Natural Products

→ Natural products are the products (or) chemicals which are derived from Living organisms such as plants, Animals (or) Micro organisms.

→ These Natural products have wide applications in various fields such as food, Medicine, Perfumery, Coloring agents, flavoring agents etc.

→ Natural products are classified into different types.

1. Alkaloids

2. Terpenoids

3. Steriods

4 Flavonoids and Iso flavonoids

5 vitamins

6. Proteins

7. Carbohydrates

8. Hormones

Alkaloids

UNIT-I

Definition:

→Alkaloids are naturally Occurring Nitrogen Containing (heterocyclic) Basic compounds which are widely distributed in plants and posses significant pharmacological Activity.

→Alkaloids means Alkali Like" Compounds.

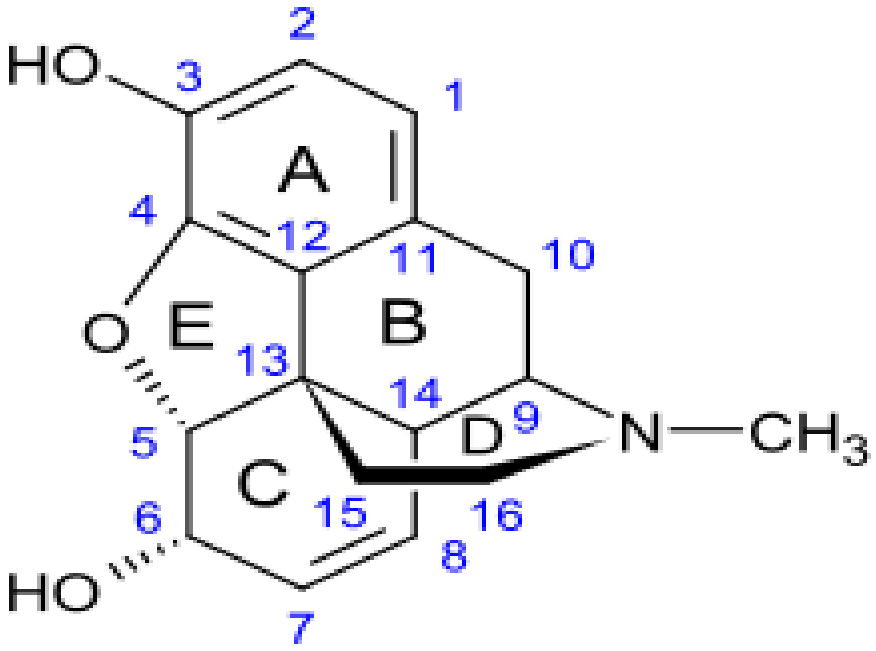
→All Alkaloids are alkaline in nature but all alkaline Compounds are not alkaloids.

→ Alkaloids are Secondary metabolites help for survival of plants.

→ there are variety of Alkaloids present in various vascular plants and have been isolated from the roots, leaves, and seeds of plants.

→Alkaloids are important Compounds of plant origin.

Ex: In 1806, Sertuerner reported the first isolation of Morphine from opium.

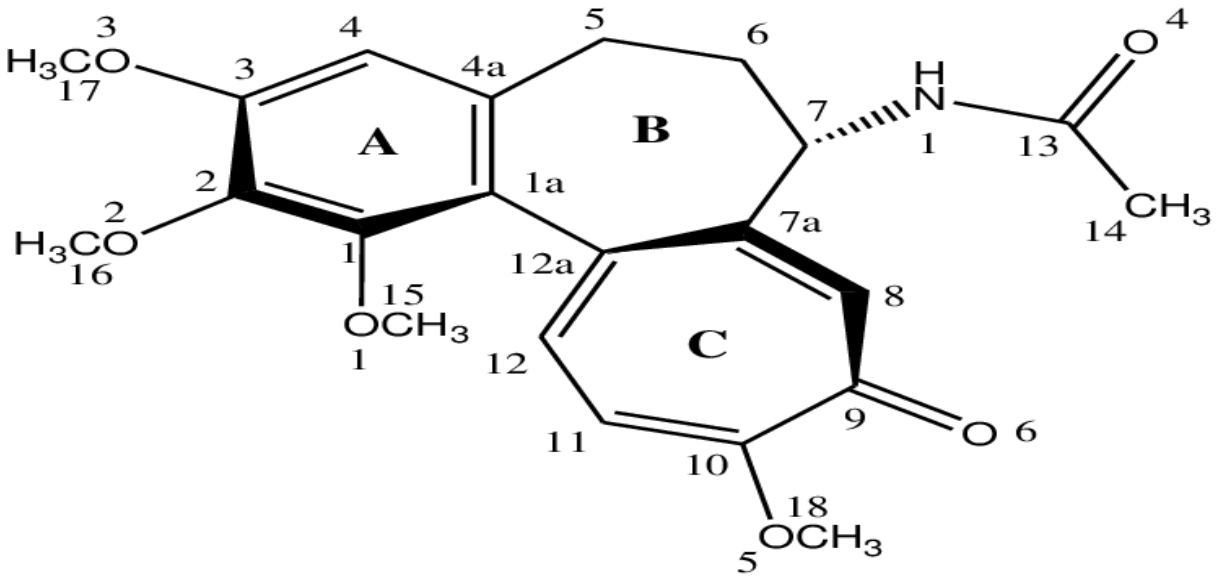


Morphine

→There are Some Exceptions for Alkaloids

Ex: Colchicine is not a heterocyclic and not basic but considered as an alkaloid.

Colchicine



→Alkaloids are defined as biological active, basic Nitrogenous compounds (generally heterocyclic ring) obtained from natural sources, mostly optically active and pharmacologically active compounds.

General properties of Alkaloids

1. Most of alkaloids are colure less solids.
2. Normally alkaloids have bitter taste and are optically active.(Levo rotatoy).
3. Generally alkaloids are insoluble in water but soluble in organic solvents.
4. Some alkaloids are liquids and soluble in water. (Ex: Coniine, Nicotine.)
5. Alkaloids contain at least one ring in the compound.
6. They contain one (or) two Nitrogen atoms in the ring and are 3° in Nature.
7. Alkaloids in small quantity act as drug but in larger quantity act as poison.
8. Alkaloid's name ends with suffix "ine". The suffix diene" indicates its isomer.

   Ex: quinine and Quinidine.

9. Prefixes epi, neo, iso used for isomers.

Alkaloid	**Source**	**Property & uses**
1. Morphine	Opium	it induces sleep, used as anesthetic to relief pain.
2. Reserpine	Rauwolfia roots	Used to treat High B.P.

General Methods of structure Elucidation

1. Molecular formula
2. Unsaturation determination
3. Nature of oxygen
4. Nature of nitrogen atom
5. Nature of Heterocyclic ring
6. Spectral Analysis
7. Synthesis

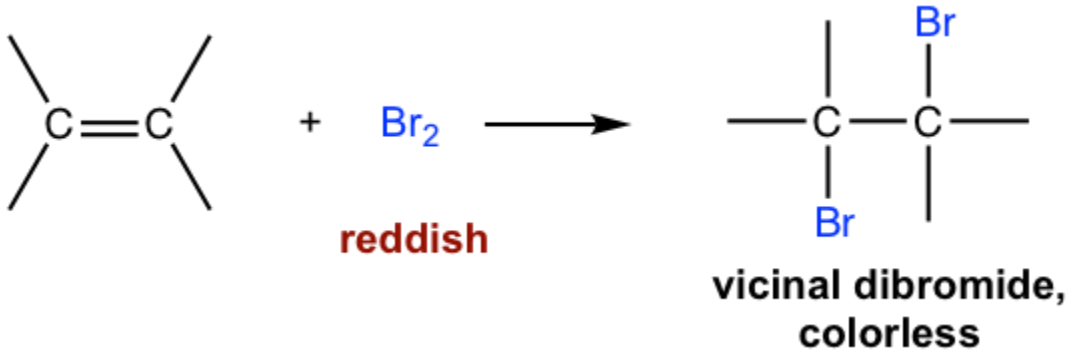
1. Molecular Formula:

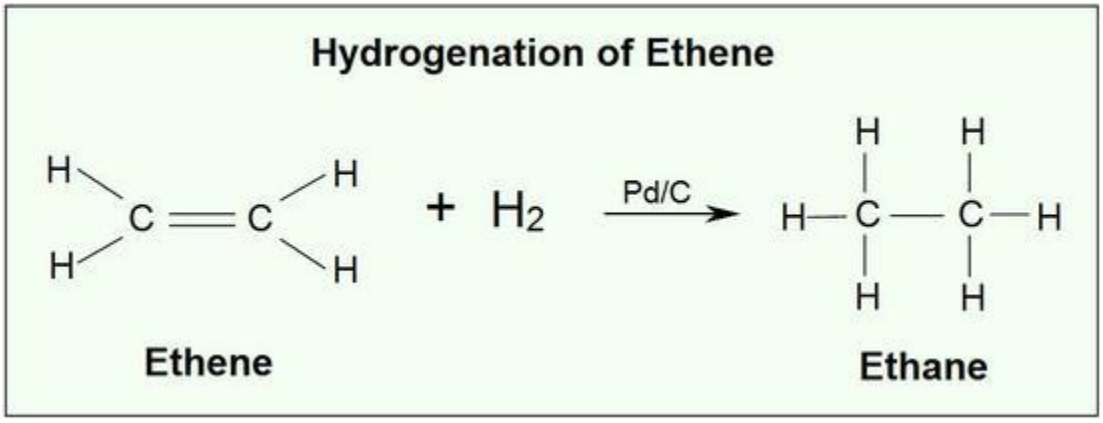
→ With Qualitative and quantitative analysis empirical formula of the Compound is determined.

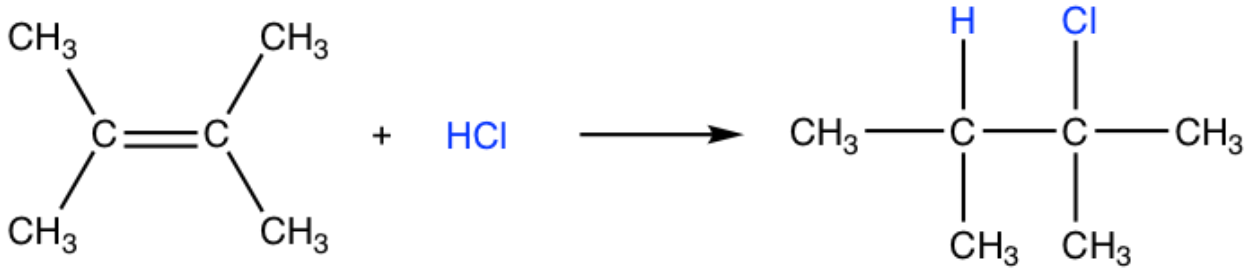
→ Molecular weight is determined by physical methods (or) by Mass spectrometry. From that molecular formula of the compound is determined.

2. Unsaturation Determination:

→Presence of unsaturation (or) double bond is determined by addition reaction with Br_2, H_2 (or) with Halo acids.







3. Nature of oxygen:

→If the alkaloid Contains oxygen atom then it may be in the form of -OH, C=0, -COOH, R – o – R^1, COOR –OCH_3, $CONH_2$, Lactones etc.

These groups are detected of

A) Hydroxyl group (-OH):

→ The IR Spectra give broad band at near 3600 cm^{-1}.

→ Presence of -OH group is detected by Acetylation (or) Benzylation reaction.

1. $R\text{-}OH + (CH_2CO)_2O \text{ ---------}\rightarrow R\text{-}O\text{-}COCH_3 + CH_3COOH$

Acetic anhydride Acetyl derivative

2. $R - OH + CH_3COCl \text{ ----------}\rightarrow R\text{-}O\text{-}COCH_3 + HCl$

Acetyl Chloride Acetyl derivative

3. $R - OH + C_2H_5COCl \text{ ----------}\rightarrow R\text{-}O\text{-}COC_2H_5 + HCl$

Benzyl Chloride Benzyl derivative

The no of -OH groups are Estimated by

1. Titration of acetyl derivative with standard alkali

$R\text{-}O\text{-}COCH_3 + NaOH$ ----------→, R-OH + CH3COONa

I mole of -OH = 1 mole of NaOH

2. Zerewittinoffs active hydrogen method

$R\text{-}OH + CH_3MgI \text{ ----------}\rightarrow R\text{-}O\text{-}MgI + CH_4$

I mole of – OH = I mole of CH_4

The nature of -OH group may be alcoholic or phenolic.

Presence of phenolic –OH group:

→The Compound is Soluble in NaOH Solution and it gives Violet (or) green color with $FeCl_3$ solution.

