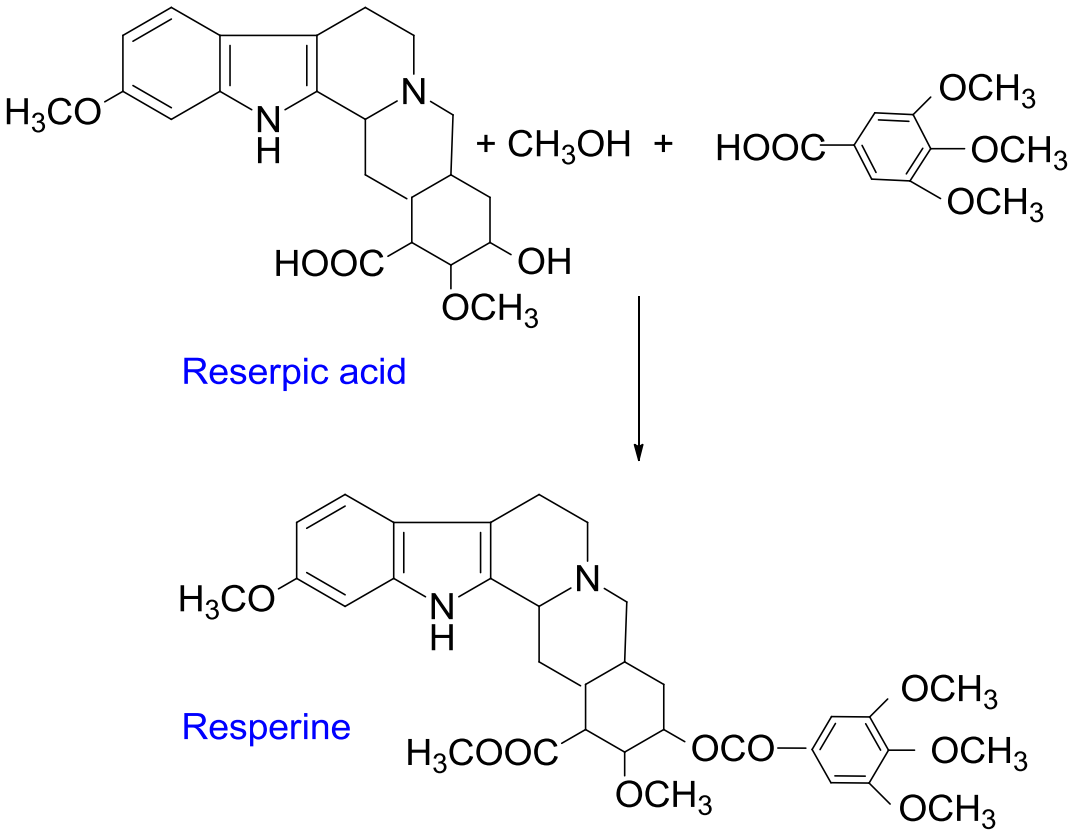
11-Hydroxy 16-methyl yobyrine

Further the COOH group and OH group are in m-position to each other. The COOH group is at C_{16}. Hence the –OH group is at C_{18}. The second methoxy group is t C_{17}.

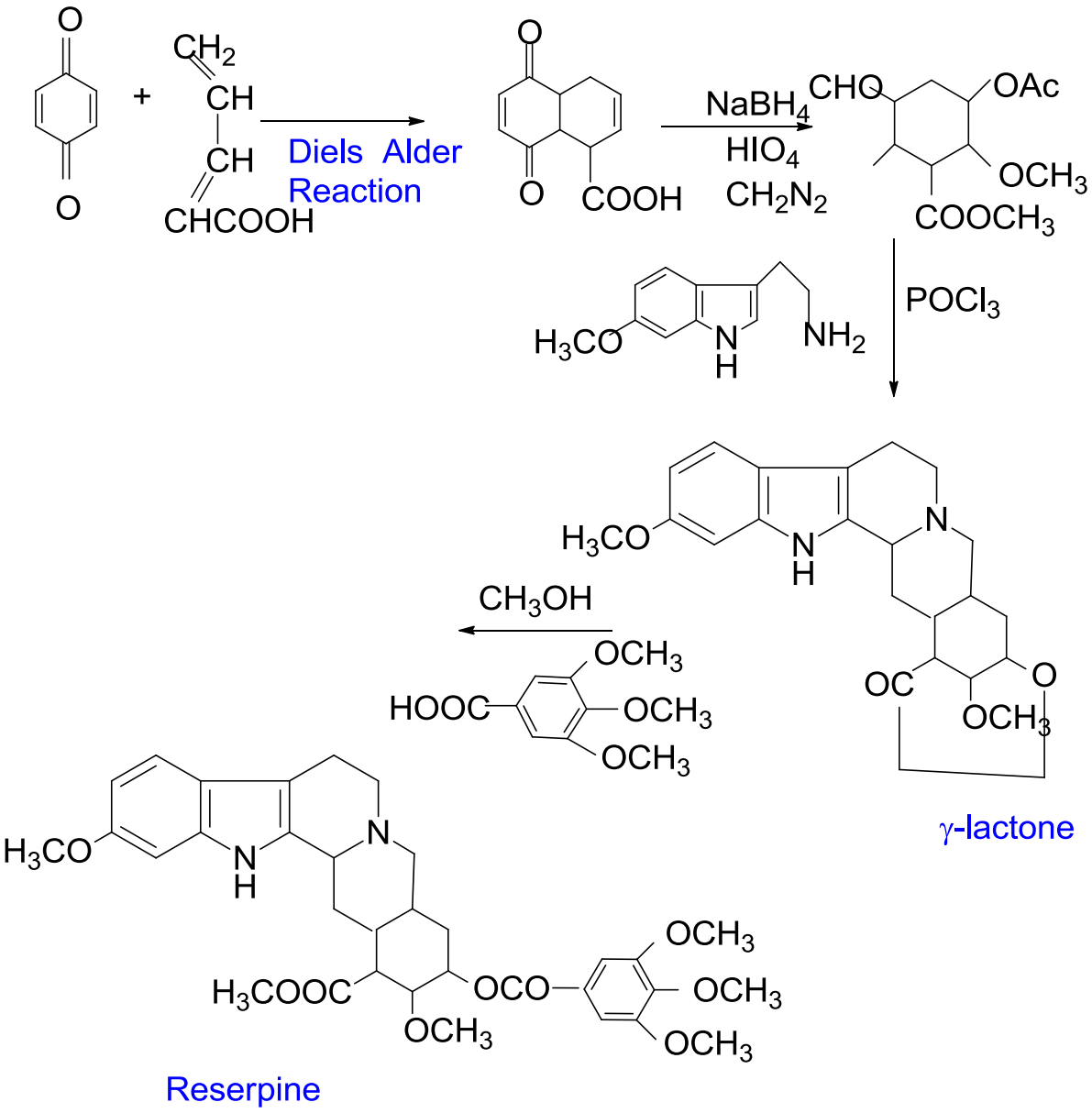


Reserpic acid

Structure of Reserpine:



Synthesis of Reserpine:



1.15 MORPHINE

1.15.1 Occurence:

Morphine was isolated from serturner plant (1806). In opium, it is present in the quantity of 10-23% along with other substances of fats, resins, proteins and so on.Codenine, Thebaine and morphine are closely related alkaloids- opium alkaloids. It belongs to phenanthrene alkaloids.Used as analgesic agent.Colourless, prismatic with a bitter taste. Soluble in alcohol and alkali solution.

1.15.2 Constitution of Morphine:

Molecular formula is $C_{17}H_{19}O_3N$.

Nature of nitrogen atom: It adds on one molecule of CH_3I to form quaternary salt, indicating the presence of tertiary nitrogen atom. By Herzig-Meyer method, revelas the presence of N-CH_3 group in morphine.

Nature of oxygen atoms: Morphine is acetylated or benzoylated forming diacetyl or dibenzoyl derivative indicating that morphine contains two hydroxyl groups.

$$C_{17}H_{17}ON(OH)_2 \xrightarrow[2CH_3COCl]{\text{Acetylation}} \underset{\text{Diacetyl Morphine}}{C_{17}H_{17}ON(OCOCH_3)_2} + 2HCl$$

$$C_{17}H_{17}ON(OH)_2 \xrightarrow[2C_6H_5COCl]{\text{Benzoylation}} \underset{\text{Dibenzoyl Morphine}}{C_{17}H_{17}ON(OCOC_6H_5)_2} + 2HCl$$

With Ferric Chloride, Morphine yields a characteristic violet colour which is soluble in NaOH to form monosodium salt which is reconverted to morphine indicating the hydroxyl group are phenolic in nature.

Morphine is treated with halogen acids, to form monohalogen derivative ie., one hydroxyl group is replaced by halogen acid. Hence, one of the hydroxyl group is alcoholic in nature.

Morphine is heated with CH_3I in the presence of aqueous KOH, it is methylated to yield codeine, $C_{18}H_{21}O_3N$. As codeine doesn't give colour with $FeCl_3$ and it is not soluble in NaOH it follows that phenolic OH in morphine is methylated.

Futher, codeine on oxidation with chromic acid, it yields codeinone, a ketone indicating that the hydroxyl group in codeine is a secondary alcoholic in nature. Codeine is a monomethyl ether of morphine.

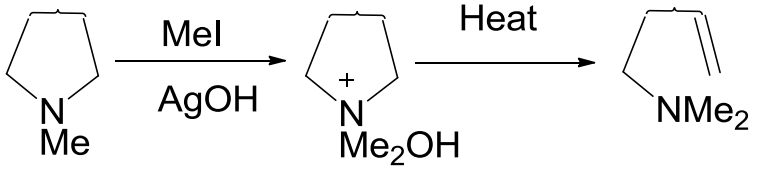
The third oxygen atom is highly unreactive indicating its nature as an ether linkage.

Presence of ethylenic Bond:

Codeine is reduced catalytically in the presence of palladium, suggesting both codeine and morphine contains one ethylenic bond.

Presence of Benzene Nucleus: On bromination, morphine forms monobromo derivative with HBr, indicating the presence of benzene nucleus.

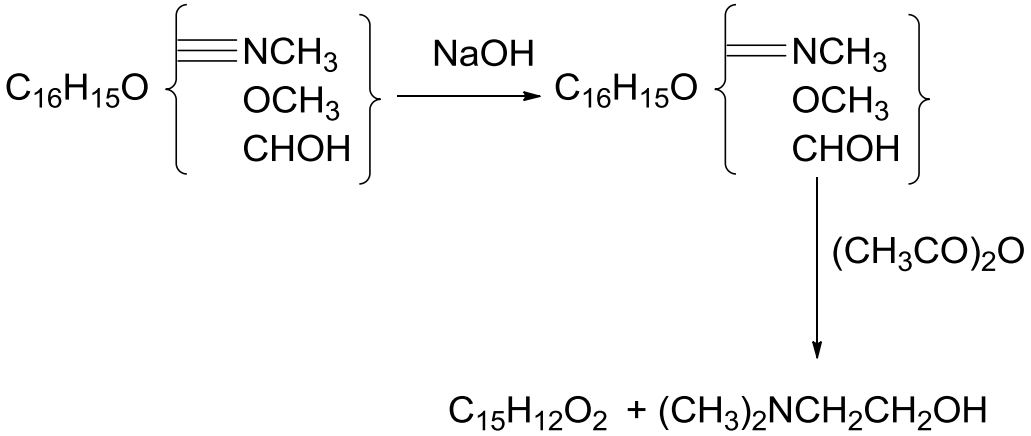
Presence of cyclic tertiary base system: Codeine on exhaustive methylation yields α-codeimethine, contains one $-CH_2$ group more than codeine and the nitrogen remains intact indicating the presence of cyclic t-amine.



Presence of Phenathrene: Morphine on distillation with Zn dust it yields a phenathrene and a number of bases.

Codeine on treating with CH_3I it yields codeine methiodide, on boiling with NaOH yields methylmorphimethine on further boiling with acetic anhydride yields a mixture of methyl morphol and ethanoldimethyl amine.

Reactions:



a) Structure of Methyl morphol:

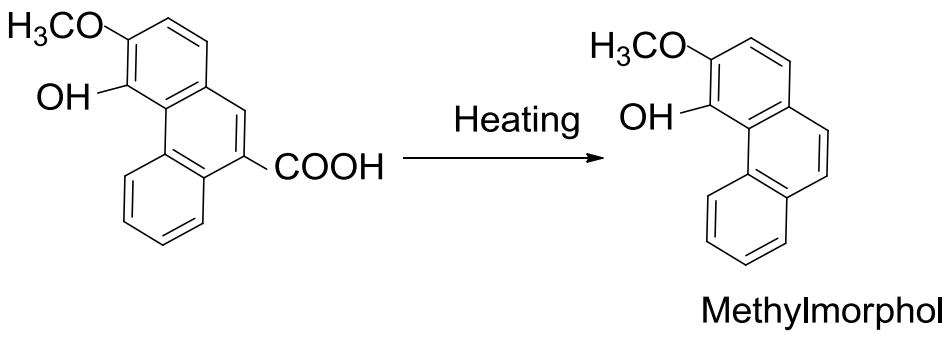
Heating the compound $C_{15}H_{12}O_2$ with HCl at 180^0c yields methyl chloride and dihydroxy phenanthrene ie morphol is obtained.

Diacetylmorphol on oxidation yields diacetyl phenanthraquinone indicating that the positions 9 and 10 are free.

Diacetylphenathraquinone on oxidation with $KMnO_4$ yields phthalic acid indicating that the two hydroxyl groups are in the same ring.

Methylmorphol is 4-hydroxy3-methoxyphenathrene.

Reactions:



Presence of $-NCH_3$ group:

The formation of ethanoldimethyl amine from methylmorphimethine revelas that both codeine and morphine contains a $N\text{-}CH_3$ group.

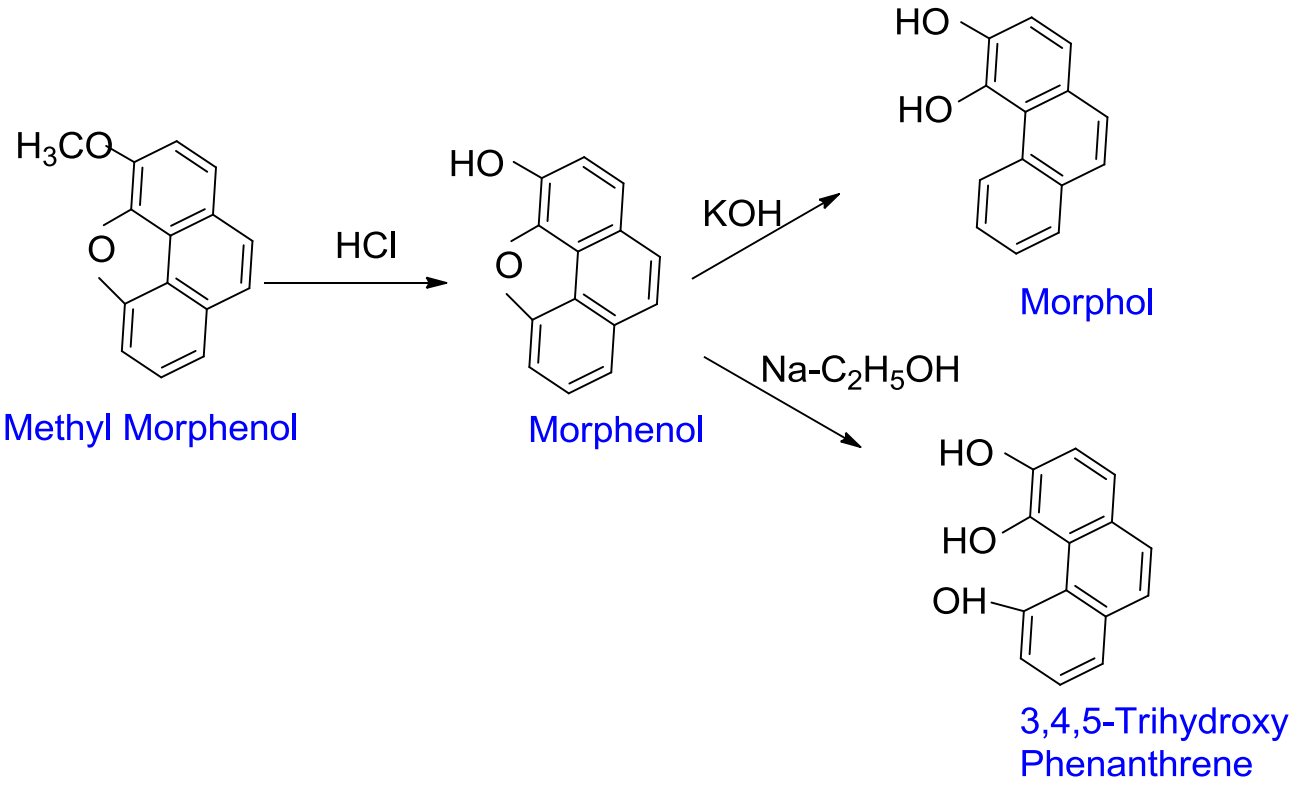
b) Structure of Morphenol:

When β-methylmorphimethine is heated with water, it yields a mixture of trimethyl amine, ethylene and methyl morphenol.

β–methylmorphimethine ⟶ Trimethyl amine + Ethylene + Methylmorphenol.

Methylmorphenol on demethylated with HCl, it yields morphenol, a compound with one phenolic hydroxyl group and an inert oxygen atom.

When morphenol is fused with KOH, it yields 3,4,5-trihydroxyphenanthrene. Also morhenol on reduction with $Na\text{-}C_2H_5OH$ it yields morphol.



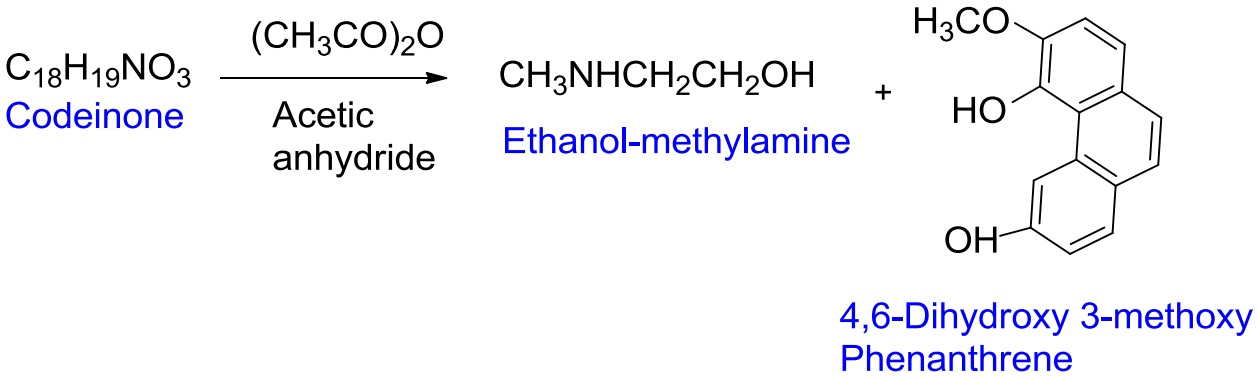
Thus, Morphenol contains an ether linkage at position 4 and 5 of the phenanthrene nucleus.

The structure of morphenol and its production from codeine reveals that the two of the three oxygen atoms (i.e) One at C3, and the other ether linkage at C_4 and C_5 of the phenanthrene nucleus.

Position of third oxygen:

Codeinone on heating with acetic anhydride yields ethanolmethyl amine and diacetyl derivative of 4,6-dihydroxy 3-methoxy phenanthrene.

Reactions:



The hydroxyl group in the 6 position must be from the oxygen of the keto group in codeinone.

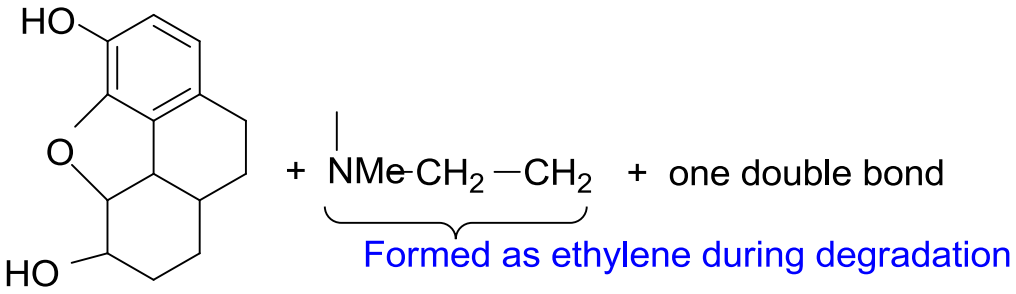
Position of **all three oxygen atoms** in morphine are : One at C_3- Phenolic; Other at C_4 and C_5 (Ether) and third (secondary alcohol) at C_6 of the phenanthrene nucleus.

c) Structure of Morphine:

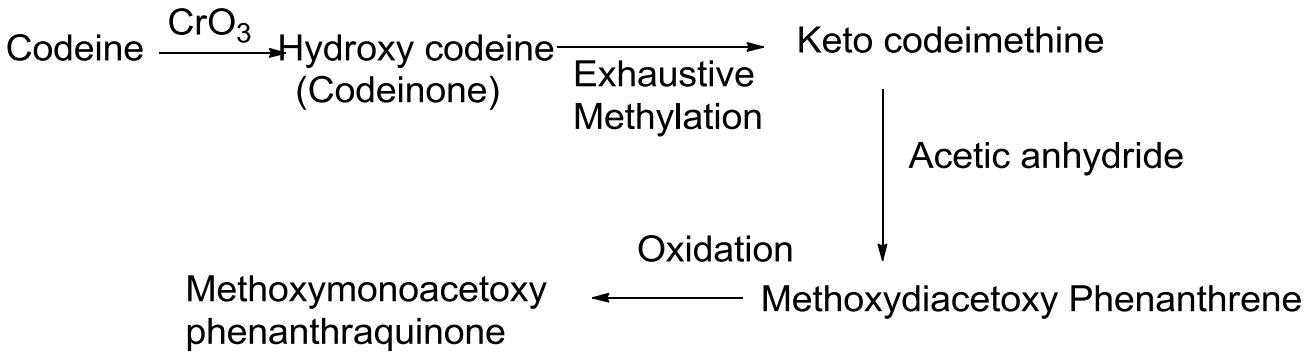
Morphine forms monobromo derivative with bromine and monosodium salt with NaOH, indicating that morphine contains a benzenoid structure.

On exhaustive methyaltion of codeimethines, ethylene and ethanol dimethyl amine is formed as the products,reveals the presence of N-CH_3 group.

Also a double bond and a tertiary nitrogen has to be present in morphine. Hence, the partial structure of morphine is,



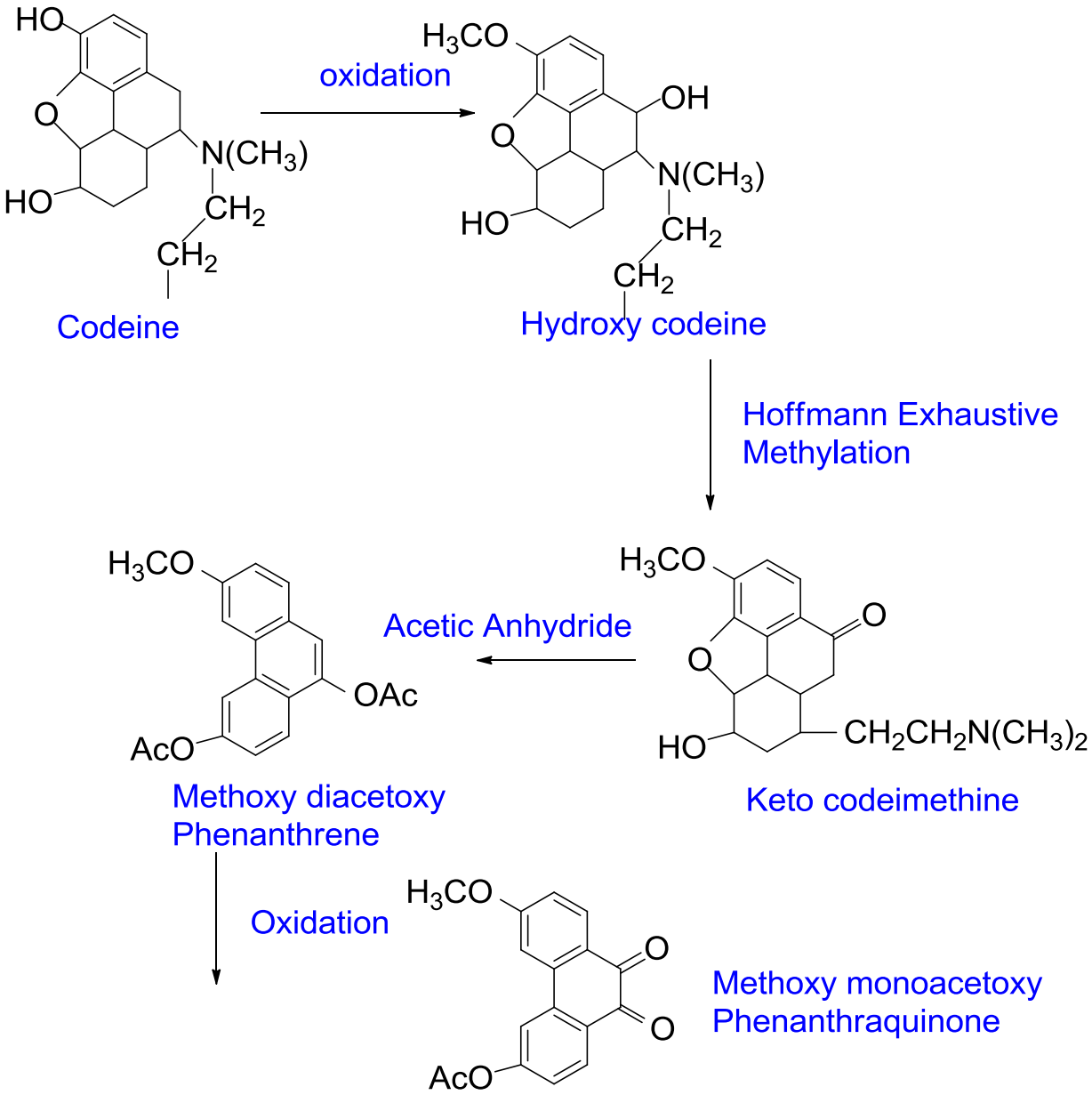
d) Point of linkage of CH_2-CH_2-N Me group:



Loss of acetyl group reveals that one of the acetoxy groups must be present either at C_9 or C_{10}. The acetyl group is inserted via ketonic group which concludes that the new hydroxyl group in hydroxy codeine is present either at C_9 or C_{10}. On the basis of steric consideration, the attachment at C_9 is most probable.

Hydroxyl group in hydroxycodeine is changed to keto group and a double bond is introduced between C_9 and C_{10} during the fission of the nitrogen ring. Nitrogen must linked either to C_9 or C_{10}. The exact point of linkage of nitrogen is at C_9, confirmed by its synthesis.

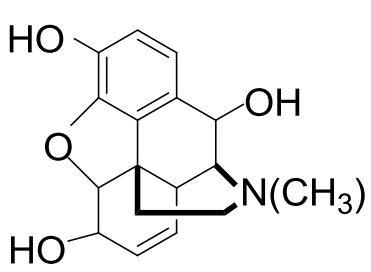
Reactions:



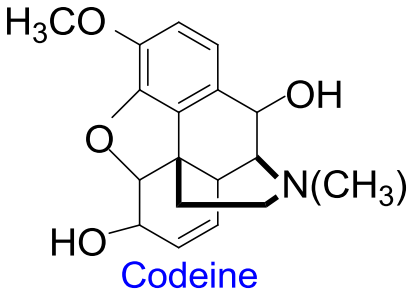
The carbon end of the side chain must be located at the angular position so that its extrusion from that position takes place during aromatisation at position C_{13} and C_{14}. The former is explained as this structure explains the formation of thebaine to thebenine.

Position of double bond: Codeine on heating with PCl_5 yields chlorocodide which on hydrolysis gives codeine, isocodenie, pseudocodeine and allopseudocodeine. The first two compound on xoidation gives the same ketone , revealing that they differ in the position of hydroxyl group at C_6. The remaining two also yields same ketone, indicating that the hydroxyl group is at position C8. These changes can be explained only if a double bond is present at C7 and C8.

The structure of Morphine and Codeine are:

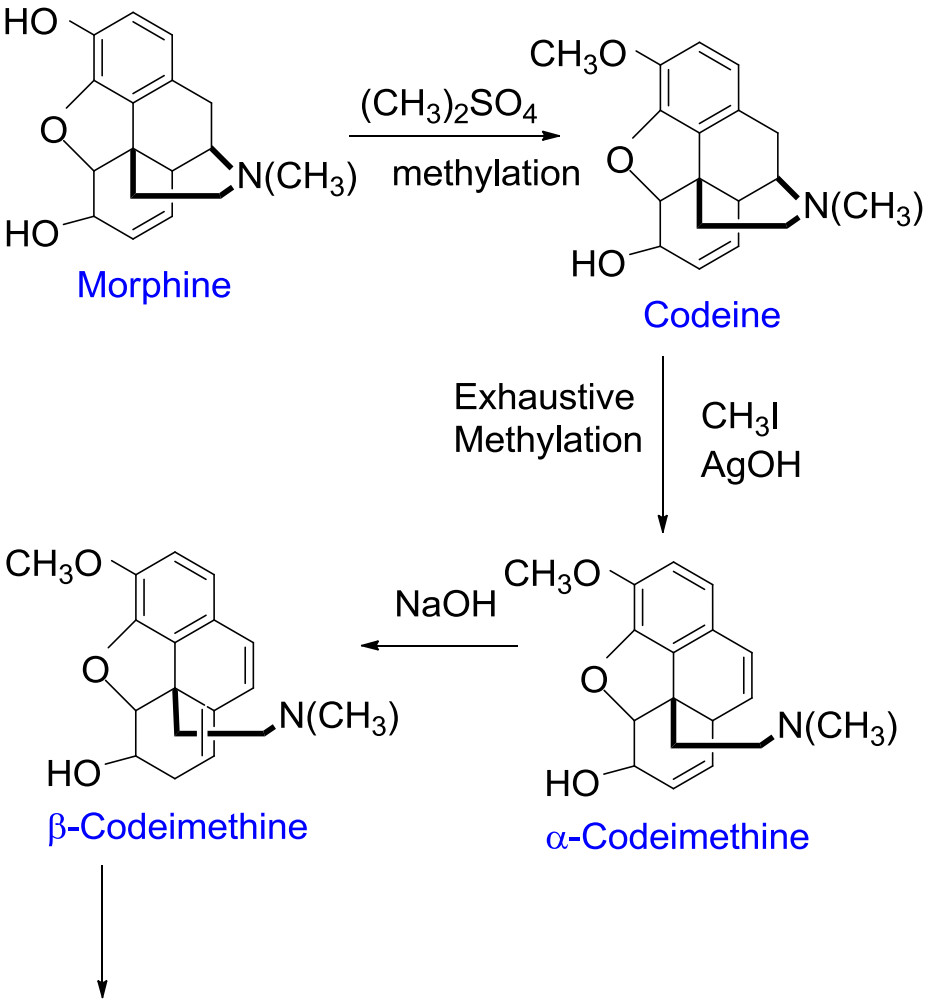


Morphine



Codeine

Reactions:

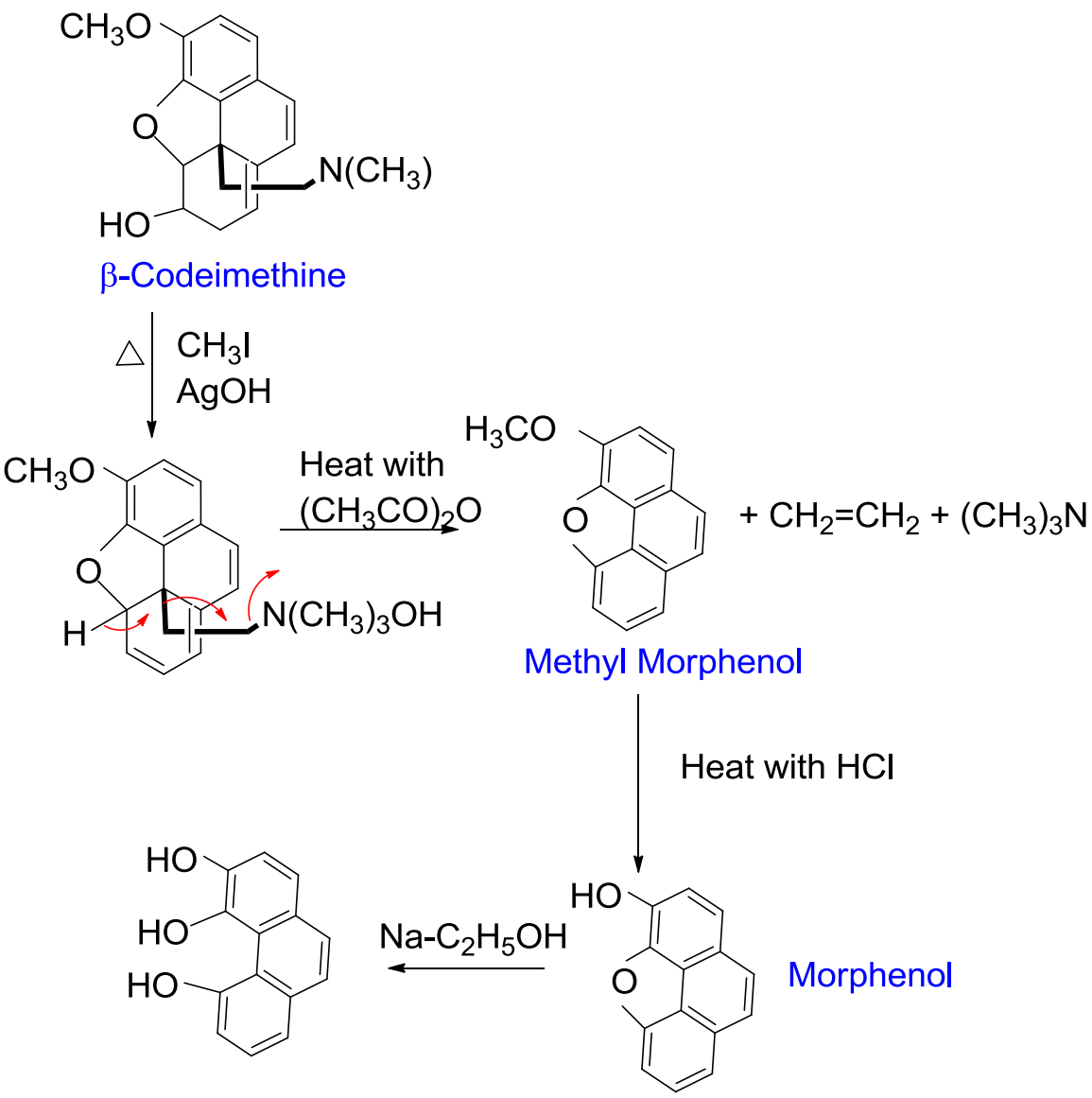


Morphine

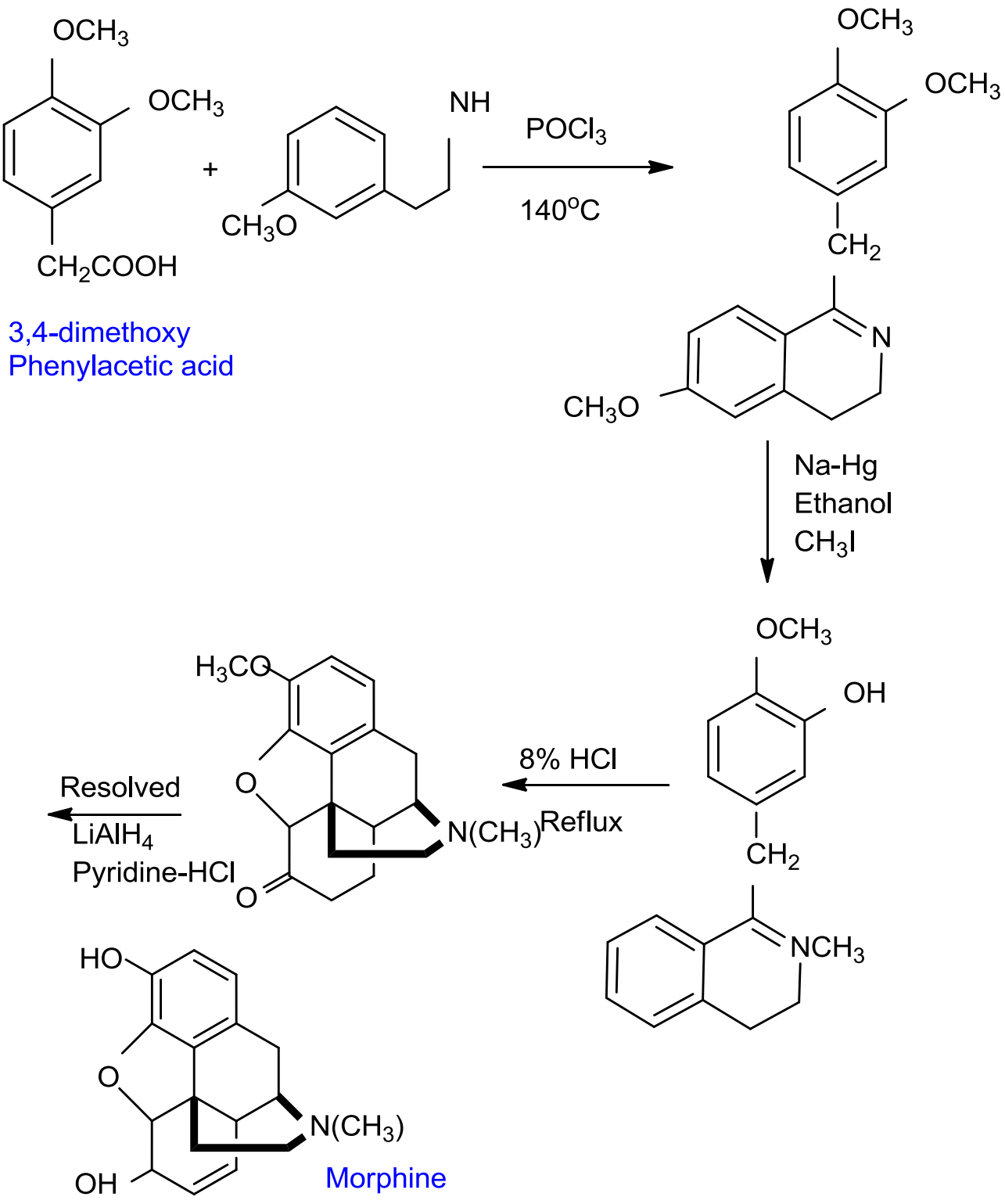
Codeine

β-Codeimethine

α-Codeimethine


CH3O
O
N(CH3)
HO
β-Codeimethine
△
CH3I
AgOH
CH3O
O
H
N(CH3)3OH
Heat with
(CH3CO)2O
H3CO
O
+ CH2=CH2 + (CH3)3N
Methyl Morphenol
Heat with HCl
HO
HO
O
Morphenol
Na-C2H5OH
HO
HO
HO

Synthesis of Morphine:



TEXT / REFERENCE BOOKS

1. Finar I. L., Organic Chemistry, 6th Edition, Pearson Education Asia, 2004.
2. S.V.Bhat,B.A. Nagasampagi, M.Sivakumar., Chemistry of Natural products, Springer Publications 2005.
3. O.P. Agarwal., Organic Chemistry-Natural Products Vol.I and II, Goel Publishing House, 1980.
4. G.R. Chatwal., Organic Chemistry-Vol II- Himalayan Books, 1985.
5. T.L. Gilchrist., Heterocylclic Chemistry, Longman Scientific and Technology, 2005.

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SCHOOL OF SCIENCE AND HUMANITIES

DEPARTMENT OF CHEMISTRY

UNIT – II - Terpenoids and Carotenoids – SCYA7201

UNIT-2

TERPENOID AND CAROTENOID

2.0 INTRODUCTION TO TERPENOIDS

Includes hydrocarbons of plant origin with the general formula$(C_5H_8)_n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives. As terpenoids contains isoprene units- Isoprenoids. The carbon skeletons of terpenoids are divisible into isoprene units. Terpene hydrocarbons are exact mutiplies of $(C_5H_8)_n$

Sesquiterpenes and Diterpenes are used as antibiotics.

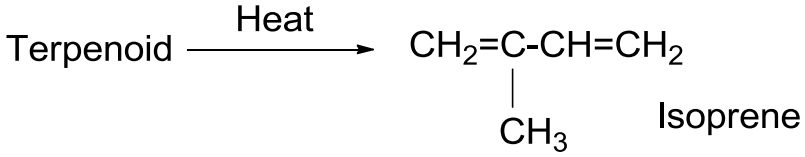
Some are used as antiseptic, insecticide etc.,

2.1 PHYSICAL PROPERTIES:

Colourless liquids-lighter than water boils between 150-180^{0}C;Highly refractive, Insoluble In water, soluble in organic solvents; Optically active.

2.2 CHEMICAL PROPERTIES:

- Unsaturated compounds with one or more double bond.
- Terpenoids undergoes addition reactions with hydrogen, halogen, halogen acids.
- Undergoes polymerisation, dehydrogenation in the ring.
- They are easily oxidised by oxidising agents.
- Labile and are stable on isomerisation with acids.
- On thermal decomposition, it yields isoprene as one of the products.