Presence of alcoholic –OH group:

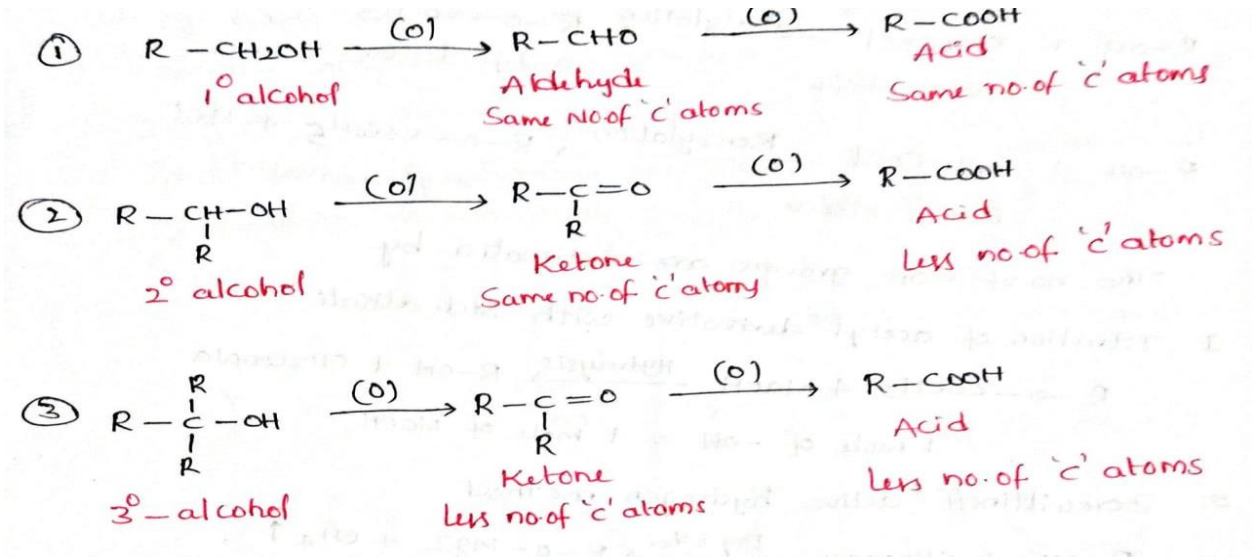
1. It shows dehydration reaction with Conc. H_2SO_4 / with P_2O_5.

2. It forms halide with thionyl chloride.

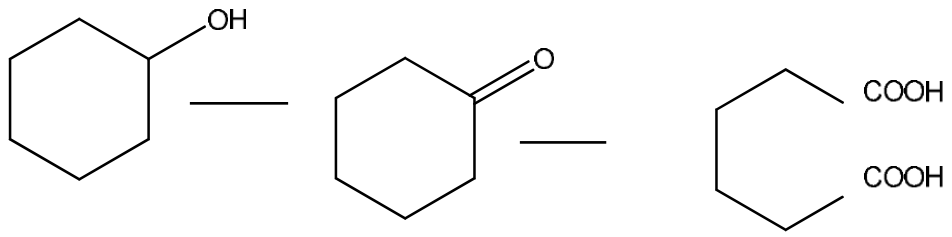
$R\text{-}OH + SOCl_2 \text{ ---------} \rightarrow R\text{-}Cl + SO_2 (\uparrow) + HCl$

→ Alcoholic –OH group may be 1^0, 2^0 (Or) 3^0 they are distinguished by Oxidation reaction.

Oxidation reaction of alcohols:



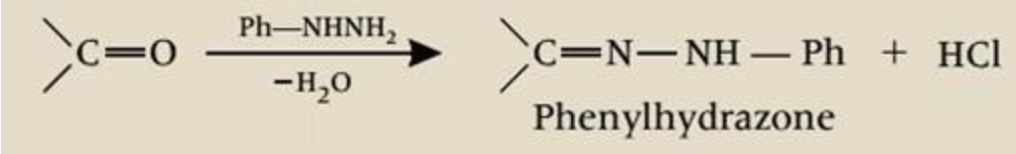
→If 2^0 – CH group is present in the ring then it gives dicarboxylic acid containing same number of Carbon atoms.



(B) Carbonyl Group (C=O):

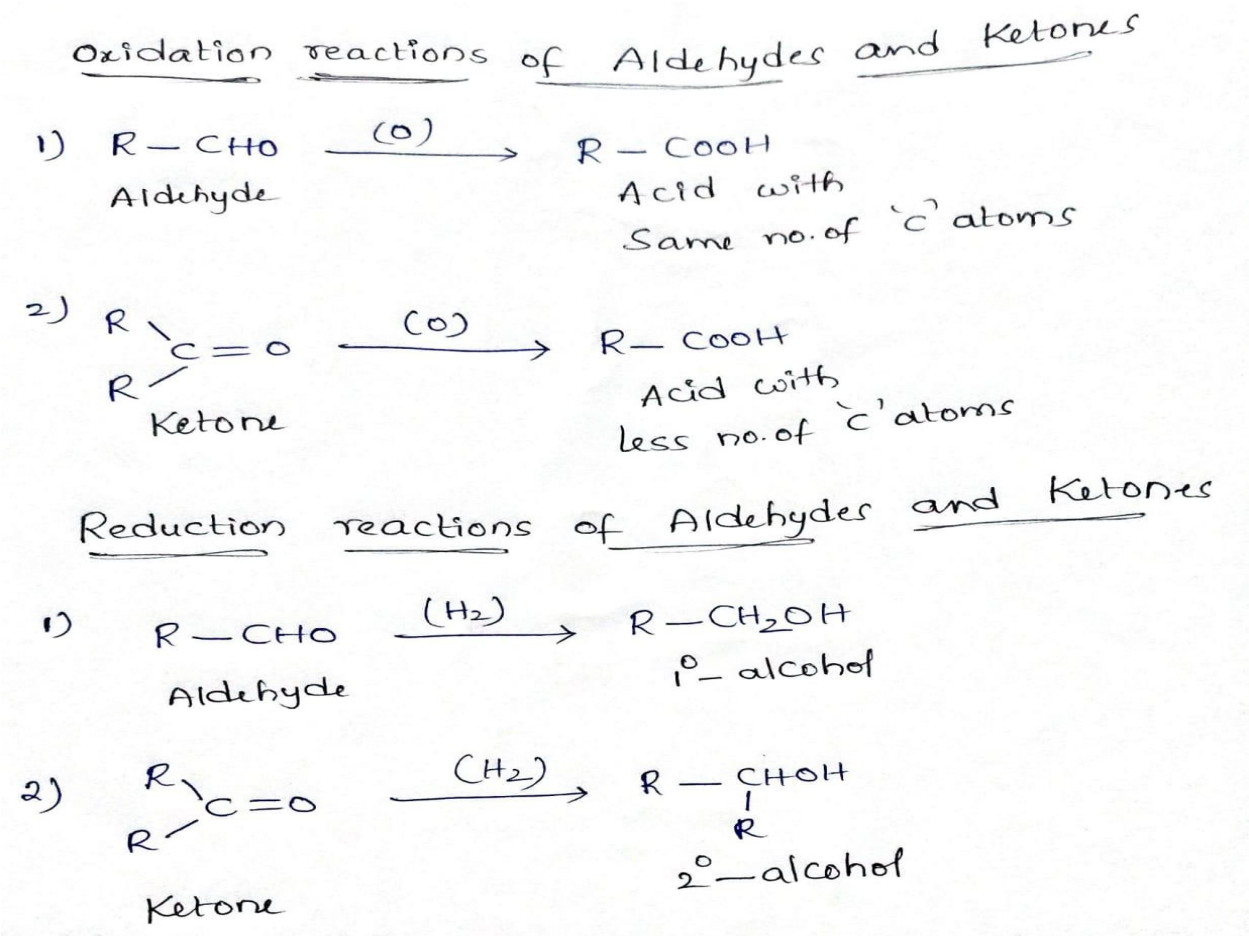
→The presence of Carbonyl Group is detected by following reactions.

The presence of aldehydes and ketones is detected by their reaction with hydroxyl amine to form the corresponding oxime.



The Carbonyl group may be an aldehyde (or) Ketone.

Oxidation and Reduction reactions of aldehyde and Ketone groups:



C) Carboxylic acid group (-COOH):

→The presence of carboxylic dead group is detected by $NaHCO_3$.

→The carboxylic acid group gives effervesce of Co_2 with $NaHCO_3$

$R\text{-}COOH + NaHCO_3 \dashrightarrow R\text{-}COONa + CO_2 (\uparrow) + H_2O$

→ The No of — COOH groups can be estimated by Titration with standard alkali

$R\text{-}COOH + NaOH \dashrightarrow R\text{-}COONa + H_2O$

I mole of – COOH = I mole of NaOH

D) Methoxy group (-OCH3):

Zeisel Method

→ the methoxy group is detected and Estimated by Zeisel's method.

$R\text{-}OCH_3 + HI \text{ ----------} \rightarrow R\text{-}OH + CH_3I$

Methyl Iodide

$CH_3I + AgNO_3 \text{ ----------} \rightarrow CH_3NO_3 + AgI (\downarrow)$

Silver Iodide

1 mole of — OCH3 = 1 mole of AgI

E) Methylene dioxyl group (-O-CH_2-O-):

→This group is detected and Estimated by oxidation with HCl (or) H_2SO_4.

$-O\text{-}CH_2\text{-}O- + HCl \text{ -------} \rightarrow HO\text{-}CH_2\text{-}OH \text{ ---------} \rightarrow H_2O + HCHO \text{ ---------} \rightarrow HCOOH$

I mole of methylene dioxyl group = 1 mole of formic acid

F) Other Oxygen Containing groups:

→These groups are detected and Estimated by alkali hydrolysis.

$R\text{-}COOR + NaOH \text{ --------} \rightarrow R\text{-}COONa + R\text{-}OH$

$R\text{-}CONH_2 + NaOH \text{ ----------} \rightarrow R\text{-}COONa + NH_3 (\uparrow)$

4) Nature of Nitrogen atom:

→Alkaloids are basic in Nature. They contain Nitrogen atom.

→ The Nitrogen atom may be 1^0, 2^0 (Or) 3^0 in nature which is detected by general reactions with Nitrous acid, acetyl chloride (or) methyl iodide.

I. Primary Amino (-NH_2) group:

→This group less commonly found in alkaloids.

$\rightarrow R - NH_2 + HNO_2 \text{ ----------} \rightarrow R\text{-}OH + N_2 (\uparrow)$

$\rightarrow R\text{-}NH_2 + 2CH_3COCl \xrightarrow{\quad} R\text{-}N(COCH_3)_2 + 2HCl$

Acetyl chloride diacetyl derivative

$\rightarrow R\text{-}NH_2 + 3\,CH_3I \xrightarrow{\quad} R\text{-}N^+(CH_3)_3I^-$

Methyl Iodide Quaternary Salt

II. Secondary Amino (-NH-) group:

$\rightarrow R\text{-}NH\text{-}R + HNO_2 \xrightarrow{\quad} R_2N^+ = N\text{-}O + H_2O$

$\rightarrow R\text{-}NH\text{-}R + CH_3COCl \xrightarrow{\quad} R_2N\text{-}COCH_3 + HCl$

$\rightarrow R\text{-}NH\text{-}R + 2\,CH_3I \xrightarrow{\quad} R_2N^+(CH_3)_2I^- + HI$

III. Tertiary Amino (-N-) group:

→ It not reacts nitrous acid (HNO_2) and Acetyl chloride (CH_3COCl).

→ It requires one mole of CH_3I to form quaternary salt.

$R_3N + CH_3I \xrightarrow{\quad} R_3N^+CH_3I^-$

The special test for 3^0 – Amines, It gives amine oxide with 30% H_2O_2.

$R_3N + H_2O_2 \xrightarrow{\quad} R_3N\rightarrow O + H_2O_2$

IV. N-methyl (N-CH_3) group:

Herzig Meyer's method

→ The N-methyl group is detected by Herzig Meyer's method.

$\rightarrow N\text{-}CH3 + HI \xrightarrow{\quad} -NH- + CH_3I$

$CH_3I + AgNO_3 \xrightarrow{\quad} CH_3NO_3 + AgI$

1 mole of $-N\text{-}CH_3$ = 1 MOLE OF AgI

The N-methyl group can be estimated by distillation With KOH

$\rightarrow N\text{-}CH_3 + KOH \xrightarrow{\quad} CH_3NH_2$

It indicates one $-CH_3$ group is attached to Nitrogen.

$\rightarrow N\text{-}(CH_3)_2 + KOH \xrightarrow{\quad} (CH_3)_2NH$

It indicates two $-CH_3$ groups attached to Nitrogen.

$\rightarrow N(CH_3)_3 + KOH \xrightarrow{\quad\quad} (CH_3)_3 N$

It indicates three $-CH_3$ groups attached to Nitrogen.

$\rightarrow -NH_2 + KOH \xrightarrow{\quad\quad} NH_3 (\uparrow)$

It indicates the presence of free amine group.

5. Nature of Heterocyclic ring

→ Nature of heterocyclic ring present in the molecule is determined by degradation methods.

→ after degradation the heterocyclic ring breaks at C-N bond and converts into unsaturated hydrocarbon.

→By Knowing the structure of hydrocarbon formed, the Nature of Heterocyclic ring present in the alkaloid Can be determined.

→Different degradation methods are as follows

1. Hofmann's Exhaustive Methylation
2. Emdes Degradation
3. Von Braun Method
4. Reductive Degradation

1. Hofmann Exhaustive Methylation:

→This is most extensively used method.

→This method involve heterocyclic ring opening with Elimination of nitrogen atom.

→This method involves following steps

1. Hydrogenation: - If Heterocyclic ring is unsaturated then it is completely reduced by Catalytic hydrogenation

2. Methylation: The saturated alkaloid is heated with Excess CH_3I to form quaternary Ammonium Salt.

3. Hydrolysis: - The Quaternary Ammonium Salt is heated with moist silver oxide to form Quaternary Ammonium Hydroxide.

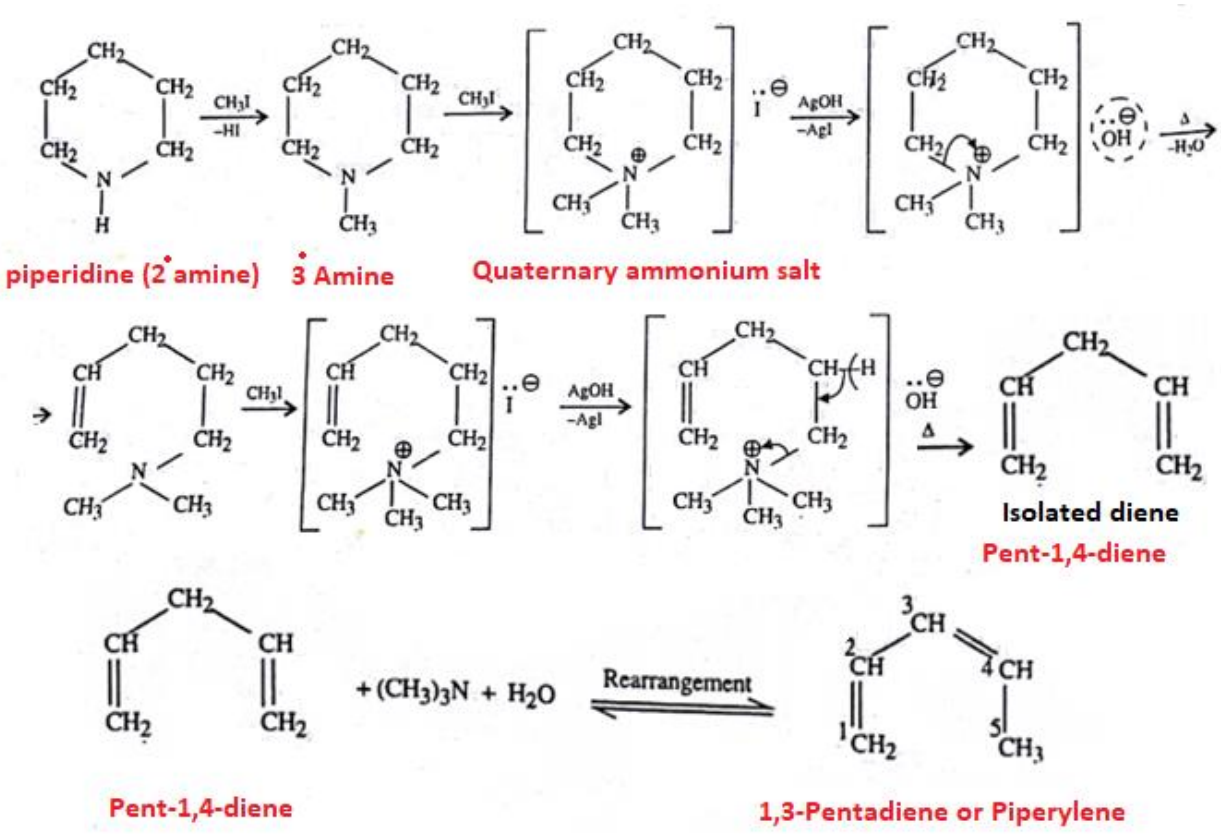
4. Dehydration: The Quaternary Ammonium Hydroxide derivative is heated then dehydration take place with β– Elimination of Hydrogen atom. The heterocyclic ring breaks at C-N bond.

5. The process is repeated till 'N' atom is completely detached from the ring.

6. The unsaturated compound formed determines the nature of heterocyclic ring in an alkaloid.

→Hoffmann's degradation method is applicable to heterocyclic rings which contain β-Hydrogen atom.

Ex: Hofmann's Exhaustive methylation of pyridine



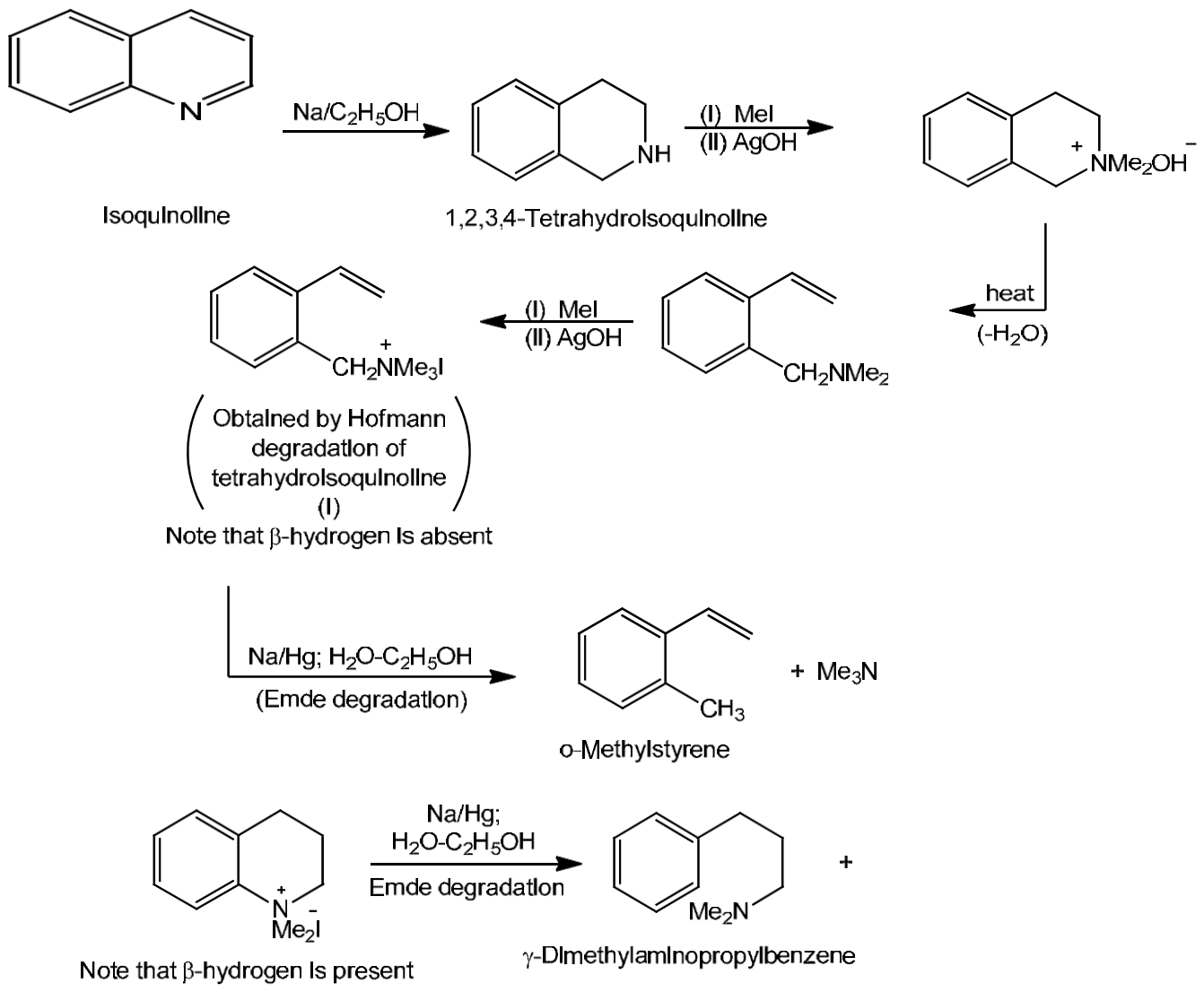
→Formation of 1, 3-pentadiene indicates the presence of 6 numbered heterocyclic ring.

2. Emdes Degradation:

→ When β- hydrogen atom not available in heterocyclic ring then this method is useful.

→ In this case steps are similar to Hofmann degradation Method up to formation of quaternary Ammonium iodide.

→The Quaternary Ammonium iodide is reduced by sodium amalgam (Na-Hg/HCl)



Isoquinoline

1,2,3,4-Tetrahydroisoquinoline

Obtained by Hofmann degradation of tetrahydroisoquinoline (I)

Note that β-hydrogen is absent

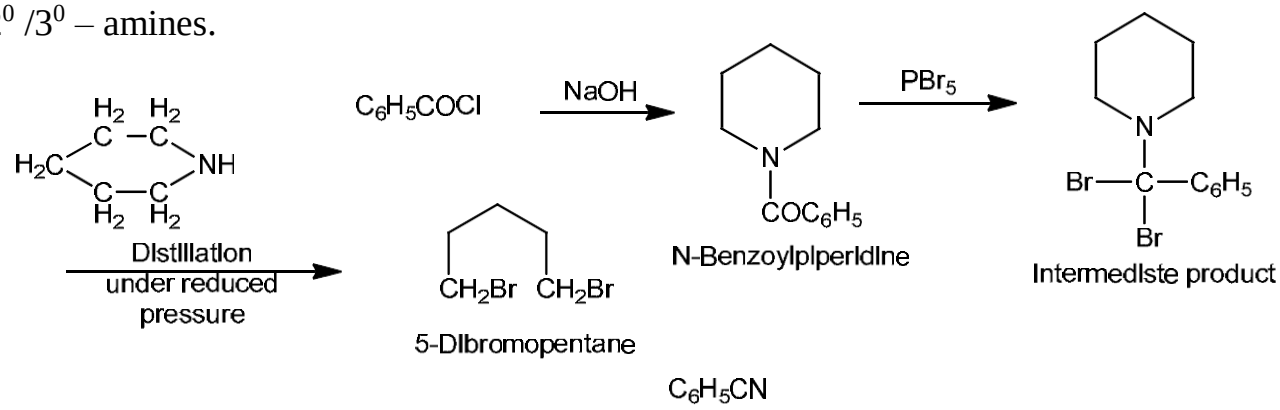
o-Methylstyrene

Note that β-hydrogen is present

γ-Dimethylaminopropylbenzene

→This method is applicable to compound, which are not degraded by Hofmanns method.

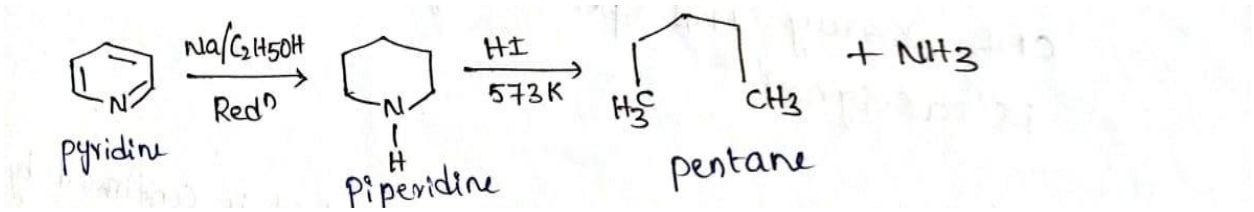
3. Von Braun method: → This method is useful for heterocyclic ring containing 2^0 /3^0 – amines.



→Formation of 1, 5 dibromo Pentane indicates the presence of 6 numbered heterocyclic ring.

4. Reductive degradation:

→In this method the heterocyclic ring is opened by heating with Conc. HI at 573K.

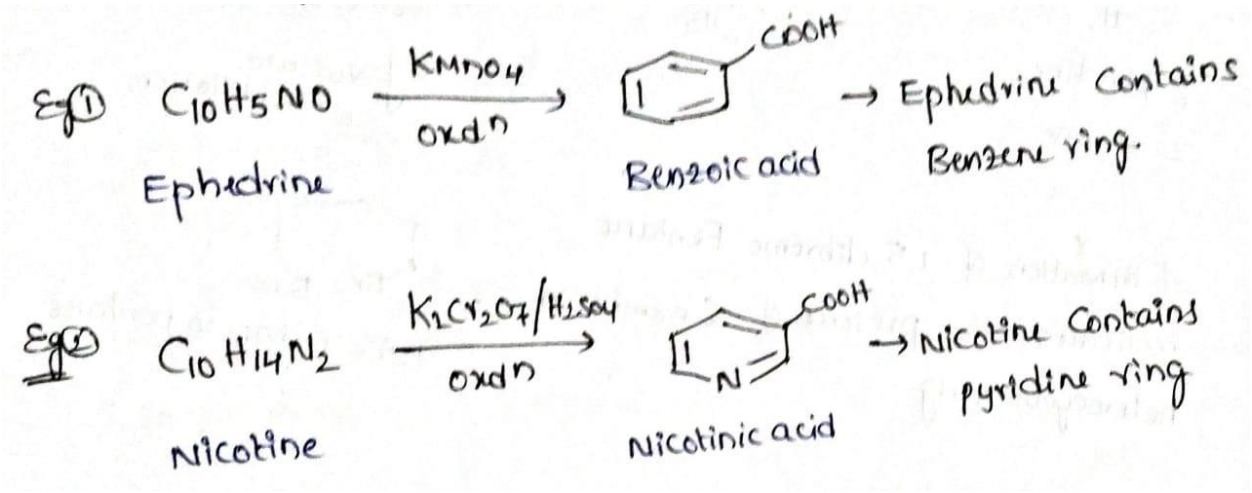


→Formation of Pentane indicates the presence of 6 member heterocyclic ring.

5. Oxidative degradation:

→oxidative degradation is carried out by using different oxidizing agents such of $KMnO_4$, H_2O_2, O_3, CrO_3, Conc-HNO_3 /H_2SO_4 etc.

→ Different products are obtained by varying strength of oxidizing agents.



6. Spectral analysis:

→With the help of spectral analysis Like UV, IR, NMR, CMR, X-ray Mass spectrometry structure of an alkaloid is assigned.

7. Synthesis:

→The proposed structure of an alkaloid is confirmed by synthesis from Known Compound.