2.3 ISOLATION:

It is carried out in two steps:

1. Isolation of essential oils.

2. Separation of terpenoids from essential oils.

1) Isolation of essential oils:

A) Steam distillation: Plant material is macerated and then steam distilled to get essential oils into the distillate. The distillate is extracted with solvents and the solvents is removed under pressure.

B) Extraction by volatile solvents:

Plant material is treated directly with light petrol at 50c. The oil is taken up by the solvent along with soluble colouring materials. Essential oils are separated by removing the solvent by distillation under reduced pressure.

C) Enfleurage Method:

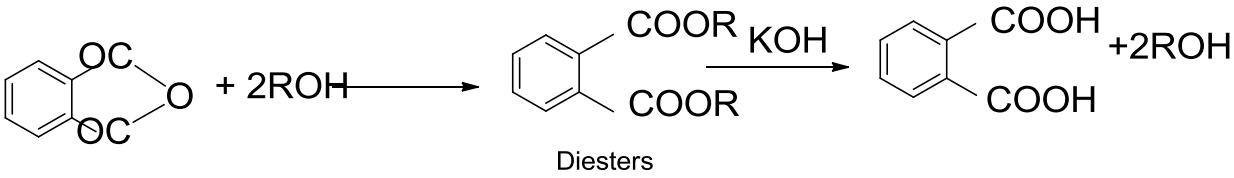
The fat is warmed at 50c. The surface of the fat is covered with flower petals until it is saturated with essential oil. The fat is digested with alcohol. The distillate is fractionally distilled to remove solvent.

2) Separation of Terpenoids from essential oils:

1) Chemical Methods:

a) Nitrosyl chloride: Essential oil containing terpenoids is treated with nitrosyl chloride in chloroform, crystalline adducts of hydrocarbons with sharp melting point is obtained.

b) Phthalic anhydride: Essential oil containing alcohols are treated with phthalic anhydride to from diesters with primary alcohol reacts more readily.



Diesters

c) Terpenoids with aldehydes and ketones are separated from essential oils by forming their adducts with $NaHSO_3$, 2,4-dinitro phenylhydrazine, semicarbazide.

2.4 PHYSICAL METHODS:

Fractional Distillation Method: Essential oils on fractional distillation gives a terpenoid hydrocarbon first, followed by their oxygenated derivatives. The residue on distillation under reduced pressure gives sesquiterpenoids.

2.5 ISOPRENE RULE:

Skeleton structures of all naturally occurring terpenoids are built of isoprene units.

Empirical formula of all naturally occurring terpenoids is C_5H_8.

Thermal decomposition of terpenoids give isoprene as one of the products.

$$(C_5H_8)_n \xrightarrow[\text{Distillation}]{\text{Destructive}} n\ C_5H_8$$

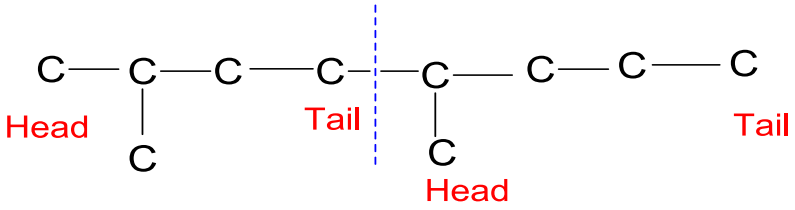
Isoprene on heated to 280^0c it polymerizes to from dipentene.

$$2C_5H_8 \xrightarrow[280^oc]{\text{Heat}} C_{10}H_{16}$$

Dipentene

Special Isoprene Rule:

The branched end of the isoprene unit is considered as head and the other end as tail. The isoprene units in terpenoids are linked in a head to tail fashion.



Limitations:

Terpenoids whose carbon content is not a multiple of 5.

Terpenoids which can't be divided to isoprene units.

Carotenoids fail to follow the rule.

2.6 GEM DIALKYL RULE:

When two alkyl groups are attached to same carbon atom is called gem-dialkyl group.

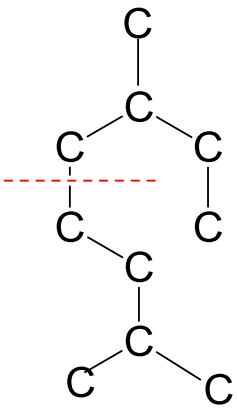
Presence of Gem-dialkyl group affects the stability of the compound.

Gem-dialkyl group tends to render the cyclohexane ring unstable where as it stabilises the three, four and five membered rings.

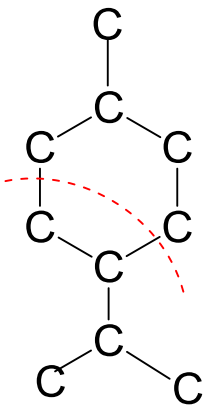
Possible structure of Terpenoids:

Monocyclic Monoterpenoids contains p-cymene structure.

P-cymene structure:



Acyclic structure



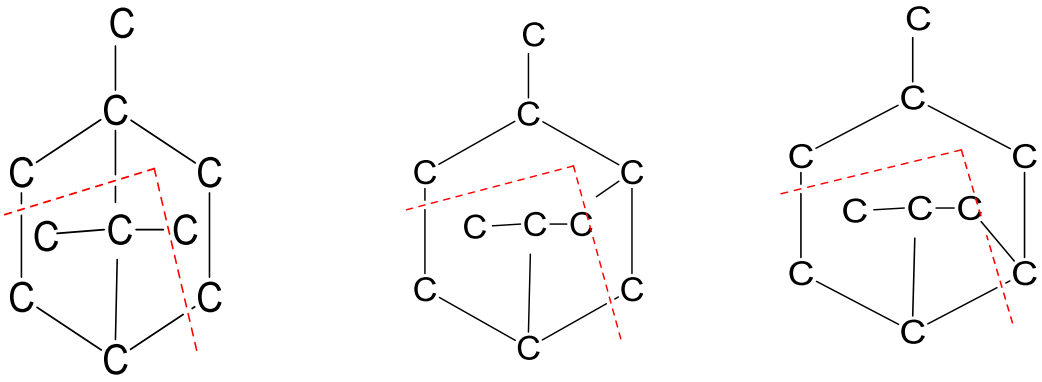
p-cymene structure.

In cyclic terpenoids:

Six membered terpenoids will have gem-dialkyl group outside the ring.

Bicyclic tyerpenoids will have gem-dialkyl group on the smaller rings.

Closure of 10 C open chain can give rise to three possible structures.



2.7 CLASSIFICATION OF TERPENOIDS:

They possess the general formula of (C_5H_8)n. The value of n forms the basis of classification.

S.No	N	No.of Carbon atoms	Class	Formula
1	2	10	Monoterpenoids	$C_{10}H_{16}$
2	3	15	Sesquiterpenoids	$C_{15}H_{24}$
3	4	20	Diterpenoids	$C_{20}H_{22}$
4	5	25	Sesterterpenoids	$C_{25}H_{40}$

Acyclic Terpenoid: Open-chain molecule.

Monocyclic Terpenoid: One ring in the molecule.

Bicyclic Terpenoid: Two ring in the molecule.

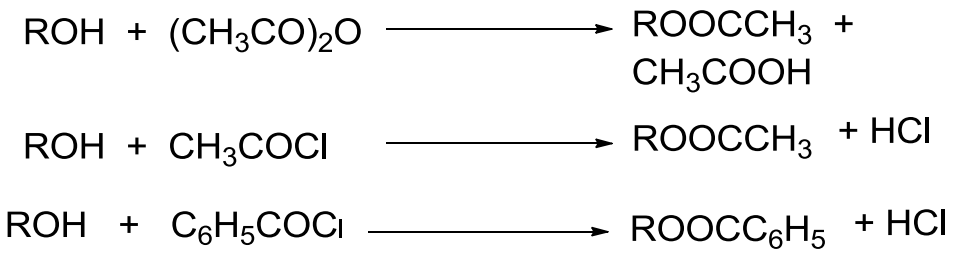
Tricyclic Terpenoid: Three rings in the structure.

2.8 STRUCTURAL DETERMINATION:

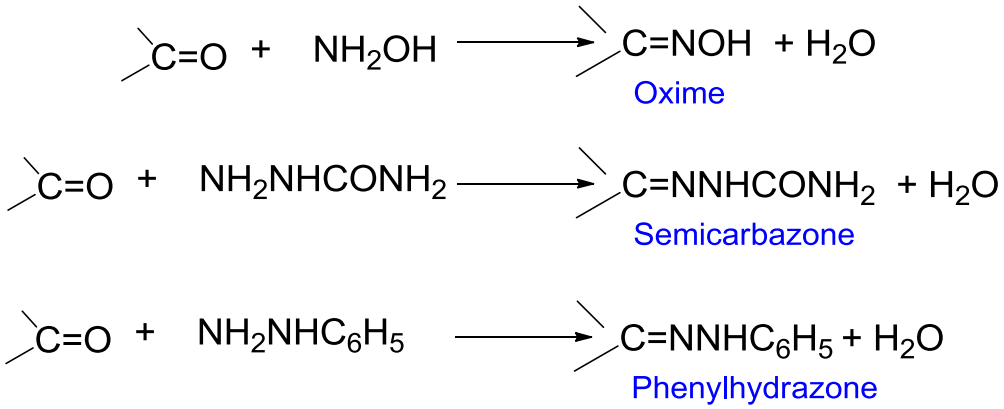
Molecular Formula- Elemental analysis helps in determining the molecular formula.

Nature of Oxygen Atom: Oxygen atom is present as hydroxy, aldehyde, keto, or carboxylic groups.

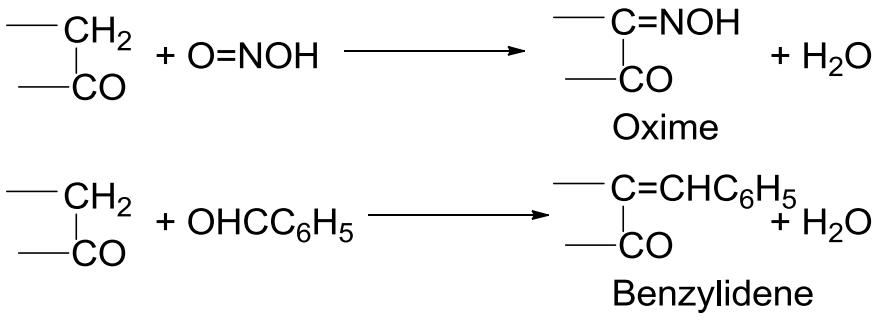
1) Hydroxyl groups can be detected Reactions:



2) **Carbonyl group:** Reaction of the terpenoid with hydroxyl amine, semi carbazide, phenylhydrazine.



Terpenoids having $-CH_2CO-$ groups forms oximes with HNO_2 acid and benzylidene derivatives with aldehydes.

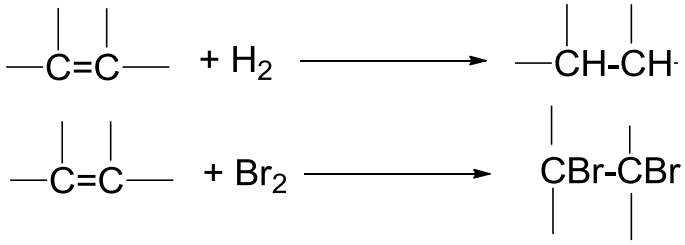


3) Unsaturation:

Presence of olefinic bonds in terpenoids is ascertained by the formation of addition compounds with hydrogen, halogen, peracids, nitrosyl chloride

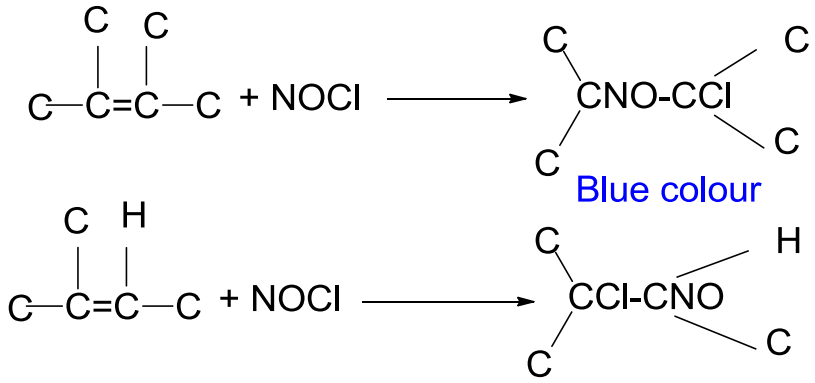
a) With hydrogen and halogens:

Terpenoid possessing olefinic linkages is hydrogenated in the presence of catalyst to form hydro compound.

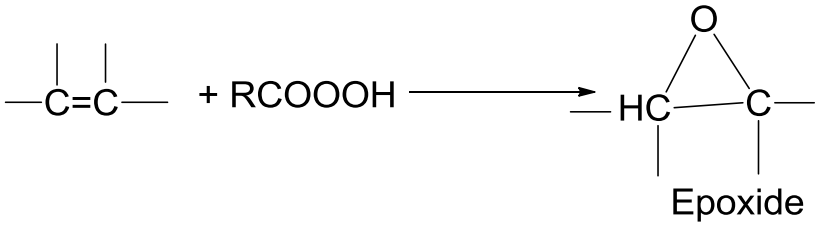


b) With Nitrosyl chloride:

Tertiary carbon atoms with olefinic linkage give blue coloured with Nitrosyl chloride.



c) Peracids forms epoxides with terpenoids with olefinic bond.



4) **Number of Rings:**

From the olefinic bond and the nature of functional groups, the type of compound can be determined.

General formula	compound
C_nH_{2n+2}	Acyclic
C_nH_{2n}	Monocyclic
C_nH_{2n-2}	Bicyclic
C_nH_{2n-4}	Tricyclic

2.9 CARVONE

Occur in Racemic form and optically active.Present in spearmint oil and caraway oil.

2.9.1 Constitution of carvone:

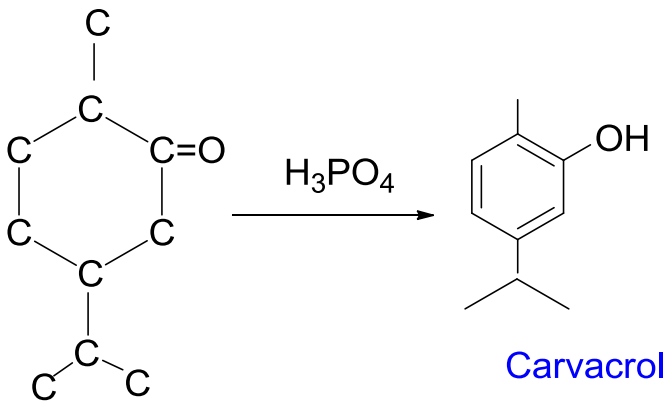
Molecular formula of the compound is found to be C10H14O.

Carvone adds four bromine atomsto yield tetrabromo derivative indicating the presence of two double bonds.

It give reactions of ketone form oxime, phenyl hydrazone.

Presence of two double bonds and a carbonyl bond indicates that the parent compound is $C_{10}H_{20}$, corresponds to monocyclic compound. Hence, carvone is a monocyclic compound.

Carvone is heated with phosphoric acid, it yields carvacrol. Formation of these compound indicates it contains p-menthane or p-cymene structure in which the keto group is at ortho position to methyl ring.



Position of two double bond:

Both carvone and limonene forms the same oxime indicates that one of the double bond is at 8 and 9. The other double bond is at 1 and 2 in case of limonene.

a) Position of other double bond at 1 and 6:

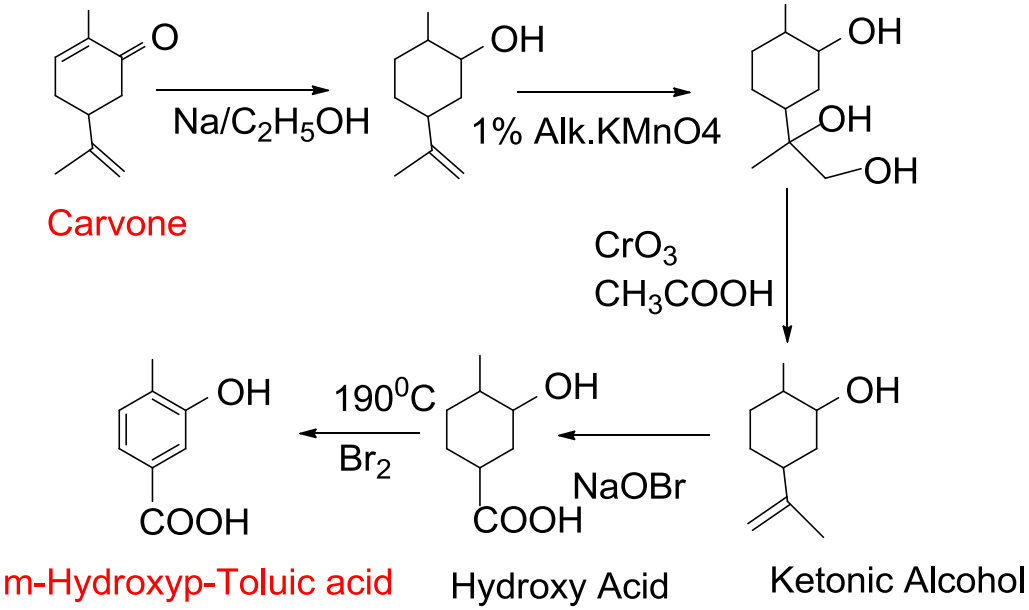
Carvone on reduction with Na/ethanol forms dihydro carveol ($C_{10}H_{18}O$), secondary alcohol and a double bond. .Dihydrocarveol on oxidation with1% alkaline $KMnO_4$ forms trihydroxy compound, $C_{10}H_{20}O_3$.

Trihydroxy compound on oxidation with chromic acid yields a ketonic alcohol with $C_9H_{16}O_2$.

Ketonic alcohol on oxidation with sodium hypobromite , loses one carbon atom to form $C_8H_{14}O_3$-hydroxy monocarboxylic acid.

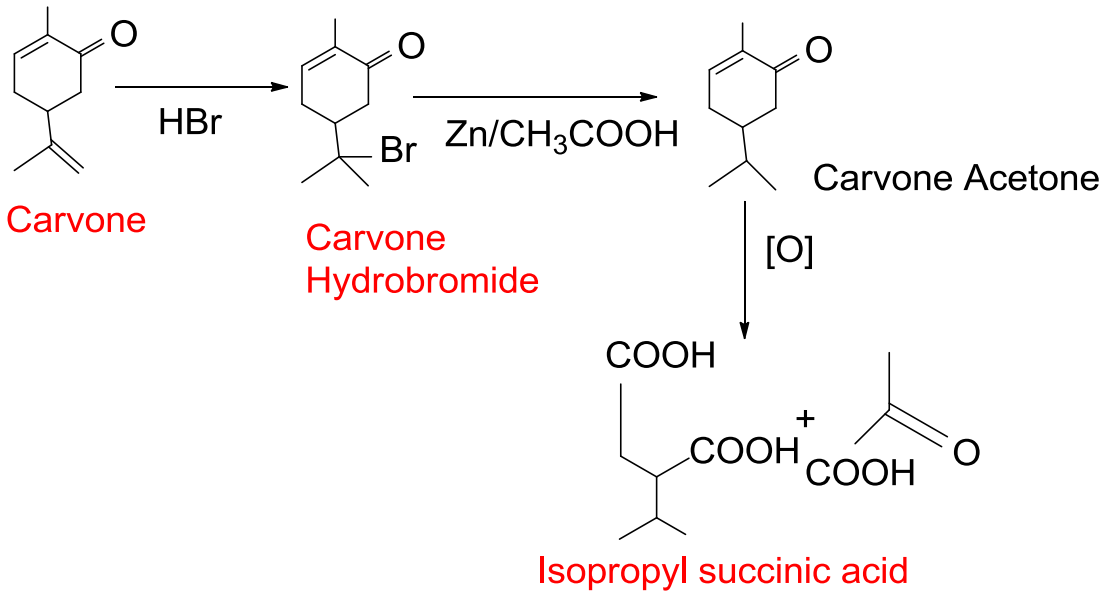
The acid on dehydrogenation with bromine at 190^{o}C, forms m-hydroxy-p-toluic acid.

Reactions to support the position of double bond at 8

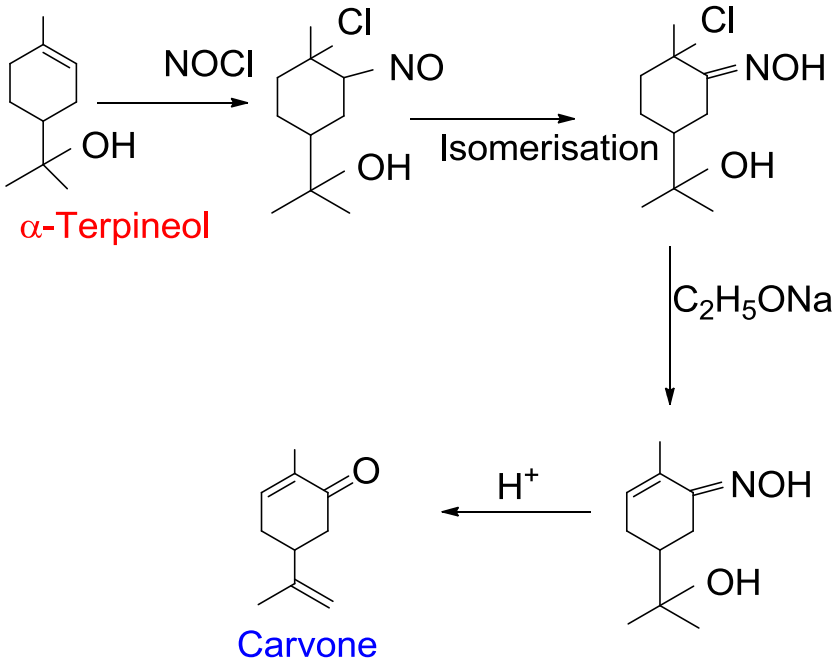


b) Evidence of double bond at position 6.

Carvone adds one mole of HBr to form carvone hydrobromide $C_{10}H_{15}OBr$, on treating with Zn /CH_3COOH yields carvone acetone . Further on oxidation with $KMnO_4$ it yields isopropyl succinic acid and pyruvic acid.



Synthesis of carvone from α-terpineol.



2.10 MENTHOL:

It is an optically active compound found in peppermint oils. It is a saturated compound with 43°C.

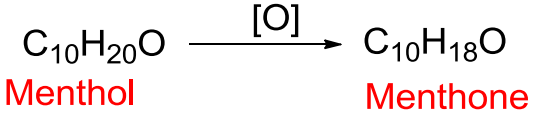
2.10.1 Constitution of Menthol

Molecular formula of menthol was found to be $C_{10}H_{20}O$.

Menthol forms esters readily with acids, it possess an alcoholic group.

Oxidation of Menthol gives a ketone, Menthone ($C_{10}H_{18}O$), indicating that the alcoholic group is secondary alcoholic group.

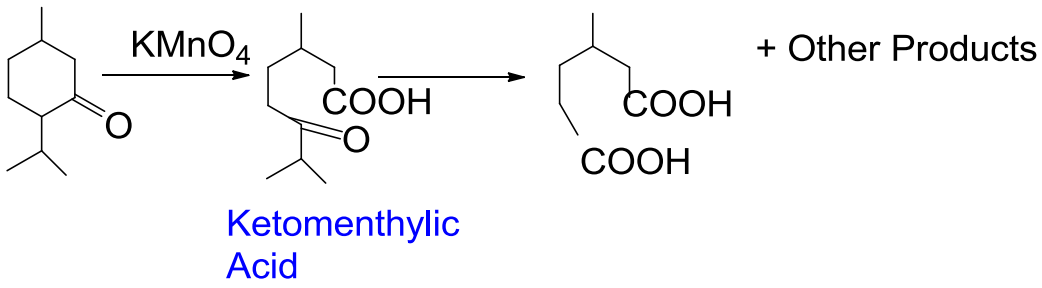
Reaction:



On dehydration followed by dehydrogenation, it yields p-cymene. Both menthol and menthone on reduction with HI gives p-menthane.

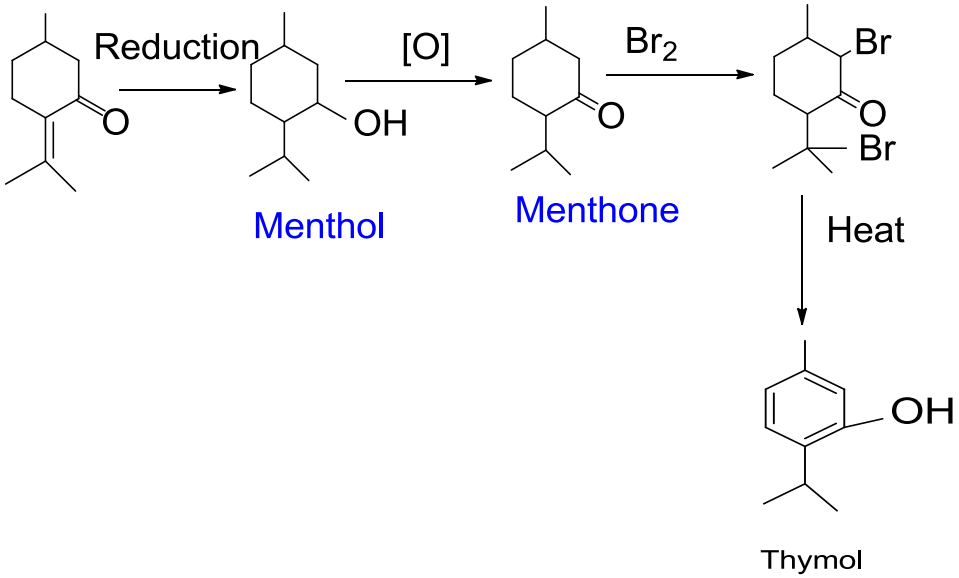
Oxidation of Menthone:

On oxidation with $KMnO_4$, it yields a keto acid $C_{10}H_{18}O_3$. one ketonic and one carboxylic group. The latter, on oxidation gives 3-methyl adipic acid.



Ketomenthylic Acid

Pulegone on reduction gives menthol .

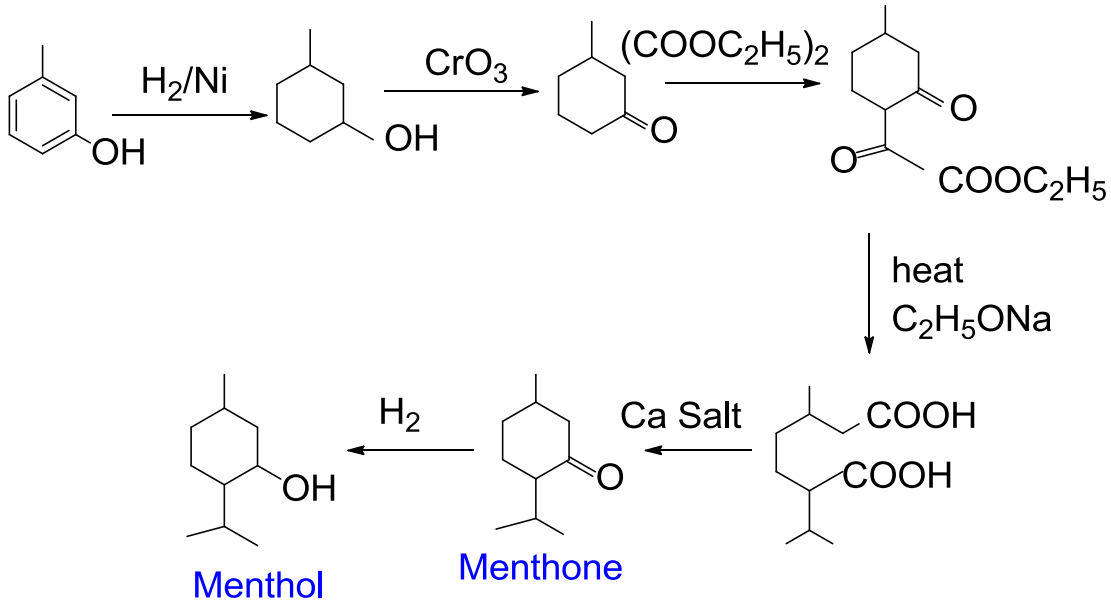


Menthol

Menthone

Thymol

Synthesis of Menthol



Menthone

Menthol

2.11 ZINGIBERENE

Monocyclic sesquiterpenoid . Optically active liquid.

2.11.1 Constitution of Zingiberene

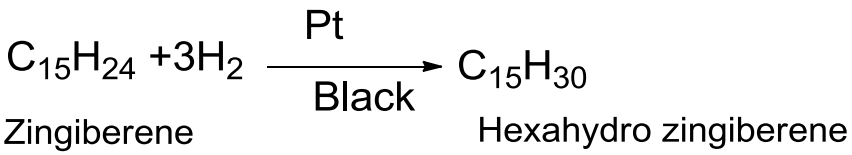
Molecular formula of zingiberene is $C_{15}H_{24}$.

It forms dihydrochloride with HCl it contains two double bonds. The molecular refraction indicates the presence of three double bonds.

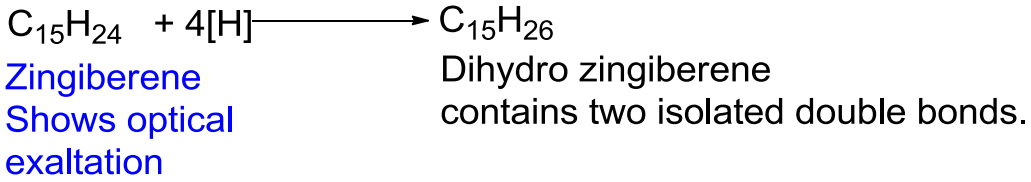
Zingiberene on reduction with hydrogen in the presence of Pt it forms hexahydrozingiberene, $C_{15}H_{30}$. The saturated hydrocarbon possesss the formula of C_nH_{2n}, zingiberene is a monocyclic terpenoid.

Zingiberene on reduction with sodium/ethanol it forms dihydrozingiberene $C_{15}H_{28}$. it indicates that two of the double bonds are conjugated.

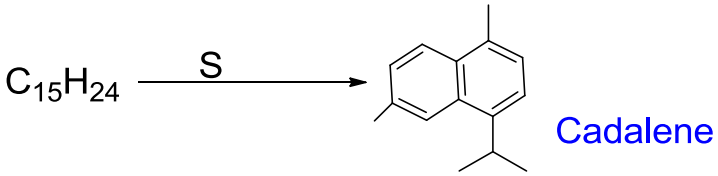
It forms an adduct with maleic anhydride.

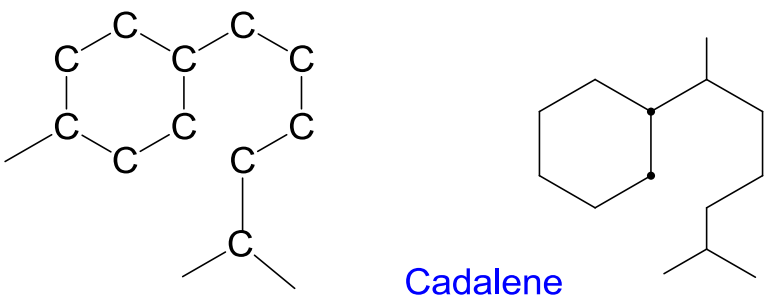


Zingiberene shows optical exaltation while dihydrozingiberene does not (68.37), indicates that the reduced form contains isolated double bonds formed by the reduction of one of the conjugated bonds in zingiberene.



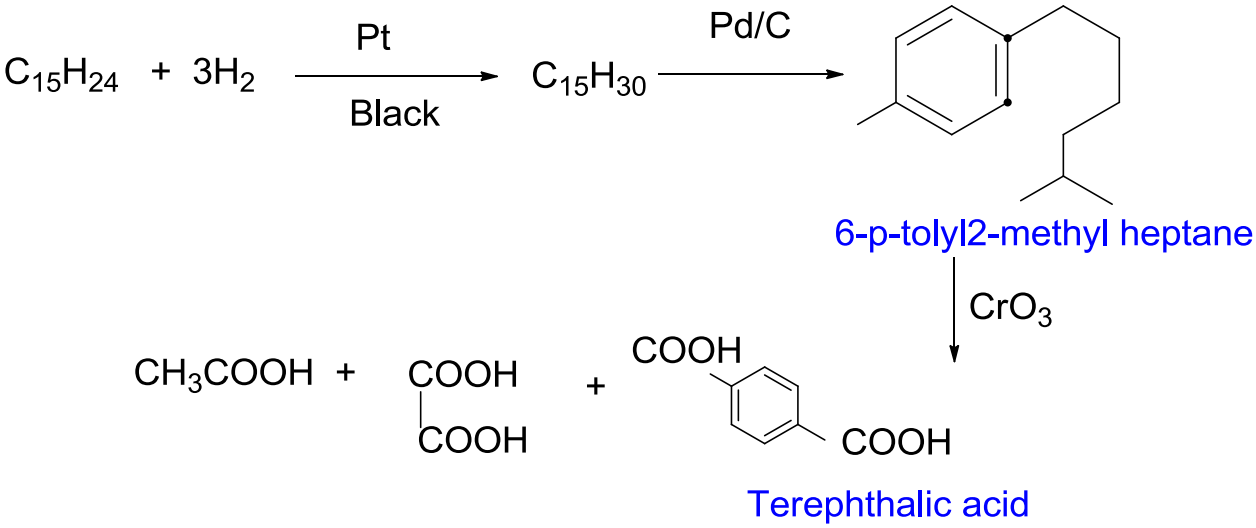
Zingiberene on heated with sulphur it forms cadalene, 1,6-dimethyl4-isopropyl naphthalene.





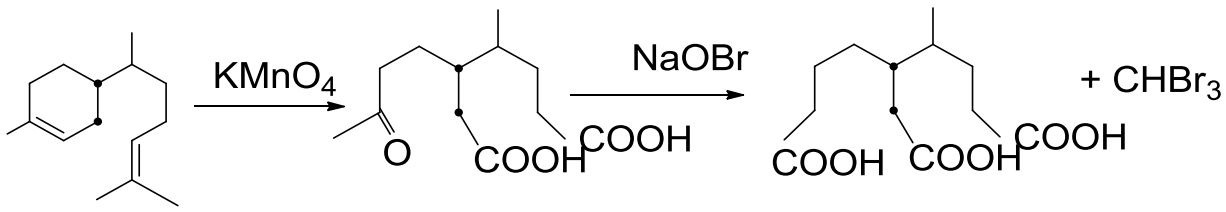
Zingiberene is ozonolysed. It yields acetone, laevulic acid and succinic acid. These products are obtained from biasbolene.

Carbon skeleton of zingiberene is confirmed by the fact that hexahydrozingiberene on dehydrogenated over palladised charcoal it yields 6-p-tolyl2-methylheptane , oxidation with chromic acid yields acetic acid, oxalic ac id and terephthalic acid.



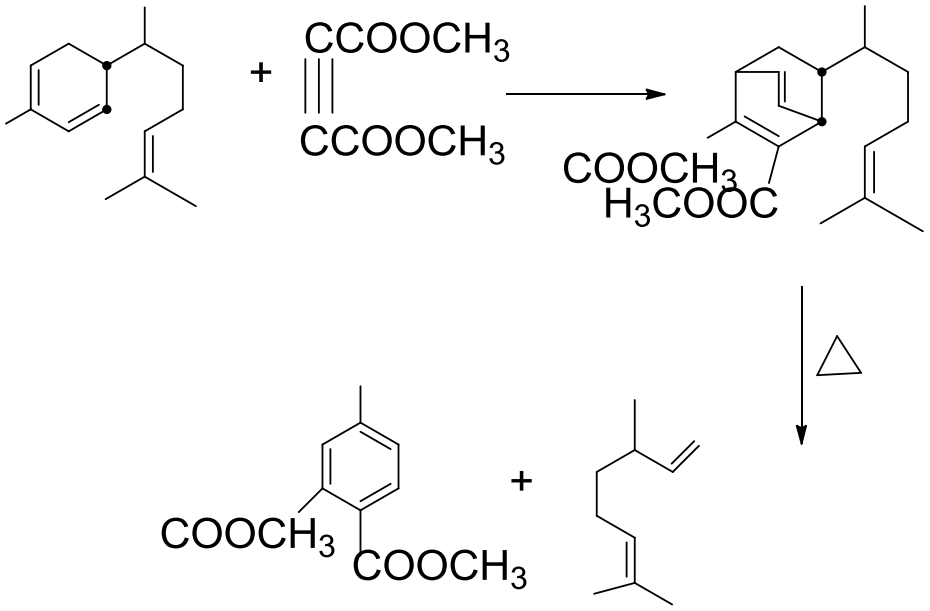
The formation of acetone as one of the product during ozonation reveals that one of the double bond is present as isopropylidene group.

Oxidation of dihydrozingiberene with $KMnO_4$, yields a ketodicarboxylic acid, $C_{12}H_{20}O_5$, on oxidation with hypobromite forms a tricarboxylic acid, $C_{11}H_{18}O_6$ (III)along with bromoform, indicates the presence of methyl ketone group (CH_3CO).

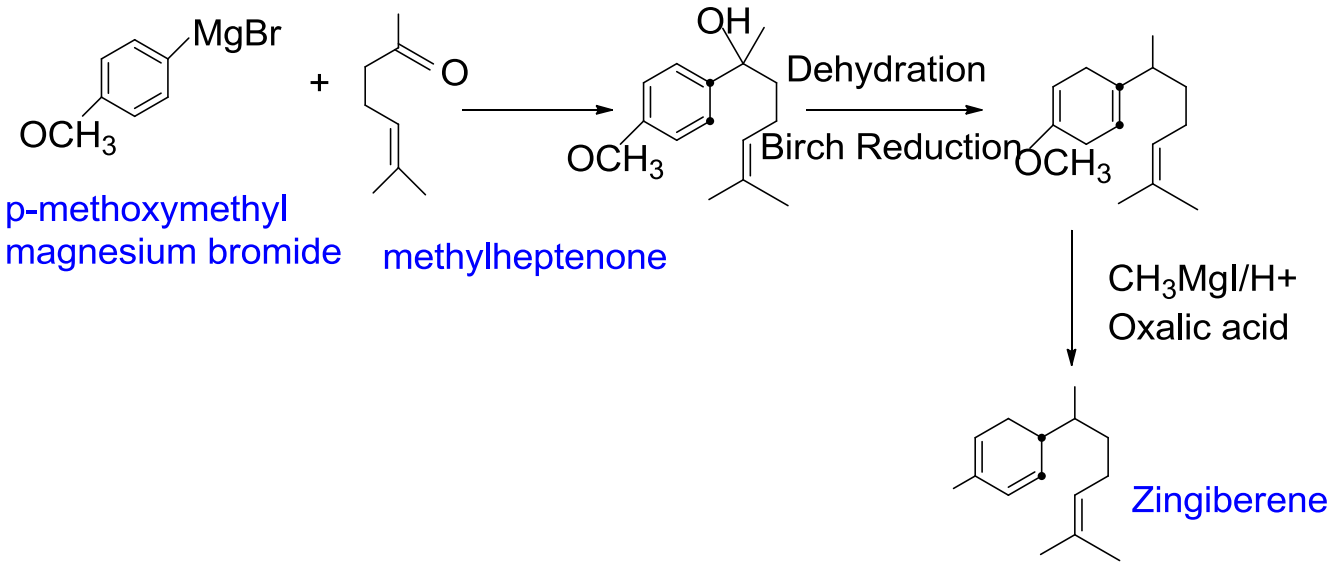


The position of third double bond can be ascertained as follows:

Zingiberene forms an adduct which on hydrolysis yields dimethyl octa 3,6-diene and methyl 4-methyl phthalate.



Synthesis of Zingiberene:



2.12 LYCOPENE

Red colouring pigment responsible for the red colour in tomatoes.

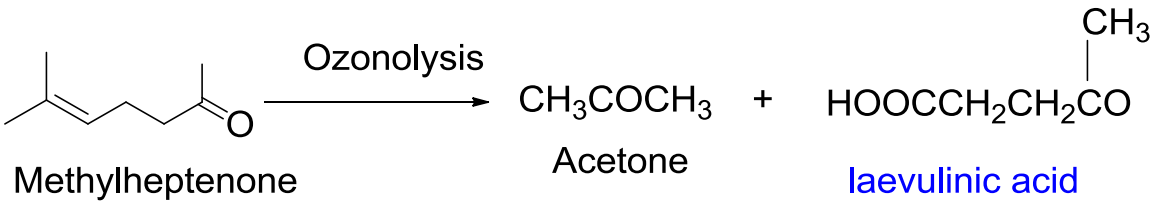
2.12.1Constitution of Lycopene

Molecular formula of the compound is $C_{40}H_{56}$.

On reduction with hydrogen with Pt as catalyst, it is converted to perhydrolycopene $C_{40}H_{82}$, by addding 13 molecules of hydrogen indicating that lycopene contains 13 double bonds.

As the molecular formula of perhydrolycopene, $C_{40}H_{82}$ (saturated lycopene) correponds to the general formula of C_nH_{2n+2} indicates that lycopene is an acyclic compound.

Lycopene on ozonolysis gives acetone and laevulinic acid. The formation of these products suggests that the terminal residue of lycopene contains methyl heptenone.