❋⁂**Classification of Alkaloids based on nitrogen heterocyclic ring**

On the basis of chemical classification, numerous classes of alkaloids are possible but we shall be mentioning the names of such classes which we are going to discuss in this chapter:

1. Phenyl ethylamine alkaloids
2. Pyrrolidıne alkaloids
3. Pyridine or piperidine alkaloids
4. Pyridine-pyrrolidine alkaloids
5. Tropane alkaloids

6. Quinoline alkaloids

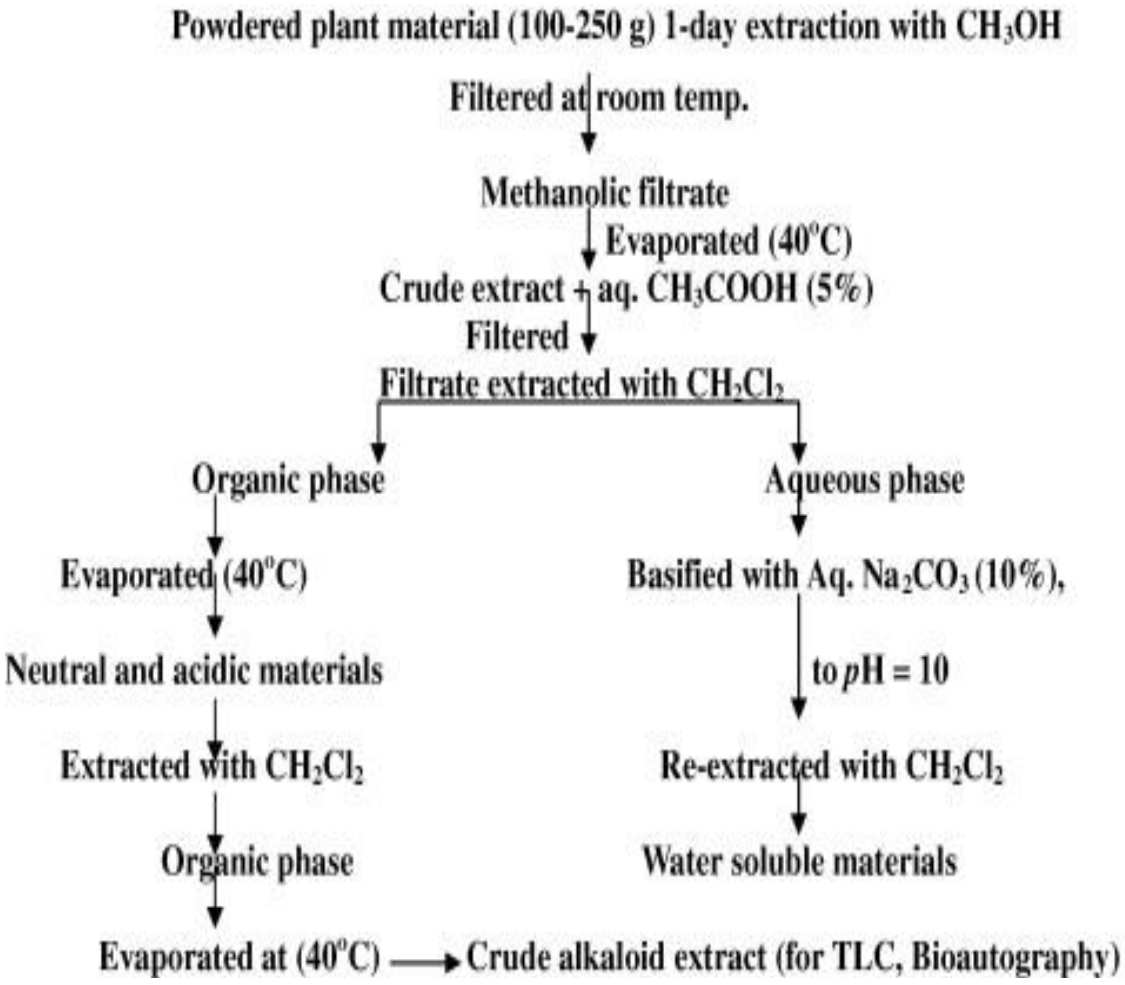
7. Isoquinoline alkaloids

8. Phenanthrene alkaloids

9. Indole alkaloids

10. Tropolone alkaloids

General isolation of alkaloids



1. Explain the structure determination, Synthesis and biosynthesis of Morphine?

→Morphine was the first alkaloid to be isolated from Serturner Plant (1806).

Structure Determination:

→ from The Analytical data Molecular formula of Morphine is $C_{17}H_{19}NO_3$.

→Double Bond Equivalence = 17 – ½ (19) + ½ (1) + 1

=17- 19/2 + 1/2 +1
= 34-19+1+2/2
= 18/2
=9

→Nature of Nitrogen

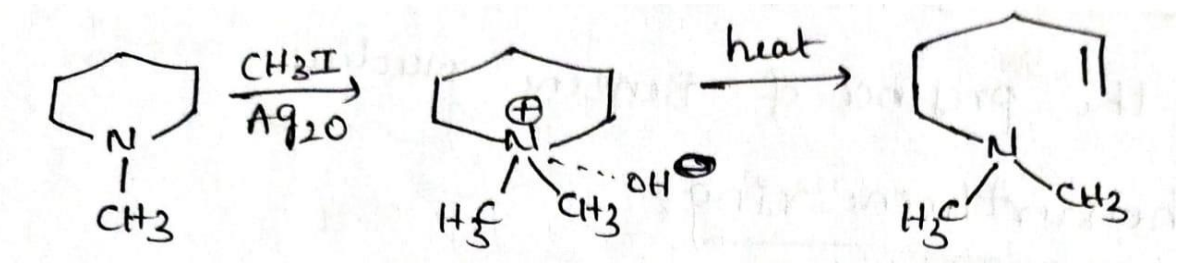
Morphine + CH_3I ----------→ Quaternary Salt
It indicates nitrogen is 3º in Nature.

Herzig-Meyer's S method

Morphine + HI ----------→ CH_3I + $AgNO_3$ --------→ AgI (↓)

It indicates the presence of one – N-CH3 group.

Presence of cyclic 3º base



→ Nature of oxygen atom:

1. Morphine undergoes Acetylation it gives diacetyl morphine.
2. Morphine undergoes Benzylation it gives dibenzyl Morphine.

- It indicates 2 -OH groups present in Morphine

Nature of -OH groups

1. Morphine+ NaOH + Neu. $FeCl_3$ ---------→ Violet coloration

It indicates 1 -OH group is phenolic -OH group.

2. Morphine + CH_3I + KOH --------→ Codeine ------→ Codeinone (Ketone)

It indicates the presence of 2°-OH group.

3. The third oxygen atom does not give any general reactions. So third oxygen presented as cyclic ketone / Cyclic Ether.

→Presence of Ethylenic bond:

Morphine undergoes catalytic hydrogenation (H_2/pd) it takes I mole of H_2.

It indicates the presence Ethylenic bond.

→Presence of Benzene Nucleus:

Morphine undergoes brominating it gives Mono bromo derivative.

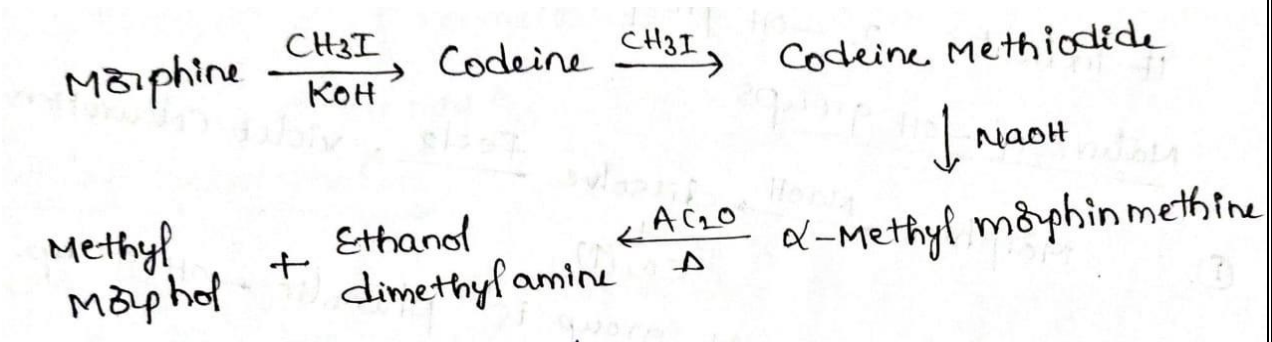
It indicates the presence of Benzene nucleus.

→ Presence of phenanthrene ring:

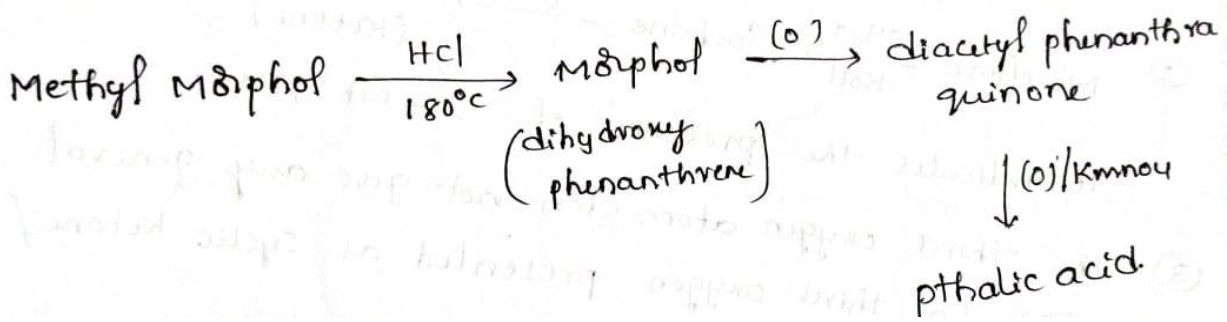
- Morphine undergoes Zn dust distillation it gives phenanthrene derivative.

So it indicated the presence of phenanthrene ring.

- Phenanthrene ring can be explained on the following reaction.



Structure of Methyl Morphol :

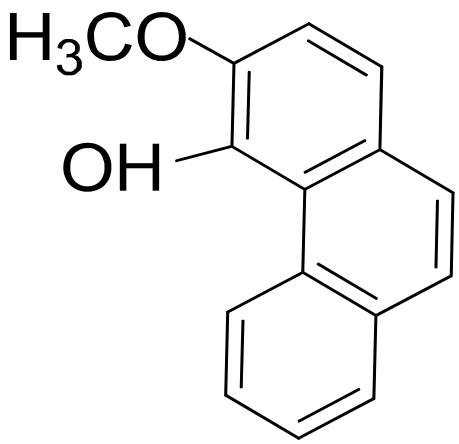


→ The formation of diacetyl phenanthra quinone indicates 9, 10 positions in phenanthrere ring are free.

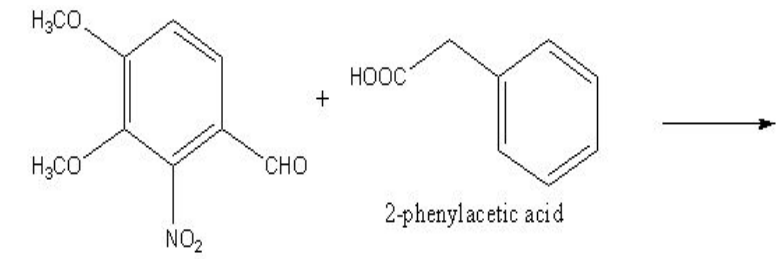
→ The formation of pthalic acid indicates that 2-OH groups are present in same ring ortho to each other.

From the above information the structure of methyl morphol is

From the above information the structure of methyl morphol is confirmed by its synthesis.