Presence of methylhepteneone at the terminal residue of lycopene is supported by the fact that controlled oxidation of lycopene with CrO_3 gives 6-methylhept5-ene 2-one.

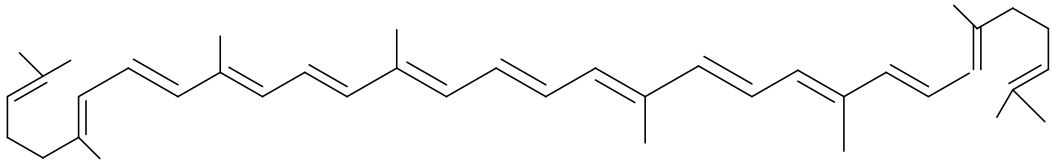
On quantitative oxidation reveals that 6-methylhept-5-ene2-one residue occurs at each end of the lycopene molecule.

Oxidation of lycopene with CrO_3 yields 8 molecules of acetic acid per molecule of lycopene, suggesting that there are $-C(CH_2)=$ groups present in the chain

Controlled oxidation of lycopene with chromic acid gives one molecule of methylhepteneone and a molecule of lycopenal, $C_{32}H_{42}O$. The lattaerc ompound on further oxidation gives another molecule of methylhepteneone and one molecule of dialdehyde, $C_{24}H_{28}O_2$.

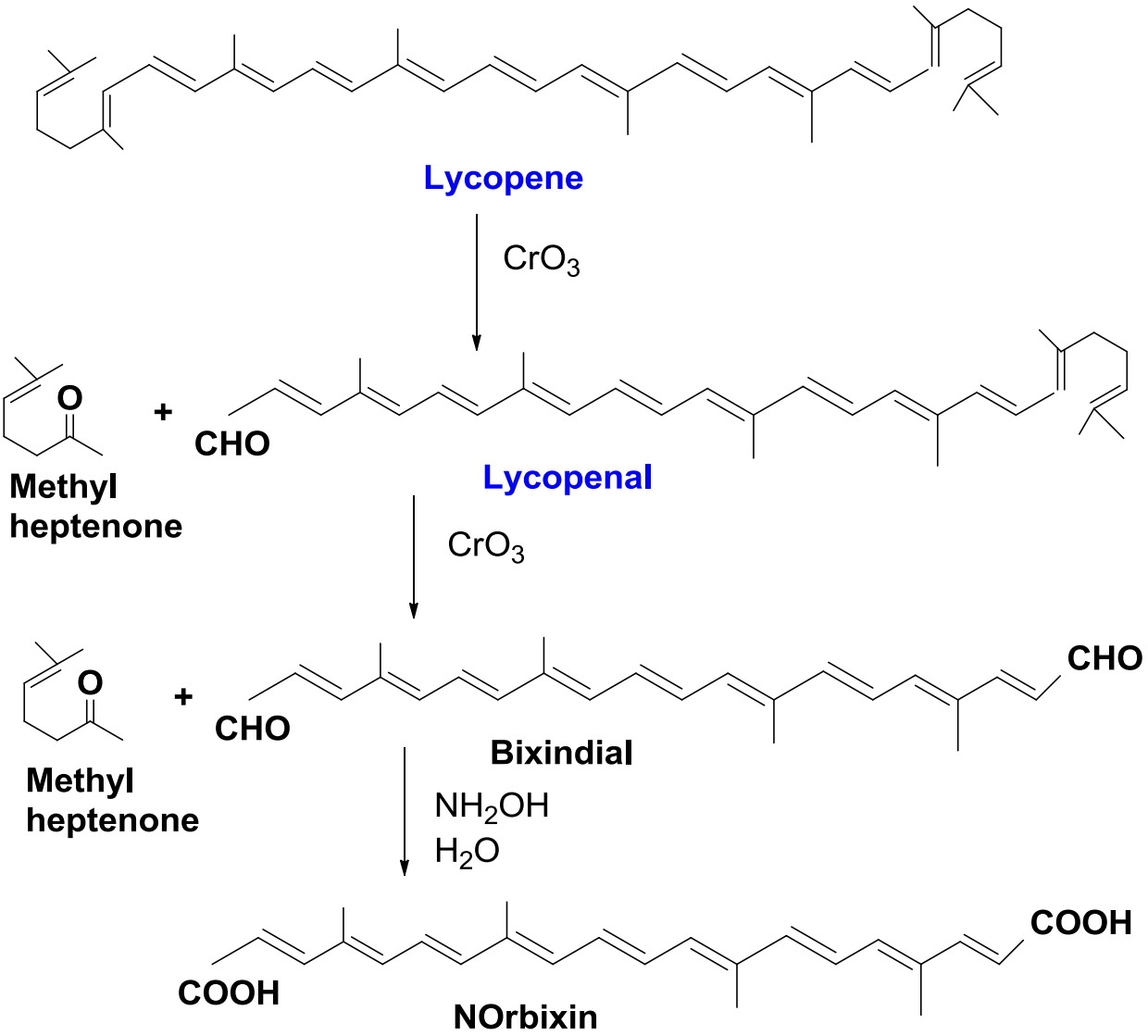
Thus the central part of lycopene is this dialdehyde and two molecules of methylhepteneone is present at the end of the chain.

The dialdehyde, $C_{24}H_{28}O_2$ is converted to dioxime which on dehydration gives dicyanide, on hydrolysis gives dicarboxylic acid , $C_{20}H_{28}O_4$ (identical to norbixin). Thus the structure of lycopene is a symmetrical one.

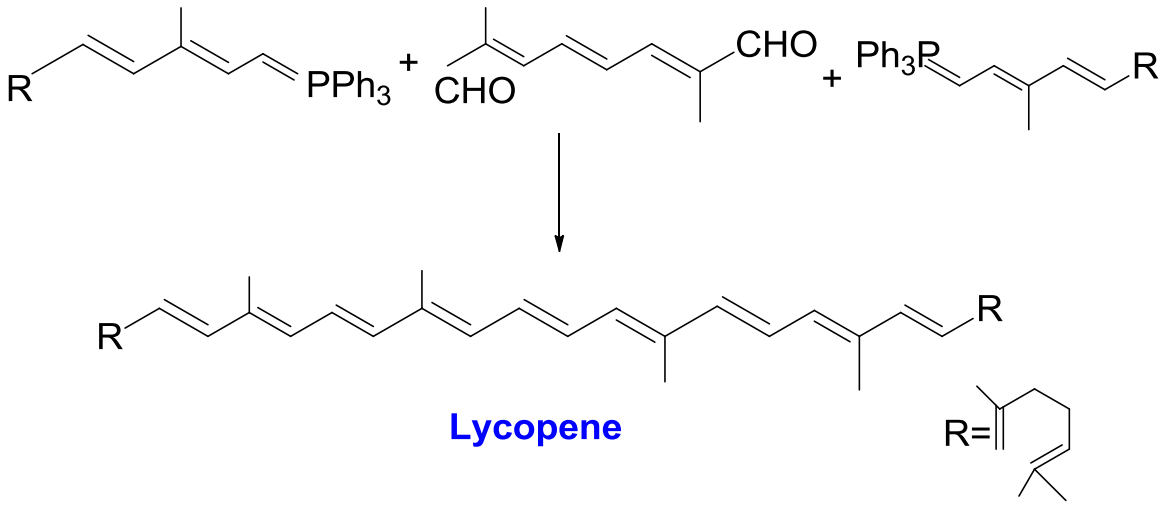


Lycopene

Reactions:



Synthesis of Lycopene



TEXT / REFERENCE BOOKS

1. Finar I. L., Organic Chemistry, 6th Edition, Pearson Education Asia, 2004.
2. S.V.Bhat,B.A. Nagasampagi, M.Sivakumar., Chemistry of Natural products, Springer Publications 2005.
3. O.P. Agarwal., Organic Chemistry-Natural Products Vol.I and II, Goel Publishing House, 1980.
4. G.R. Chatwal., Organic Chemistry-Vol II and Vol. II- Himalayan Books, 1985.
5. T.L. Gilchrist., Heterocylclic Chemistry, Longman Scientific and Technology, 2005.

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SCHOOL OF SCIENCE AND HUMANITIES

DEPARTMENT OF CHEMISTRY

UNIT – III - Proteins & Lipids – SCYA7201

UNIT-3

PROTEINS & LIPIDS

3.0 INTRODUCTION: PROTEINS

Protein was derived from the Greek word 'proteus' meaning first was coined by Mulder in 1839. Proteins are biopolymers containing large number of amino acids joined to each other by peptide bonds.

3.1 CHARACTERISTICS OF PROTEINS:

- Amphoteric: Like amino acids, they possess free -NH_2 and –COOH groups. Hence they react with both acids and bases.
- They are colourless except chromoproteins, tasteless and odourless.
- They don't have sharp melting point and are optically active.
- They are insoluble in alcohol and wtaer but soluble in dilute acids and alkalies.
- They are large molecular weight compounds.
- Large hydrophilic colloids and cant pass through vegetable or animal membrane.
- Protein has a characteristic isoelectric point at which its ionisation and solubility is minimum used in isolation and identification of proteins by electrophoretic methods.

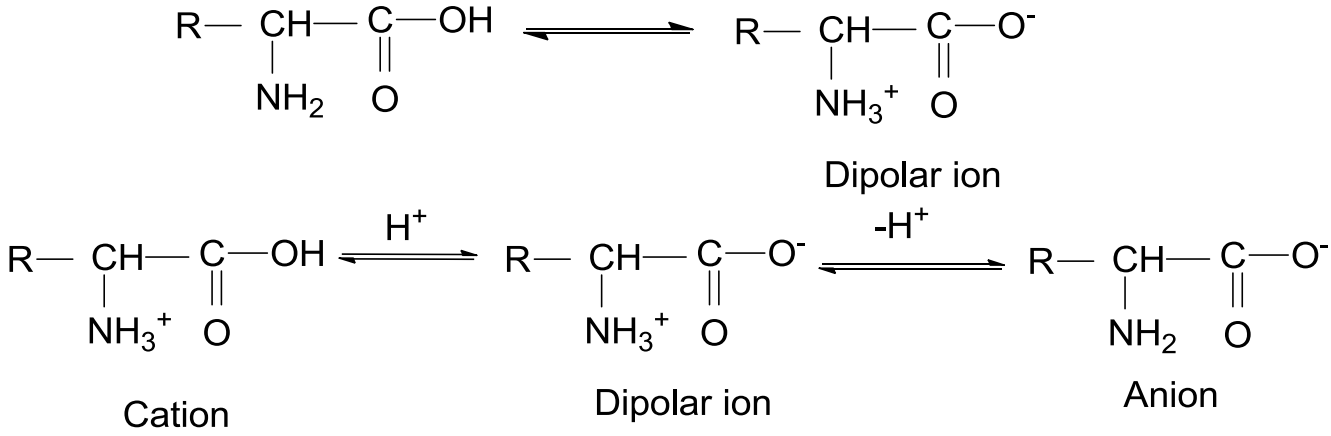
3.2 ZWITTER ION-DIPOLAR ION

Amino acids exists as charged molecules in solutions as they contain both acid and basic group in the same molecule. Hence, transfer of a proton from COOH to amino group takes place as an internal acid-base reaction forming inner salt or ampholyte or zwitter ion

A zwitter ion is a doubly charged ion containing both positive and negative charge.

Isoelectric Point:

The predominant form in which amino acid exists is dependent on the pH of the solution.



When an acid is added, the equilibrium is shifted in favour of cation and as base is added, the equilibrium is shift in favour of anion. For each aminoacids, there exists a particular pH at which the concentration of cation and anion will be equal. The dipolar ion

has no net charge and these ions don't migrate under the influence of electric current – isoelectric point.

3.3 DENATURATION AND RENATURATION:

Denaturation refers to the changes in the properties of protein and is followed by coagulation. It is caused by

Physical agents- Mechanical shaking, heat ,cooling, freezing and ionizing radiations

Chemical agents: Acetone, alcohol, salicylates, urea, detergents etc.,

Proteins precipitated by these reagents- Denatured and the process is knbown as Denaturation. Denaturation leads to unfolding of the protein chain causing disorganization.

On denaturation, proteins 1) Decrease in their solubility; 2) Decrease in shape and size of the molecule.3) Change in optical rotation 4)Change in the conformation of unfolding of protein

Denaturation is of two types:
Irreversible Denaturation: Denatured protein can't be brought back to its original state.

Eg: curdling of milk and Boiling of egg

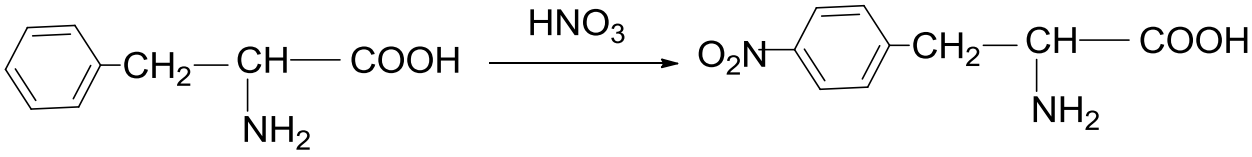
Reversible Denaturation or Renaturation or Refolding: The process of regaining the properties of protein by a denatured protein.

Eg: Trypsin becomed denatured on exposing to 80-90^{o}c and its activity is restored by cooling to 37^{o}c.

3.4 COLOUR REACTIONS OF PROTEIN

(i) Biuret Test: To the protein solution added NaOH and a dilute solution of $CuSO_4$. A violet colour may be obtained due to the formation of biuret ($NH_2CONHCONH_2$) as an intermediate.

(ii) Xanthoproteic Test: When a protein solution is warmed with HNO_3 acid, a yellow colour is obtained.

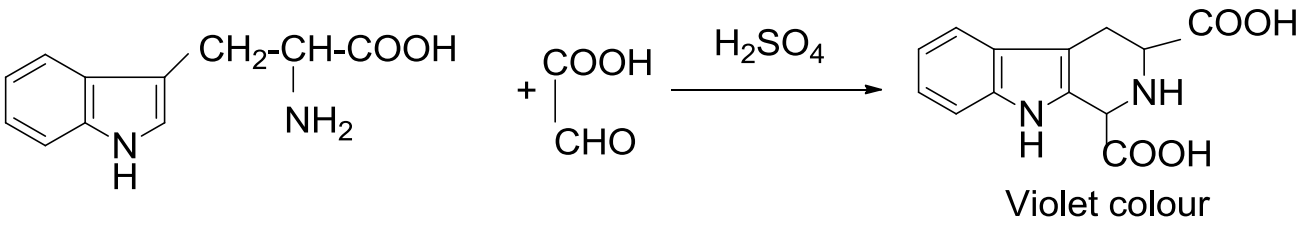


(iii) Millon's test: When million's reagent (mercurous and mercuric nitrate in nitric acid) is added to a protein solution a white p[pt is formed which turns red on heating due to the presence of phenolic(-OH) in the molecule.

(iv) Lead acetate: Protein containing cysteine gives black colour.

(*v) Hopkins-cole Test:*

Concentrated sulphuric acid is added along the sides of the test tube containing a solution of protein containing glyoxalic acid, a violet colour indicates the presence of protein.



Violet colour

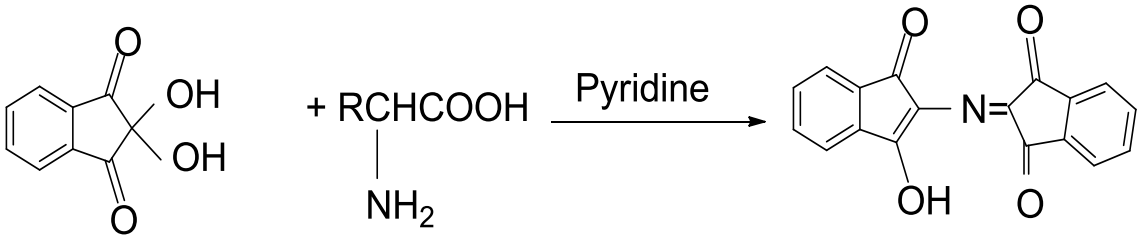
(vi) Folin's Test: Protein is treated with alkaline phospho molybdo tungstic acid to form blue indicating the presence of tyrosine.

(vii) Nitroprusside Test: Proteins on treating with sodium nitroprusside in NH_4OH gives red colour due to Cysteine.

(viii) Sullivan's Test: Proteins on treating with naphthoquinone gives red colour.

(ix) Molisch's Test: Protein on heating with α-naphthol in conc.H_2SO_4 gives a violet colour at the junction- Carbohydrate.

(x) Ninhydrin Test: When a pyridine solution of protein is heated with ninhydrin a violet colour is formed. The colour product indicates the presence of free NH_2 and COOH group in the molecule.



Violet/Blue coloured

3.5 CLASSIFICATION OF PROTEIN:

3.5.1 Based on Solubility:

a) Fibrous proteins:

Thread like structures made up of linear molecules which are arranged parallel to the axis. Held by inter-molecular hydrogen bonding. Insoluble in water, but soluble in concentrated acids and alkalis. Highly resistent to protelytic enzymes. Chief structura materials.

Eg: Keratin in skin, hair, nail and wool.

b) Globular proteins:

Molecules of proteins are folded into compact units and assumes a spherical shape. Soluble in water, dilute acids, alkalis and salts. They serve as a regulator of various metabolic activities.

Eg: Enzymes,Hormones and Haemogolbin.

3.5.2 BASED ON HYDROLYSIS

a) Simple Proteins (Homoproteins): Proteins which on hydrolysis yields α-aminoacids.

(i) Albumins: Properties: Soluble in water, Precipiated from full saturated solution of $(NH_4)_2SO_4$, Coagulated by heat. Examples- Egg albumin, lacatalbumin, ovalbumin, serum albumin.

(ii) Globulins: Properties: Insoluble in water, soluble in dilute salt solution, acids and alkalis, coagulated by heat, precipitated by half saturated solution of $(NH_4)_2SO_4$.Examples: Serum globulin, Vegetable globulin.

(iii) Prolamins: Properties: Insoluble in water or salt solution, soluble in dil.acids, alkalies and in 70-90% ethanol. Examples: Zein,Hordein.

(iv) Glutelins: Properties: Insoluble in water or salt solution, soluble in dil.acids and alkalies, coagulated by heat.Examples: Glutenin, Oyrzenin.

(v) Scleroproteins: Properties: Insoluble in water or salt solution, soluble in conc.acids and alkalis.Examples: keratin, fibroin.

(vi) Basic proteins: It is of two types.

Histones: Properties: Soluble in water, dilute acids, insoluble in NH_3, Not coagulated by heat.

Examples: Nucleohistones, Haemoglobin.

(vii) Protamines: Properties: Soluble in water, dilute acids. Not coagulated by heat. Examples: Salmine from whale sperm.

b) Conjugated Proteins (Heteroproteins): Proteins which contain non-protein part called prosthetic group along with protein substances.

Depending on the prosthetic group, they are

(i) Nucleoproteins: The prosthetic group is nucleic acids. Eg: Nuclein, Chromatin.

(ii) Glycoproteins: These are proteins having carbohydrate (<4%) in their prosthetic group. Eg: egg albumin, serum albumin. They are also known as mucoproteins is the carbohydrate content is >4%.Eg: Ovomucoid, mucin.

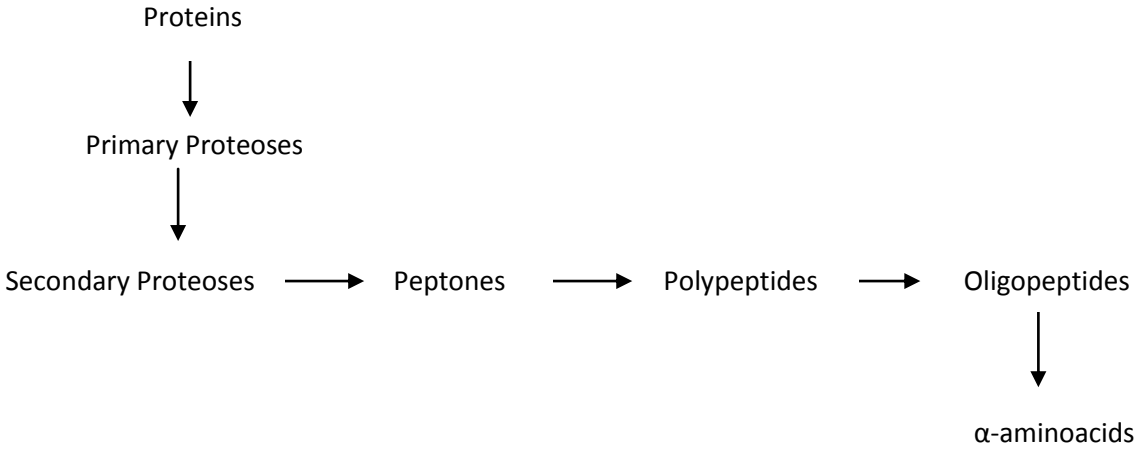
(iii) Lipoproteins: These are water soluble proteins in which the prosthetic group is lecithin and choline Eg: Lipovitellin.

(iv) Phosphoproteins: The prosthetic group is phosphoriic acid Eg: Caesin.

(v) Chromoproteins: The prosthetic group is chromophoric group. Eg: falvoproteins, cytochromes.

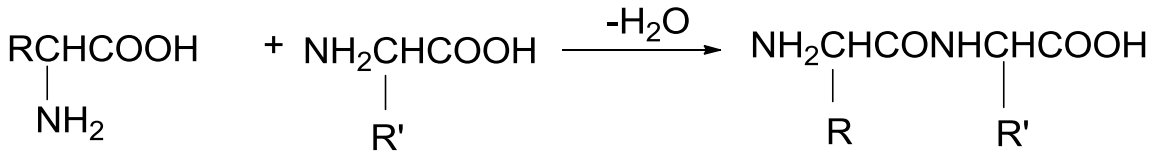
(vi) Metalloproteins: These are conjugated proteins in which the metal is an integral part of the structure. Eg: haemoglobin, Haemocyanin.

c) Derived Proteins: Whe proteins are hydrolysed by acids, alkalies, enzymes the degradation products are known as derived proteins.



3.6 PEPTIDES

When two amino acids combine with each other, and inter molecular reaction takes place between the carboxylic group and the amino group of another amino acid resulting in an amide linkage-peptide linkage and the molecule is peptide. Peptide bond is a chemical, covalent bond.



Peptide molecule there is a free amino group and free carboxylic groups at the terminal end. The free amino group attached to the aminoacid is known as N-terminal amino acid or N-terminal end.The free carboxylic acid group attached to the aminoacid is known as C-terminal amino acid or C-terminal end.

3.6.1 CLASSIFICATION OF PEPTIDES

a) Based on the number of amino acid, peptides are classified as

Oligopeptides: Peptides containing 2-25 amino acids in their structure. Polypeptides: Peptides containing 25-100 aminoacids in their structure.

Proteins: Peptides with more than 100 aminoacids.

b) Based on the structure:

Linear peptides:

a) Homomeric: Aminoacids are the only products on hydrolysis eg: salamine, Glycyl alanine

b) Heteromeric: Other products in addition to amino acids eg: Glutathione.

Cyclic Peptides:

Homodetic: Peptides with only peptide linkage. Eg: TRH(Thyrotropin releasing Hormone)

Heterodetic- structures contains other linkages like disulphide or ester linkages. Eg: Oxytocin.

Peptide containing ester linakges –peptolides.

3.7 STRUCTURE OF PROTEIN

Proteins are long polypeptide chains consisting of amino acids as their principle constituent.

- Isolating the proteins from the source.
- Primary structure of proteins deals with the number, nature and sequence of amino acids in its polypeptide chain
- Secondary structure of Protein deals with the arrangement of peptide chain in space to form coils or sheets.
- Tertiary structure of protein refers to folding of the peptide chain to form 3-dimensional structure.
- Quaternary structure deals with the association of various polypeptide chains to form protein.

3.7.1 Isolation of Protein:

The animal/vegetable source is treated with solvents like 10% NaCl, 1% Na_2CO_3, 0.1% NaOH. The solution is further treated w ith alcohols, $(NH_4)_2SO_4$ where proteins are precipitated.

3.7.2 Primary Structure of protein:

It refers to the nature, number and the sequence of amino acids in the polypeptide chain. Isolated protein may contain a single polypeptide chain or more polypeptide chain (subunits). The subunits are separated. Determination of amino acid composition by hydrolysis of polypeptide chain. End-group analysis is carried out to determine the nature of N- and C-terminal groups. Sequencing of amino acids in the polypeptide chain.

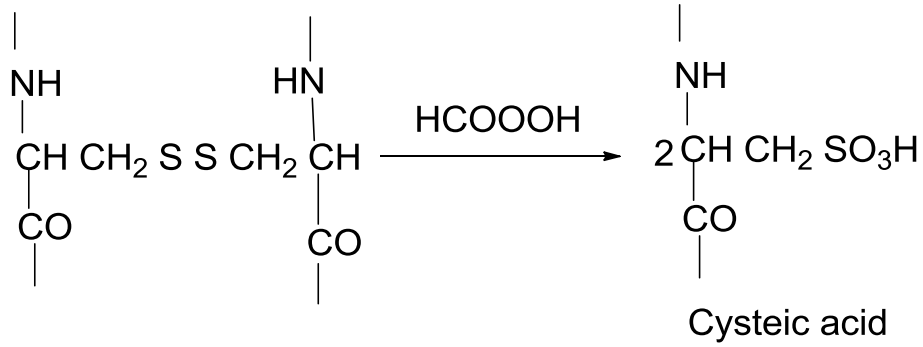
Ascertaining the isolated protein contains single polypeptide or more:

If the isoelectric point is less than the pH 5.5-6.2- more acidic groups are present in the molecule.

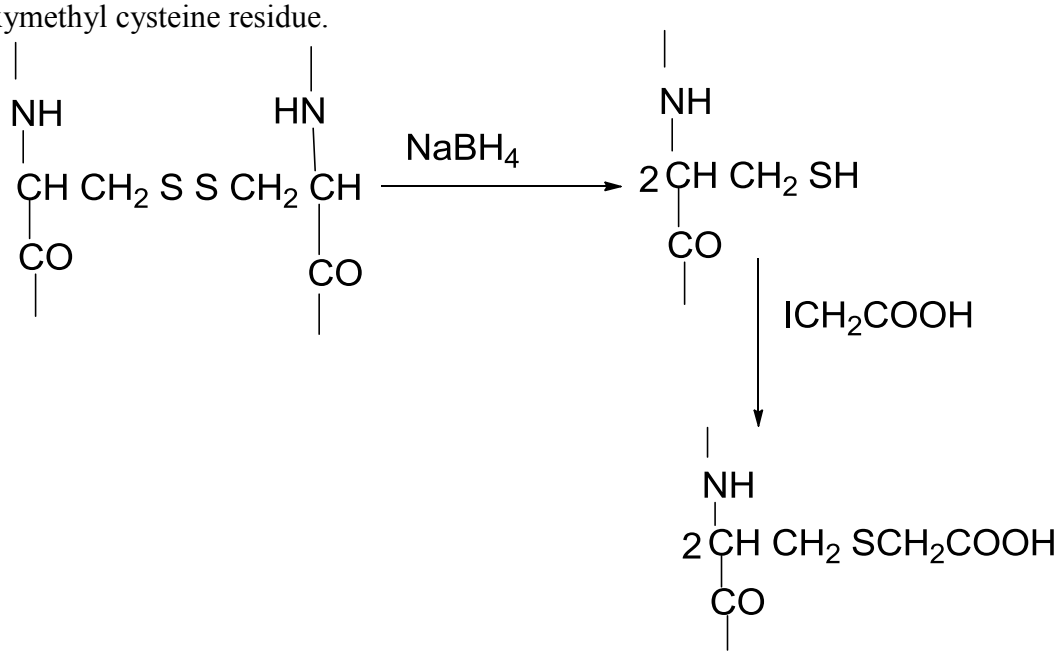
If the isoelectric point is neutral pH 5.5-6.2, indicates the cyclic structure of protein.

Negative test towards ninhydrin indicates the cyclic structure.

Cyclic structures contains large amount of cysteines. The subunits are held by disulphide linkages. These linkages are splitted before the primary structure is determined.



The disulphide bond may be reduced by $NaBH_4$ and the products are treated by iodoacetic acid. The position of the disulphide linkages are deduced from the position of carboxymethyl cysteine residue.



Protein is completely hydrolysed into its constituent amino acids (L configuration)

3.7.2.1 Hydrolysis:

1) Acid hydrolysis: It is carried out with 6N HCl at 110^{o}c for 12-96 hrs. It largely destroys tryptophan, cysteine, serine and threonine. Glutamine and asparagine are hydrolysed to glutaric acid and asparatic acid.

2***) Alkaline Hydrolysis:*** It is acrried out with 5N $Ba(OH)_2$ at 100^{o}C. Destroys-arginine, cystenine .

3) Enzymatic Hydrolysis: slow and usage of several enzymes it is converted to amino acids.

Eg: Trypsin- splits peptide bond in which CO group is a part of lysine, arginine residue.

Chymotrypsin- splits the peptide bonds of phenyl alanine, tyrosine and tryptophan.

Pepsin- Splits the NH bond of leucin, glutamic acid.

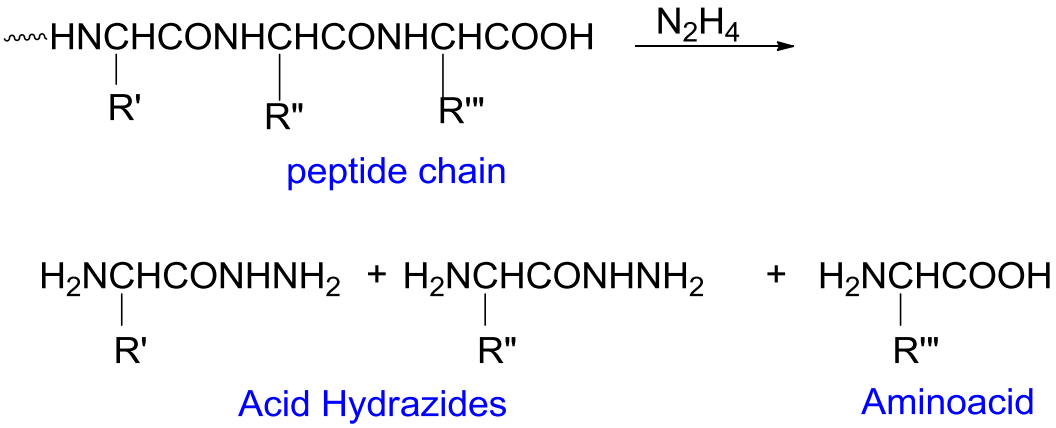
Papin- splits the co group of glycine, arginine and lysine.

Molecular weight is determined by physical methods like osmotic pressure, viscosity measurements.

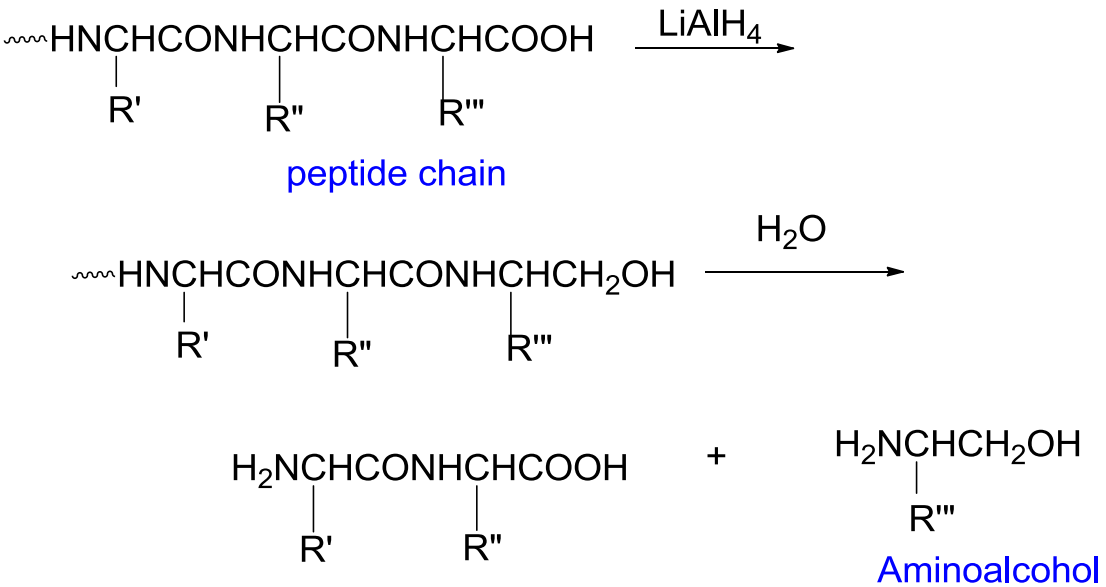
3.7.3 Terminal group analysis:

3.7.3.1 C-Terminal analysis

a) Hydrazinolysis: Protein or peptide is heated with hydrazine at 100°c, converts all the amino acid residues to amino acid hydrazides and free amino acids. The mixture of products is subjected to chromatography, the free amino acids are eluted and can be identified.



b) Reduction : Reduction with $LiAlH_4$:It converts the free carboxyl group to primary alcohols which on hydrolysis produces a mixture of amino acids and an amino alcohol, separated by paper chromatography.

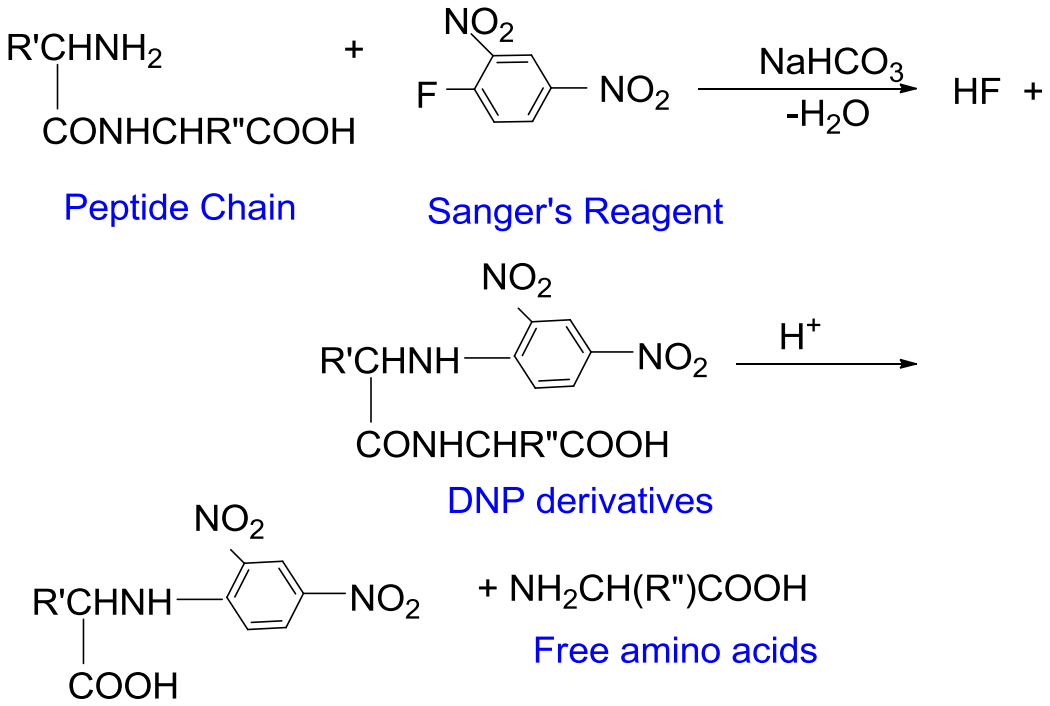


c) Enzyme Carboxypeptidase: Attacks the peptides only at the end which contains the free carboxyl group. When this terminal acid residue is liberated, the new free carboxyl group is attacked. After a given time, successive amino acids are liberated, helps in identifying the sequence.

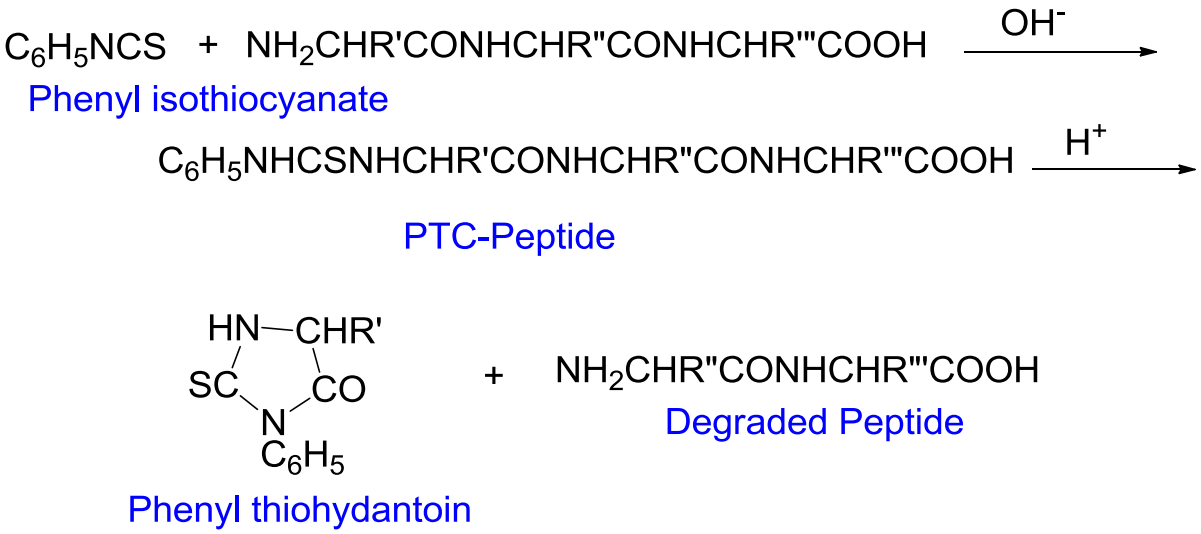
3.7.3.2 N terminal analysis

a) Enzyme leucine amino peptidase- attacks the peptides only with free NH_2 group and proceeds to attack in succession each new terminal amino group.

b) DNP method(Sanger's method)- the aminoacids in the peptide chain reacts with 1-fluoro 2,4-dinitro benzene in the presence of mild alkaline solution of Na_2CO_3 at room temperature to form 2,4-dinitro phenyl derivatives , on hydrolysis produces DNP-aminoacids and a mixture of free amino acids.



c) Edman Method: Reaction between phenyl isothiocyanate and the peptide to form phenyl thiocarbamyl (PTC-peptide) in the presence of dil. alkali. On treating with HCl, the PTC-peptide is converted to a phenyl thiohydantoin and a peptide.



3.7.4 Sequence of amino acids:

Suppose an hexapeptide whose aminoacids are A,B,C,D,E,F. the N-terminal analysis shown to be C. The hexapeptide can be written as C(A,B,D,E,F). Partial hydrolysis ofd the peptide results in C-A, (B,E), (B,D), C(A,E), (B, D, F) and (B,D,E,F).

The sequence can be written as C-A-E-B-D-F.

Significance: paleogenetics

Haemoglobin consists of two α and twoβ chains. Each β chain consists of 146 amino acids. Replacement of one glutamic acid side chain in the 6th position by valine results in sickle-cell anemia.

Hbnormal- val. Hist, leucine, threnonine, proline, glutamic acid

Hb sickle cell- val. Hist, leucine, threnonine, proline, valine.

Evolution:

β chain of haemoglobin of horse differs from man at 26 sites while that of pig at 10 sites. Gorilla at one site.

3.7.5 Secondary Structure of protein:

The aminoacids in the protein are linked by covalent bonds and they can assume infinite number of shapes due to free rotation about the single bond. But, each protein has only a single three-dimensional conformations. It deals with the manner in which the protein chain is coiled/folded.Proteins exists in two conformations:

a) Pleated structure- β structure.

b) Helical structure-α Helix.

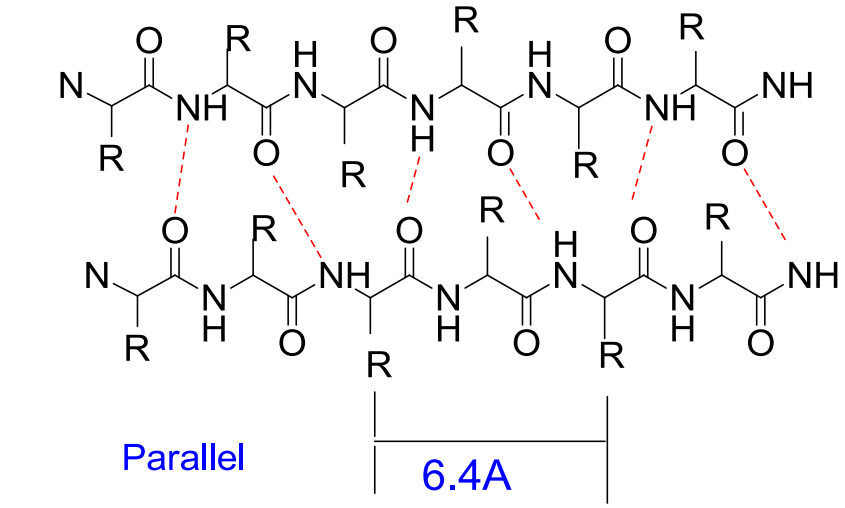
a) Pleated sheet:

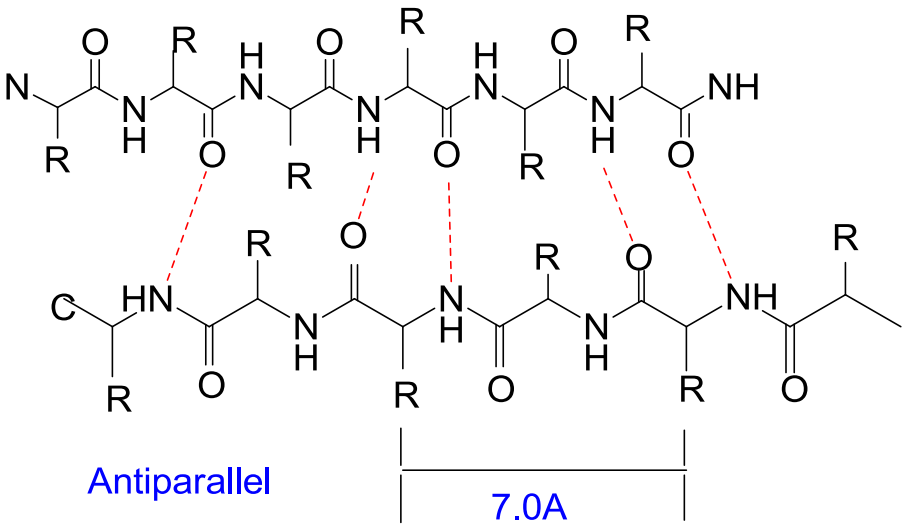
Peptide chains are fully extended to form flat zig-zags. The chains lie side by side to form a flat-sheets. Each chain is held by hydrogen bonds with a repeat distance of 7.2Å(the distance between two alternate amino acid residues).The presence of small and medium sized residues results in slight contraction of the peptide chain leading to pleated structure with a shorter distance between alternate amino acid residue- beta arrangement.

Two types of pleated structure is possible:

Parallel – chains run in the same direction.

Antiparallel-chains run in the opposite direction





In silk fibroin, has a repeat distance of 7.0 and approaches more closely to a fully extended flat-sheet structures. The structures are held by strong inter-molecular hydrogen bonding. Since the amino acids present are glycine, alanine and serine which has small side chains permits for maximum hydrogen bonding.

When wool is stretched , the α-keratin is converted to β-keratin. The helixes are uncoiled and the chain are stretched to give sheet structure. The hydrogen bonds in the helix is replaced by hydrogen bonds between the chains. larger side chain, results in a shorter distance in the peptide chain of 6.4Å

b) Helical/spiral (α helix)

The polypeptide chain with larger or bulkier side chains is coiled to form a helix, spiral structure. The helix is coiled in a right-handed fashion, termed as α helix.Helical form, hydrogen bonding occurs between different parts of the same chain, intra-molecular hydrogen bonding- holds the helix together. Hydrogen bonding takes place between the co group of one residue with the NH group of the 4^{th} amino acid residue, prevents free rotation so that the helix is rigid. Helix have 3.7 amino acid residue per complete turn.

X-ray analysis shows that the distance between any two successive turns in the helix (pitch/ gyre) is 5.0-5.5 Å The distance between any two like atoms in the helix is 1.5Å The diameter of the helix is 10 Å. At least three hydrogen bonds is broken for the free rotation to takes place. Large no. of hydrogen bonds maintains a stable structure. The side chains in the helix are projected away from the coil. The amino acids proline, hydroxy proline doesn't fit into the helical structure as these amino acids doesn't carry hydrogen and the α-helical structure ends at this point and proceeds in some other configuration. Thus proline along with serine and asparagine, glycine are known as helix-breakers. This structure is found in both fibrous and globular proteins.

3.7.6 Tertiary Structure of Protein:

Entire molecule folds to produce a specific shape is given by tertiary structure. The fibrous proteins have large helical content and are rigid rod-like molecules. It contains no tertiary structure because these polypeptide chains contains only one conformation (α helix). But they have quaternary structure as they composed of two or more polypeptide molecules aggregated into a specific conformational pattern. In globular proteins, the polypeptide chains consists of helical sections which are folded about the random coil to give spherical shape.

The folding of the entire molecule involves

1) Hydrogen bonding: It occurs between the carbonyl oxygen of one peptide bond and hydrogen atom of the amino group of other peptide bond. The bond is weak since large no.of them are involved for stabilisation. The strength of the bond is maximum when the atoms are linear.

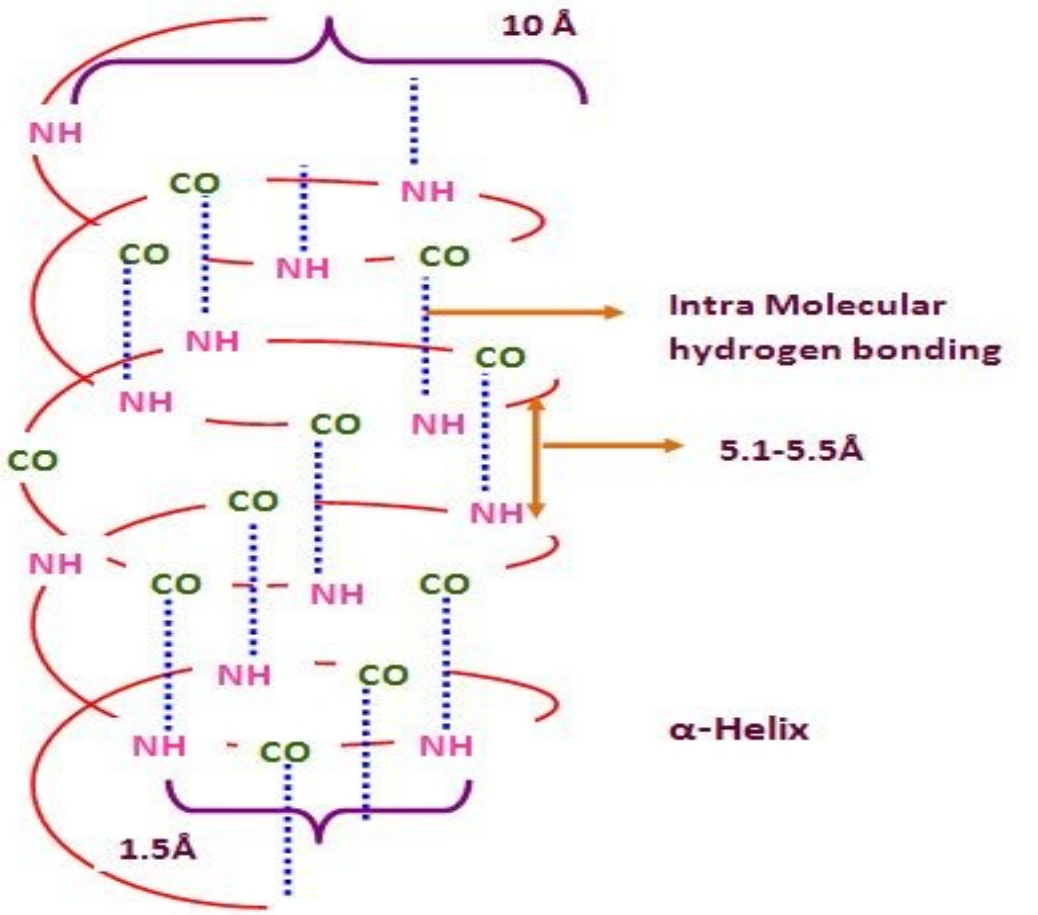


Fig 3.1 Secondary structure of Protein: α-Helix

2) Electro static forces (Ionic): The forces may be repulsive or attractive depending on the charges of the polar groups. The two polar groups are carboxylate (COO^-) and ammonium ($-NH_3^+$). When like charged polar groups are close together, the repulsive force will de stabilise the conformation. When unlike charged polar groups are close together, the attractive force will stabilize the conformations called as salt-linkages or Ionic bonds. The charges on the polar groups depends on the pH of the solution.

3) Covalent Bonds: The presence of disulphide bonds both in inter-intra peptide chain will affect the conformation.

4) Hydrophobic bonds: The different hydrophobic groups in the molecule comes in contact with one another through folding of the chain making it less access able to water. Hydrophobic bonds increases the stability of the structure. The folding takes place in which the hydrophobic side chains lie inwards while hydrophilic groups lie on the surface of the polypeptide chain.

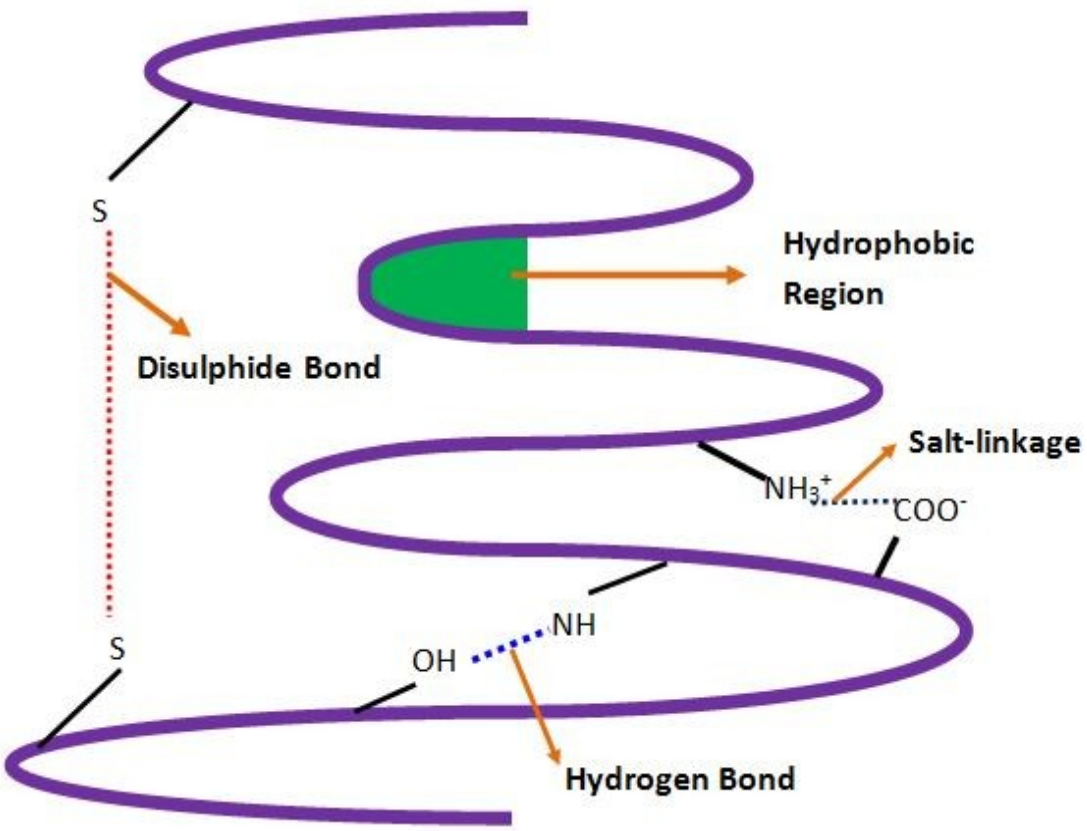


Fig 3.2 Tertiary structure of Protein

3.7.7 Quaternary Structure:

Protein contains several polypeptide chains is know as oligomeric and the individual chains are known as protomers or sub units. If the subunits are identical, they are known as homogeneous quaternary structure.

Eg: Isoenzymes of lactic dehydrogenase.

If the subunits are not identical they are heterogeneous quaternary structure.

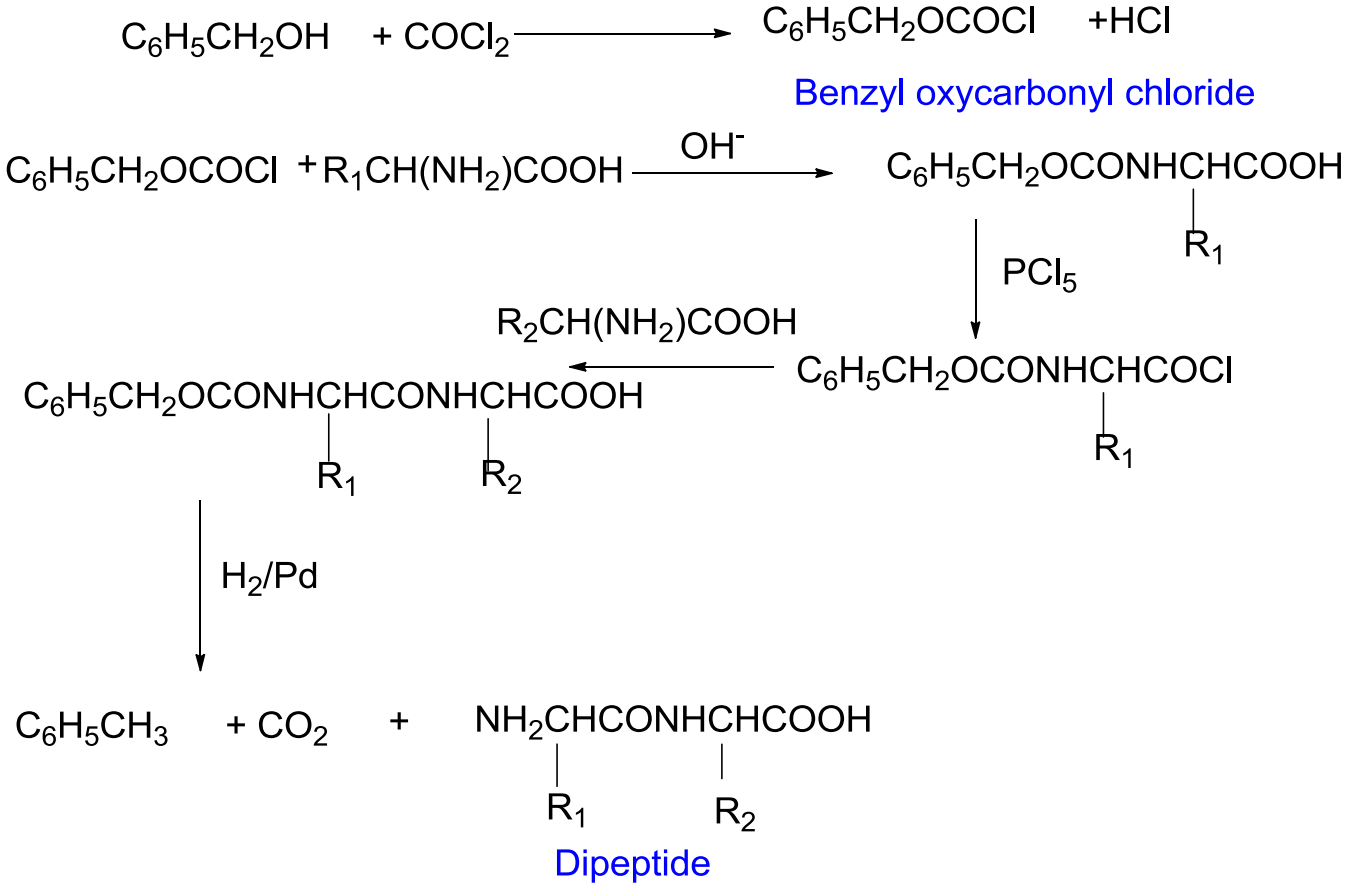
Eg: Haemoglobin consists of 4 polypeptide chains two α-chain with 141 amino acid residues and two β chains with146 amino acid residues.

3.8 SYNTHESIS OF POLYPEPTIDES:

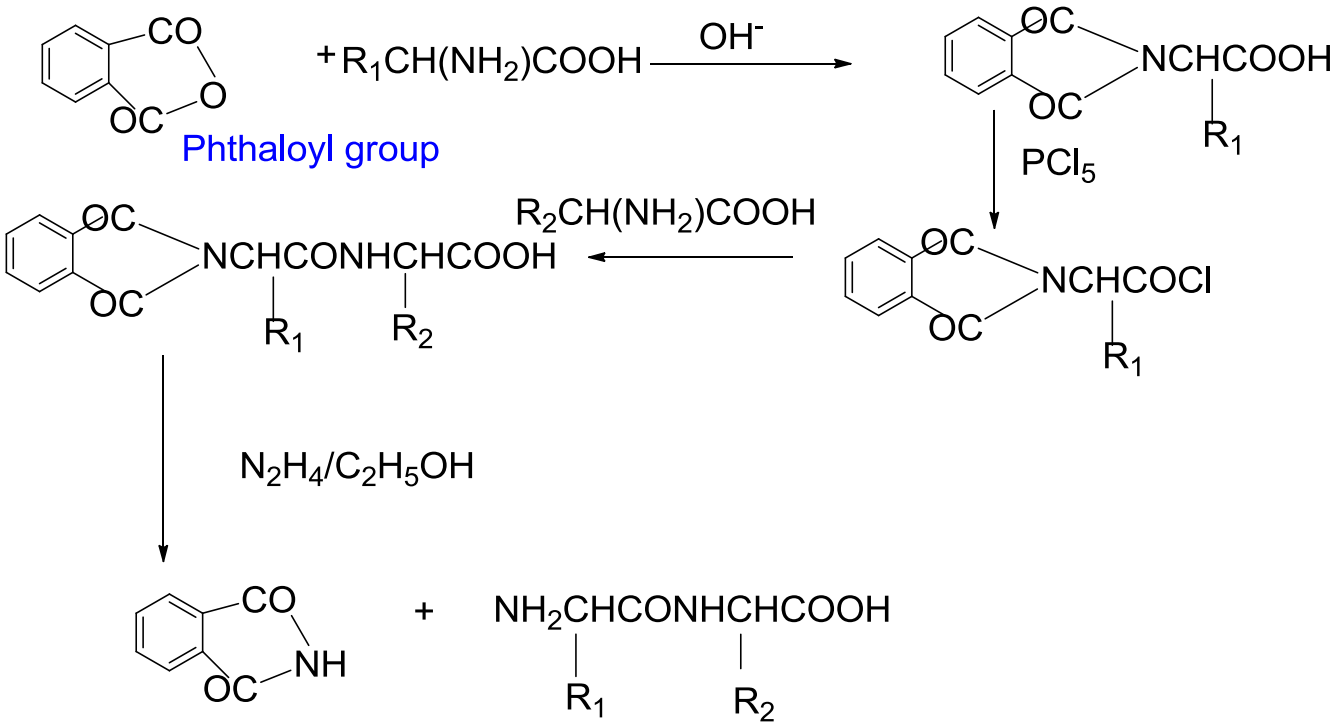
1) Bergmann's Synthesis:

In this method, benzyloxycarbonyl chloride is used as an aminop protecting group which is prepared by the action of carbonyl chloride on benzyl alcohol in toluene solution.

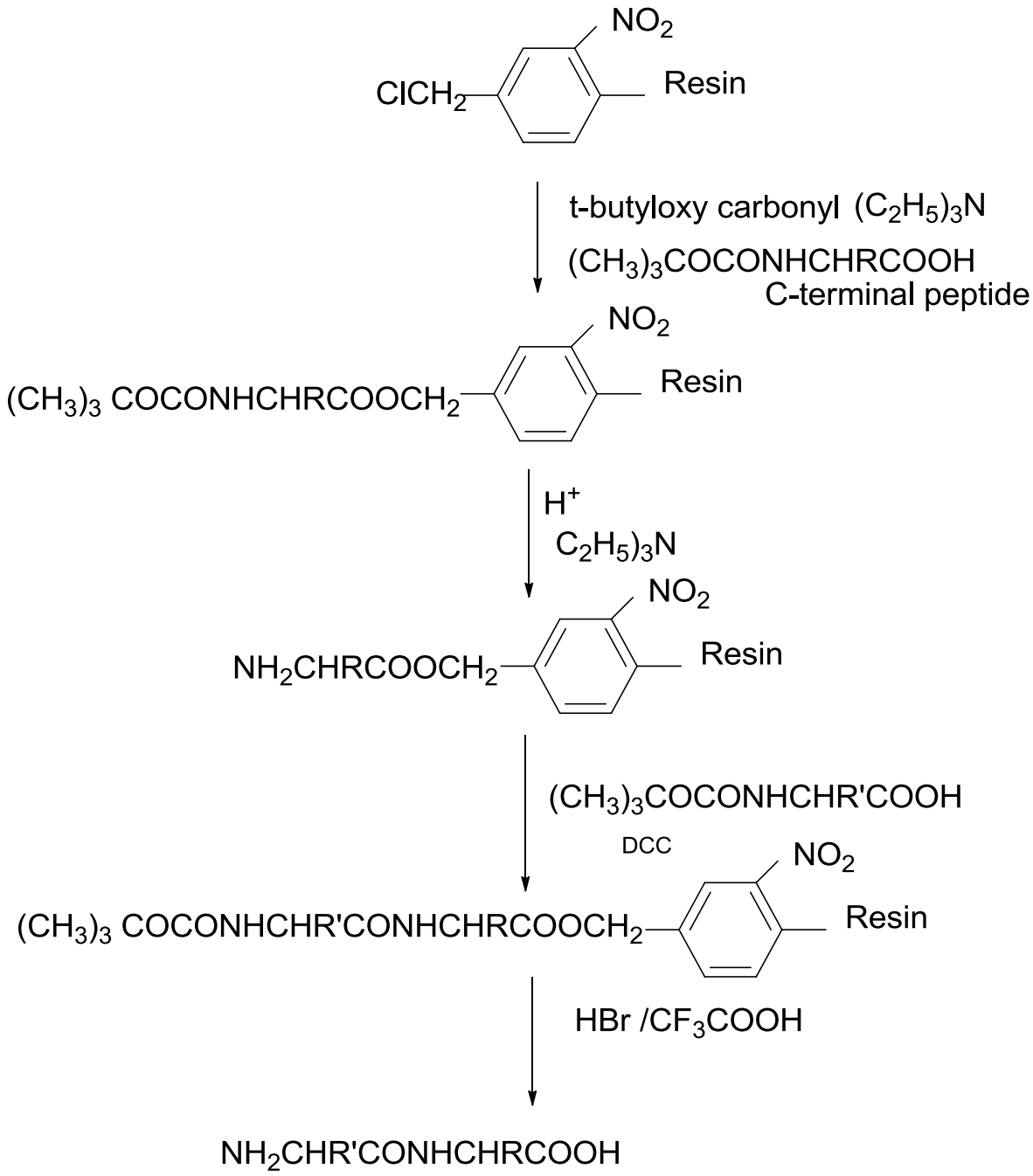
Polypeptide is synthesized by reacting benzyloxy carbonyl chloride with first amino acid followed by treating with PCl_5 to acid chloride which is then treated with second amino acid to form derivative which on reduction forms a dipeptide.



2) Sheehan's method: Phthaloyl group is used as an amino protecting group.



3) Solid phase synthesis: An amino acid is covalently linked to an insoluble resin as a support for the peptide. The resin is a copolymer of styrene and DVB which are chloromethylated and nitrated. A suitable N-acyl derivative of amino acid is synthesized and refluxed to form an insoluble benzylester derivative. Then a second N-acyl aminoacid is reacted to form dipeptide.



3.9 LIPIDS

Naturally occurring organic compounds insoluble in water but soluble in non polar organic compounds like $CHCl_3$, CCl_4. The term 'lipid' was used by 'Bloor' in 1943.

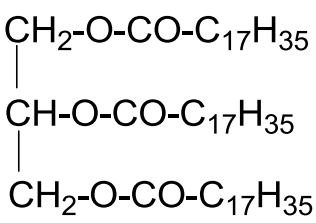
3.9.1 CLASSIFICATION OF LIPIDS:

a) Simple lipids; b) Compound lipids; c) Derived lipids

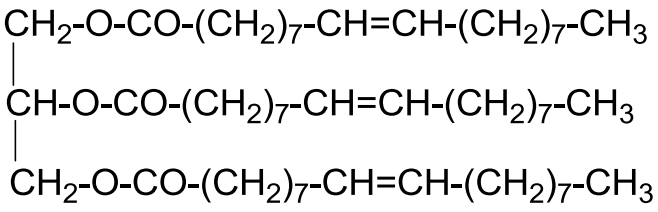
A) Simple Lipids (Homolipids): These are esters which on hydrolysis yields fatty acids and glycerol. Eg: Fat, oils and waxes

3.10 FATS AND OILS:

They are triglycerides with oil being liquid at room temperature. Fats are solids at room temperature.



Stearin



Olein
Unsaturated

3.10.1 Physical properties:

Colourless, odourless and tasteless; Lighter than water and are immiscible with water; Soluble in benzene, petroleum ether; Readily forms emulsions when agitated with water; Non-volatile but decoposes on strong heating. Animal fat contains cholesterol, unsaturated alcohol while vegetable fat contains phytosterol.

3.10.2 Extraction of oil and Fat:

The following procedure are used for the extraction process:

Rendering: Animal fat are extracted by chopping the liver, kidney and boiling with a water or steam. The fat melts and floats at the surface.

Pressing: Seeds containing vegetable oil is crushed by rollers and pressed in hydraulic press . The oil separates leaving the cake , used as cattle feed

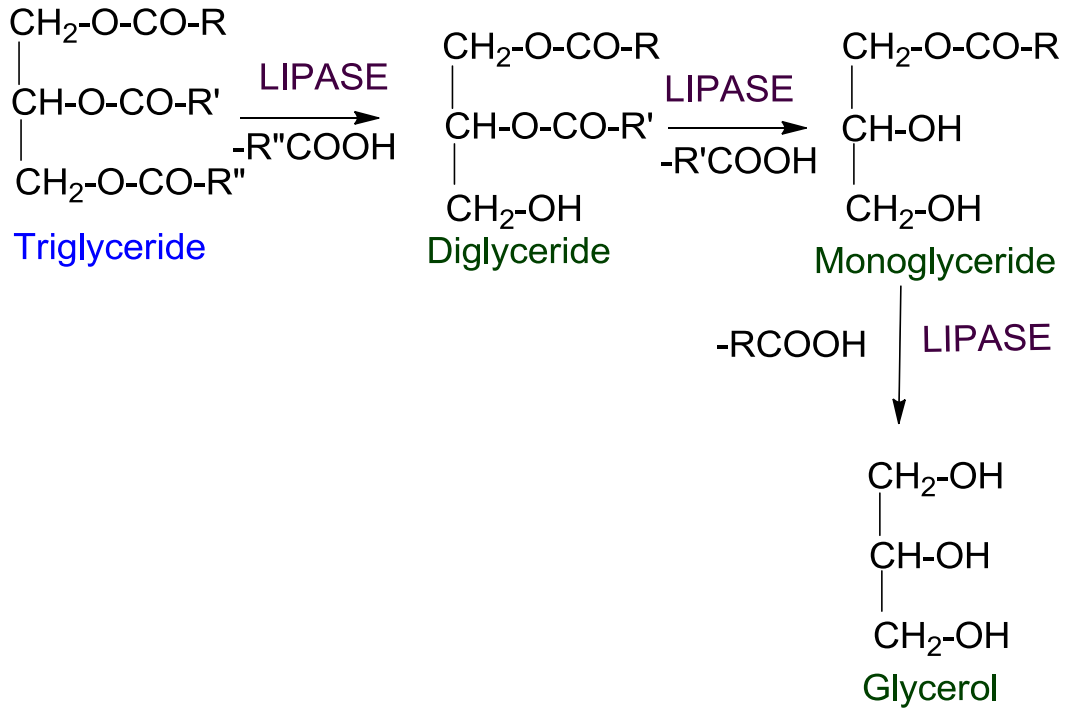
Solvent extraction: The pressed cake is crushed and treated with organic solvents like CCl_4, benzene, ether.

Refining: The crude oil is treated with alkali and bleaches at 70-80^{o}c with animal charcoal, Fuller's earth and deodorized by passing superhaeted steam. The oil is quickly cooled and packed.

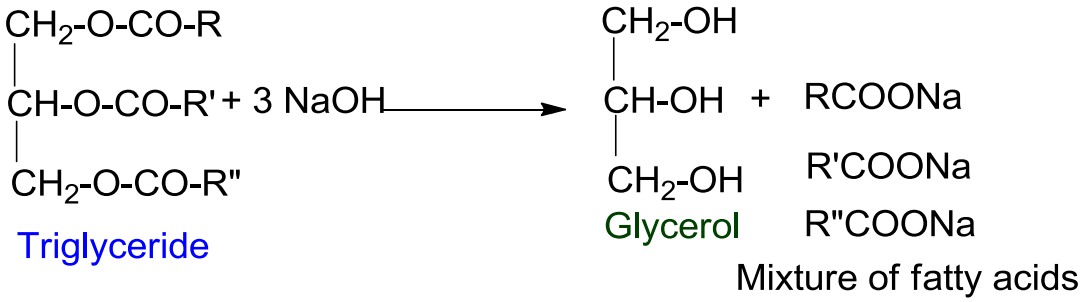
3.10.3 Chemical Properties:

i) Hydrolysis: oils and fats can be hydrolysed to glycerol and fatty acids by heating with acids, alkliies or by enzyme lipase.

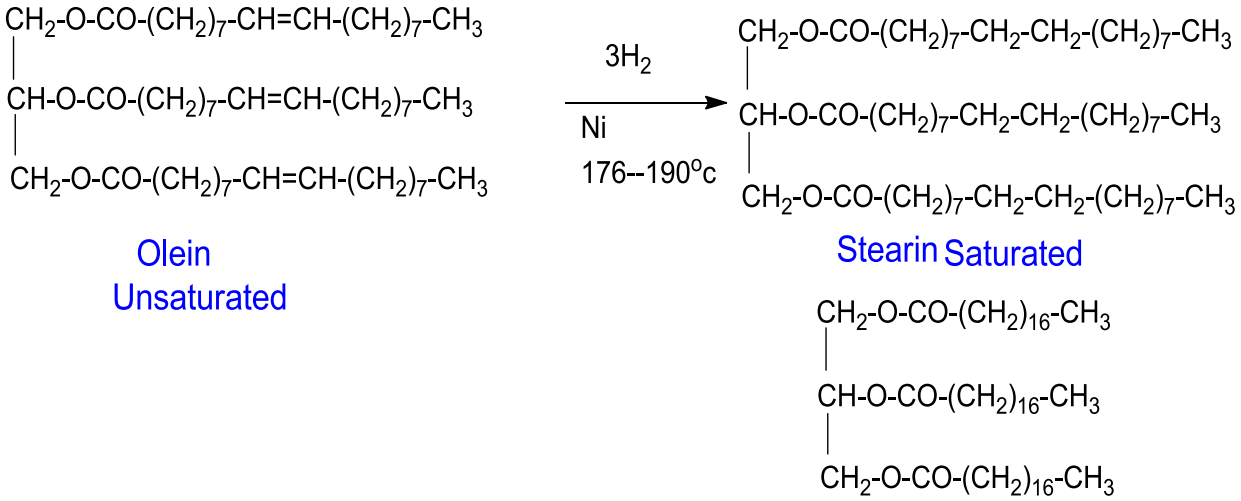
Enzymatic Hydrolysis: Lipases brings out the splitting of fats in steps to glycerol at 0-40^{0}c.



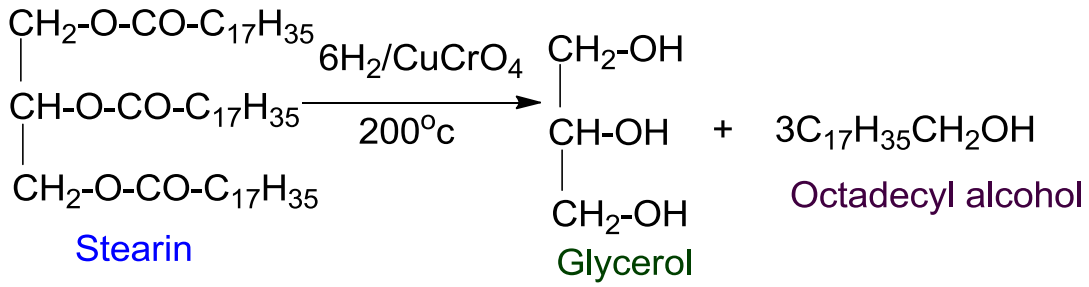
ii) Saponification: Hydrolysis of fats and oils in the presence of alkali is known as sapnofication.



(iii) Hydrogenation: When hydrogen is passed through oil/fat in the presence of a catalyst, the unsaturated glycerides is converted to saturated glycerides known as vegetable ghee or margarine.



(iv) Hydrogenolysis: When hydrogen is passed under pressure of 200 atm. Pressure in the presence of copper chromium catalyst it splits into glycerol and higher aliphatic alcohols.



(v) Drying of Oil: Oil with PUFA have the ability to absorb oxygen from atmosphere and then polymerizes to form a hard, translucent coating and are used in making paints. Such oils are called drying oils.

(vi) Rancidification: When fats are allowed to exposed to air, they undergoes slow decomposition and develop unpleasant smell. The process is known as rancidification. During rancidification, the chemical changes takes place are:

a) Enzymatic Hydrolysis: Enzymes produced by microorganisms produces bad smelling due to lower fatty acids and is known as hydrolytic rancidity.

*b) Aerial Oxidation:*Fats and oils produces peroxides on aerial oxidation which on hydrolysis produces aldehydes and ketones with unpleasant odour.

c) β- oxidation of saturated fatty acids produces ozonide which on hydrolysis followed by decarboxylation forming ketones possessing unpleasant odour. Both ethe oxidation process is known as oxidative rancidity.

3.10.4 Analysis of Oil and Fats

(i) Acid Value: It indicates the amount of free fatty acids present in an oil or fat. High acid valu indicates that the fat/oil is stored in improper conditions.

Acid value is the number of milligrams of KOH required to neutralize the free organic acid present in 1g of oil/fat.

Acid value= (Vol.of KOH)x N/10 x56.01/(wt. of oil/fat).

Determination: Dissolved a weighed qualtity of fat/oil in alcohol and titrated against N/10 KOH using phenolphthalein as indicator.

(ii) Saponification Value: It is defined as the number of mgs of KOH required to completely neutralize the fatty acids resulting from complete hydrolysis of 1g of fat/oil.

Determination: Refluxing a weighed amount of the fat/oil in known excess of standard caustic potash and back titrating the excess alkali with N/2 HCl using phenolphthalein as indicator. The titration is repeated for blank solution without a sample oil.

Saponification Value = (Vol.of HCl req.blank- Vol.of HCl req. sample) xN/2X56.01/ (wt. of oil/fat)

It gives an idea about the molecular weight of an oil/fat. They are inversely related to each other. High saponification value indicates that the fat contains low molecular weight compounds and vice versa.

(iii) Ester value: The difference between saponification value and acid value is known as ester value.

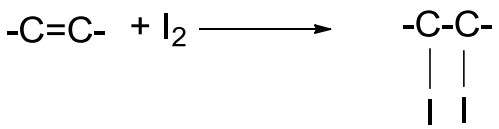
(iv) Reichert-Meissel value (RM Value): It is a measure of volatile fatty acids in fat/ghee used to mainly determine the purity of ghee/ butter..

It is defined as the number of ml of N/10KOH solution required to neutralize volatile, water soluble distillate of 5g of hydrolysed fat/oil.

(v) Acetyl value: It is a measure of alcoholic groups present in an oil/fat. It is defined as the number of mgs of KOH required to neutralize acetic acid liberated by the saponification of 1g of completely acetylated fat/oil.

(vi) Polenske Value: It is defined as the ml of N/10 KOH required to neutralize water insoluble fatty acids obtained from the distillate of 5gm of hydrolysed fat/oil.

(vii) Iodine Value: It is defined as the number of grams of iodine that combine with 100g of fat/oil.



It is a measure of degree of unsaturation of fat/oil. high iodine value indicates high degree of unsaturation in oil/fat.

Determination: A weighed amount of oil is dissolved in 15ml of pure $CHCl_3$ or CCl_4 in iodine flask. It is then treated with 25ml of wij's reagent (dissolved 8gms of ICl_3 in 250ml of glacial acetic aid with 9g of I_2 in 500 ml of galcial acetic acid). The flask is kept in dark for 30 minutes. The solution is then mixed with 15ml of 10% KI and titrated against N/10 sodium thio sulphate using starch as indicator. A balnk determinationis carried out in the absence of oil/fat.

Iodine value= (Volume of thio for balnk-vol.of thio for sample) x N/10X127x100/ (wtof oilx1000)

3.11 WAXES

They are esters of fatty acids with higher monohydric alcohol like cetyl, myricyl alcohol. They differ from paraffin waxes which are mixture of higher alkanes. They are insoluble in water and soluble in fat solvents. They are not easily digestible and are used as protective agent for plants and animals.

1) Bees Wax: It is secreted by honey bee contains myricyl palmitate $C_{15}H_{31}COOC_{30}H_{61}$. It is used in boot polishes, cosmetics and pharmaceutical preparations.

2) Spermaceti wax: It is a white, lustrous, translucent component of spermoil obtained from the head of whale sperm. It contains cetyl palmitate $C_{15}H_{31}COOC_{16}H_{33}$. It is used as a lubricant in delicate instruments like watches, cosmetics and candles.

3) Carnauba wax: It is found in the leaves of carnauba palm of Brazil, contains myricyrl cerotate $C_{25}H_{51}COOC_{30}H_{61}$. It is used in boot polishes, varnishes.

4) Lanolin: It is also known as 'wool wax' obtained from the wool of the sheep. It consists of cholesterol esters of fatty acids ranging from valeric to palmitic acids. Used in onitments

5) Chinese wax: It is the secretion of insect coccus plea, hardest wax contains ceryl cerotate $C_{25}H_{51}COOC_{26}H_{53}$. It is used in sizing, polishes and candles.

6) Bay Berry wax: Green waxy fat contains glycerides of lauric, palmitic and myristic acid used for candles.

7) Candellia wax: Vegetable wax used for electrical insulation.

3.12 COMPOUND LIPIDS

These are lipids which contains additional groups like phosphorus, carbohydrate and sulphur together with fatty acids and alcohols. Compounds contains 'P' are phospholipids and those containing 'S' are sulpholipids while those containing carbohydrate- Glycolipids.

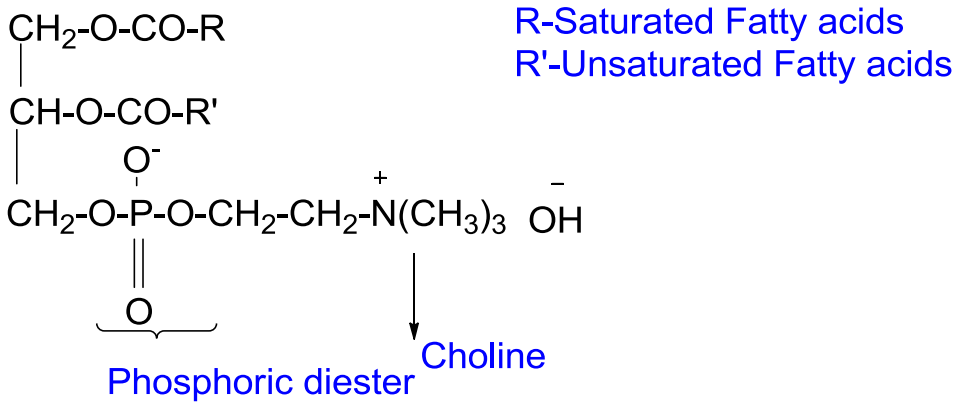
3.12.1 Phospholipids

Lipids having alcohol, fattyacids and phosphoric acids. They are regarded as fattyacids in which one of the fatty acids is replaced by phosphoric acid.

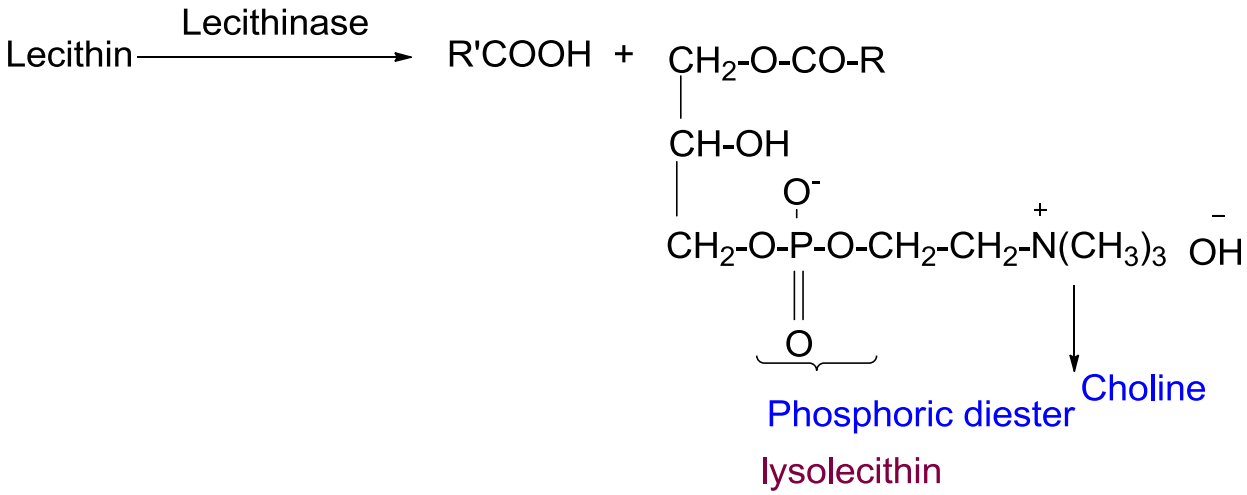
a) Lecithin (Phosphatidyl Choline):

They are glycero phospholipids in which the alcohol is glycerol. Two of the –OH groups are esterified with either saturated or unsaturated fatty acids. The third –OH group is not esterified but rather bya phosphate group which is esterified by a nitrogen based alcohol, Choline. When hydrolysed they yield glycerol, fatty acids, choline and phosphoric acids. The fatty acids present in lecithin are stearic, palmitic, oleic, linoleic and arachidonic acid.