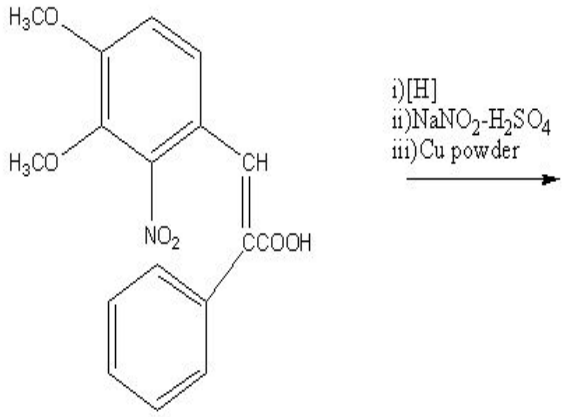


Methyl morphol further confirmed by:-

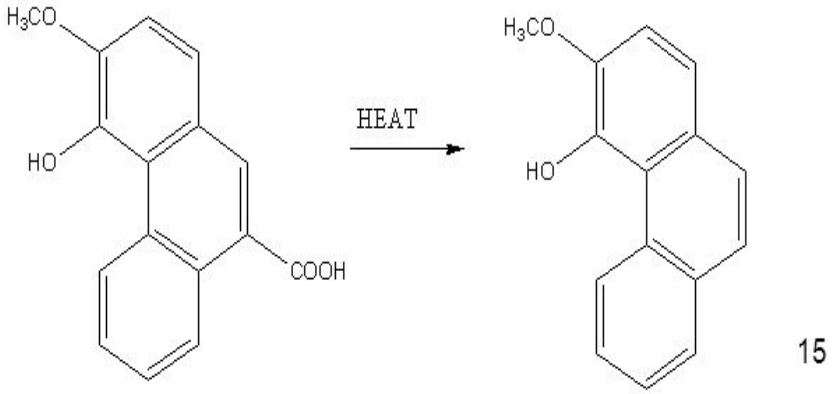


3,4-dimethoxy-2-nitrobenzaldehyde

2-phenylacetic acid



3,4-dimethoxy-2-nitro pheny cinnamic acid



15

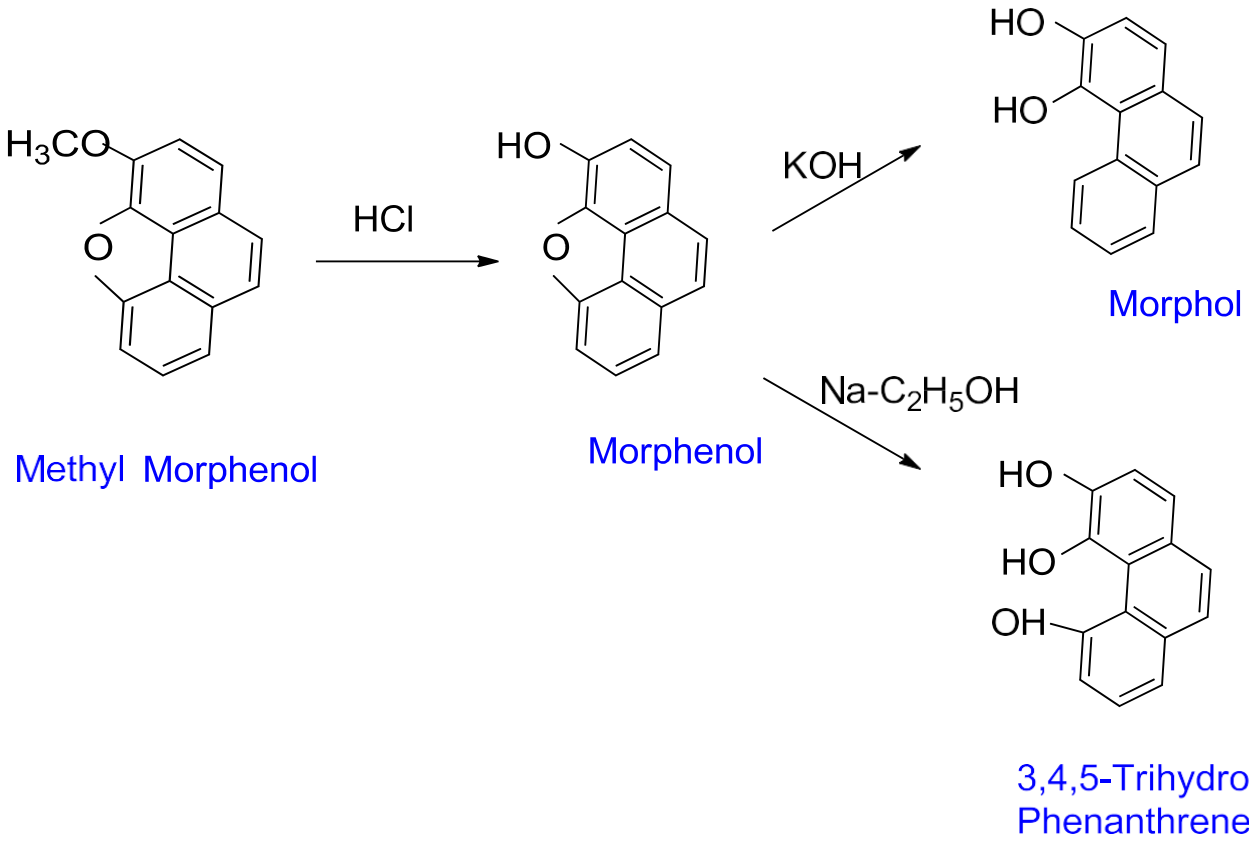
METHYL MORPHOL

Position of Oxygens:

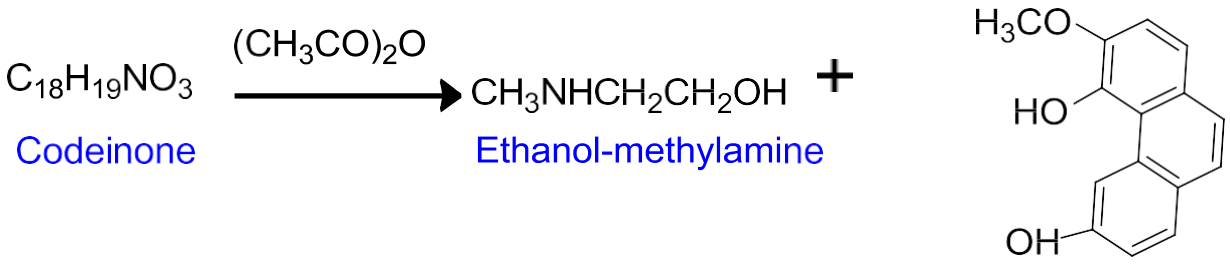
→ the position of two oxygen atoms can be detected by elucidating the structure of morphenol.

Structure of Morphenol:

β–methylmorphimethine → Trimethyl amine + Methylmorphenol. + Ethylene

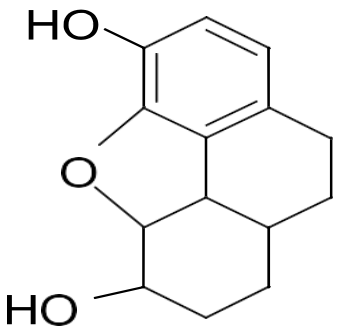


→From the structure of morphenol the position of two oxygen atoms in morphine can be obtained i.e. one at C_3 and other oxygen present as ether in between C_4 and C_5 positions of the phenanthrene nucleus.

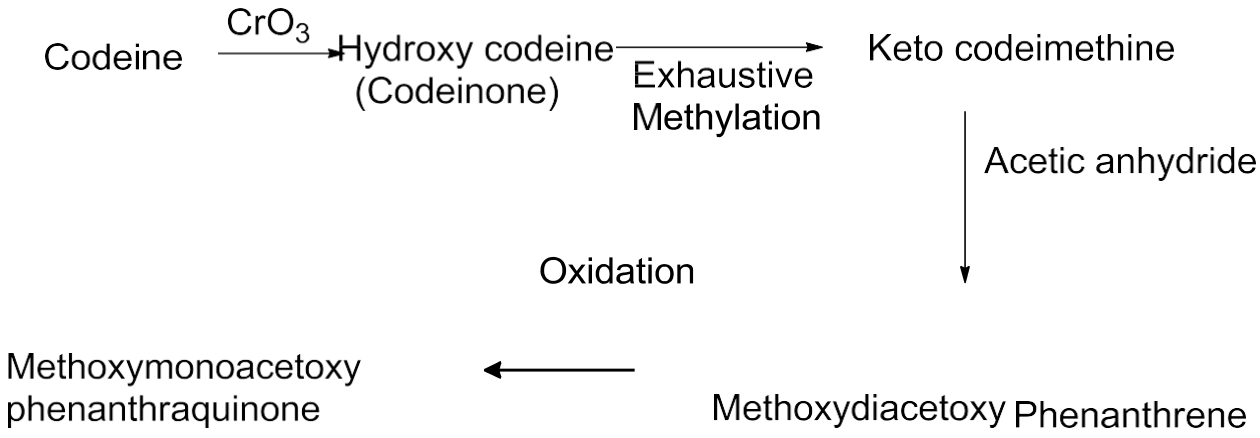


→From the above reaction the third oxygen atom present at C_6 position of phenanthrene nucleus.

→from all of the above information the partial structure of morphine is



Point attachment of Nitrogen atom:



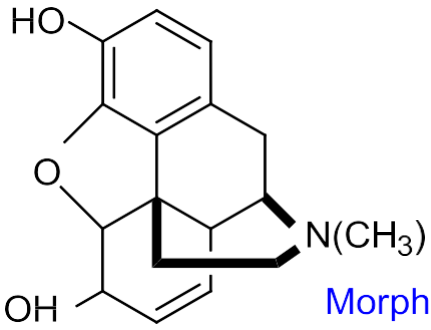
→ From the above degradation reaction the N-atom is attached at C_9 or C_{10}. This is confirmed by synthesis of morphine.

Position of Double bond:

Codeine on heating with PCl_5 yields chlorocodide which on hydrolysis gives codeine, isocodenie, pseudo codeine and allopseudocodeine. The first two compound on oxidation 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

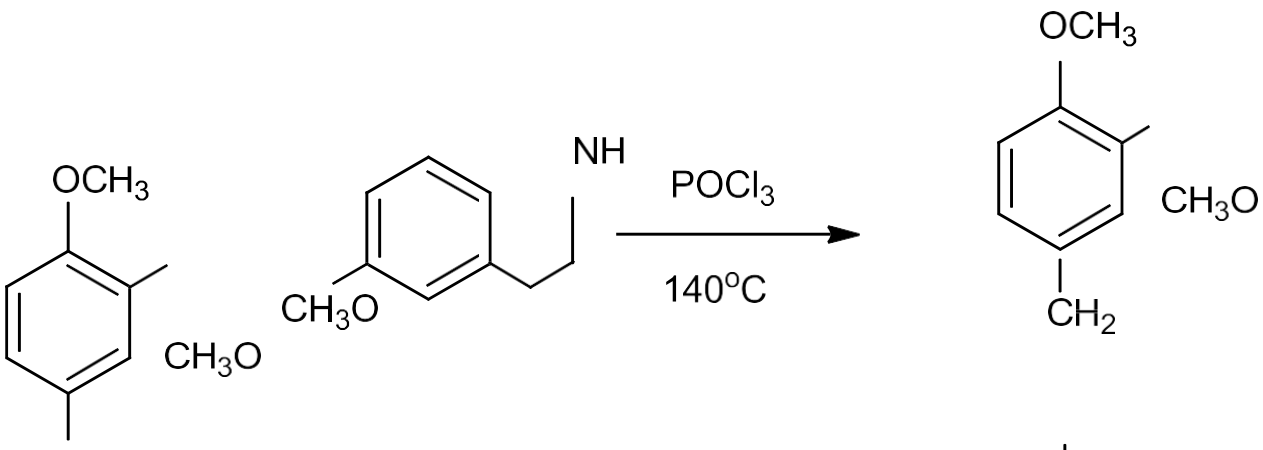
that the hydroxyl group is at position C8. These changes can be explained only if a double bond is present at C7 and C8.

From all of the above information the structure of Morphine is

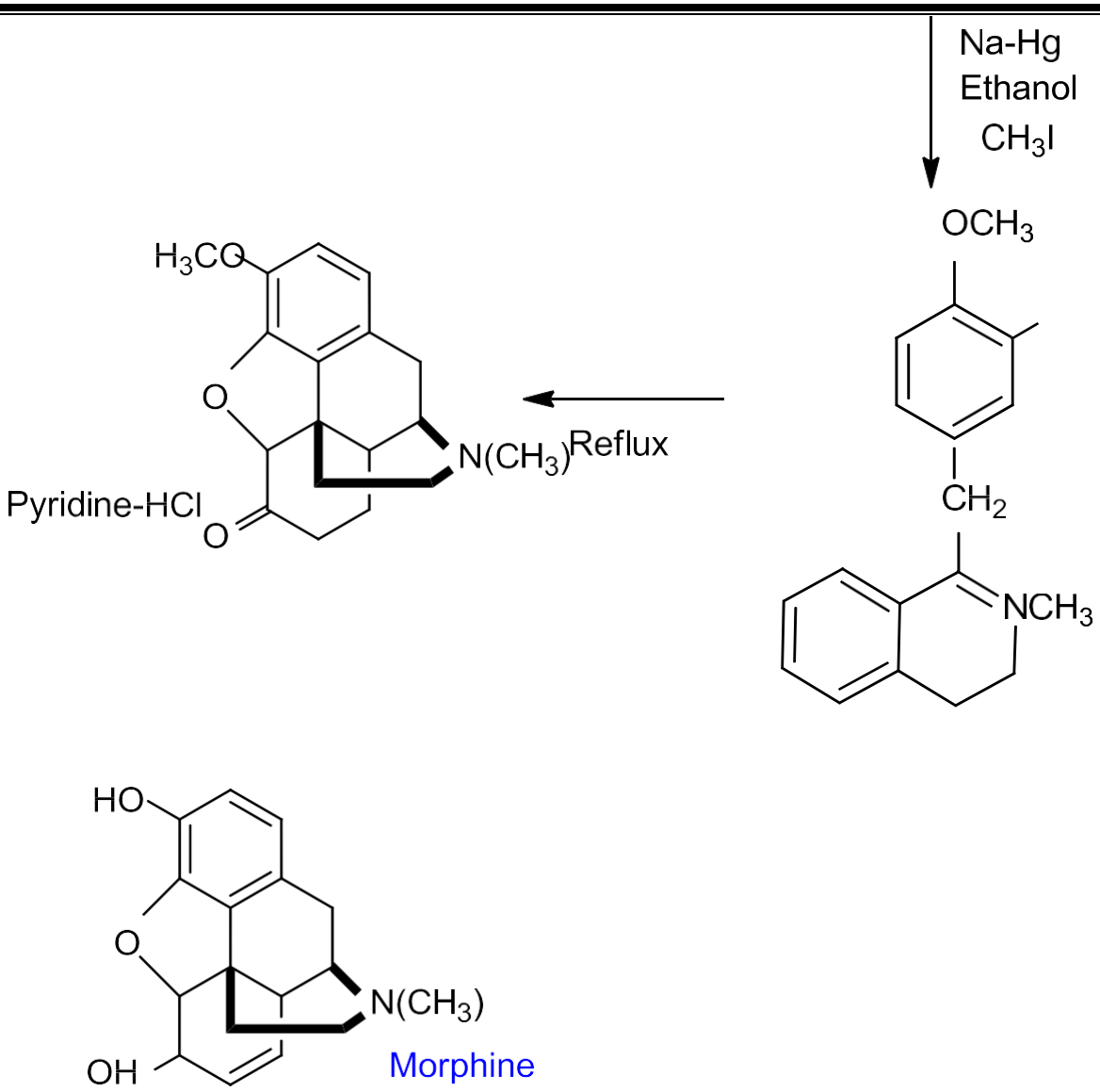


Morphine

Synthesis of Morphine:



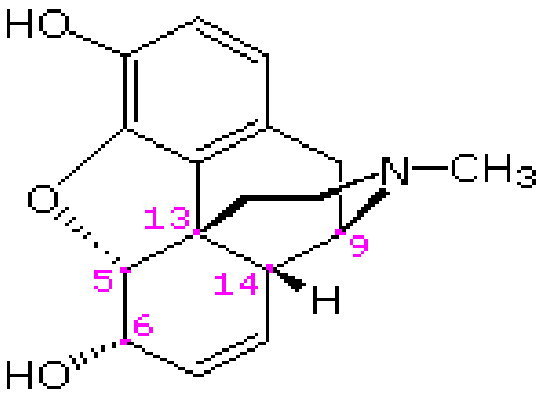
3,4-dimethoxy
Phenylacetic acid



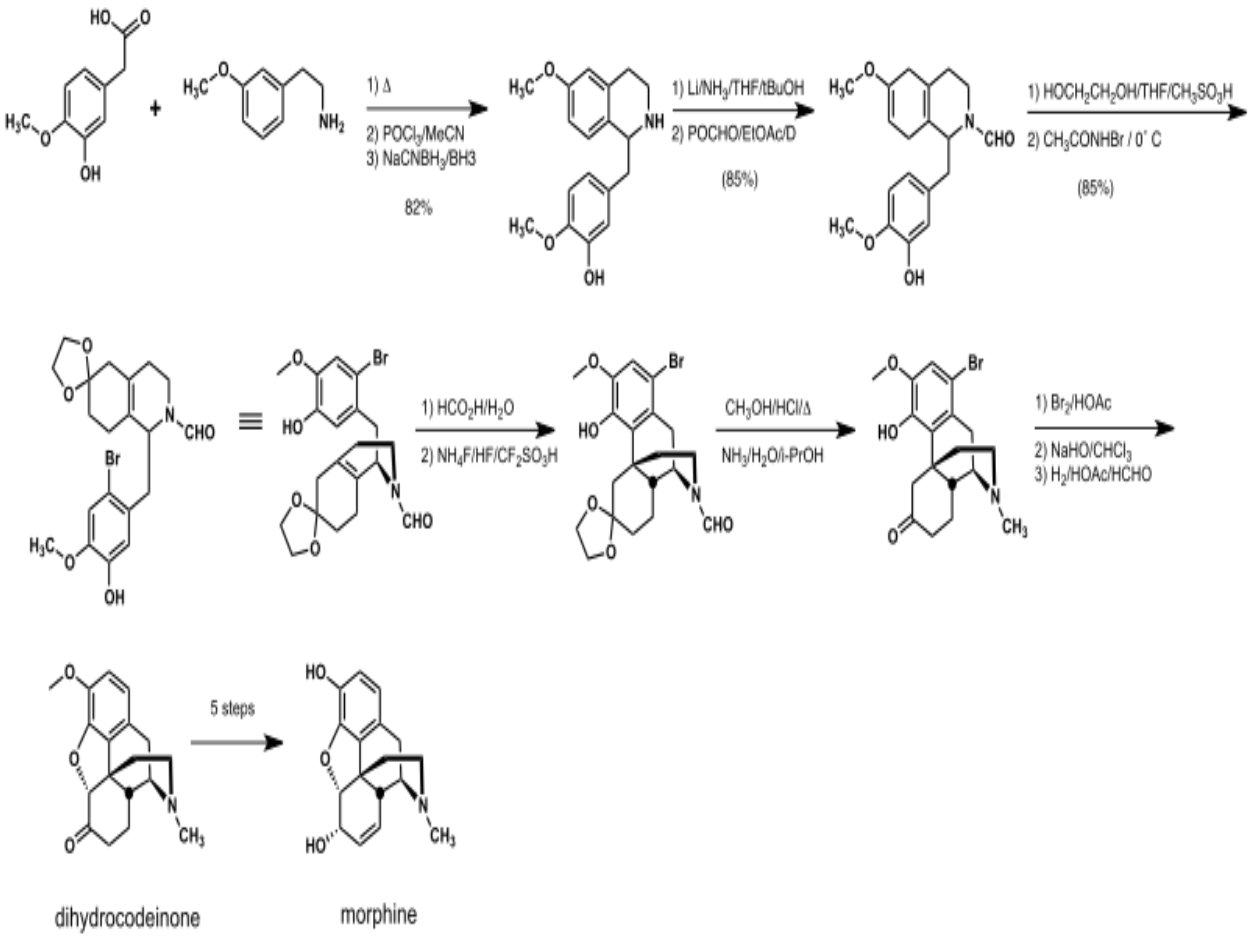
Stereochemistry of Morphine:

→ Morphine has five asymmetric carbon atoms: C $_5$, C $_6$, C $_9$, C $_{13}$, and C $_{14}$.

→ Emde concluded that three of the five asymmetric carbon atoms (C $_5$, C $_6$, C $_9$) rotate the plane of polarized light to the left (-) and the remaining two (C $_{13}$, C $_{14}$) do so to the right (+).



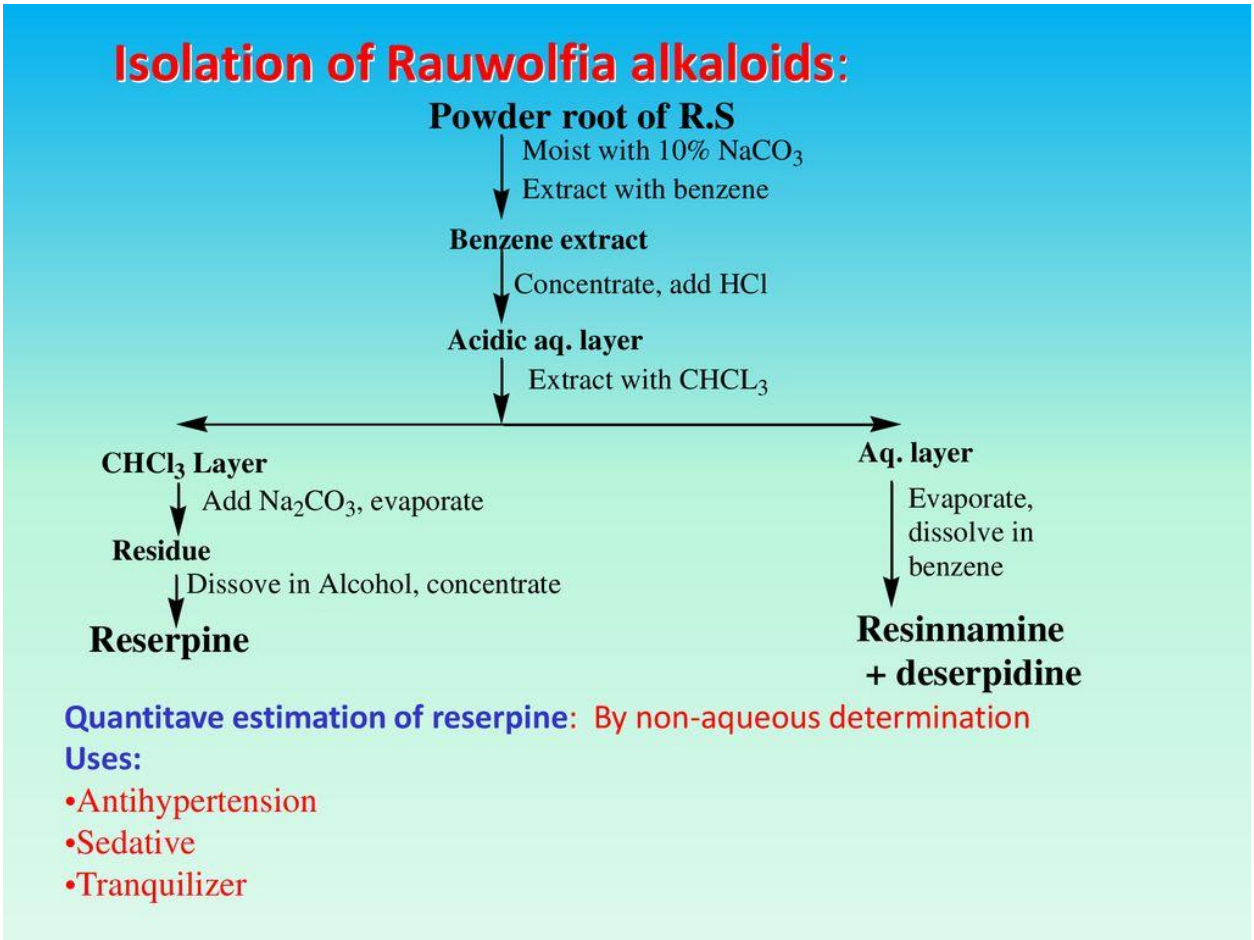
Bio synthesis of Morphine:



2. Explain the Structure Determination, Synthesis and Bio synthesis of Reserpine?

→Reserpine is the main constituent of Rauwolfia species.

→Reserpine is mainly used for the treatment of hypertension, headache, asthma and dermatological disorders.



Structure Determination:

→Molecular formula of Reserpine 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.

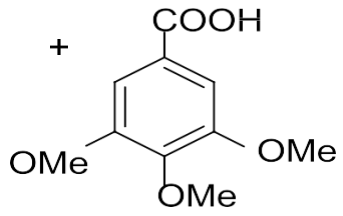
→**Nature of N atom:** It is a weak base indicating both the nitrogen is present in the ring. It doesn't have a hydroxyl group but forms monoacetyl derivative indicating one of the nitrogen as NH group. Other nitrogen is an 3^0 N.

→**Hydrolysis:**When reserpine is hydrolysed with alkali solution it yields a mixture of methyl alcohol, 3,4,5 trimethoxy benzoic acid and reserpic acid

$$C_{33}H_{40}N_2O_9 + 2H_2O \xrightarrow{NaOH} CH_3OH + C_{22}H_{28}N_2O_5$$

($C_{22}H_{28}N_2O_5$).

Reserpic acid



3,4,5- Trimethoxy benzoic acid

→ The two alcoholic groups (one in CH_3OH and another in Reserpic acid) in its hydrolysis products reveals that Reserpine is 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.

→ In order to explain the structure of Reserpine, we must know the structure of Reserpic acid.

Structure of Reserpic Acid:

→ Molecular Formula of Reserpic acid 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.

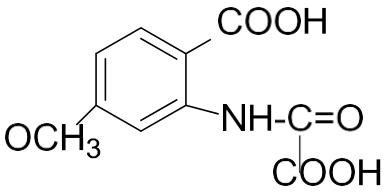
$C_{19}H_{29}N_2$ { 2 OCH_3, 1 -COOH, 1-OH }

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

$C_{15}H_{20}N_2$ { 2 OCH_3, 1 $-CH_2OH$, 1-OH }

→**Oxidation of reserpic acid:**

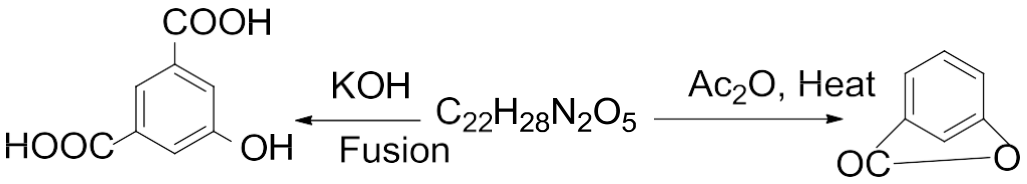
On oxidation with $KMnO_4$ it yields 4-methoxy N-oxalyl anthranilic acid as one of the oxidation products, confirming the presence of one indole nucleus in reserpic acid. Moreover, it reveals that one of the methoxy groups is in m-position to NH group.

$C_{22}H_{28}N_2O_5$ $\xrightarrow[\text{Oxidation}]{KMnO_4}$ 

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.

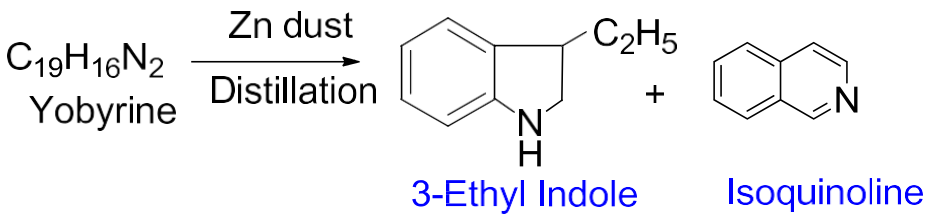


γ-lactone

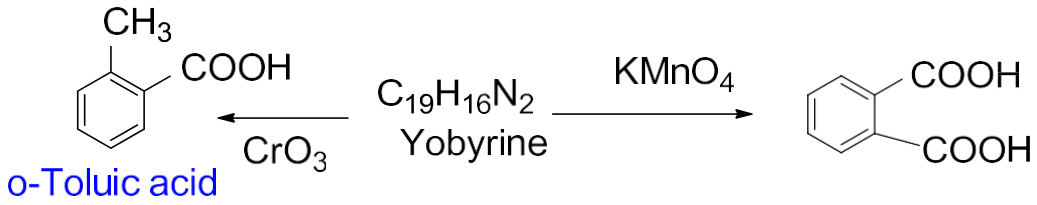
→**Dehydrogenation:** when methyl reserpate is dehydrogenated with Se it yields a hydrocarbon with $C_{19}H_{16}N_2$.This hydrocarbon is obtained by dehydogenation of yohimbine with Se hence called as Yobyrine.