They are yellowish grey solids, rapidly darken in colour when exposed to air. They are soluble in ether and alcohol not in acetone. At physiological pH, they are equally ionised and carries no net charge. Hence, it is a neutral phospholipids. They occur in two forms: α and β lecithin.

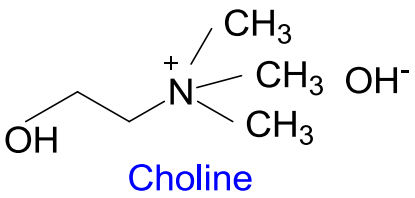


Enzymatic hydrolysis by lecithinase yields lysolecithin and fatty acids.



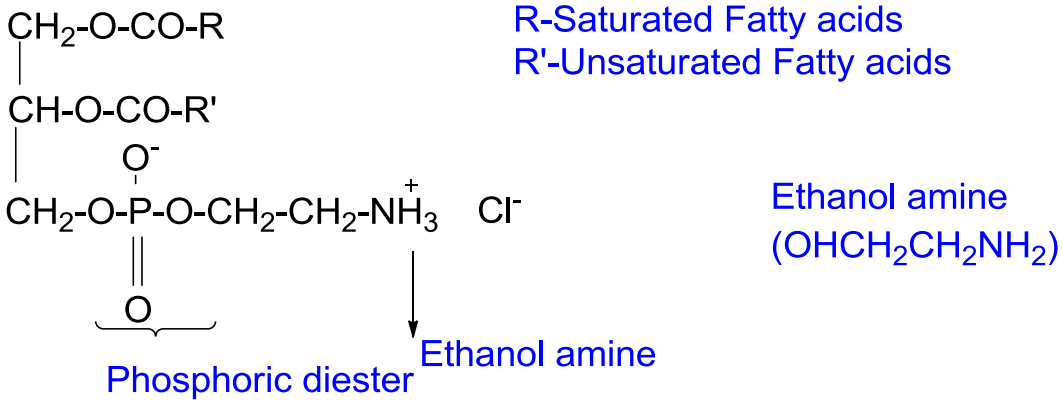
Choline: It is a trimethyl hydroxy ethyl ammonium hydroxide which is a strong quaternary base like NaOH. It is a component of lecithin and sphingomylein. It is present in liver, kidney, heart, milk and in egg yolk. It act as a methyl donor in trans methylation reactions.

Acetyl choline, the acetylated derivative of choline is plays an important role in the transmission of nerve impulses.

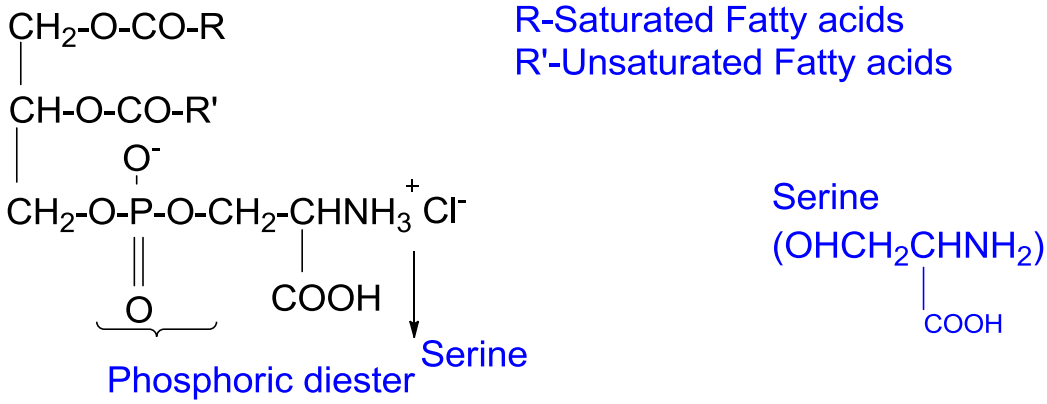


b) Cephalins:

They occur along with lecithin in animal and plant cells. They are similar in structure like lecithin except the nitrogen base attached to the phosphoric acid is not choline but ethanol amine or serine. Both α and β cephalins are known.

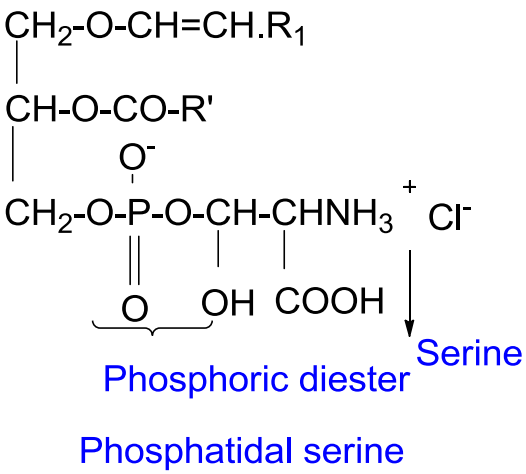


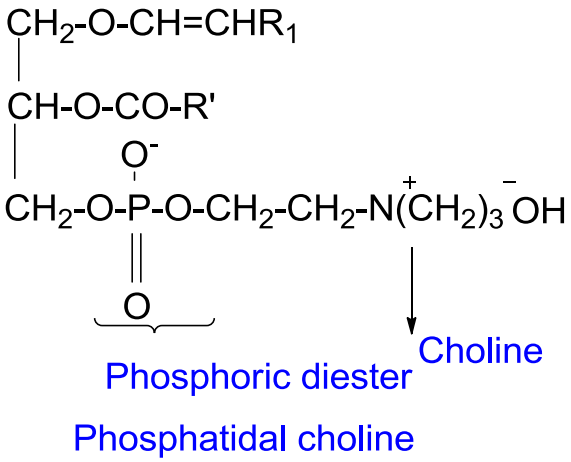
Phosphatidyl serine

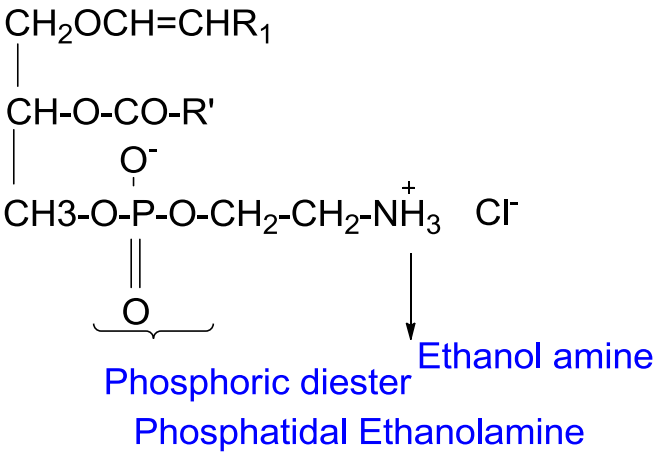


c) Plasmologens (Phosphoglyceracetals):

Plasmalogens constitute 10% of the phospholipids of the brain and muscle. Structurally, these resemble lecithins and cephalins but one of the fatty acids is replaced by an unsaturated ether. The nitrogen base can be choline, serine or ethanol amine.

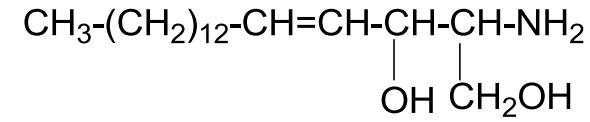




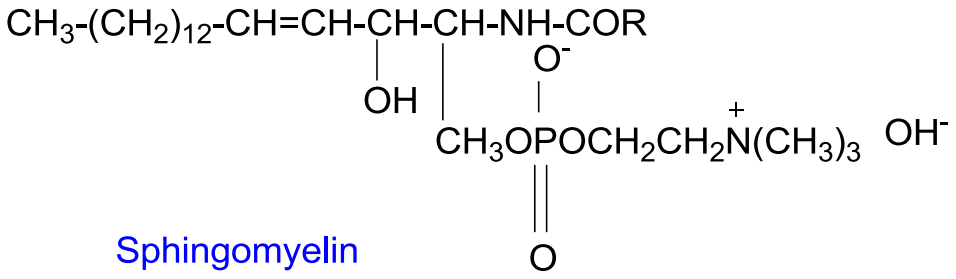


d) Sphingomyelins:

They are found in brain and nerve tissues. They contain a fatty acid, phosphoric acid, choline and complex amino alcohol called sphingosine. The saturated fatty acid is attached to the amino group of the sphingosine by an amide linkage. The phosphoric acid is attached to one of the hydroxyl group of sphingosine. The combination of fatty acids with sphingosine is known as ceramide. They are white crystalline solids, opalescent suspensions in water. They are associated with multiple sclerosis and Niemann-Pick disease.



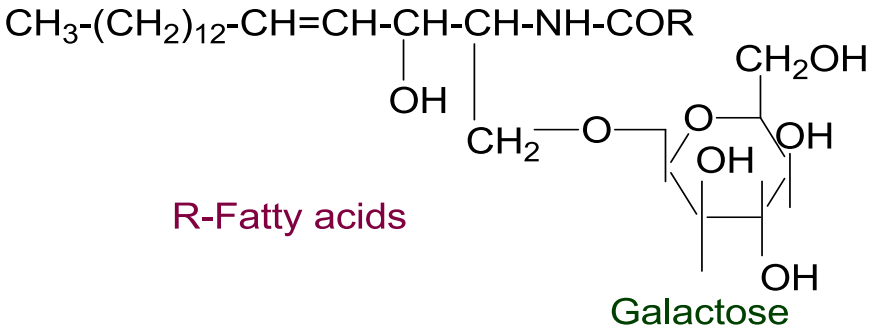
Sphingosine (1,3 dihydroxy 2-amino Octdec-4-ene)



Sphingomyelin

3.12.2 Glycolipids (cerebrosides):

These are compounds of fatty acids with carbohydrates and contain nitrogen but no phosphoric acid. They are important consitutent of brain and constitute 8% of the total solid matter.The structure of cerebroside contains a high molecular weight fatty acids, sphingosine and either galactose or glucose instead of choline but no phosphoric acid. They have no electrical charge and the polar groups are neutral. Cerebroside contains ceramide (N-acyl sphingosine) in their structure. The sphingosine carries galactose by glycosidic linkage on its primary alcohol group and the fatty acid by amide linkage on its primary amino group.



R-Fatty acids

Galactose

Cerebroside

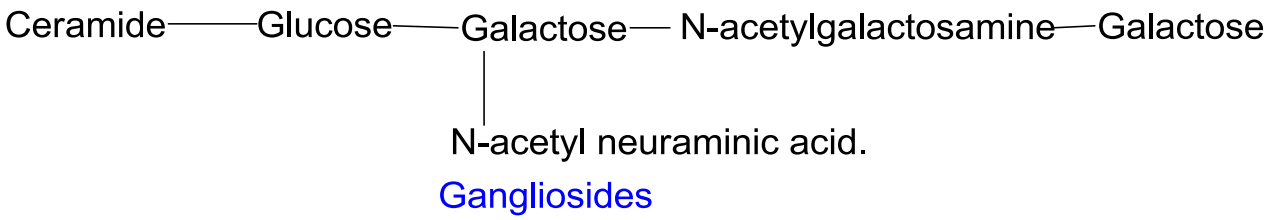
Individual cerebrosides are differentiated on the basis of fatty acid component as

- Kerasin –contains saturated C24 fatty acid – lignoceric acid.
- Phrenosin(Cerebron)- contains 2 –hydroxy lignoceric acid- cerebronic acid.
- Nervon- Unsaturated homologue of lignoceric acid.

In Gaucher's disease, a deficiency of enzyme glucosyl ceramide hydrolase prevents the cleavage of ceramide trihexoside, leads to the accumulation of the latter thereby affecting lungs and bone marrow.

b) Gangliosides:

These are found in ganglion cells of nervous tissues and make up to 6% of the membrane lipids. The structure of gangliosides is similar to cerebrosides in that they contain a ceramide linked to carbohydrate (glucose or galactose) In additrion it contains N-acetyl galactosamine and N-acetyl neuraminic acid.



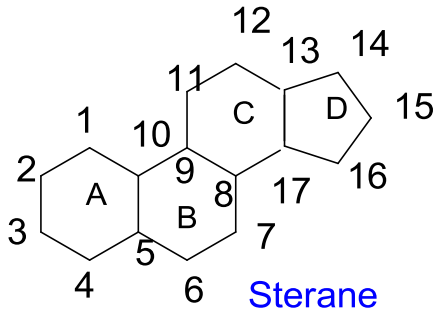
Gangliosides

3.13. DERIVED LIPIDS:

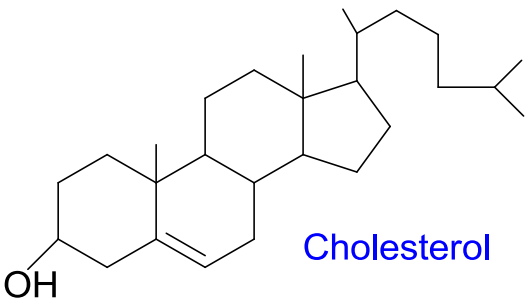
It includes the hydrolysis products of simple, compound and various compounds like steroids, fatty acids, ketones.

a) Steroids:

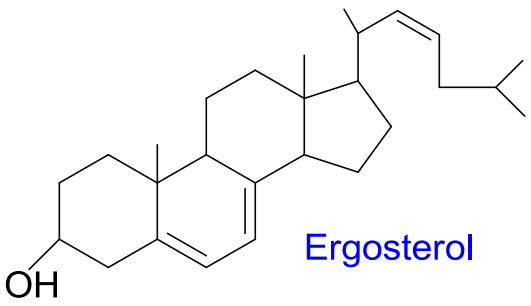
These are solid materials and contains no fatty acids thus they are non- saponifiable. They can't be hydrolyzed by heating with alkali. Steroids are considered as derivative of fused and fully saturated ring system called cyclopentanoperhydro phenanthrene or sterane. It contains 3 cyclohexane rings fused in a non-linear and a terminal cyclopentane ring.



Sterane



Cholesterol



Ergosterol

TEXT / REFERENCE BOOKS

1. Finar I. L., Organic Chemistry, 6th Edition, Pearson Education Asia, 2004.
2. S.V.Bhat,B.A. Nagasampagi, M.Sivakumar., Chemistry of Natural products, Springer Publications 2005.
3. O.P. Agarwal., Organic Chemistry-Natural Products Vol.I and II, Goel Publishing House, 1980.
4. G.R. Chatwal., Organic Chemistry-Vol II- Himalayan Books, 1985.
5. T.L. Gilchrist., Heterocylclic Chemistry, Longman Scientific and Technology, 2005.

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SCHOOL OF SCIENCE AND HUMANITIES

DEPARTMENT OF CHEMISTRY

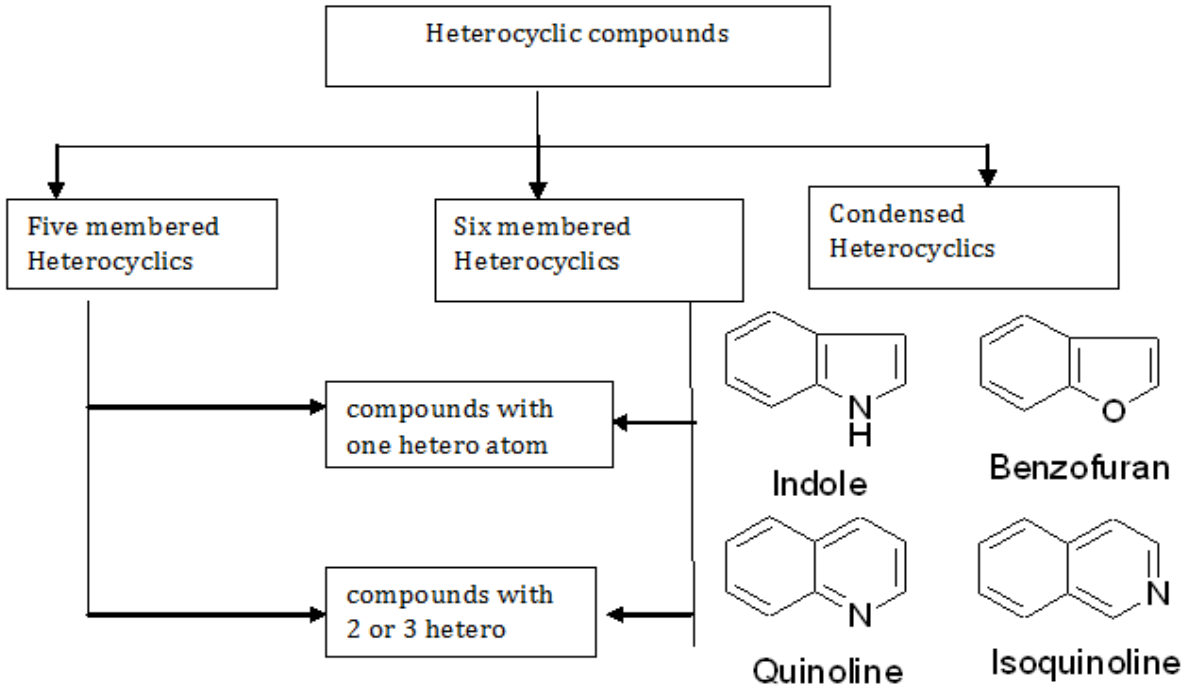
UNIT – IV - Synthesis of Heterocyclic compounds – SCYA7201

UNIT-4

HETEROCYCLIC COMPOUNDS

4.0 INTRODUCTION

Heterocyclic compounds are compounds with at least one heteroatom as the ring member which are relatively stable and exhibits aromatic character.

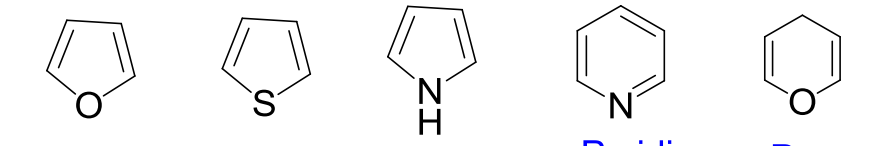


4.1 NOMENCLATURE

Rings with one hetero atom follows the following rule:

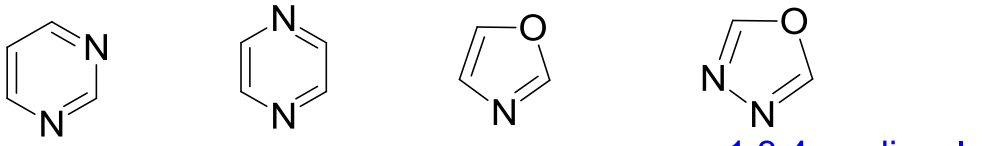
Hetero atom	Prefix	No.of atoms	Suffix	UnSaturated Other Atoms N	UnSaturated N	Saturated Other Atoms	N
N	Aza	3	Ir	ene	ine	Ane	dine
O	Oxa	4	Et	e	e	Ane	dine
S	Thia	5	Ol	e	e	ane	dine

P	Phospha	6	In	e	e		
As	arsa	7	epin	e	e		



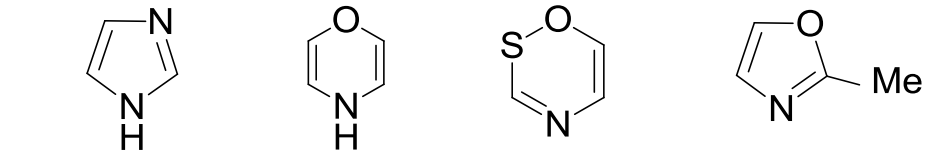
Furan /Oxole | Thiophene /Thiole | Pyrrole /azole | Pyridine /Azine | Pyran /Oxine

Rings with more than one heteroatom, the order of priority is O>S>N.



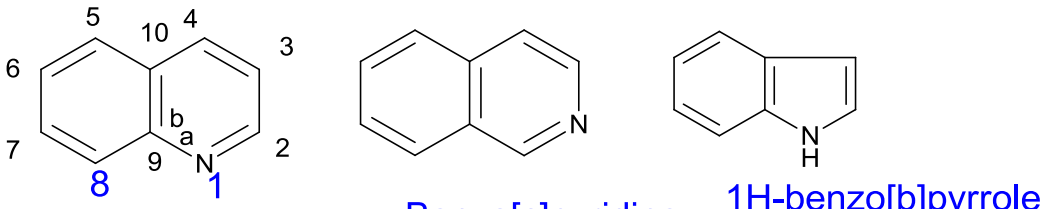
1,3 diazine pyrazine | 1,4 diazine pyrimidine | 1,3 oxazole | 1,3,4oxadiazole

A saturated heteroatom with an extra-hydrogen attached is given priority over an unsaturated form of the same atom.



1-H 1,3-diazole Imidiazole | 1-H 1,4 Oxazine | 1,2,4-oxathiazine | 2-methyl 1,3 oxazole

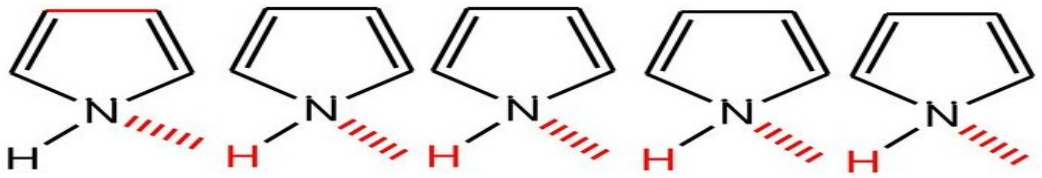
Systems where two rings share a common single or double bond, which are said to be fused rings.A common case is where a benzene ring is fused to a heterocyclic ring. The name begins with the prefix “benzo.” The point of attachment is indicated by a letter that defines the “face” of the heterocycle involved Thus, the1,2- position on the heterocyclic ring is always the “a- face,” 2,3- is the “b-face,” 3,4- is the “c-face,” and so on.



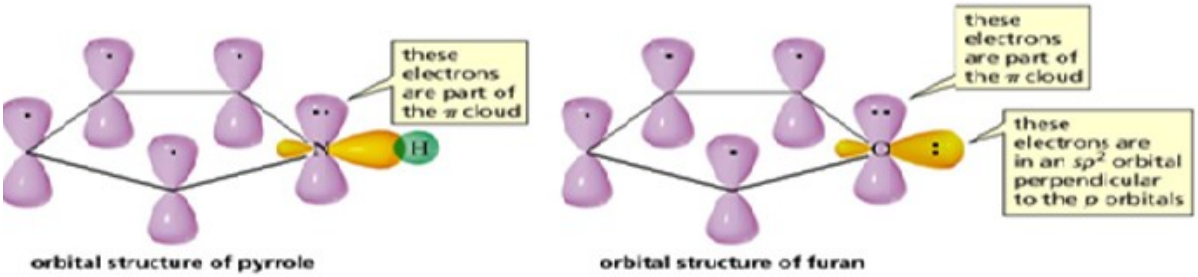
Benzo[b]pyridine Quinoline | Benzo[c]pyridine isoquinoline | 1H-benzo[b]pyrrole Indole

4.2 GENERAL CHARACTERISTICS

Pyrrole, furan and thiophene are colorless liquids of boiling points 126°, 32°, and 84° respectively. Pyrrole has a relatively high boiling point as compared to furan and thiophene, this is due to the presence of intermolecular hydrogen bonding in pyrrole.



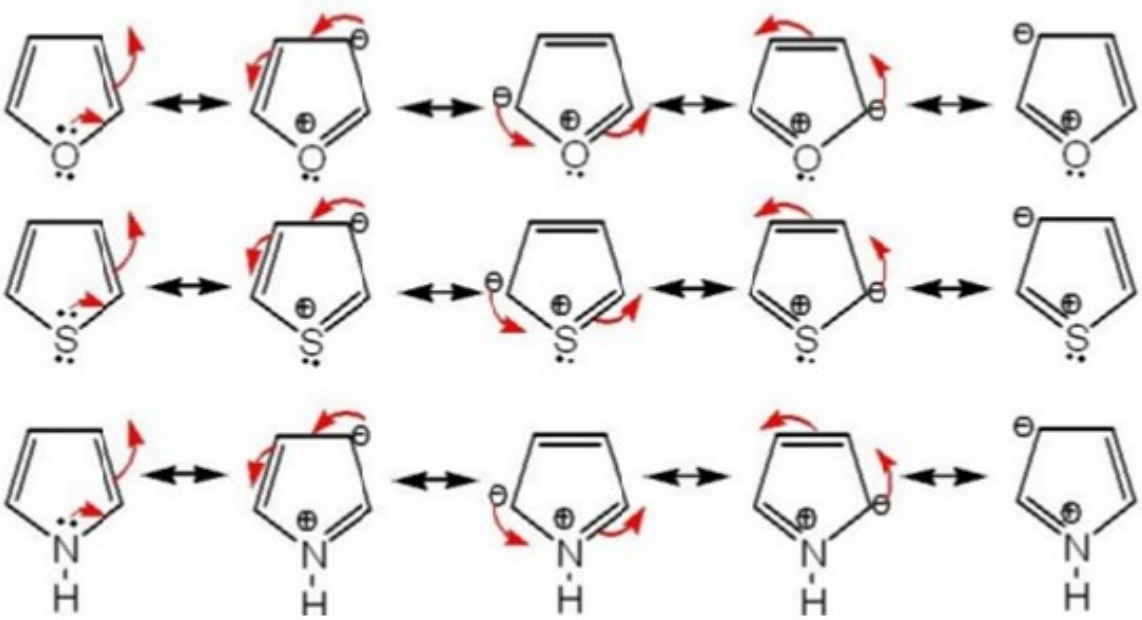
Pyrrole furan and thiophene are aromatic because:1) they fulfill the criteria for aromaticity, the extent of delocalization of the nonbonding electron pair is decisive for the aromaticity, thus the grading of aromaticity is in the order of: furan< pyrrole < thiophene< benzene .oxygen (3.44), nitrogen (3.04) and thiophene (2.56).



orbital structure of pyrrole

orbital structure of furan

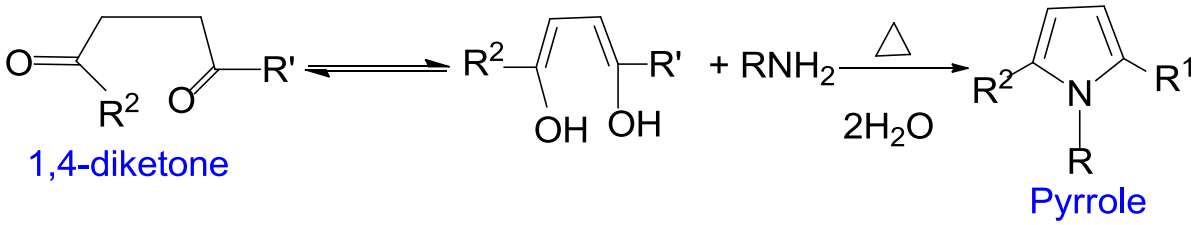
They tend to react by electrophilic substitution due appearance of –ve charge on carbon atoms due to delocalization as shown in the following resonance structures.



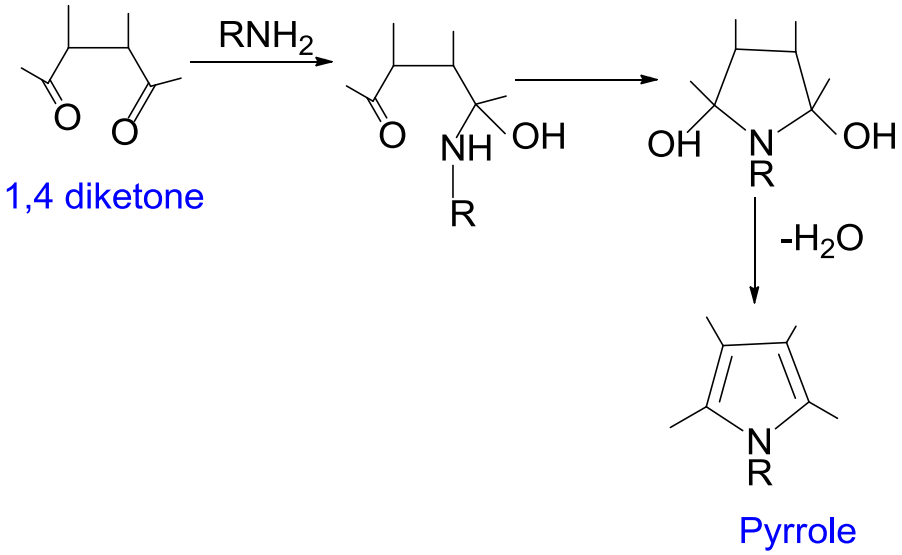
4.3 PYRROLE

4.3.1 Synthesis:

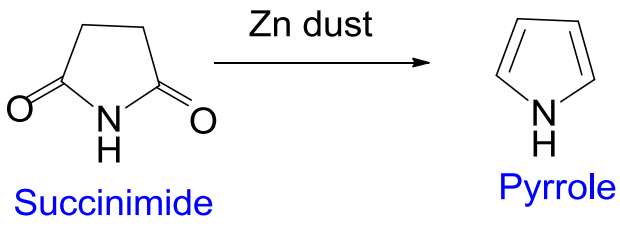
1) Pall-Knorr synthesis: Heating diketone with ammonia or aliphatic amine.



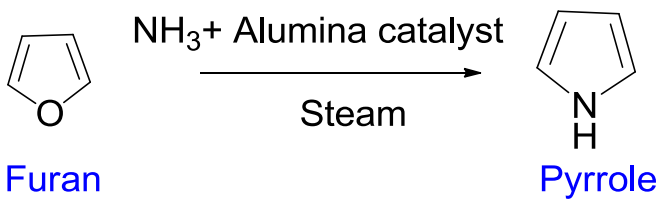
Mechanism:



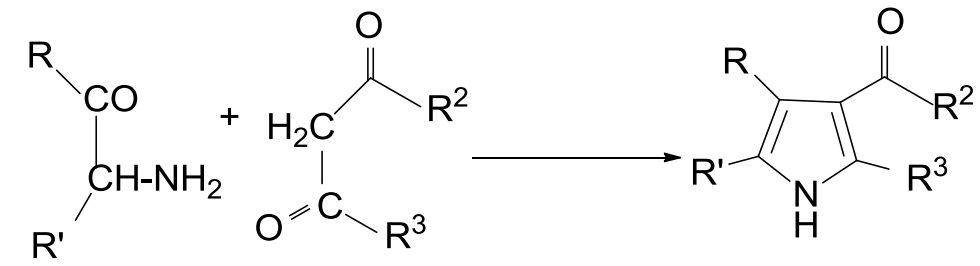
2) Pyrrole is obtained by distillation of succinimide over zinc dust



3) By heating a mixture of furan, ammonia, steam over the presence of alumina catalyst



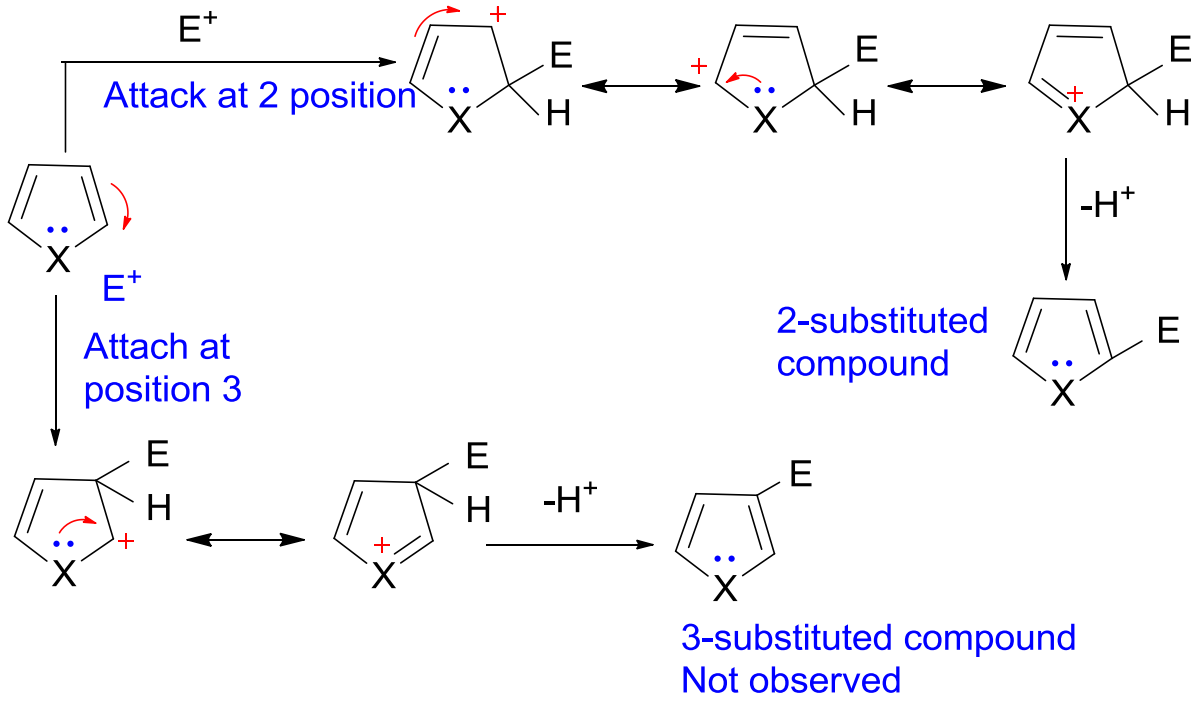
4) Knorr-Pyrrole synthesis: Reaction between α- amino ketone and β-keto ester.



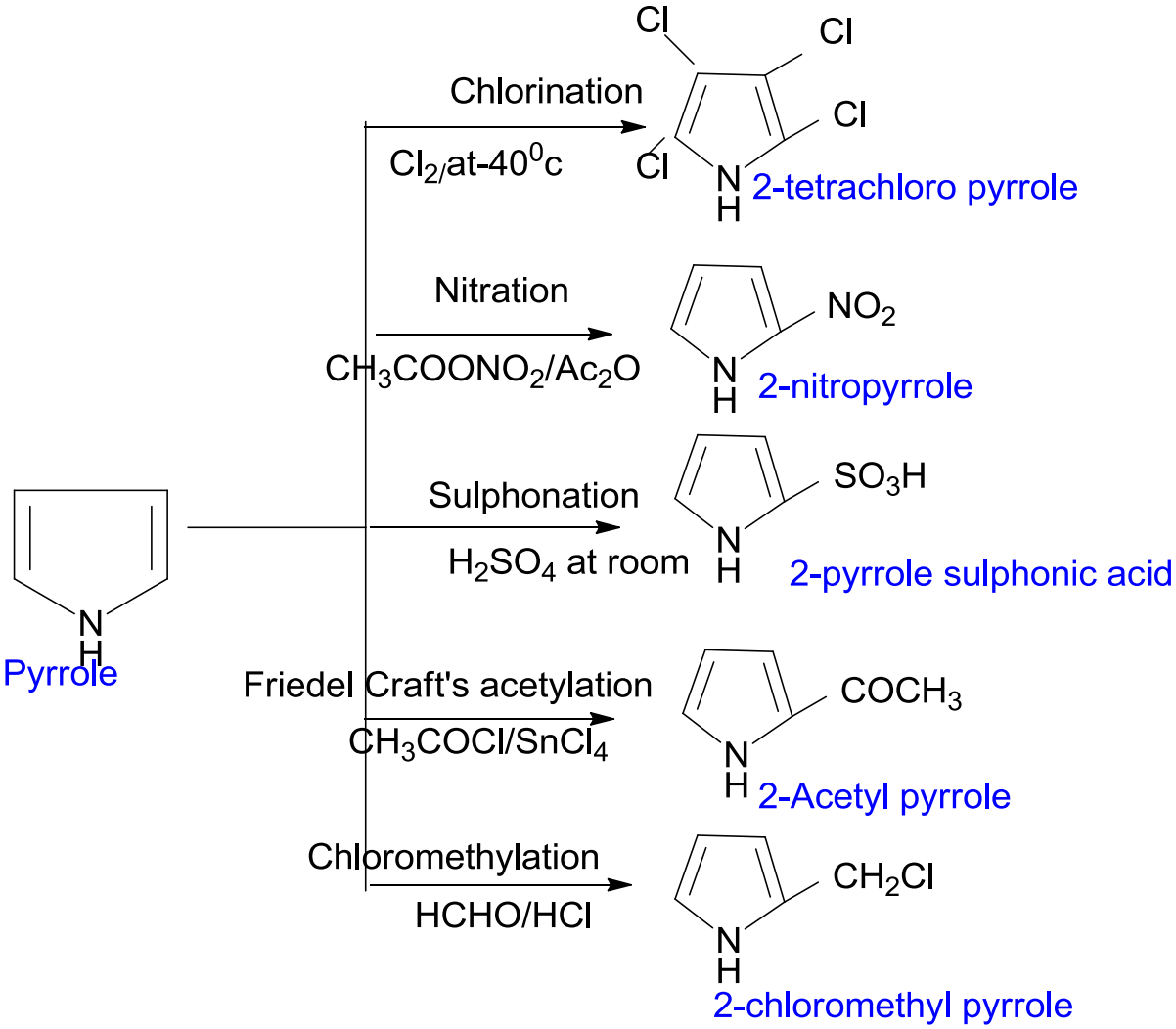
α-aminoketone β–dicarbonyl derivative

4.3.2 Electrophilic Substitution:

Electrophilic substitution in 5 membered ring occurs at C2 and not at C3. The resonance structures are more at C2 than at C3.



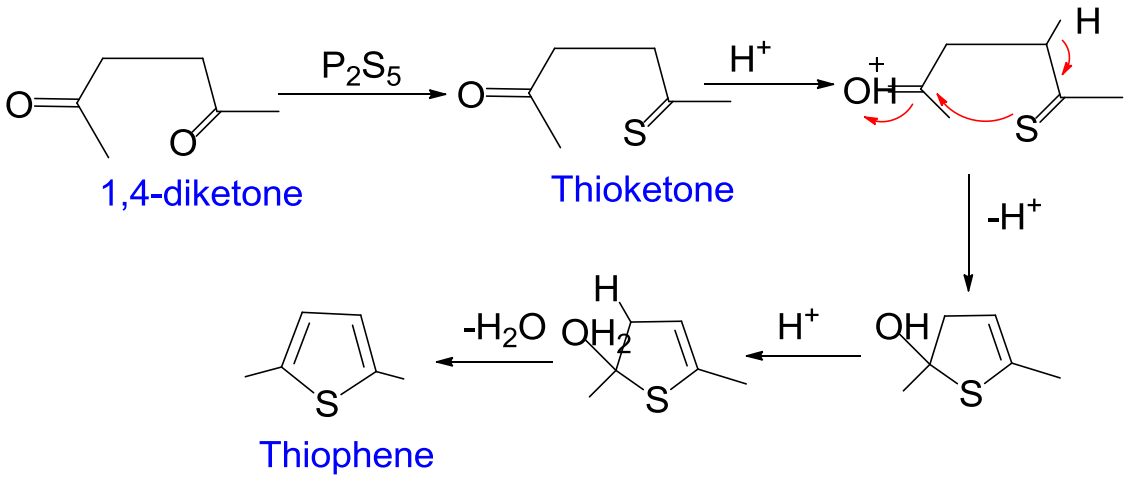
Electrophilic reactions in Pyrrole



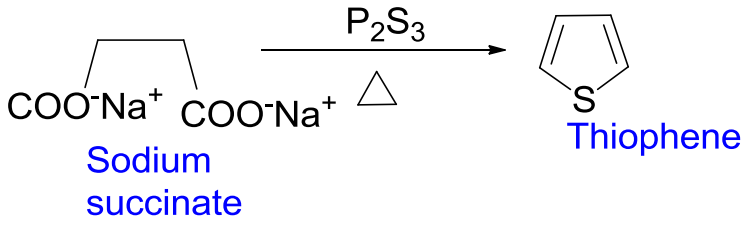
4.4 THIOPHENE

4.4.1 Synthesis:

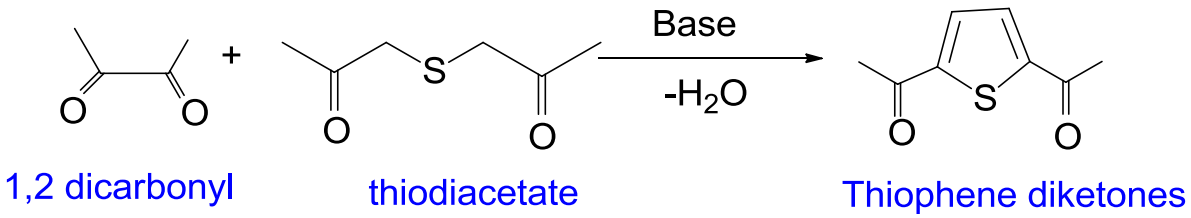
1) Pall-Knorr synthesis: Heating diketone with Phosphorus penta sulphide.



2. Laboratory Synthesis: Heating a mixture of sodium succinate with phosphorus tri sulphide

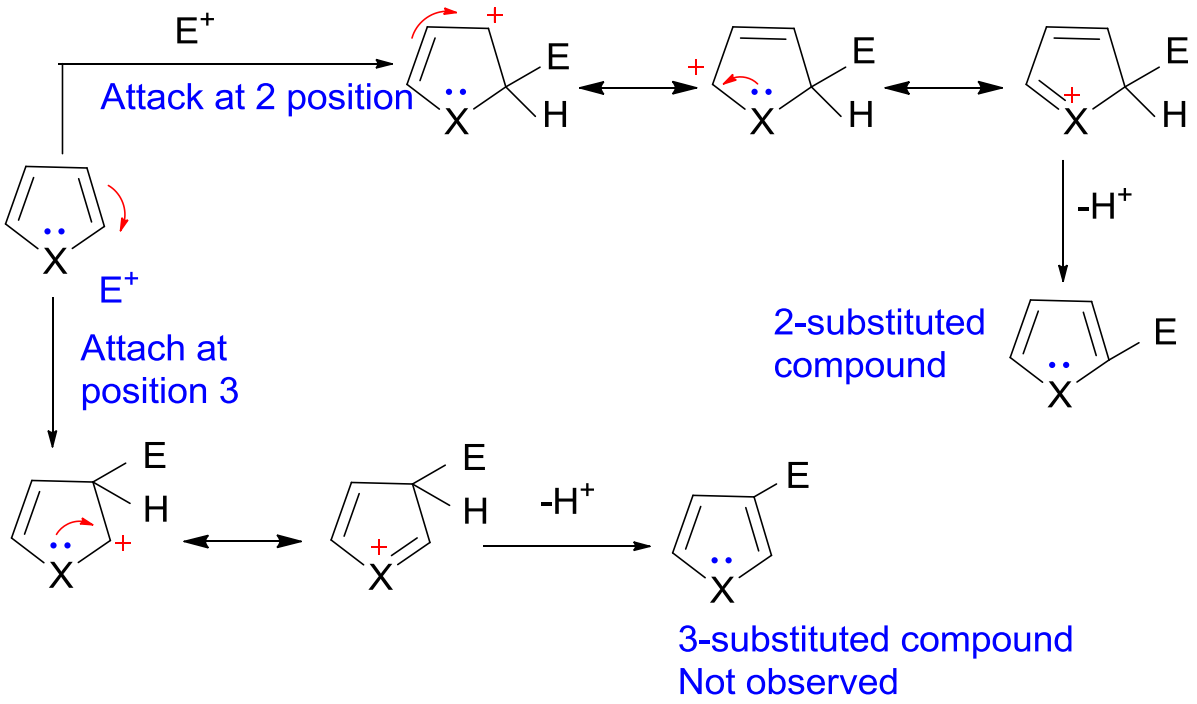


3. Hinsberg Synthesis: Condensation between 1,2 dicarbonyl and thiodiacetate in the presence of a base gives thiophene 2,5 diacids(-diketone)

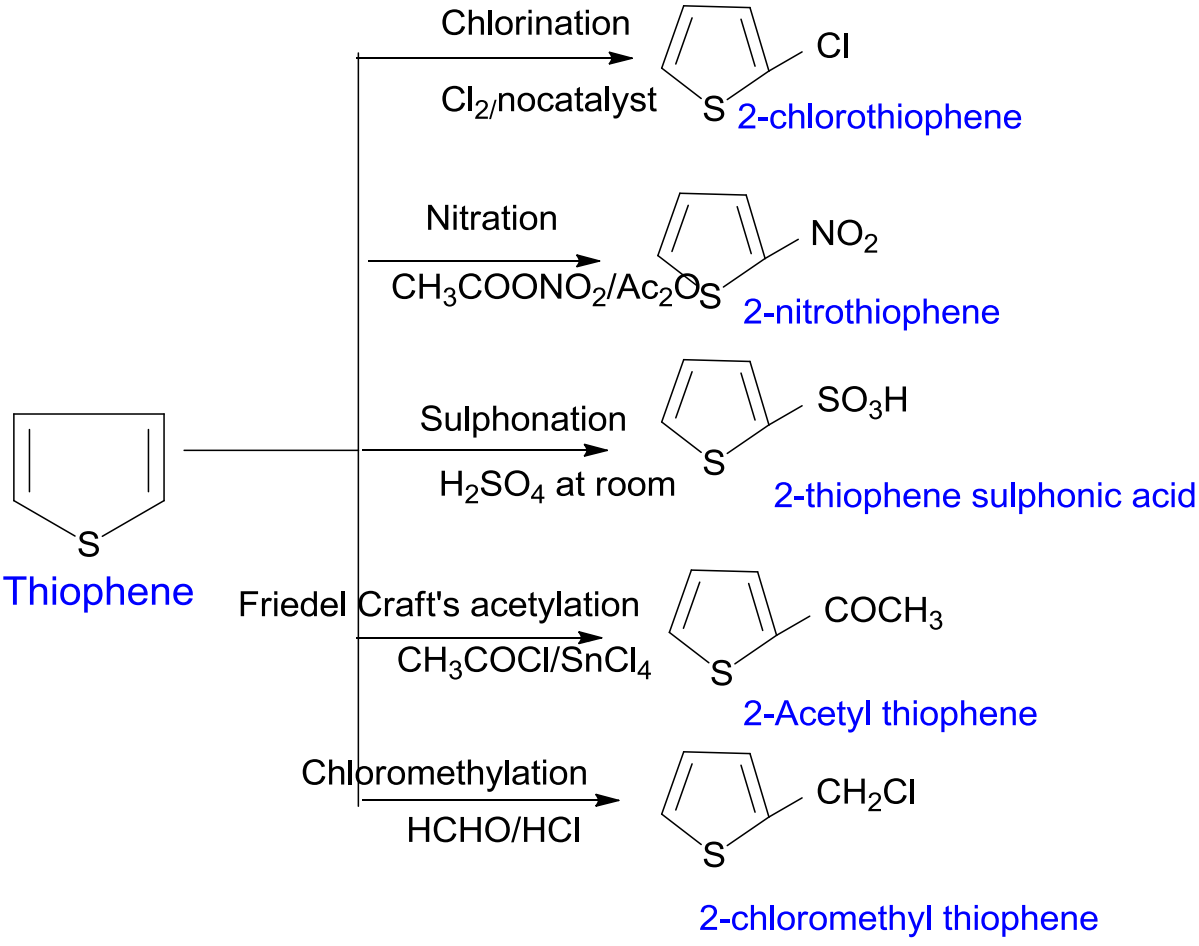


4.4.2 Electrophilic substitution:

Electrophilic substitution in 5 membered ring occurs at C2 and not at C3. The resonance structures are more at C2 than at C3.



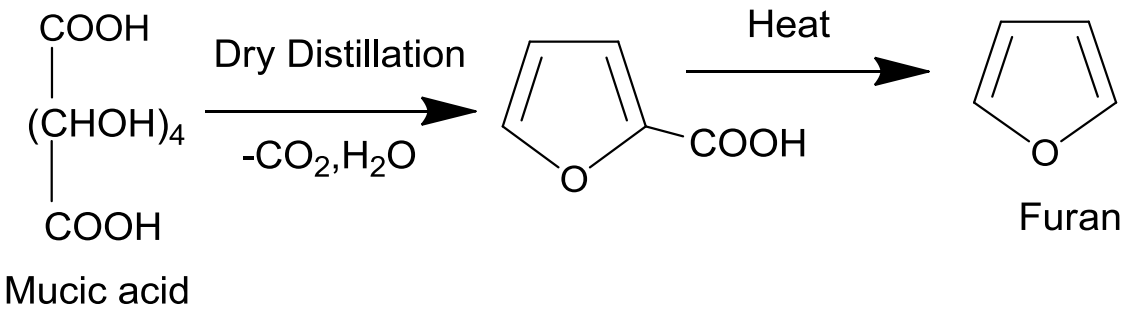
Electrophilic substitution reactions of thiophene:



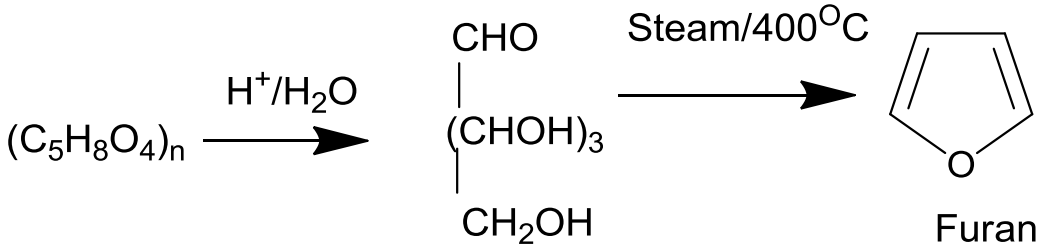
4.5 FURAN

4.5.1 Synthesis:

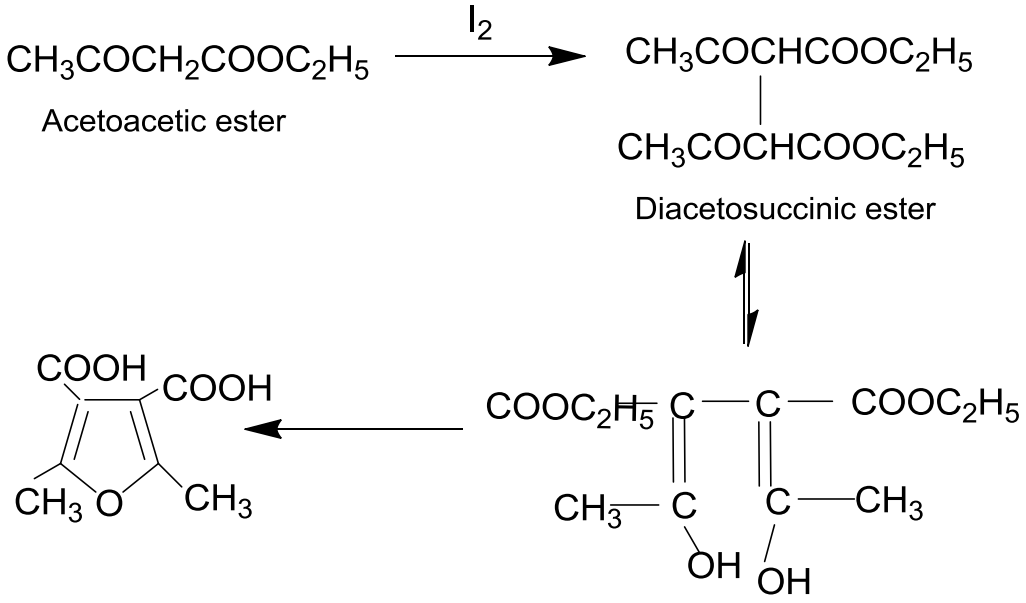
1)Dry distillation of mucic acid followed by decarboxylation



2) Pentosan are hydrolysed to xylose followed by dehydration and cyclization to furfural, steam distillation to furan.

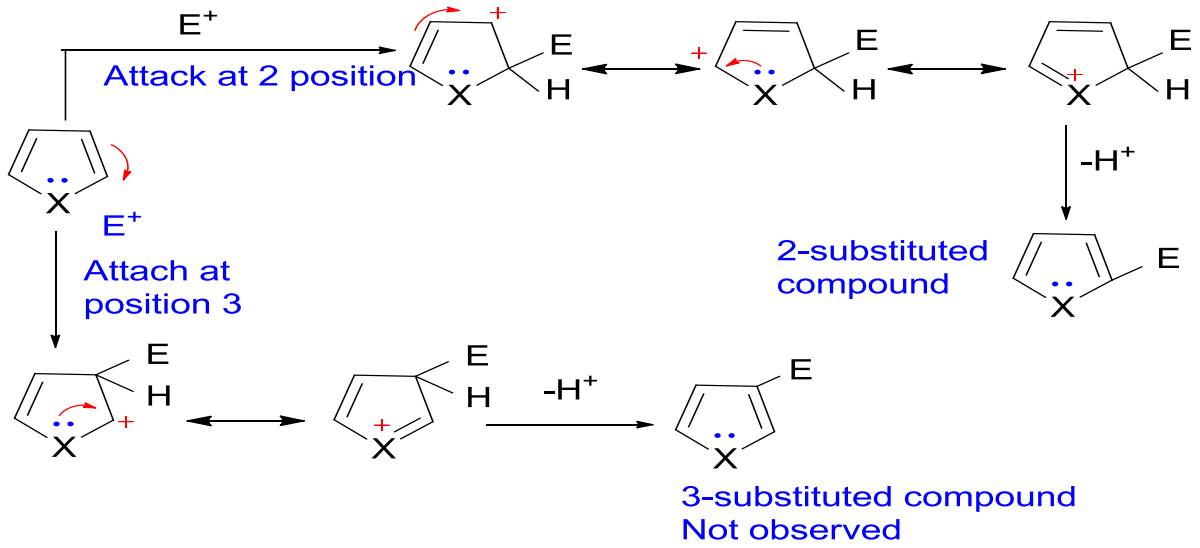


3) Furan derivatives is obtained from acetoacetic ester.

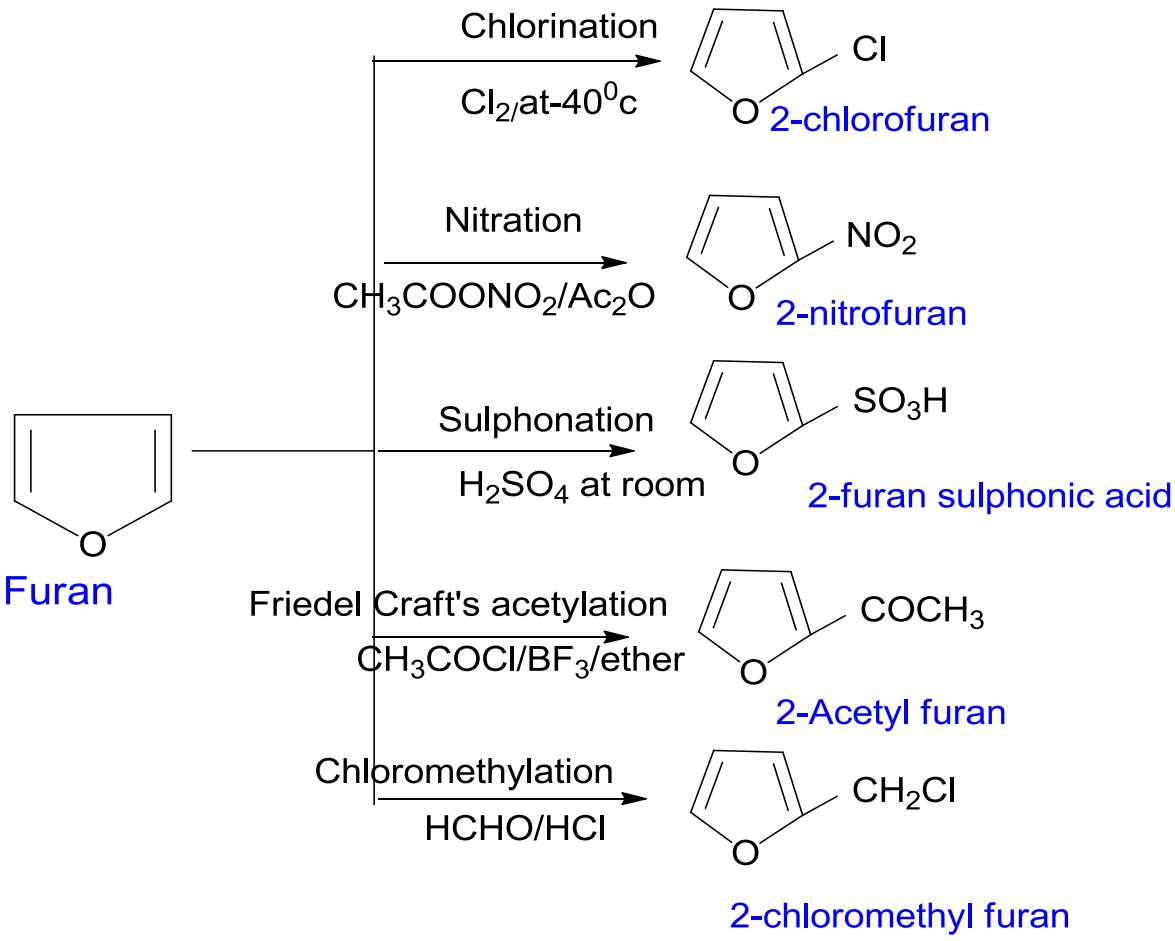


4.5.2 Electrophilic substitution

Electrophilic substitution in 5 membered ring occurs at C2 and not at C3. The resonance structures are more at C2 than at C3.



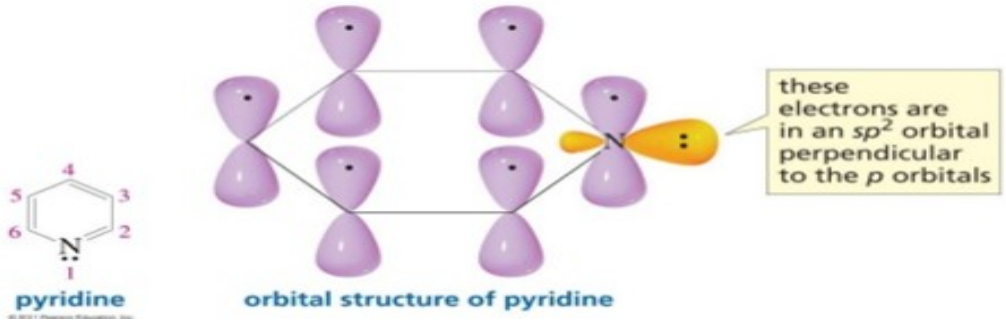
Electrophilic substitution at position 2.



4.6 SIX-MEMBERED RING (PYRIDINE)

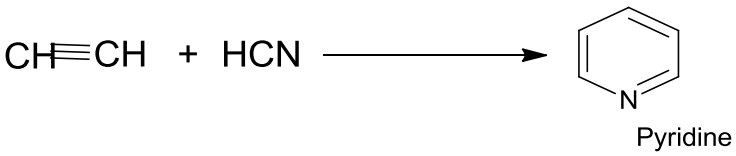
Pyridine is a 6 membered ring , with one heteroatom, Nitrogen.Aromatic in nature.

Carbon are in sp^2 hybridisation, planar in nature.

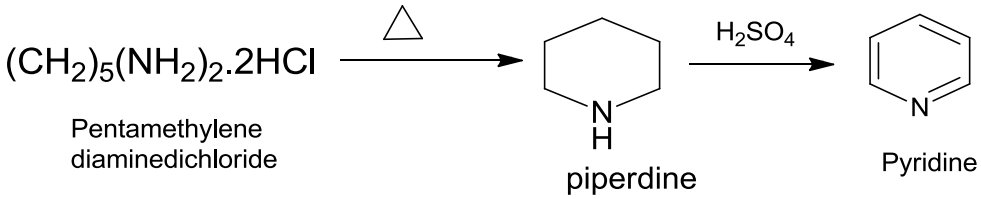


4.6.1 Synthesis:

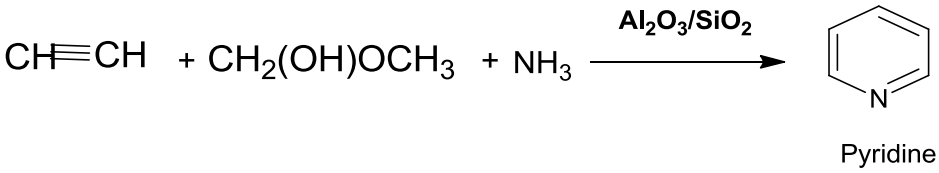
1) Passing acetylene and HCN through a red hot tube.



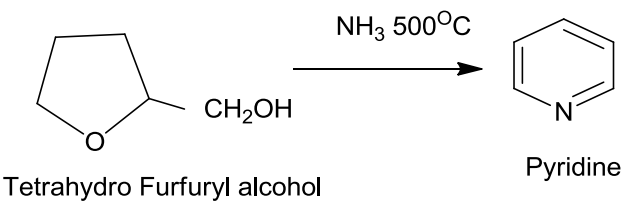
2) Heating pentamethylene diamine hydrochloride followed by heating with conc.H_2SO_4



3) Passing acetylene, HCHO and NH_3 over alumina catalyst.



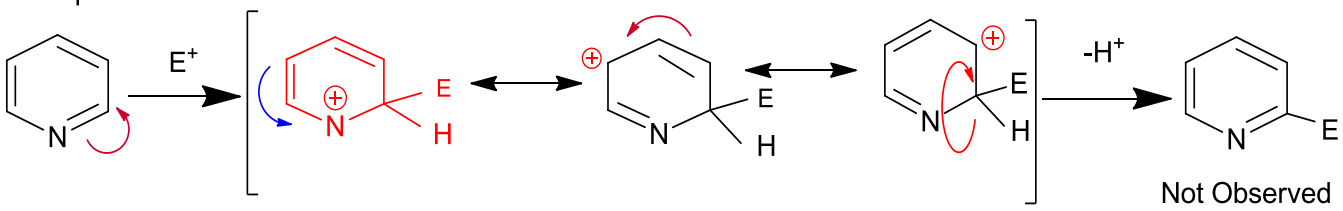
4) Heating tetrahydrofurfuryl alcohol with NH_3



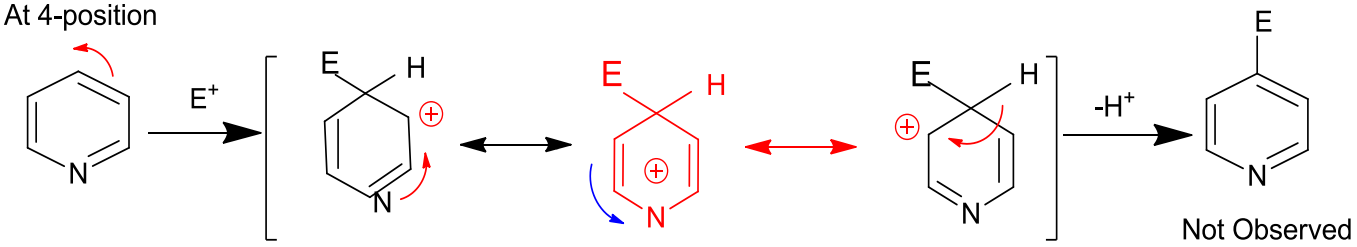
4.6.2 Electrophilic substitution-Π electron excess reaction

The attack of 2 and 4 position involves the formation of positive charge on the highly electronegative nitrogen atom. The cations from these structures are highly unstable. Hence, substitution occurs only at 3-position.

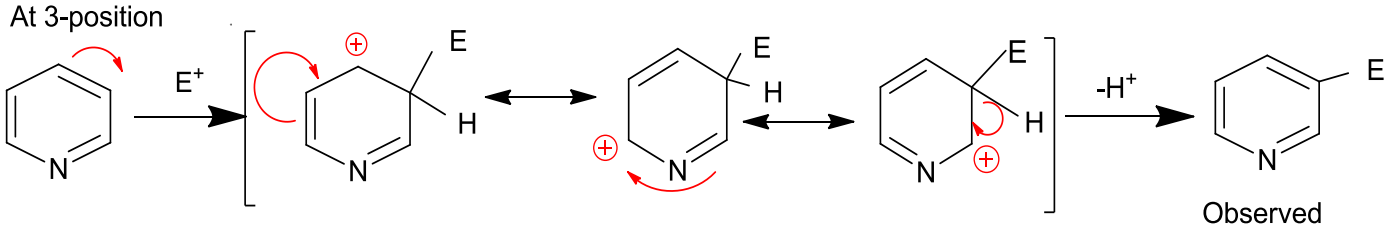
At 2-position



At 4-position



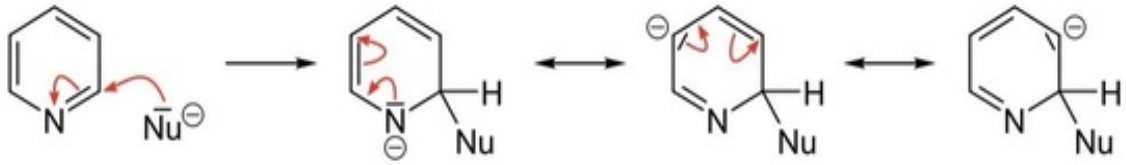
At 3-position



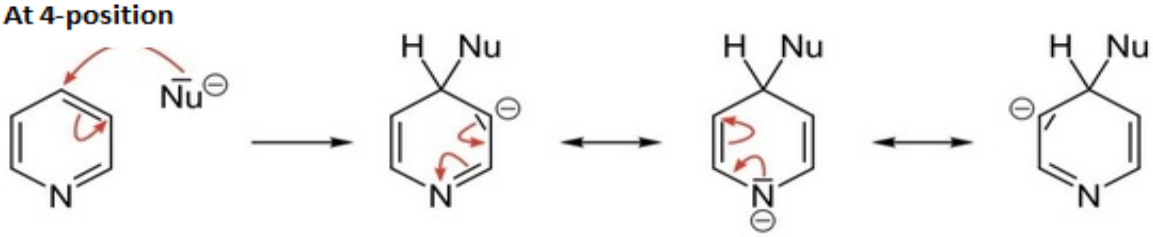
4.6.3 Nucleophilic substitution-Π electron deficient

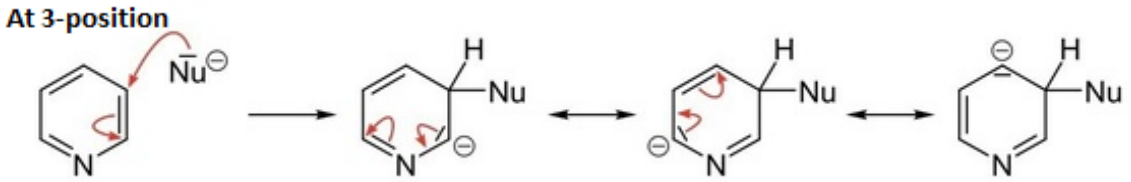
The electron density at position 2 and 4 is less.The negative charge on nitrogen is more resonance stabilized. Nucleophilic substitution occurs at 2 and 4 position.

At 2-position

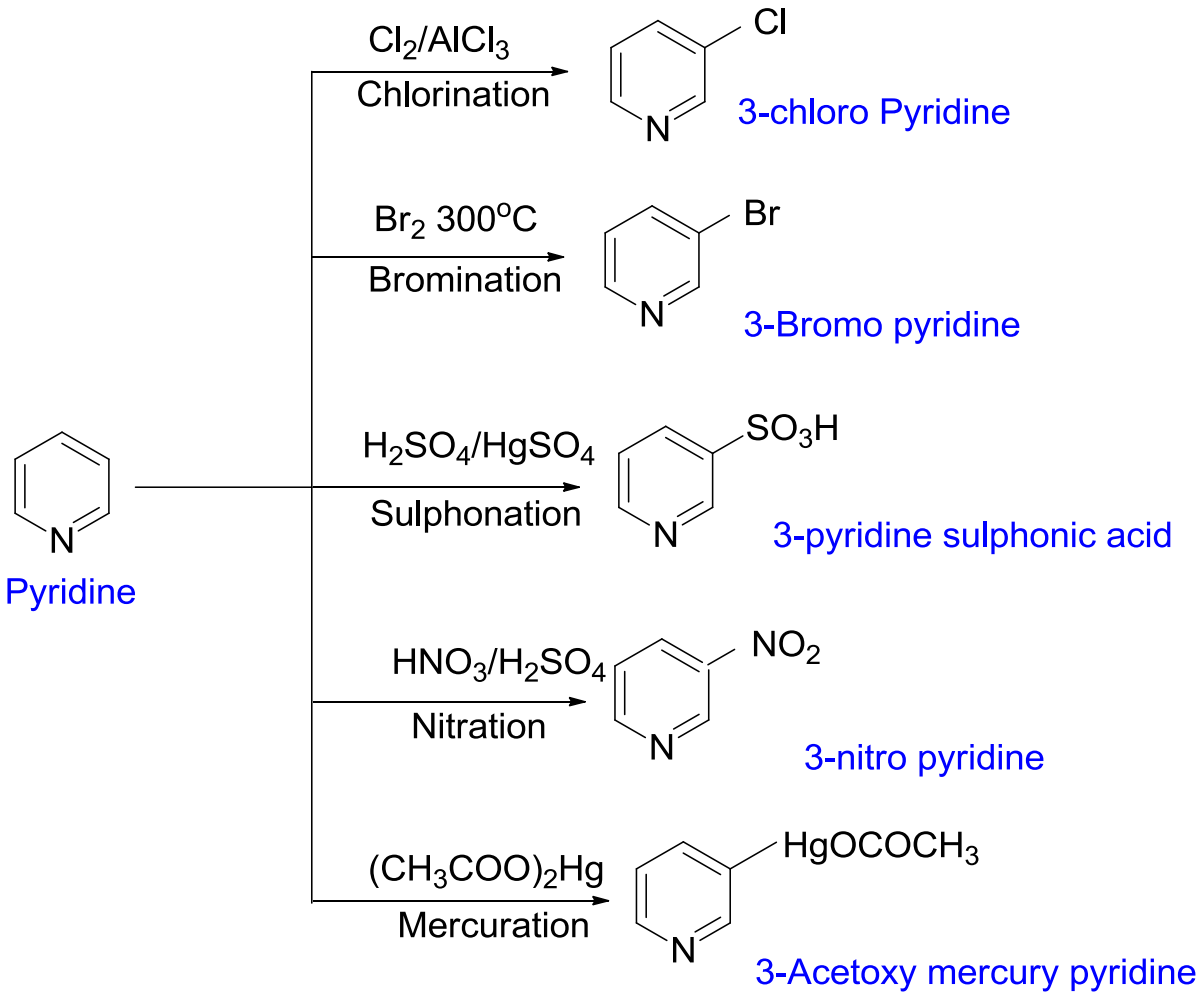


At 4-position

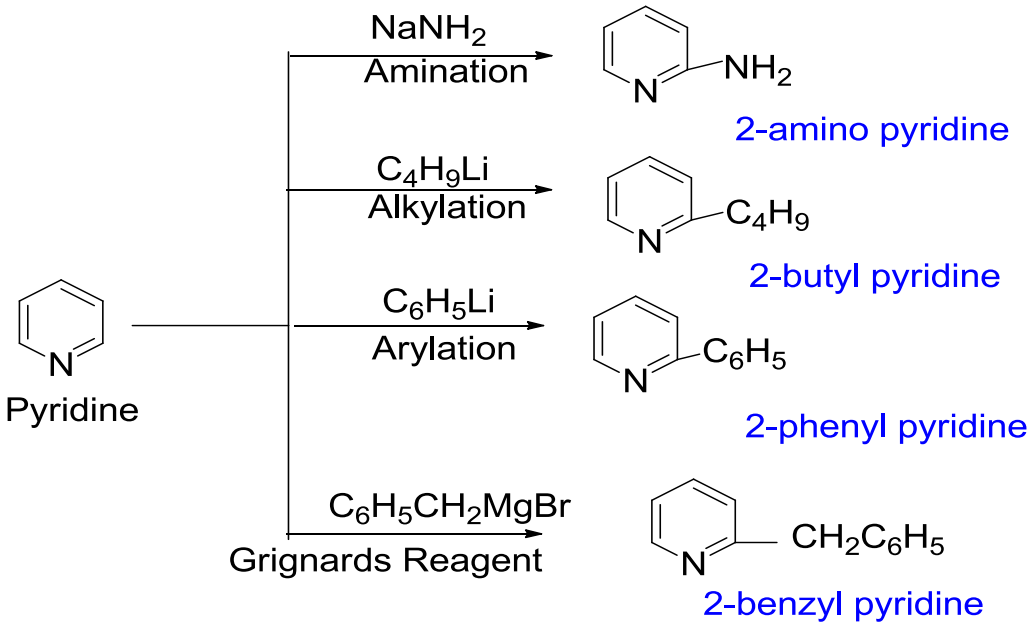




Electrophilic substitution-Π electron excess reaction

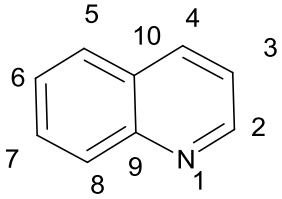


Nucleophilic substitution-Π electron deficient

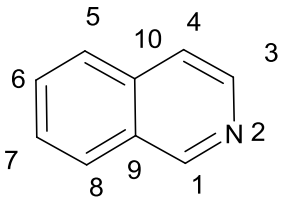


2.7 QUINOLINE AND ISOQUINOLINE

Quinoline: Isoquinoline:



Benzo[b]pyridine



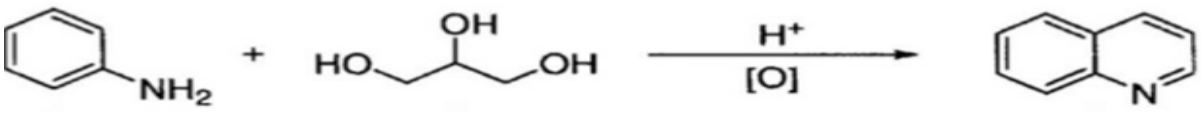
Benzo[c]pyridine

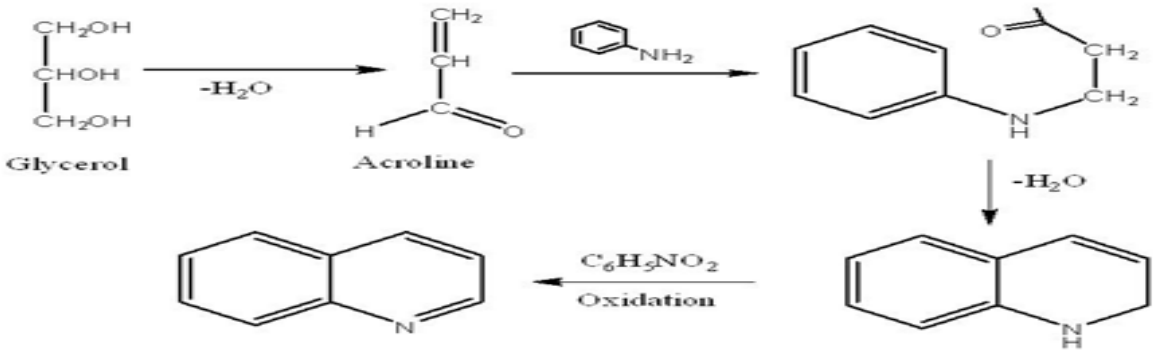
Aromatic; sp^2 hydridization; 10e-s; Planar Basic in nature.

4.7.1 Synthesis of quinoline

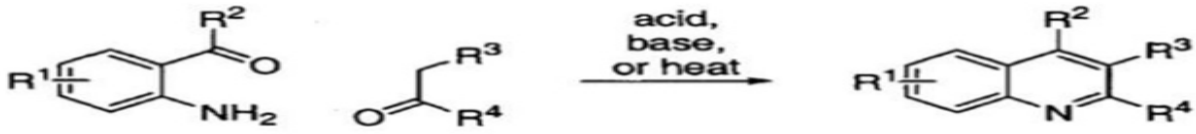
1) Skraup synthesis:

Reaction of aniline and glycerol in the presence of acid and oxidant.

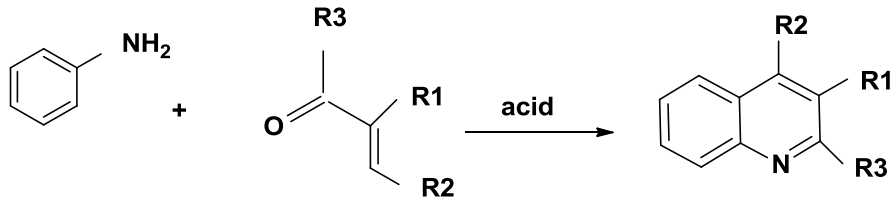




2) Friedlander's synthesis: Condensation of O-aminobenzaldehyde and aldehyde in the presence of alkali.

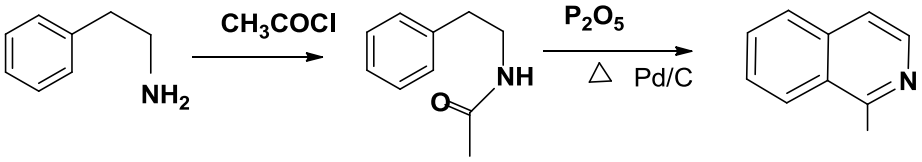


3) Doebner-Miller synthesis: Condensation of aniline with α,β- unsaturated carbonyl compound.

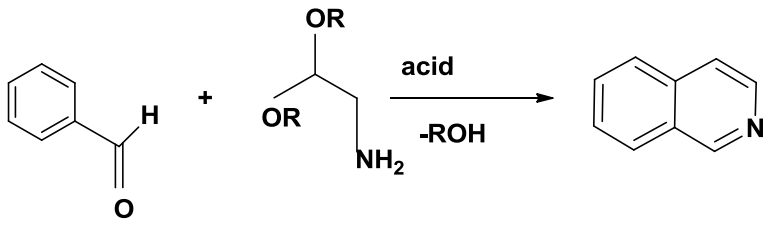


4.7.2 Synthesis of Isoquinoline

1) Bischler-Napieralski synthesis: Reaction of 2-aryl ethanamine with acylchloride followed by cyclization and reduction.

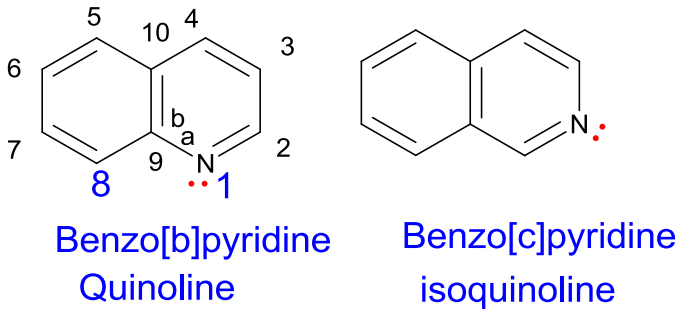


2) Pomeranz-Fritsch synthesis: Reaction of benzaldehyde with dialkoyethylamine.



4.7.3 Resonance structures

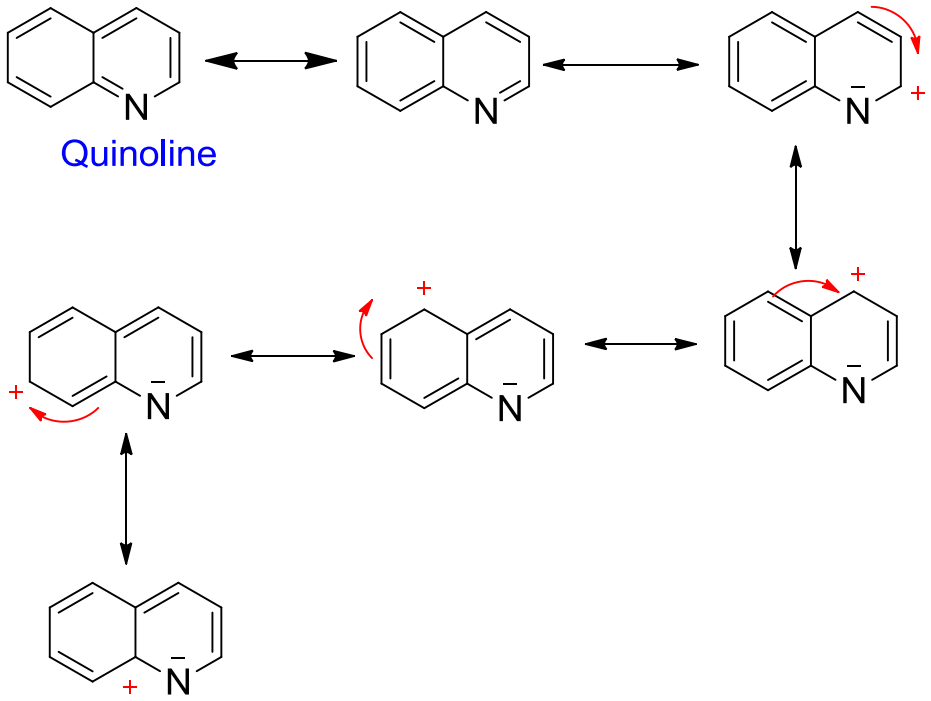
Quinoline and Isoquinoline contains a pyridine ring fused to a benzene ring.



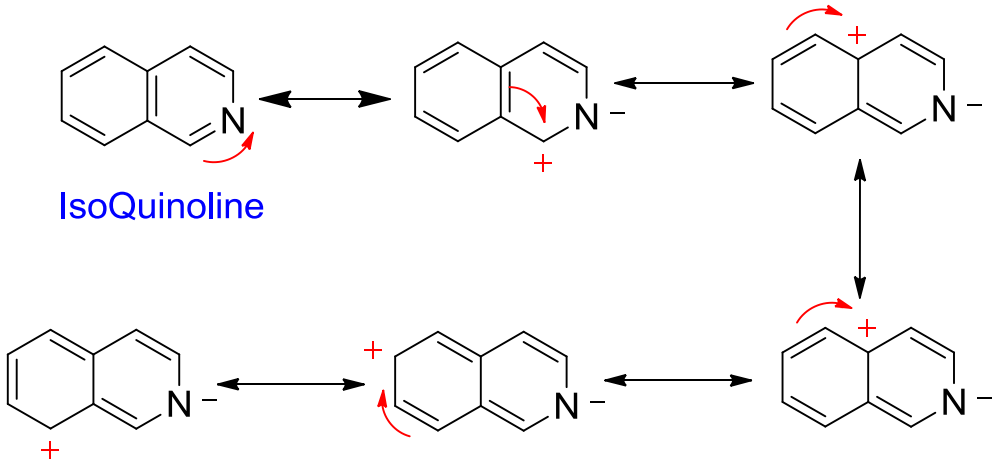
The Nitrogen has a deactivating effect on the ring towards electrophilic substitution.