Structure of Yobyrine:

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

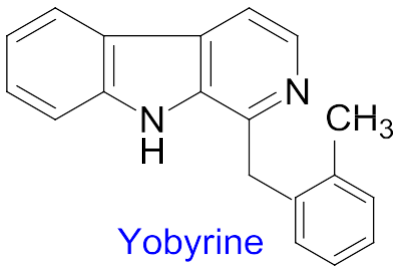


→ When yobyrine is oxidised with $KMnO_4$, it yields phthalic acid. On oxidation with CrO_3 it yields o-toluic acid.

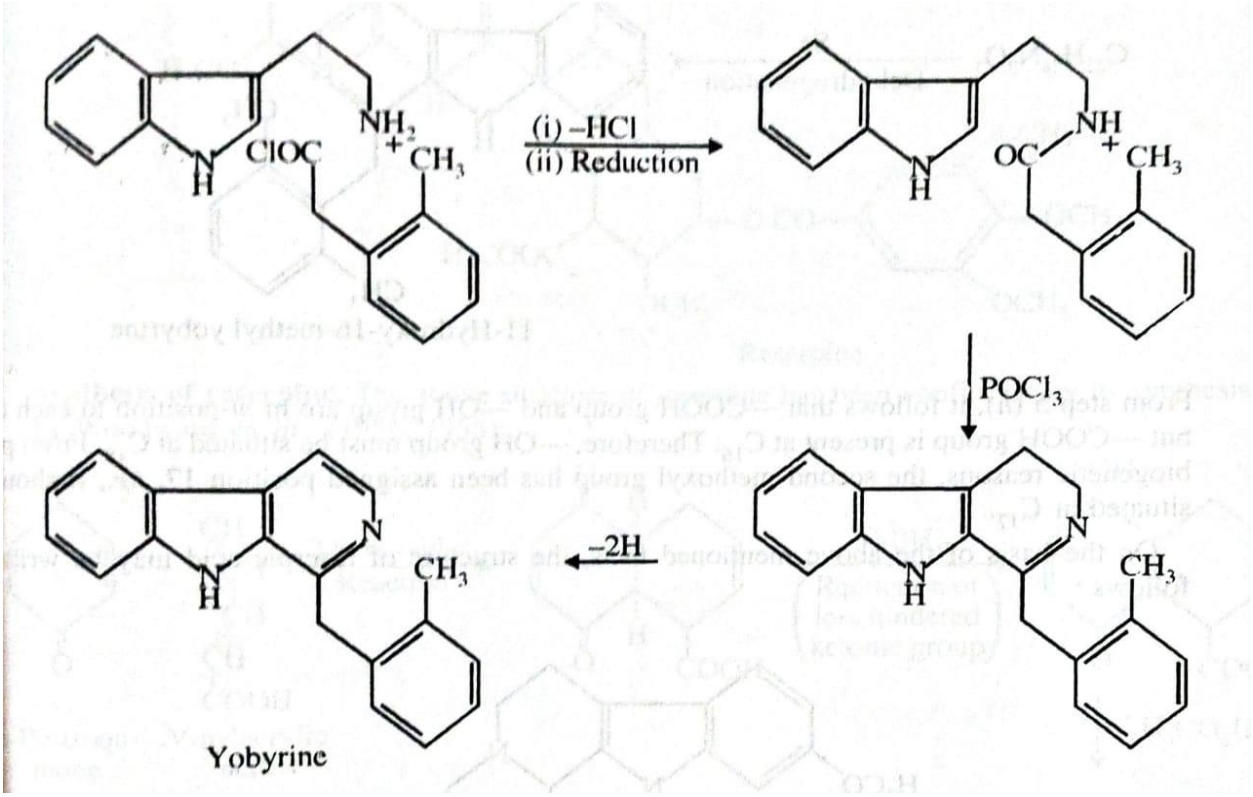


→Yobyrine gives condensation products with aldeydes indicating the presence of pyridine ring with a $–CH_2$ substituent adjacent to nitrogen atom.

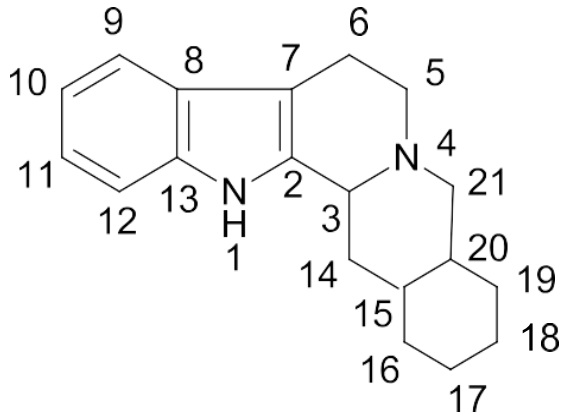
Thus the structure of yobyrine is



→ The structure of yobyrine is confirmed by its synthesis.

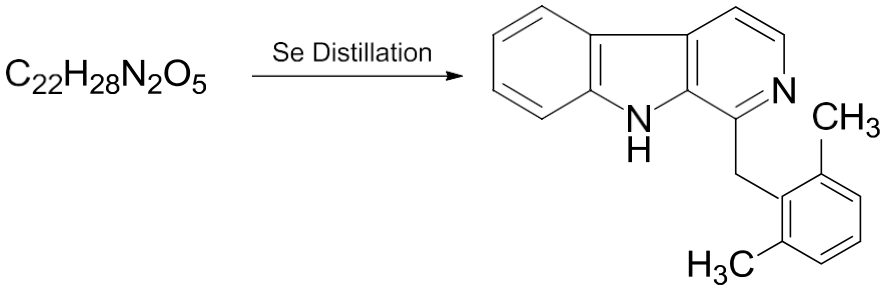


→ From the above information the partial structure of resrpic acid is



→ **Position:**

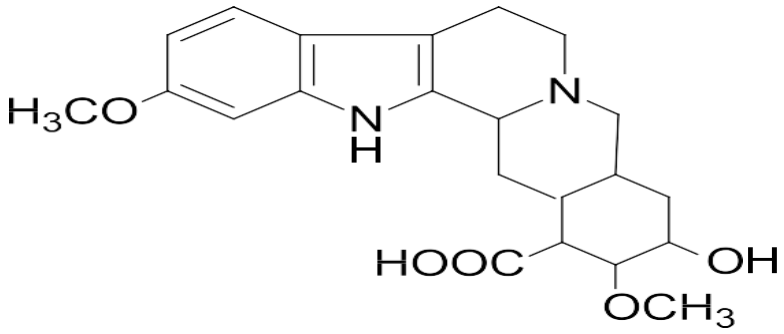
→Reserpic acid on dehydrogeation yields 11-hydroxy 16-methyl yobyrine indicating that – COOH group is present at C_{16}. Further, one of the methoxy groups is present in the m-position to the NH group of the indole.



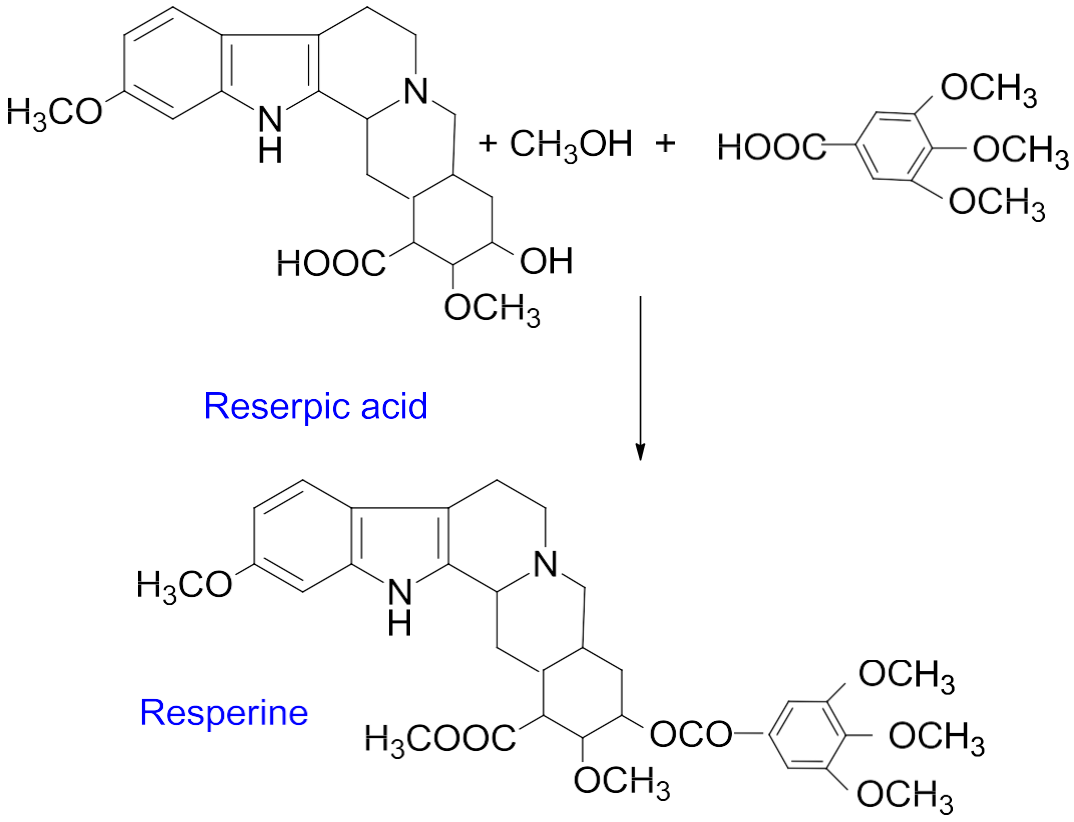
11-Hydroxy 16-methyl yobyrine

→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}.

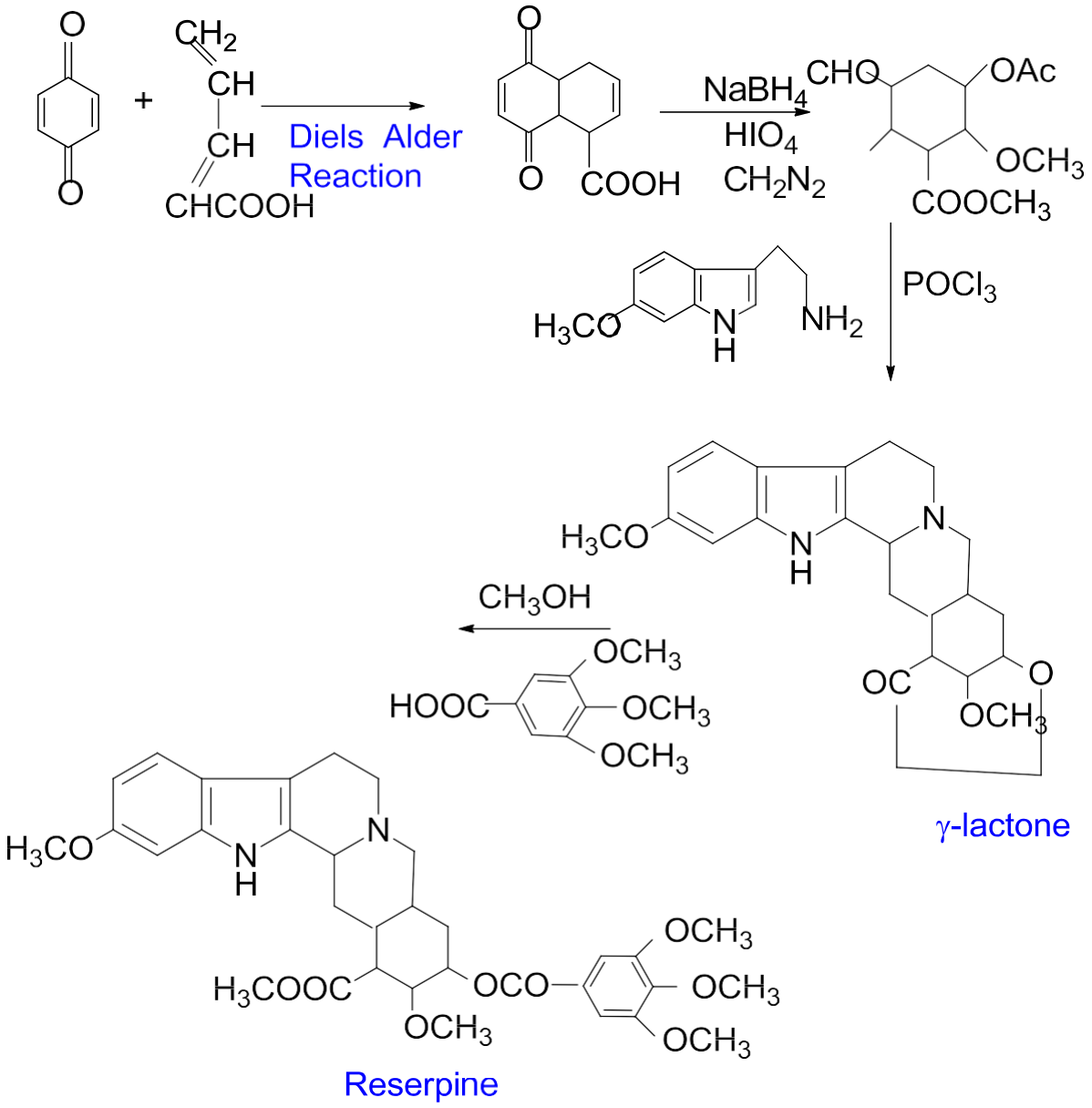
→From all of the above information the structure of Reserpic acid is