Hence, it takes place less vigorously at position 5 and 8 in the benzene ring.Nitrogen lone pair is not released into the aromatic system. The nitrogen withdraws electrons makes it an Π electron deficient systems.

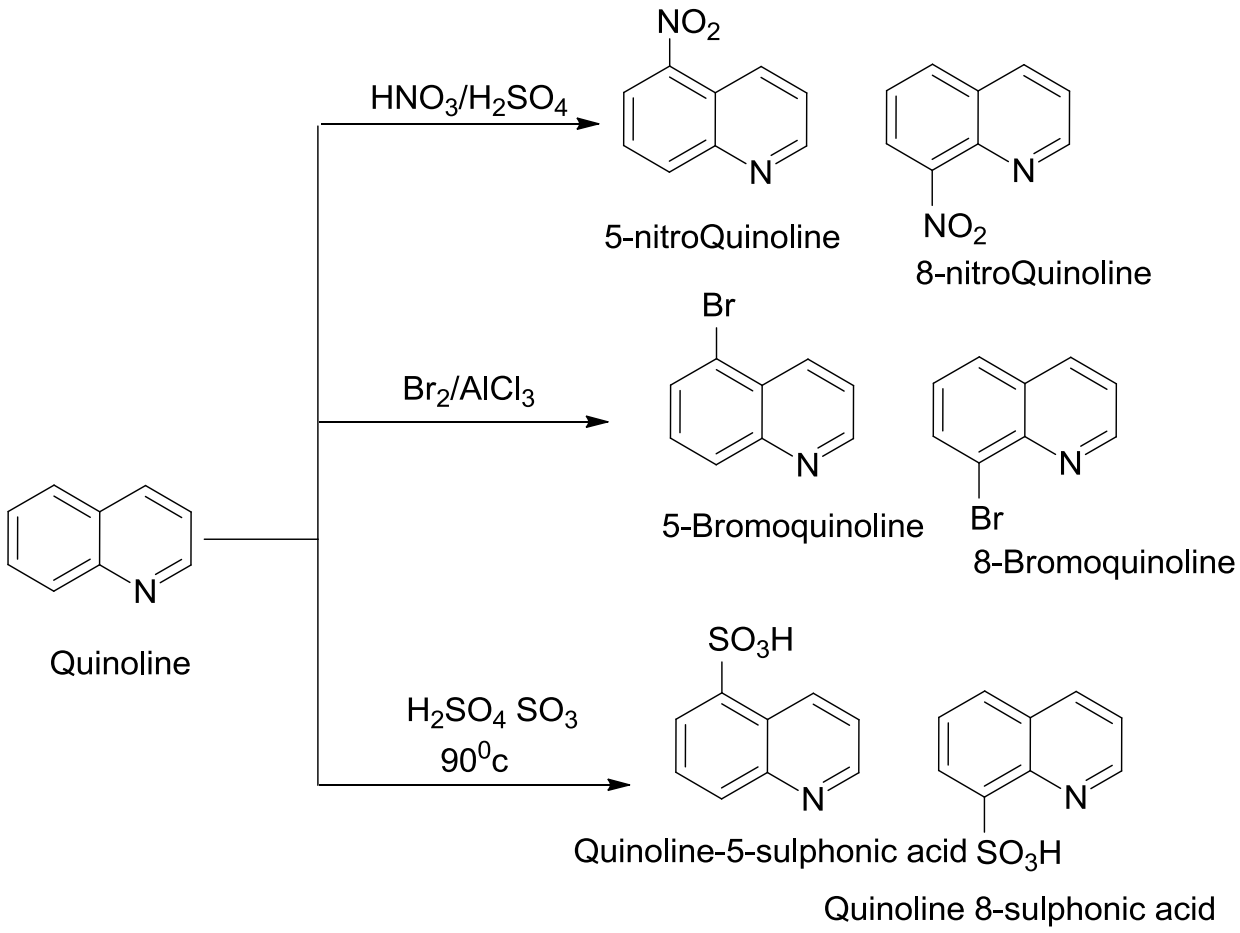
Quinoline:



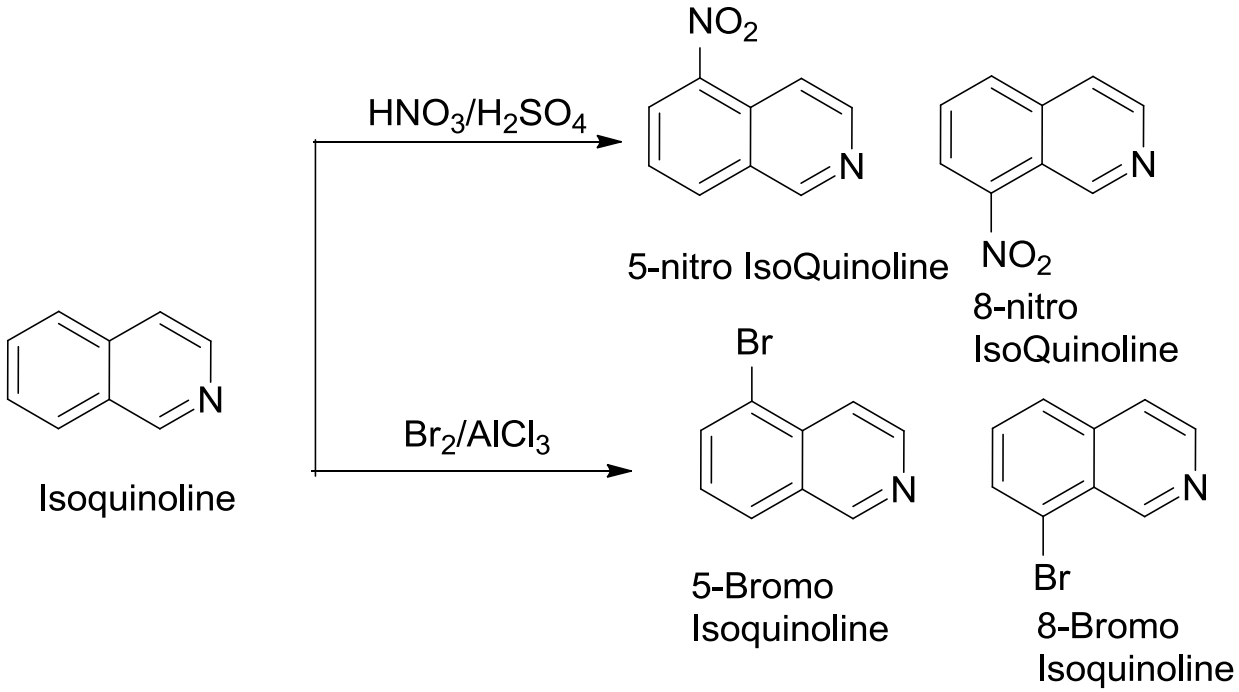
Isoquinoline



Electrophilic substitution at 5 and 8 position

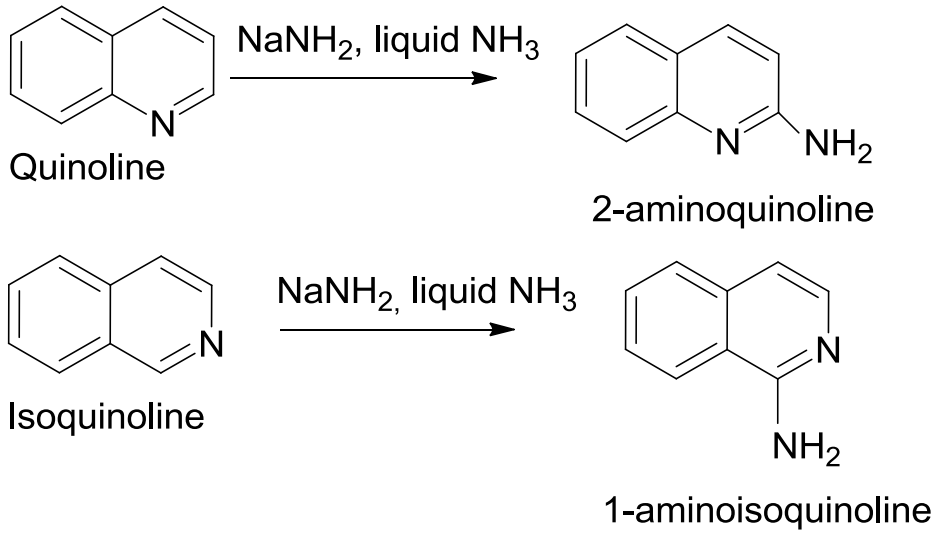


Isoquinoline



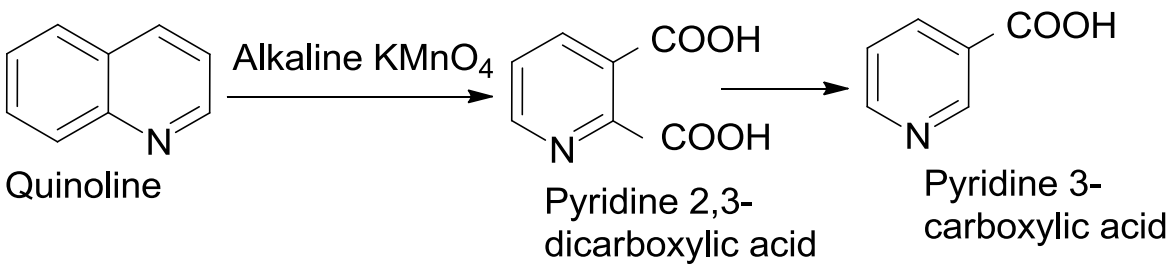
4.7.4 Nucleophilic Substitution

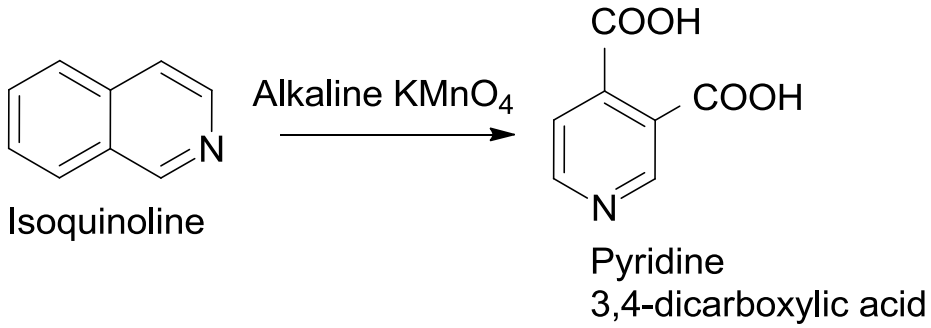
Quinoline and Isoquinoline undergoes facile nucleophilic substitution same as in pyridine. Quinoline gives 2-aminoquinoline while isoquinoline gives 1-aminoisoquinoline.



4.7.5 Oxidation

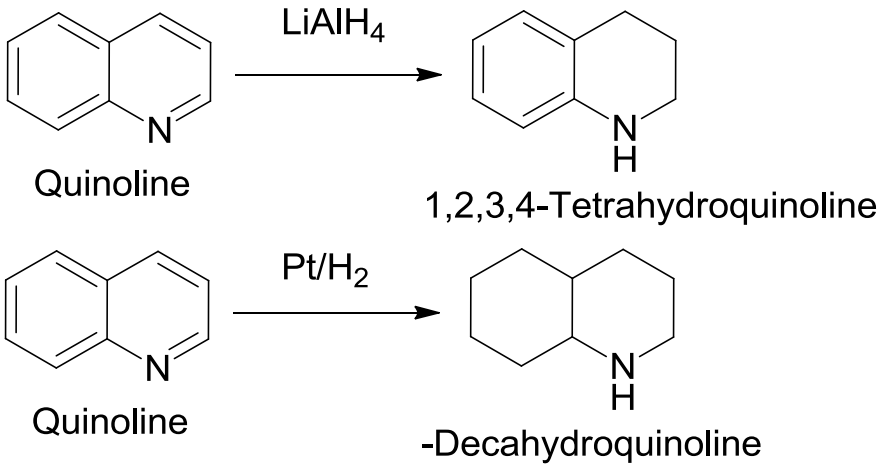
Quinoline and isoquinoline undergoes oxidative cleavage with alkaline $KMnO_4$ to form pyridine 2,3 and pyridine 3,4-dicarboxylic acids.



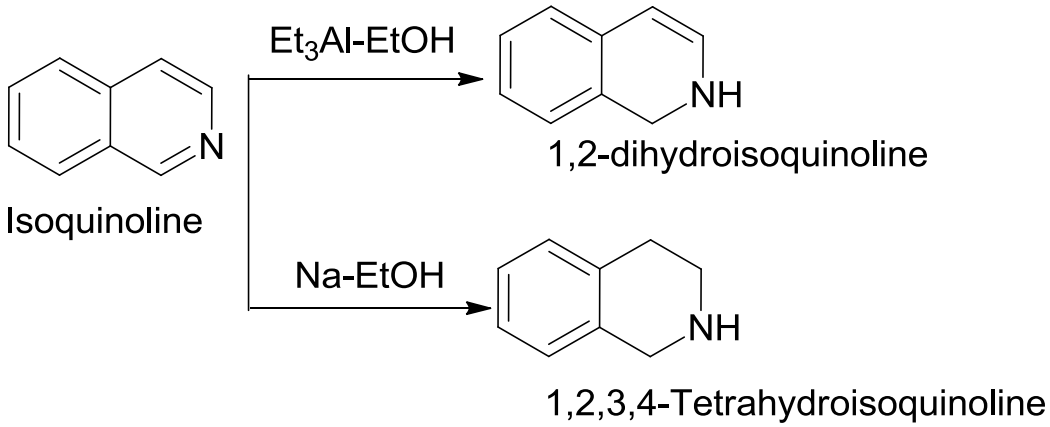


4.7.6 Reduction

Quinoline on reduction give 1,2,3,4-tetrahydro quinoline.



Isoquinoline on reduction gives 1,2-dihydo and 1,2,3,4-tetrahydro isoquinoline.



TEXT / REFERENCE BOOKS

1. Finar I. L., Organic Chemistry, 6th Edition, Pearson Education Asia, 2004.
2. S.V.Bhat,B.A. Nagasampagi, M.Sivakumar., Chemistry of Natural products, Springer Publications 2005.
3. O.P. Agarwal., Organic Chemistry-Natural Products Vol.I and II, Goel Publishing House, 1980.
4. G.R. Chatwal., Organic Chemistry-Vol II- Himalayan Books, 1985.
5. T.L. Gilchrist., Heterocylclic Chemistry, Longman Scientific and Technology, 2005.

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SCHOOL OF SCIENCE AND HUMANITIES

DEPARTMENT OF CHEMISTRY

UNIT – V - Antibiotics and Vitamins – SCYA7201

UNIT-5

ANTIBIOTICS AND VITAMINS

5.0 INTRODUCTION

ANTIBIOTICS is defined as a chemical substance produced by or derived from living cells which are capable in small concentration of inhibiting life processes or even destroying the microorganisms.

5.1 CHARACTERISTIC OF ANTIBIOTICS

1) It must have been aproduct of metabolism although it can be synthesized.
2) It must be effective at low concentrations.
3) It must antagonise the growth and or survival of one or more species of the microorganisms.
4) It must be effective against a pathogen and doesn't cause any significant toxic effects.
5) Its stability must be high so that it can be isolated and proceeded.
6) It must be stored for a long time period without any appreciable loss in activity.
7) It must exhibits proper concentration level between successive dosages.
8) It must be completely eliminated from the body soon after its administration has been stopped.

5.2 CLASSIFICATION OF ANTIBIOTICS:

(I) First classification: It is divided into two types

a) Broad spectrum antibiotics:These are used as curative agents against several ailments Eg: Penicillin, Chloramphenicol.

b) Narrow spectrum antibiotics:These are highly specific in action Eg: Bactivacin, Nystatin.

(II) Second Classification:

It is based on the type of bacteria the antibiotic can destroy as Gram-Positive antibiotic and Gram-Negative antibiotic.

In Gram-staining Method, The bacterial smear is treated with a solution of crystal violet followed by treatment with iodine solution. The smear is then treated with alcohol. The bacteria which retain the colour of crystal violet is known as Gram-positive bacteria. The bacteria which loses the colour of crystal violet and get stained by safranin and appears red is known as Gram-Negative bacteria.

Gram-Positive bacteria: Pneumococus, Streptococcus.

Gram-Negative bacteria: E.Coli, Typhoid Bacillus, V.Cholerae.

(III)Third Classification:

It is based on the chemical structure

a) Penicillins: Derived from aminoacids- Eg: Penicillins, Cephalosporins.

b) Aminoglycosides: They contains a sugar molecule glycosidically linked to aminoacid

Eg: Streptomycin, Neomycin

c) Chloramphenicol and its analogues.

d) Tetracycline: They contain four, six-membered fused ring system Eg: Tetracycline, Aureomycein.

e) Macrolides: They contain a lactone ring. Eg: Erythomycin.

f) Polypeptides: They contain a polypeptide chain Eg: Tyrothyricin.

g) Polyenes: They have a conjugated polyenes Eg: Amphotericin.

5.4 CHLORAMPHENICOL (CHLOROMYCETIN)

It is abroad spectrum antibiotic isolated from the species, Streptomyces Venezuelae.

5.4.1 Production and Isolation of Chloramphenicol:

The culture of Streptomyces sp is added to the culture medium in a 50 Gallon tank. the culture tank contains maltose, peptone, sodium carbonate, NaCl and antifoaming agents. In the tank, the culture is allowed to grow for 24 hrs followed by growth in aseptic conditions for 72 hrs. The medium is concentrated and the culture is extracted with ethyl acetate and removed by distillation. The culture is concentrated with sulphuric acid, alkali where crystals of chloramphenicol is obtained. It is recrystallized by ethylene dichloride or water.

5.4.2 Properties of Chloramphenicol

It occurs as a fine powder or greyish white crystals.

It has a bitter taste with sharp melting point of 150^{o}c.

It is soluble in alcohol.

5.4.3 Constitution of Chloramphenicol

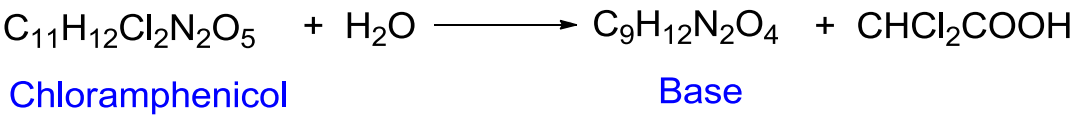
1)Molecular formula of chloramphenicol is $C_{11}H_{12}Cl_2N_2O_5$.

2) Presence of Nitro group: Reduction of chloramphenicol with Sn/dil.HCl followed by diazotisation and reaction with β-naphthol gives an orange-red dye. The presence of nitro group is further confirmed by reducing catalytically the compound with Pd to form a product whose absorption spectrum is similar to p-toluidine. Hence, chloramphenicol is a p-nitrobenzene substituted compound and its chlorine is present in the side chain.

3) Absence of free amino or carbonyl groups: It doesn't give colour reactions with Fehling's solution/Tollen's reagent.

4) Presence of two hydroxyl group: On acetylation witha cetic anhydride/ pyridine chloramphenicol yields diacetyl derivative.

5) Hydrolysis: On hydrolysis with either acids/alkalies, chloramphenicol yields dichloroacetic acid and an optically active base.



Structure of a Base:

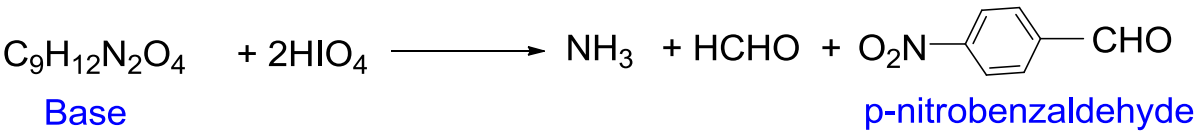
1) It is an optically active base and its molecular formula is $C_9H_{12}N_2O_4$.

2) Presence of amino group: The base undergoes diazotisation and forms a orange-red dye with β-naphthol.

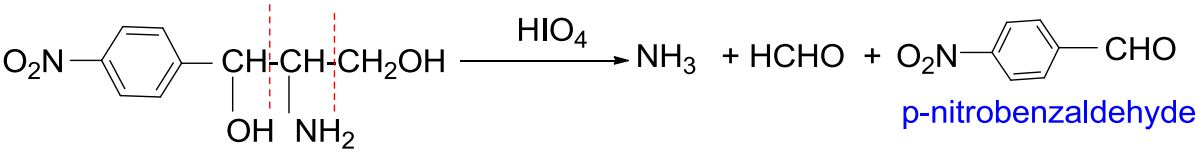
3) When acetylated with acetic anhydride/pyridine it forms a triacetyl derivative indicating the presence of two hydroxyl group and an amino group. Further, the base doesn't give colour reactions with neutral $FeCl_3$ indicating that hydroxyl groups are alcoholic and not phenolic.

4) When treated with methyl dichloroacetate it yields dichloroacetamide.

5) When oxidised with periodic acid, it consumes two equivalents of acid to yield p-nitro benzaldehyde, HCHO and ammonia. The reactiobn reveal that it contains a propyl group, present at para position to nitro group with an amino group on the second carbon atom.

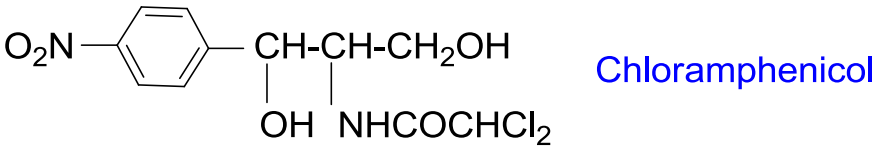


6) The formation of products shows that the base is 2-amino1-p-nitrophenyl propane 1,3-diol.

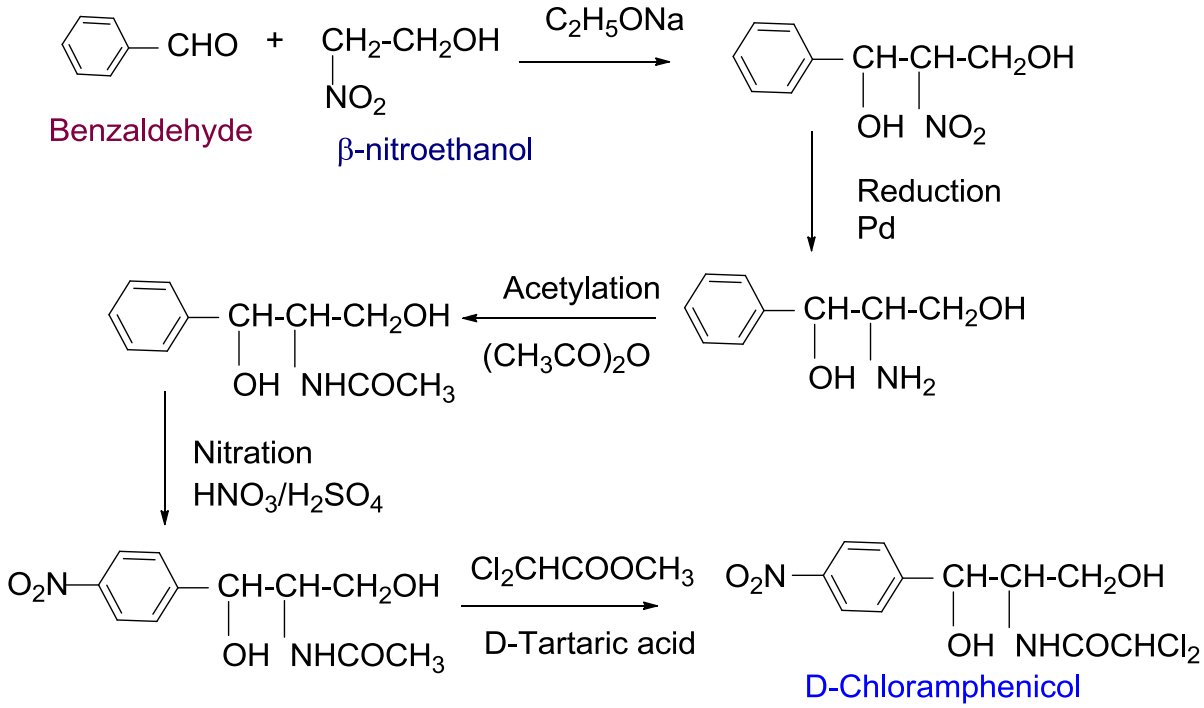


Structure of Chloaramphenicol:

As chloramphenicol does not react with periodic acid reveals that the amino group is blocked by chloroacetic acid. Hence, the structure of chloramphenicol is D(-)Threo dichloro acetamino 1-p-nitrophenyl propane 1,3-diol.

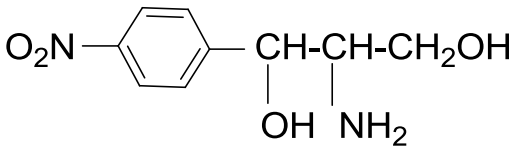


Synthesis of Chloramphenicol



5.4.4 Structure and Activity:

The base 2-amino 1-phenyl propane 1,3 diol nucleus is essential for its antibiotic activity.



Nitro group in the phenyl ring of chloramphenicol is replaced by -CN, -$CONH_2$, -COOH, -NH_2,-OH, the molecule exhibits antibacterial activity.

Heterocyclic ring in the place of phenyl ring in the parent antibiotic showed decreased activity.

The dichloroacetyl group is essential for antibacterial action, replacing the chloro group by dibromo has only 80% bacterial activity.

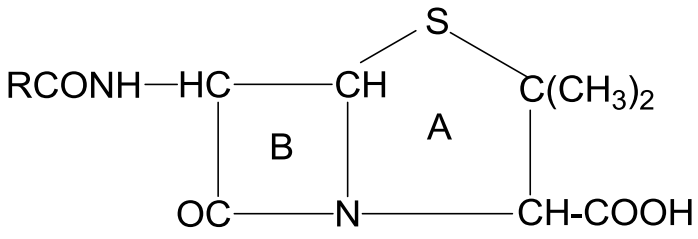
Only D-Threo isomers exhibits antibacterial activity.

5.4.5 Uses:

Used to combat pneumonia, enteric fever, dysentery, whooping cough, Haemophilus influenza. It destroys the bacteria by interfering with protein synthesis.

5.5 PENICILLINS

Penicillin is given to the mixture of compound with molecular formula $C_9H_{11}N_2O_4SR$ and differ only in the nature of 'R'. The general structure of Penicillin is



Penicillin

The thiazolidine ring 'A' is attached to β-lactum ring (B) through the side chain R-CO. Any modification either in thiazolidine ring or β-lactum decreases its anti-bacterial activity.

5.5.2 Production of Penicillin:

Submerged Culture Method: In this process, Penicillium Chrysogenum is used for the culture and the medium contains corn steep, $CaCO_3$, phenyl acetic acid, dipotassium phosphate. The culture and the medium are taken in large tanks and are agitated by sterile air by maintaining 24^oC for 2-3 days. The mould grows from the bulk of the liquid as globular pellicles with mycelium. The mycelium is then filtered and extracted with alcohol at acidic pH. It is then freeze-dried in vacuum and is administered by injection.

5.5.3 Constitution of Penicillin:

1)Molecular formula of Penicillin is $C_9H_{11}N_2O_4SR$

2) Presence of carboxylic acid: Penicillin forms mono-sodium salt indicating the presence of one carboxylic group.

3) Presence of amino or thiol group: Penicillin forms a dye with naphthol and salt with mercuric chloride indicating the presence of free amino group or thiol group.

4) Hydrolysis: Penicillin on hydrolysis with either dil acids or alkalis, it gives an equivalent amount of an amine, Pencillamine and an aldehyde, Penillolaldehyde with a loss of CO_2.

$$\underset{\text{Penicillin}}{C_9H_{11}N_2O_4SR} + 2H_2O \longrightarrow \underset{\text{Penicillamine}}{C_5H_{11}NO_2S} + \underset{\text{Penillolaldehyde}}{C_3H_4NO_2R} + CO_2$$

a) Structure of Penicillamine:

1)Molecular formula of Penicillamine is $C_5H_{11}NO_2S$.

2) Penicillamine gives blue colouration with neutral $FeCl_3$ and colour reaction with sodium nitroprusside indicating the presence of thiol group (-SH).

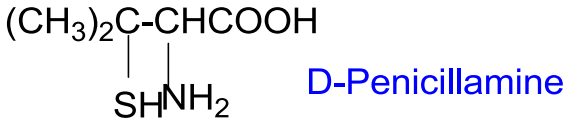
3) Electrometric Titration reveals that penicillin has three pKa Value of 1.8, 7.9 and 10.5 which corresponds to -COOH, -NH_2 and -SH groups respectively. The presence of these groups indicates that Pencillamine is a substituted cysteine.

4) D-Penicillamine reacts with acetone to form isopropylidene derivative (contains no free amino or thiol group) and is reconverted into Penicillamine on hydrolysis. these reactions suggests that the amino group and thiol group are present on the adjacent carbon atom.

5) Penicillamine on treatment with bromine water yields sulphonic acid, a characteristic reaction of thiol group.

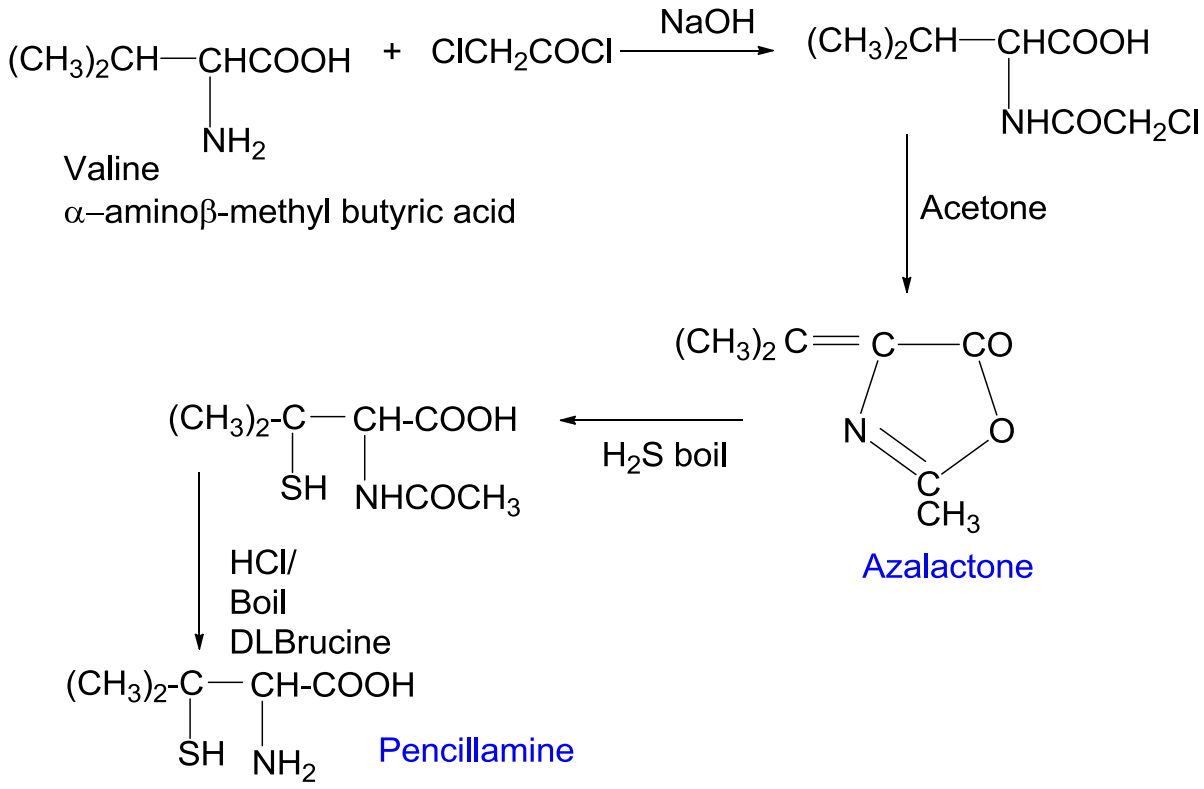
6) Kuhn-Roth Method: D-penicillamine give avery low value of 0.2 moles indicates that the molecule contains a isopropyl group and not a methyl end.

Thus, the Structure of D-Penicillamine is



D-Penicillamine

Synthesis of D-Penicillamine:



Valine
α–aminoβ-methyl butyric acid

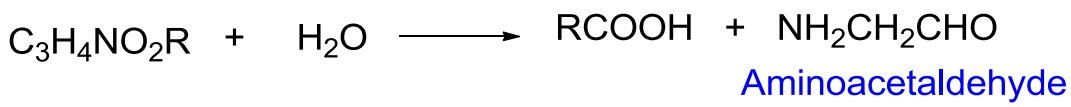
Azalactone

Pencillamine

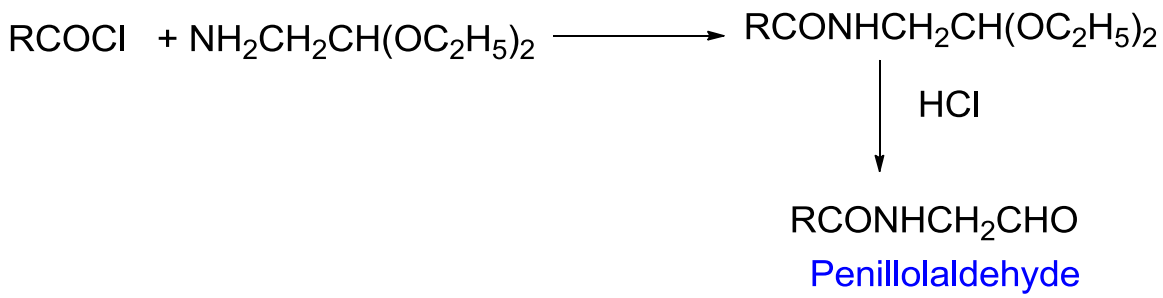
b) Structure of Penillolaldehyde:

1) Molecular formula of Penillolaldehyde is C3H4NO2R.

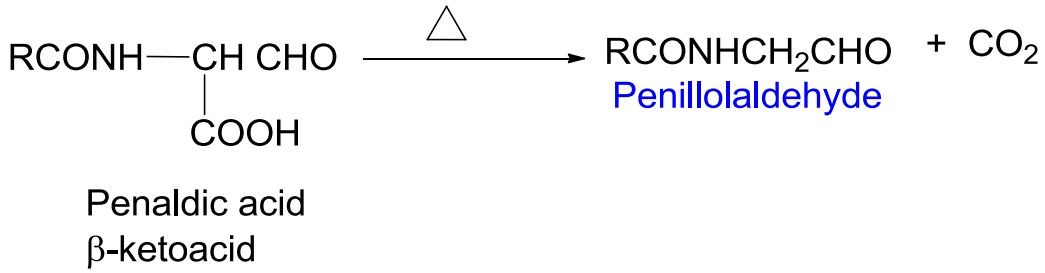
2) On hydrolysis, Penillolaldehydes yields substituted acetic acids and amino acetaldehyde. Hence, Penillolaldehyde are acyl derivatives of aminoacetaldehyde.



3) Penillolaldehyde is prepared from acid chloride and aminoacetal.



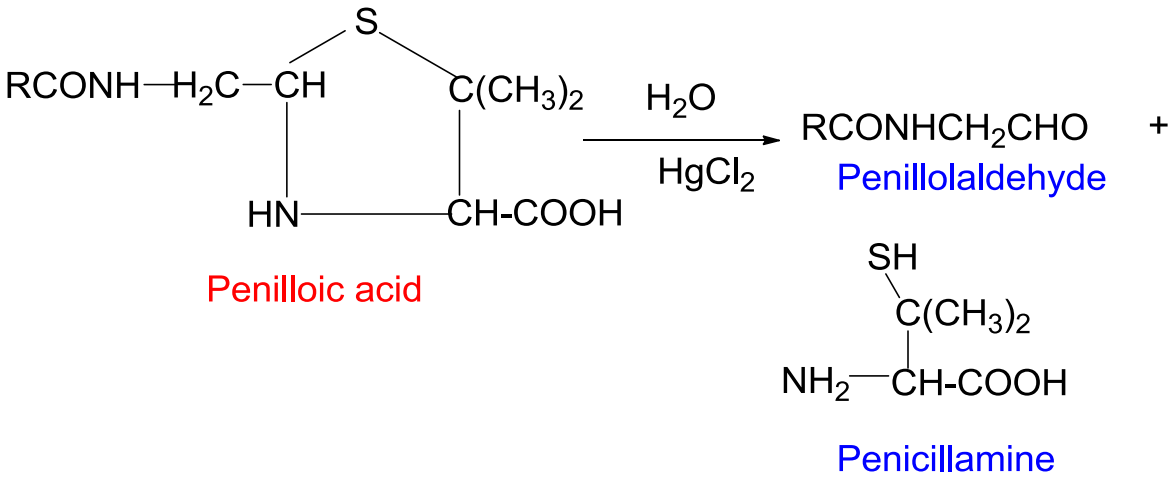
4) Penicillin on hydrolysis gives penicillamine, CO_2, penillolaldehyde. The formation of CO2 indicates that an unstable acid is formed, β-ketoacid which undergoes decarboxylation to yield CO_2. The β-ketoacid is Penillolaldehyde carboxylic acid.



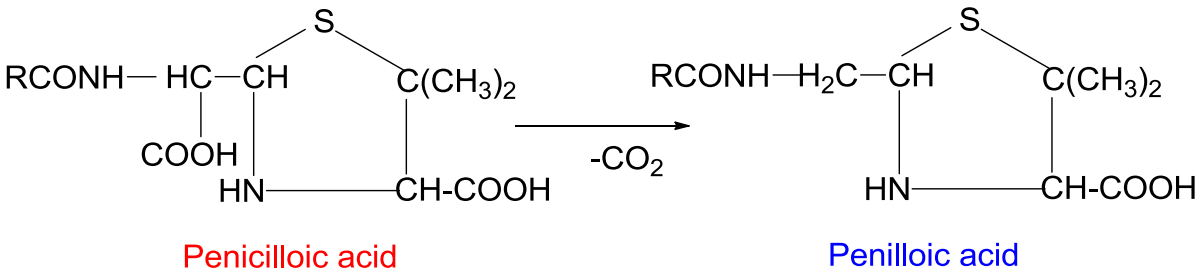
d) Mode of linking of Penicillamine and Penilloaldehyde:

When Penicillin is hydrolysed with dil. alkali or by enzyme Penicillinase it yields Penicilloic acid, a dicarboxylic acid which eliminates CO_2 to form mono carboxylic acid, Penilloic acid. In Penicilloic acid, one of the carboxylic acid is in β-position to the electron attracting group (thio).

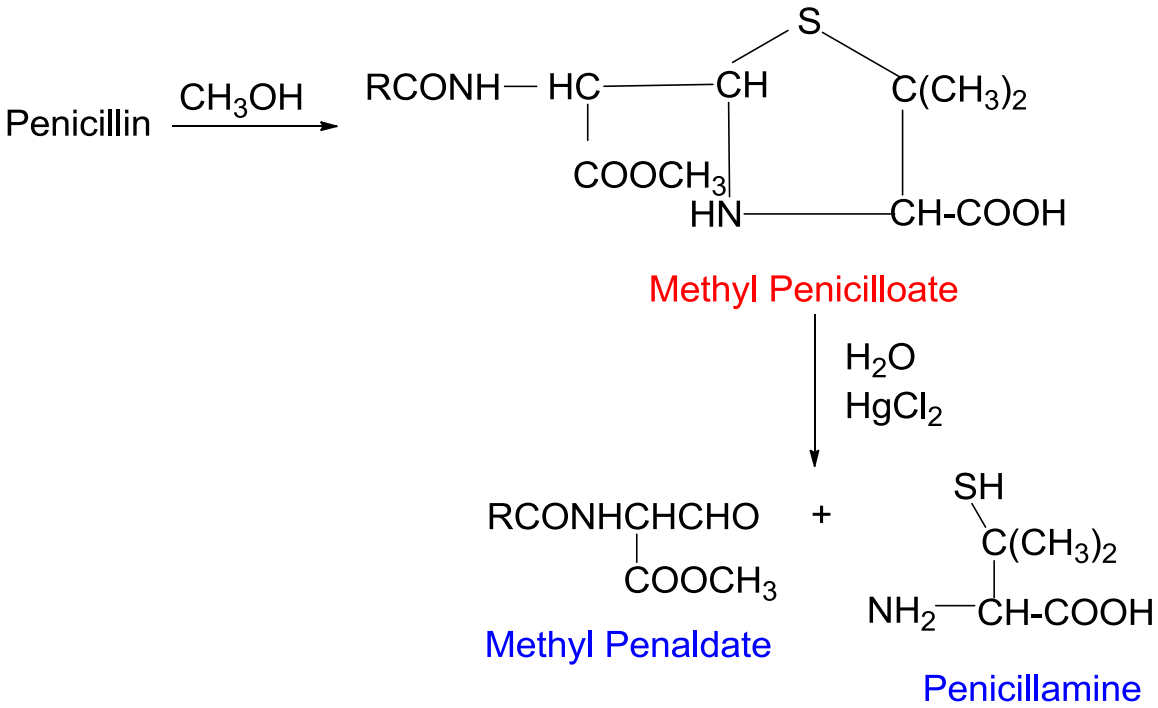
Penilloic acid on hydrolysis with mercuric chloride yields Penicillamine and Penilloaldehyde. The reaction is a characteristic of thizolidine ring. Both Penilloic acid and Penicillin possesss neither free amino group nor carboxylic acid. Hence, The structure of Penilloic acid is,



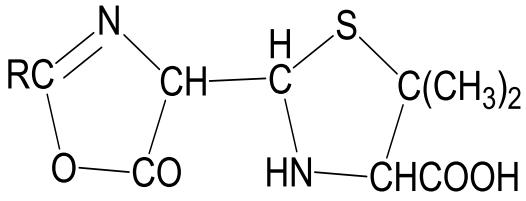
The structure of penicilloic acid is given as,



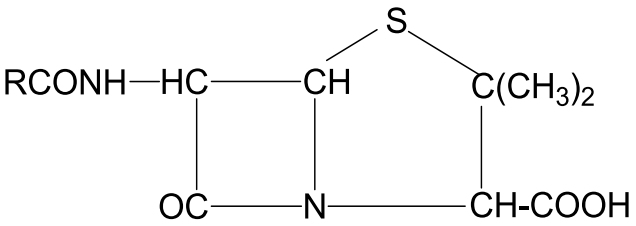
The structure of Penicilloic acid is confirmed by the reaction of penicillin with methanol to form methyl penicilloate which on hydrolysis with $HgCl_2$ forms methylpenaldate.



From the above disc ussion, it can be suggested that Penicillin can possess either a oxazolone structure or a β-lactam structure.

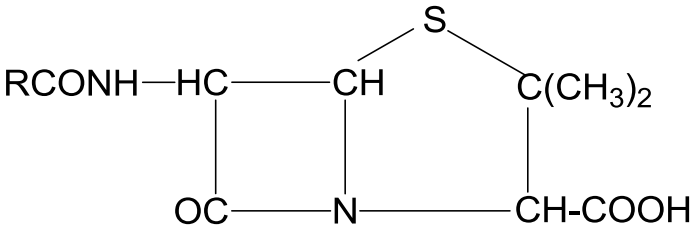


Oxazolone



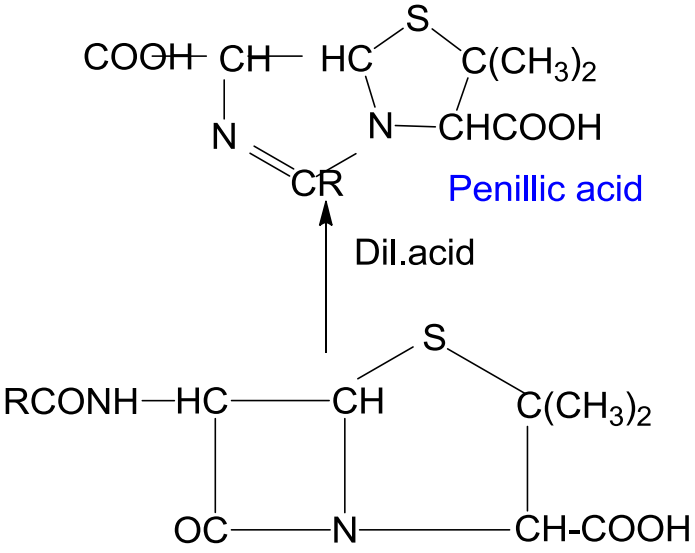
β–lactum

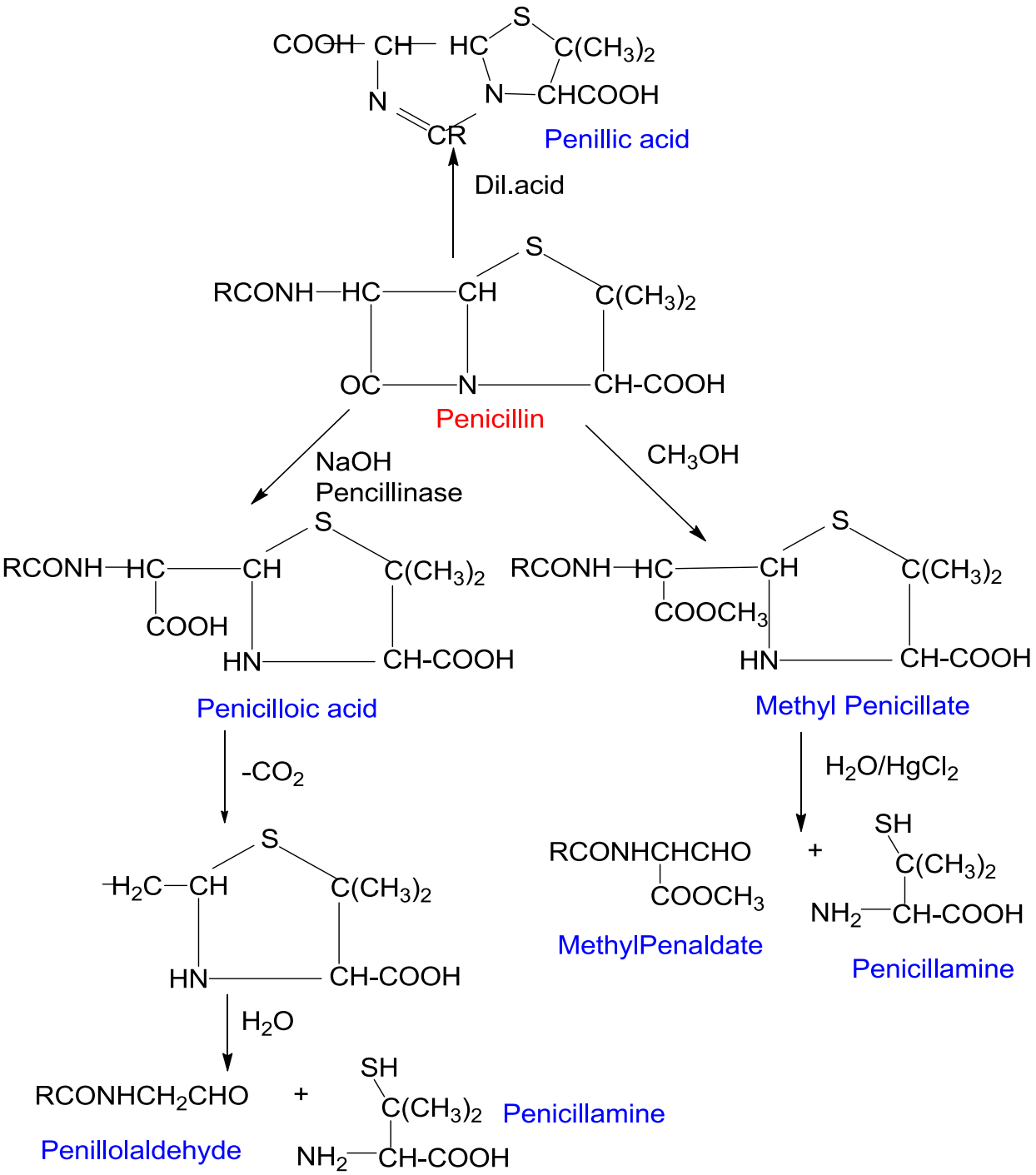
The structure of Penicillin is confirmed by IR spectrum. The IR spectrum of oxazolone shows a characteristic carbonyl bond at 1825cm^{-1} which is absent both in Penicillin structure and in β-lactam structure. Hence, Penicillin possess a β- lactum structure with a thiazolidine ring. Thus , the structure of Penicillin is



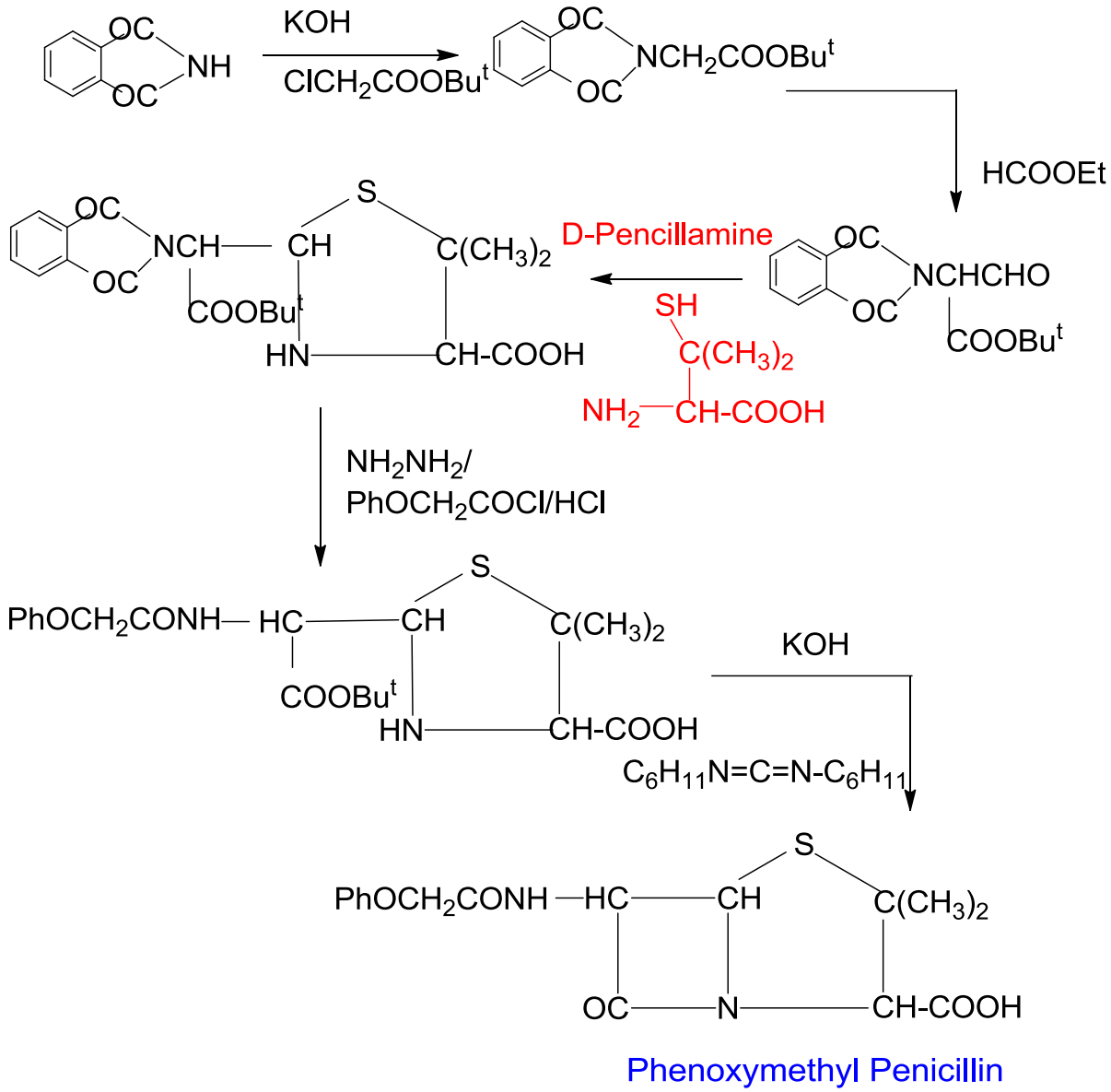
Penicillin

Reactions of Penicillin:

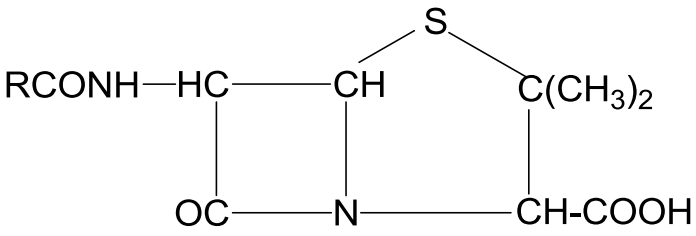


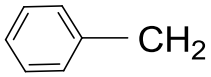
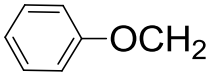
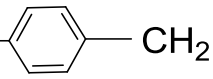

COOH CH HC S C(CH3)2
N N CHCOOH
CR
Penillic acid
Dil.acid
RCONH HC CH S C(CH3)2
OC N CH-COOH
Penicillin
NaOH
Pencillinase
CH3OH
RCONH HC CH S C(CH3)2
COOH
HN CH-COOH
Penicilloic acid
RCONH HC CH S C(CH3)2
COOCH3
HN CH-COOH
Methyl Penicillate
-CO2
H2O/HgCl2
H2C CH S C(CH3)2
HN CH-COOH
RCONHCHCHO
COOCH3
MethylPenaldate
+
SH
C(CH3)2
NH2 CH-COOH
Penicillamine
H2O
RCONHCH2CHO
Penillolaldehyde
+
SH
C(CH3)2
NH2 CH-COOH
Penicillamine

Synthesis of Penicillin:



Based on the 'R' group in the structure of Penicillin is



S.No	R group	Name
1	2-Pentenyl $CH_3\,CH_2CH{=}CHCH_2$	Penicillin-F
2	Benzyl 	Penicillin-G
3	Phenoxymethyl 	Penicillin-V
4	p-Hydroxybenzyl 	Penicillin-X

5.5.4 USES:

The action of Penicillin is bactericidal. It is active against Gram positive Bacilli Than Gram-Negative bacilli. They are non-toxic to mammalian cells.

5.6 STREPTOMYCIN:

5.6.1 Introduction:

It is an antibiotic isolated from Streptomyces grises. It is effective against Gram-Negative and Gram-positive organisms. It is used in the treatment of tuberculosis, meningitis and pneumonia.

5.6.2 Production and Isolation of Streptomycin

The culture from streptomyces grises is allowed to grow in the medium (Glucose, peptone, NaCl, steep liquor) in large vats at 24-28^{o} C for 3-4 days. The mycelium is separated and streptomycin is extracted from the filtrate by adsorption on charcoal. The pure form is obtained as sulphate.

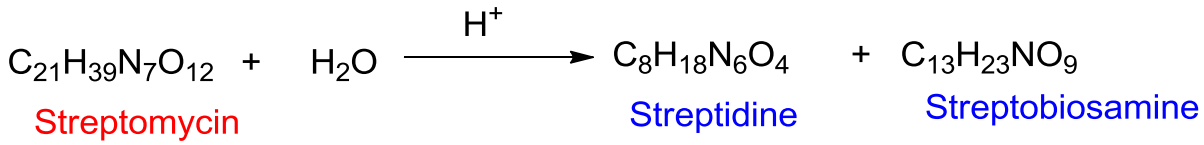
Properties: It is a colourless solid, laevorotatory.

5.6.3 Constitution of Streptomycin

1.The molecular formula of streptomycin is $C_{21}H_{39}N_7O_{12}$
2.Nature of nitrogen atom : streptomycin forms trihydro chloride indicates that three nitrogen atoms are strongly basic

$$\underset{\text{Streptomycin}}{C_{21}H_{39}N_7O_{12}} + 3HCl \longrightarrow \underset{\text{Crystalline}}{C_{21}H_{39}N_7O_{12}.3HCl}$$

3.Hydrolysis : On hydrolysis it forms an equivalent mole of streptidine $C_8H_{18}N_6O_4$ and streptobiosamine $C_{13}H_{23}NO_9$

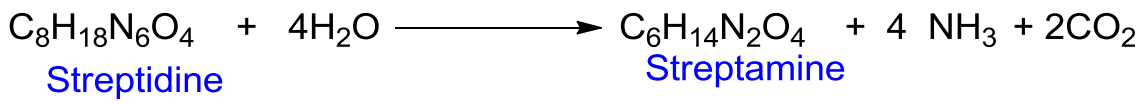


a) Structure of streptidine

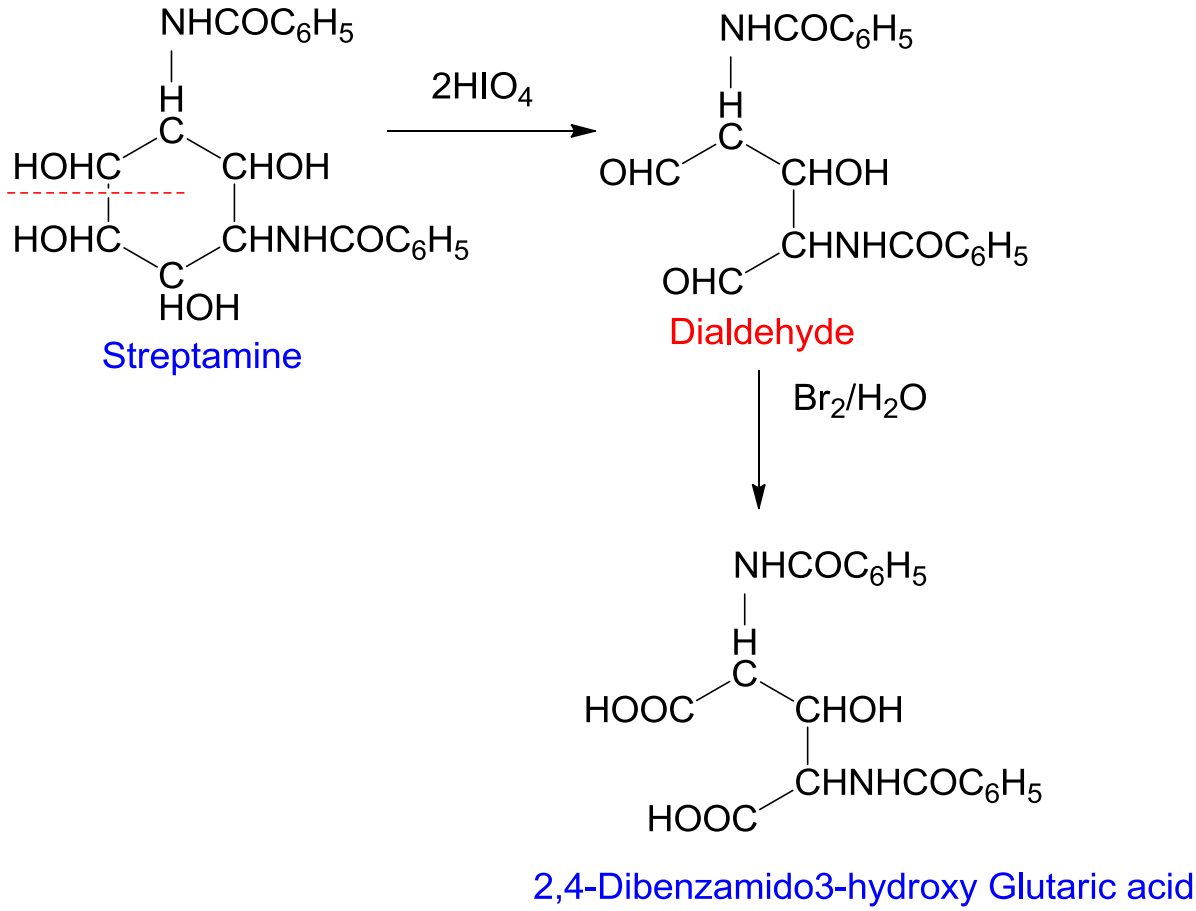
1.Molecular formula of streptidine is $C_8H_{18}N_6O_4$

2.Streptidine on oxidation with $KMNO_4$ it yields two molecules of guanidine indicates that streptidine has two guanido groups

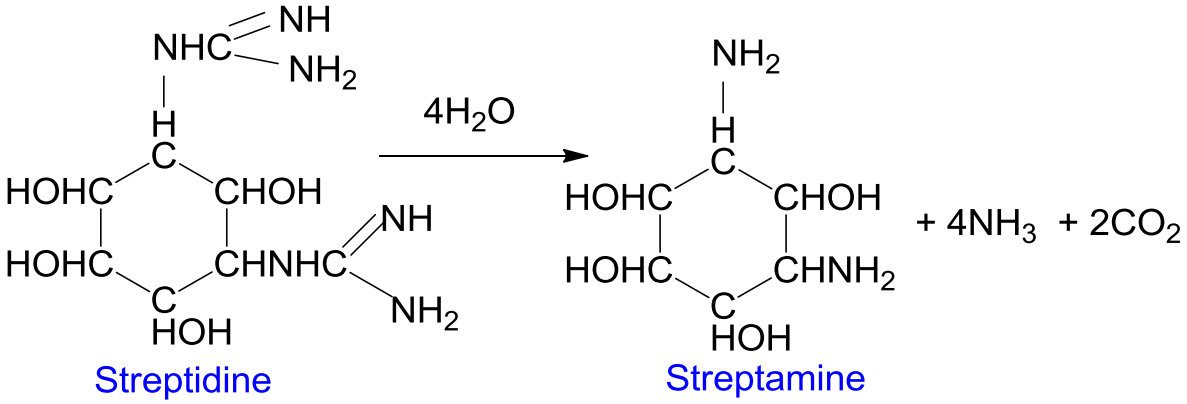
3.Streptidine on alkaline hydrolysis it yields streptamine and NH_3



4.Streptamine on benzoylation forms N,N –Dibenzoyl streptamine on oxidation with HIO_4 it forms dialdehyde which on oxidation with Br_2 /water forms 2,4 Dibenzamido 3-hydroxyglutoric acid



5. Streptamine is obtained by alkaline hydrolysis of streptidine



Streptidine Streptamine

b) Structure of streptobiosamine

1. The molecular formula of comopound is $C_{13}H_{23}NO_9$
2. Streptomycin tri hydrochloride on reaction with CH_3OH in HCL it yields streptidine dihydro chloride and streptobiosamine dimethyl acetate (I)

$$C_{21}H_{39}N_7O_{12}.3HCl + 3CH_3OH \longrightarrow C_8H_{18}N_6O_4.2HCl + H_2O$$

Streptomycin — Strepidine dihydrochloride

$C_{13}H_{22}NO_7(OCH_3)_4.HCl$

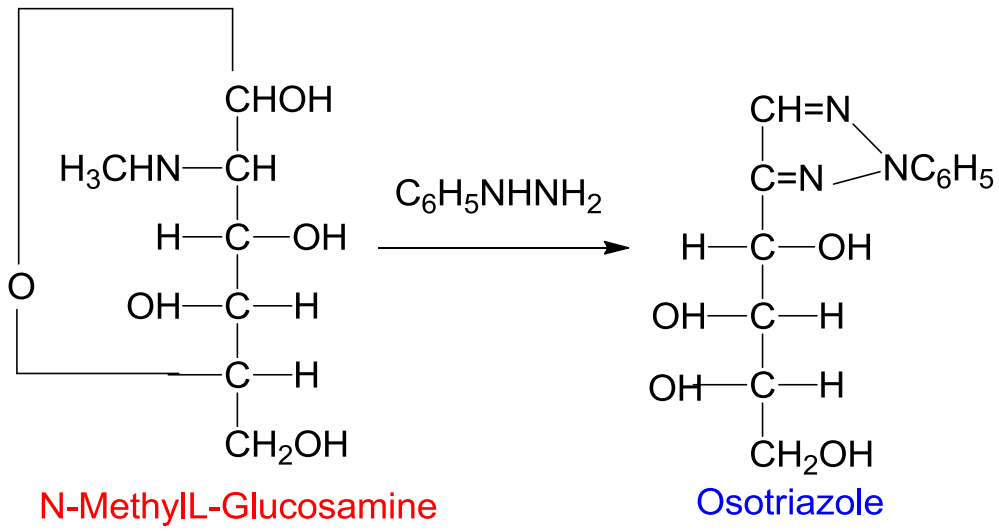
Methylstreptobiosamine dimethyl acetal (I)

3. When compound I undergoes alkaline hydrolysis it yields methyl acetal indicating the presence of methyl amino group. The compound I on reaction with acetic anhydride in HCL medium ,it forms N-methyl glucosamine and a hexose sugar streptose .

$$C_{13}H_{22}NO_7(OCH_3)_4.HCl \xrightarrow{(CH_3CO)_2O} \text{N-Methyl Glucosamine} + \text{Streptose.}$$

c) Structure of N-methyl glucosamine

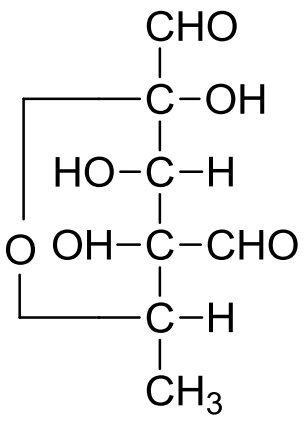
When N-methyl glucosamine is treated with phenyl hydrazine it forms hydrazone phenylosotriazole which is L-glucosamine .Thus the structure of glucosamine is



N-MethylL-Glucosamine Osotriazole

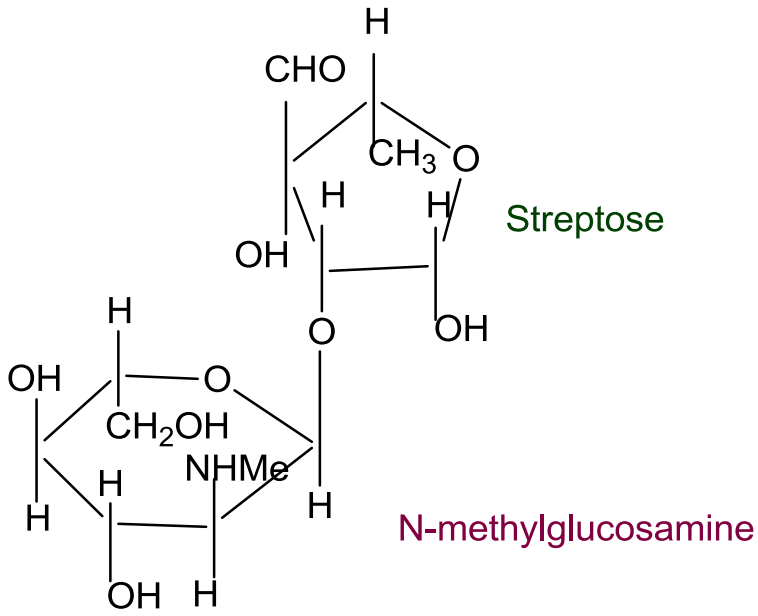
d) Structure of streptose:

1. Streptomycin on hydrolysis with alkali yield maltol which contains furanose ring structure. The maltol can be formed only if the aldehydic group present at position I of the streptose is involved in glycoside linkage.
2. Streptobiosamine on oxidation with Br_2/H_20 it yields monolactone on further oxidation with HIO_4 consumes 3 moles of HIO_4 indicating the presence of 3 hydroxyl groups .Hence the structure of streptose is given by



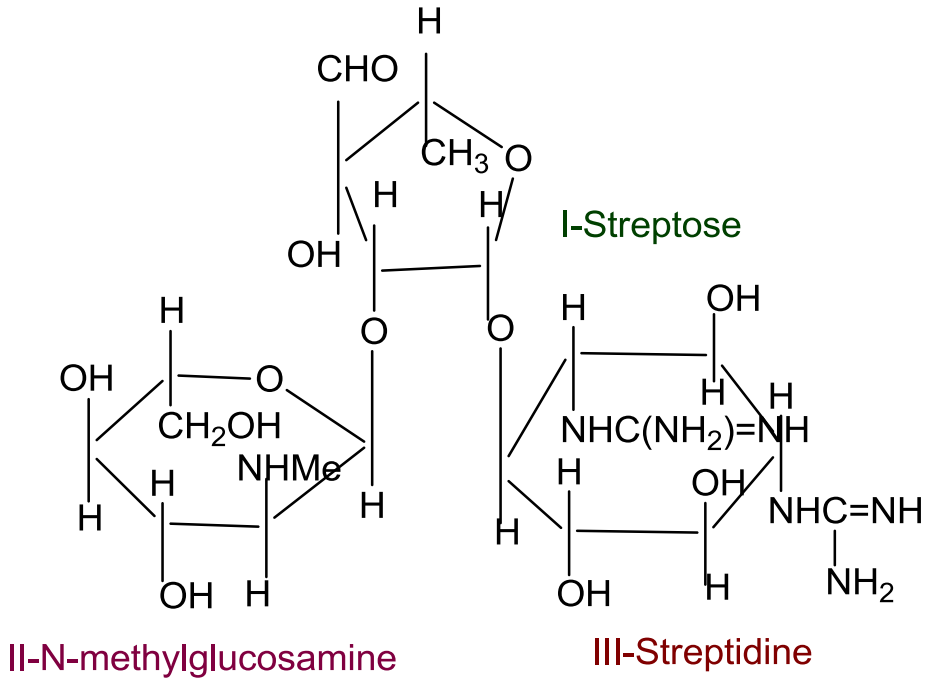
Streptose

The structure of streptobiosamine is given by



Streptobiosamine

The carbon atom C_1 of streptose is linked glycosidically to C_4 of streptidine fragment to form the structure of streptomycin



Streptomycin

The following linkages in streptomycin is

1. A β- L – glucoside linkage is present between streptose (I) and streptidine (III)
2. An α -L-glycosidic linkage is present between streptose (I) and N- methyl glucosamine.

5.6.4 USES

Streptomycin exerts its effects on tubercle bacilli and interferes with the RNA metabolism by blocking the formation of mononucleotides.

5.7 VITAMINS

5.7.1 Introduction:

It is an essential dietly factor required by organism in small amounts whose absence results in deficiency

Classification of vitamins

a. Fat soluble group : Include vitamin A,D,E and K
b. Water soluble group : Include vitamin B and C

Provitamins: These are biological inactive compounds which are similar to vitamins and can be converted to vitamins in viro. Such compounds are called provitamins.

1.β –carotene is the provitamin for vitamin A

2.Erosterol is the provitamin for vitamin D

5.7.2 VITAMIN A

There are two vitamins A1 and A2 .VitaminA or A1 is retinol while Vitamin A2 contains more conjugation than Vitamin A1.

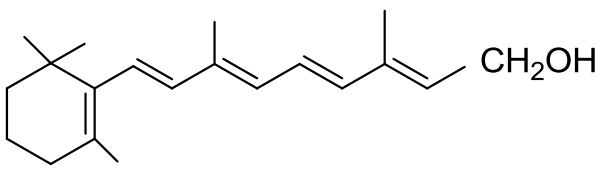
It is a fat soluble vitamin occurs as esters in fats in fish livers and in blood .The other sources are green vegetables ,tomatoes, cabbage which is present as carotenes and are converted to retinol by invivo.

5.7.2.1 Deficiency:

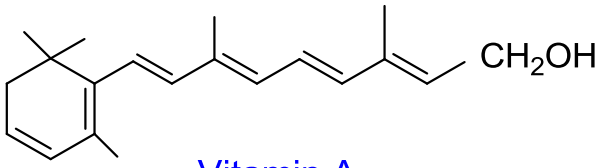
1. It influence the growth in animals and increase the immunity. Its deficiency causes night blindness with prolonged deficiency it may lead to hardening of conjuctive softening of corona known as Xerophthalmia (complete blindness).

It may also lead to dryness of skin hair

Excess of vitamin A is injurious leads to bone fragility nausea weakness and dermatitis.



Vitamin A_1 (Retinol)



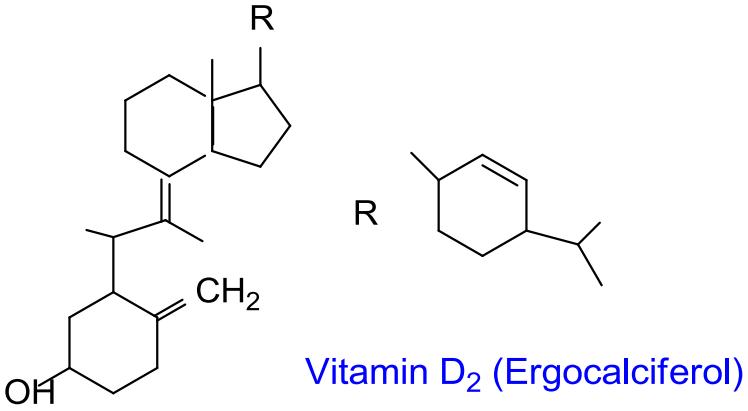
Vitamin A_2

5.7.3 VITAMIN D

It represents a group of fat soluble vitamins which are structurally related to sterols. There are 5 vitamins which are structurally related to sterols. There are 5 vitamins like D_1,D_2,D_3,D_4,D_5 .All 5 vitamin D is prepared by irradiation of specific sterols,provitamins mostly ergosterol. Vitamin D_2 is also called ergocalciferol, found in cod liver oil and other fresh liver oils,hens eggs and milk of mammals.

5.7.3.1 Deficiency:

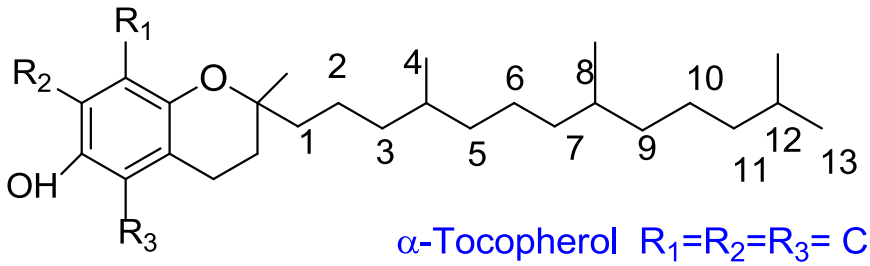
Deficiency of vitamin D_2 causes rickets a disease occur in children. The disease is characterized by a softening and bending of bones. It is also known as antirachitic factor. The main function of D_2 is to control calcium and phosphorus metabolism.



Vitamin D_2 (Ergocalciferol)

5.7.4 VITAMIN E: (Tocopherols Gr.tokos=child brith,photo-to bear)

It is a fat soluble vitamins consisting of a group of eight compounds collectively known as tocopherols.these are α,β,ϒ,δ,€, ,ɳ. The most biologically active compound is α-tocopherol where β and ϒ -tocopherol exhibit half the activity.



α-Tocopherol $R_1=R_2=R_3= CH_3$

β-Tocopherol $R_1=R_3= CH_3$; R_2-H

γ-Tocopherol $R_1=R_2= CH_3$; R_3-H

δ-Tocopherol $R_2=R_3= CH_3$; R_1-H

It occurs in wheat germ oil ,cotton seed oil soyabean oil,palm oil and rice.

5.7.4.1 Deficiency:

1.It causes antisterility.

2.It also causes increase in the number of leucocytes (i.e) WBC of the blood ,causing anaemia.

3.It also increases concentration of RNA and DNA in the bone marrow.

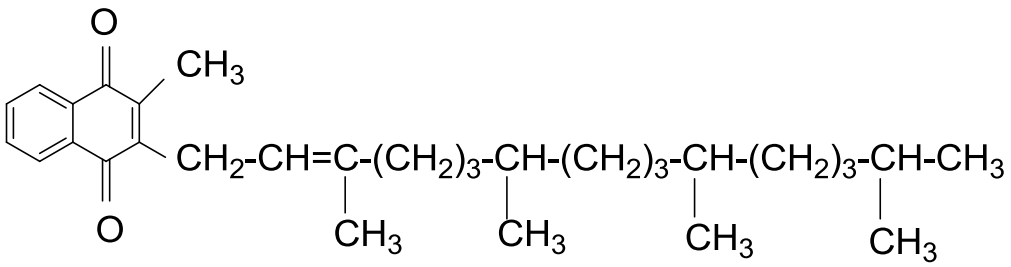
4.It also causes increased excretion of pentose in urine which is due to degeneration of muscles.

5.7.5 VITAMIN K

The term vitamin k refers to two compounds vitamin k_1 and k_2 .These vitamin occurs in all greeny leafy vegetables like spinach,cabbage,carrots etc.

Deficiency: Both are antihaemorrhagic vitamins and are involved in blood clotting.

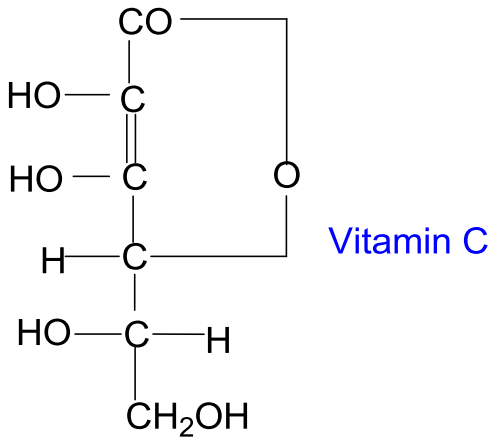
They are helpful in blood clotting by activating the formation of blotting-clotting enzyme for thrombin synthesis. The deficiency of the vitamin lengthens the time of blood clotting.



Vitamin K_1

5.7.6 VITAMIN C (Ascorbic acid)

It is a water soluble vitamin ,widely distributed in both plants and animals. It occurs in citrus fruits like lemons, oranges, beans ,tomatoes and in small quantities occurs in milk and blood.



Vitamin C

5.7.6.1 Deficiency:

It causes disease scurvy (tendency to haemorrhage and structural changes in cartilage, bone and teeth.) in infants and adults

It severe deficiency it leads to swelling and bleeding of gums and teeth.

It also cause anaemia ,lengthens the time for coagulation of blood and causes blood capillary to become fragile.

5.8 VITAMIN B COMPLEX

It is a group of water soluble vitamins found in yeast,liver and rice polishing. The group includes vitamine B_1 –Thiamine , B_2 –Riboflavin, B_3 –pantothenic acid ,B_4 – Folic acid , B5-nicoticnic acid, B_6 –pyridoxine, B_{12}- cyanocobalamine.

A) VITAMIN B_1(THIAMINE)

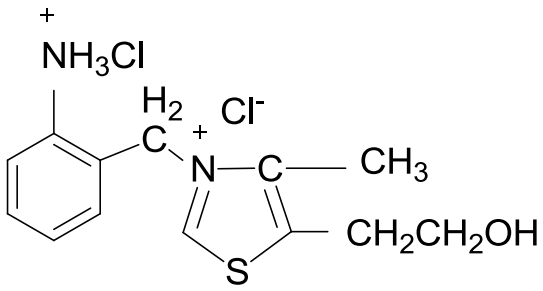
It is a water soluble vitamin occur in the outer coats of seeds including cereal grains like rice, wheat etc. it also occur in yeast,milk,groundnuts,eggs all green vegetables ,fruits.

Deficiency: A deficiency of this vitamin in man causes beri-beri (type of paralysis).

There are two Types of Beri-beri

a.Dry beri-beri : It causes weakness in muscles, loss of weight, neuritis,pain in the arms and legs and decrease in blood pressure.

b.wet beri-Beri: Severe deficiency of thiamine leads to edema,impaired cardiac function and in some cases the entire nervous systems is affected.



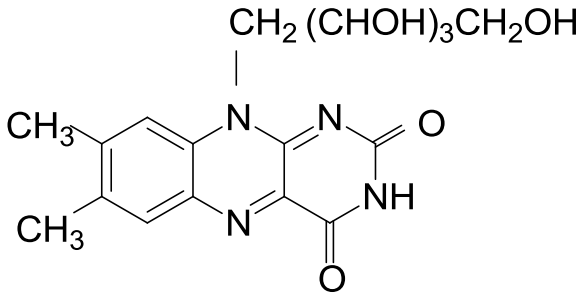
Vitamin B_1- Thiamine Hydrochloride

B) VITAMIN B_2(RIBOFLAVIN)

It is chemically related to yellow water soluble pigments known as flavin and is isolated from milk, lactoflavin or Riboflavin.

It is widely distributed in plants and animals in yeast ,vegetables, egg white milk, liver,kidney ,meat etc.

Deficiency : Signs are dark red tongue,dermatitics. In adults in reduces growth and causes general weakness.



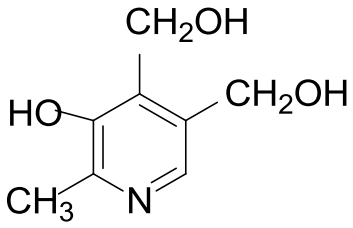
Vitamin B_2-Riboflavin

C) VITAMIN B_6(PYRIDOXINE OR ADERMINE)

It is a water soluble vitamin composed of these vitamins namely pyridoxine,pyridoxal and pyridoxamine .These vitamins are interconvertible and in general the pyridoxine is referred as vitamin B_6. It occurs abundantly in cereals ,grains,molasses and yeast.

Deficiency: It causes nervousness, insomnia, irritability stomatitis (inflammation of the mucous membrane of the mouth) and also causes general weakness.

It is employed to stop nausea ,vomiting in pregnancy and for treatment of epilespsy acne or dermatitis.



Vitamin B_6- Pyridoxime

D) VITAMIN B_{12}(CYANOCOBALAMIN)

It is a water soluble vitamin containing cobalt .It occurs in milk, cheese, eggs, meat, liver of sheep ,horse , pig, fish etc. It is a red crystalline substance, soluble in water .

Deficiency : It is an essential growth factor and its deficiency causes pernicious anaemia followed by degradation of spinal cord.

TEXT / REFERENCE BOOKS

1. Finar I. L., Organic Chemistry, 6th Edition, Pearson Education Asia, 2004.
2. S.V.Bhat,B.A. Nagasampagi, M.Sivakumar., Chemistry of Natural products, Springer Publications 2005.
3. O.P. Agarwal., Organic Chemistry-Natural Products Vol.I and II, Goel Publishing House, 1980.
4. G.R. Chatwal., Organic Chemistry-Vol II- Himalayan Books, 1985.
5. T.L. Gilchrist., Heterocylclic Chemistry, Longman Scientific and Technology, 2005.