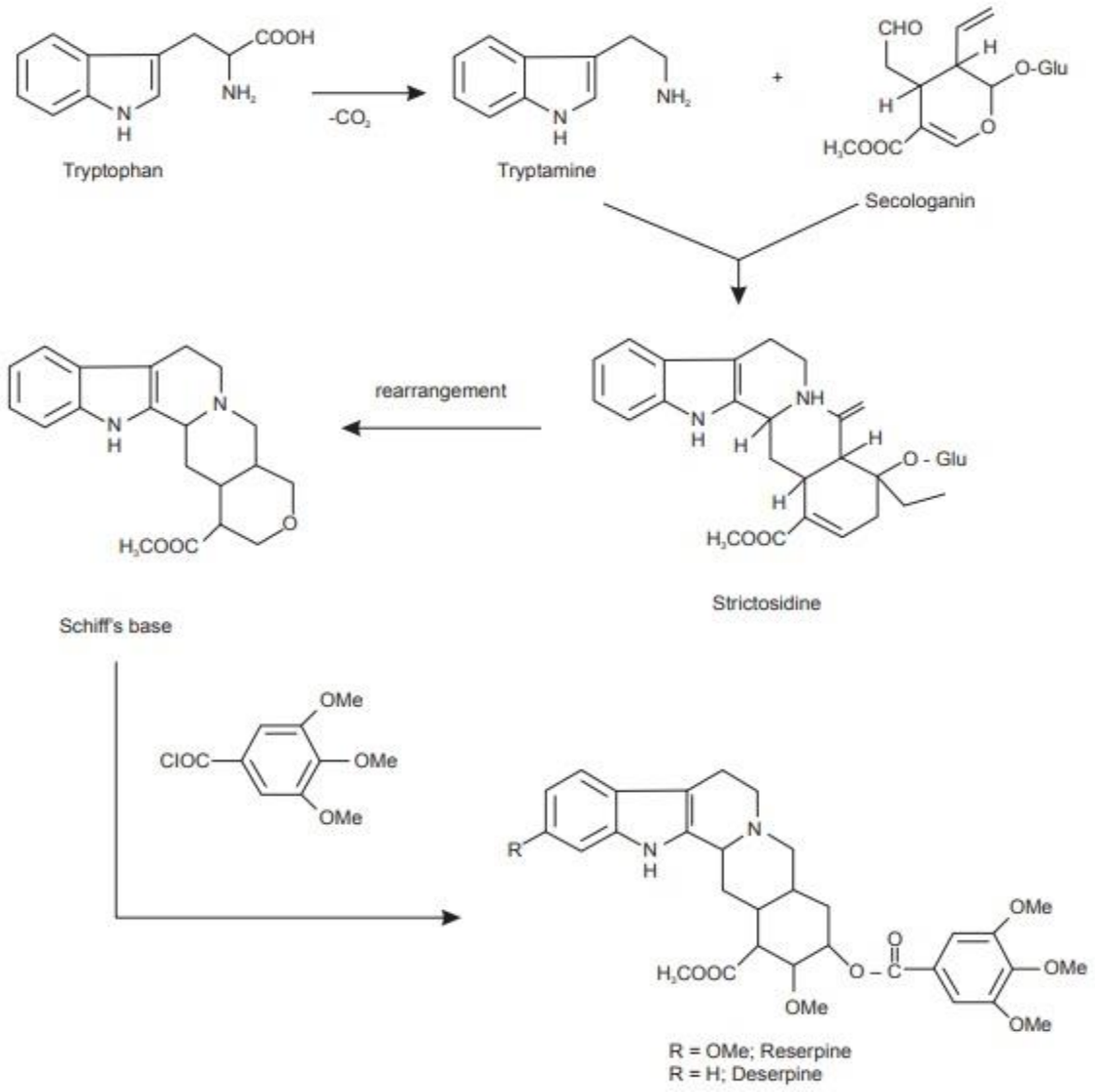
Structure of Reserpine:



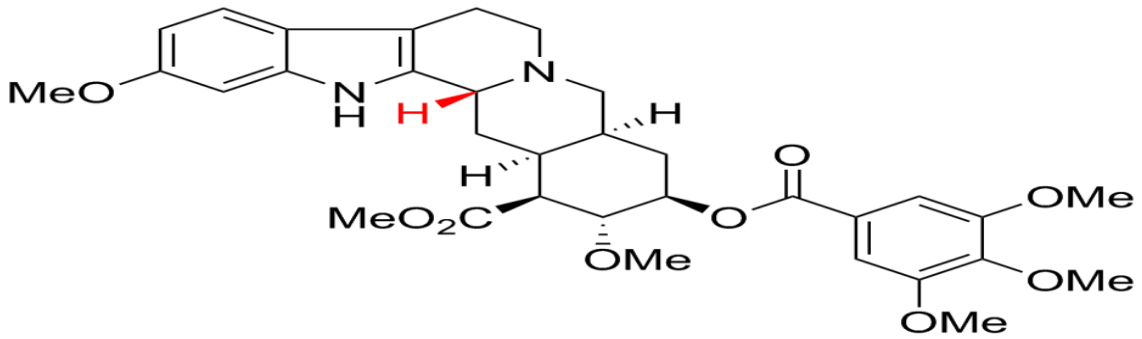
Synthesis of Reserpine:



Bio Synthesis of Reserpine:



Stereo chemical Structure of Reserpine:



Reserpine

3. Explain Structure Determination, Synthesis and Bio synthesis of Strychnine

Structure Determination:

→ From the analytical data molecular formula of strychnine is $C_{21}H_{22}N_2O_2$.

→ $D.B.E = 21 - \frac{22}{2} + \frac{2}{2} + 1$

= 21- 11 + 1 + 1

= 12

→ **Nature of Nitrogen atom:**

→ Strychnine is treated with CH_3I to give Quaternary salt.

It indicates the presence of one 3^0 – N atom.

→ Strychnine + KOH + Hyd ----------→ Strychnic acid + Carboxyl group

It indicates the presence of - N – CO – type linkage.

→ Strychnine + HNO_2 ----------→ Nitroso compound

It indicates the presence of 2^0 – Amino group.

→From the above reactions strychnine contains amide linkage.

→**Nature of Oxygen:**

→From the formation of strychnic acid one of the oxygen atom present in amide linkage.

→The second oxygen does not give any reactions. So 2^{nd} oxygen may be present in ether linkage.

→Strychnine + HI -------→ deoxy strychnine + Hyd --------→ deoxy strychnic acid

→From the above reaction proved that 2^{nd} oxygen must be present in ether linkage.

→**Presence of double bond:**

→Strychnine undergoes catalytic reduction gives dihydro strychnine.

It indicates the presence of one double bond.

→Presence of keto group:

→Strychnine undergoes electrolytic reduction gives strychnidine.

→Now strychnidine undergoes catalytic reduction gives dihydro strychnidine.

It indicates the presence of keto group.

→Presence of – N-CO- CH_2- group:

→Strychnine undergoes condensation with C_6H_5CHO gives benzylidene derivative.

It indicates the presence of reactive methylene (-CH_2-CO-) group.

→But strychnine also having Amide linkage (- N-C=O).

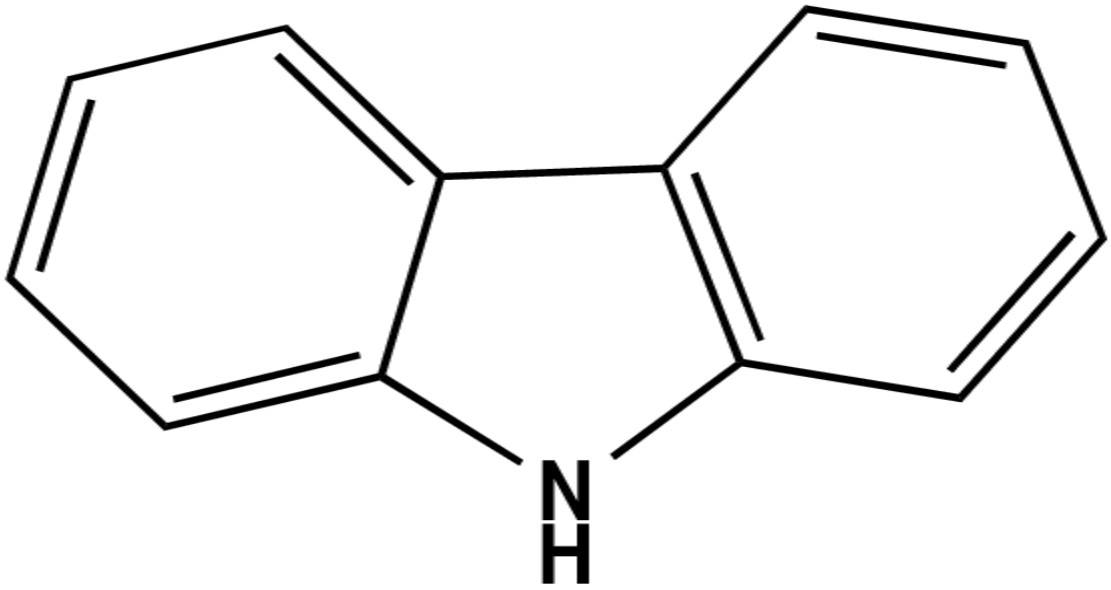
→Therefore strychnine contains **– N-CO- CH_2- group.**

→Presence of Carbazole Nucleus:

1 .Strychnine is treated with $K_2Cr_2O_7$ and 80% H_2SO_4 gives deep purple color.

2. Hydrogenated Carbazole is treated with $K_2Cr_2O_7$ and 80% H_2SO_4 gives deep purple color.

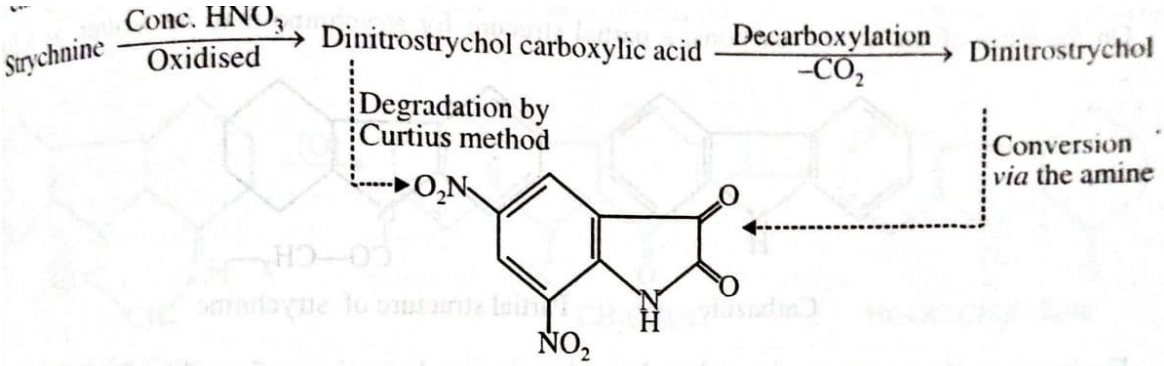
→The formation of deep purple color in both reactions indicates that strychnine having carbazole nucleus.



→Strychnine + lime distillation ----------→ Indole + Carbazole

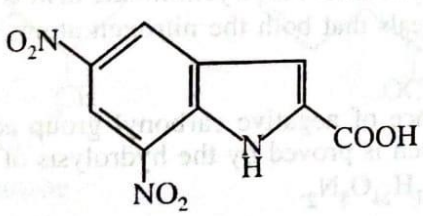
→From the above reaction strychnine does not have ether linkage.

→Presence of Indole Nucleus:

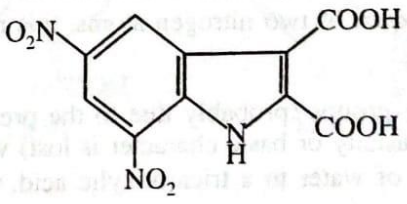


Dinitroisatin

On the basis of the above facts, dintrostrychol and dinitrostychol carboxylic acid may be assigned structures I and II respectively.



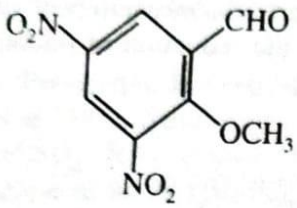
(I)



(II)

→The formation of dinitroisatin indicates the presence of indole nucleus.

The above structures I and II are confirmed by the synthesis of dinitrostrychol.

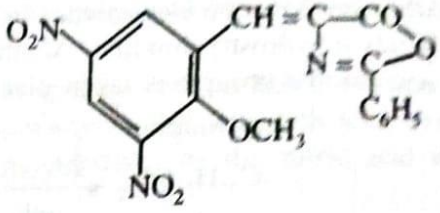


Hippuric acid

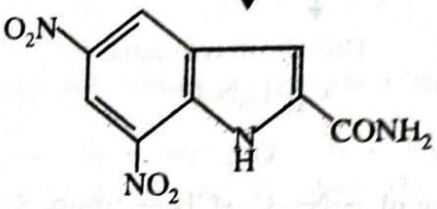
$C_6H_5C = N.CH_2.COOH$

OH

(Enolic form of hippuric acid)

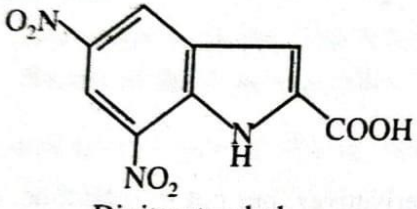


C_2H_5OH | NH_3



HCl

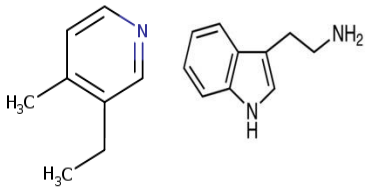
Hydrolysis



Dinitrostrychol

→Position of 3^0- N atom:

Strychnine + soda lime (dst) ----→ Indole + Carbazole + β-collidine + Tryptamine

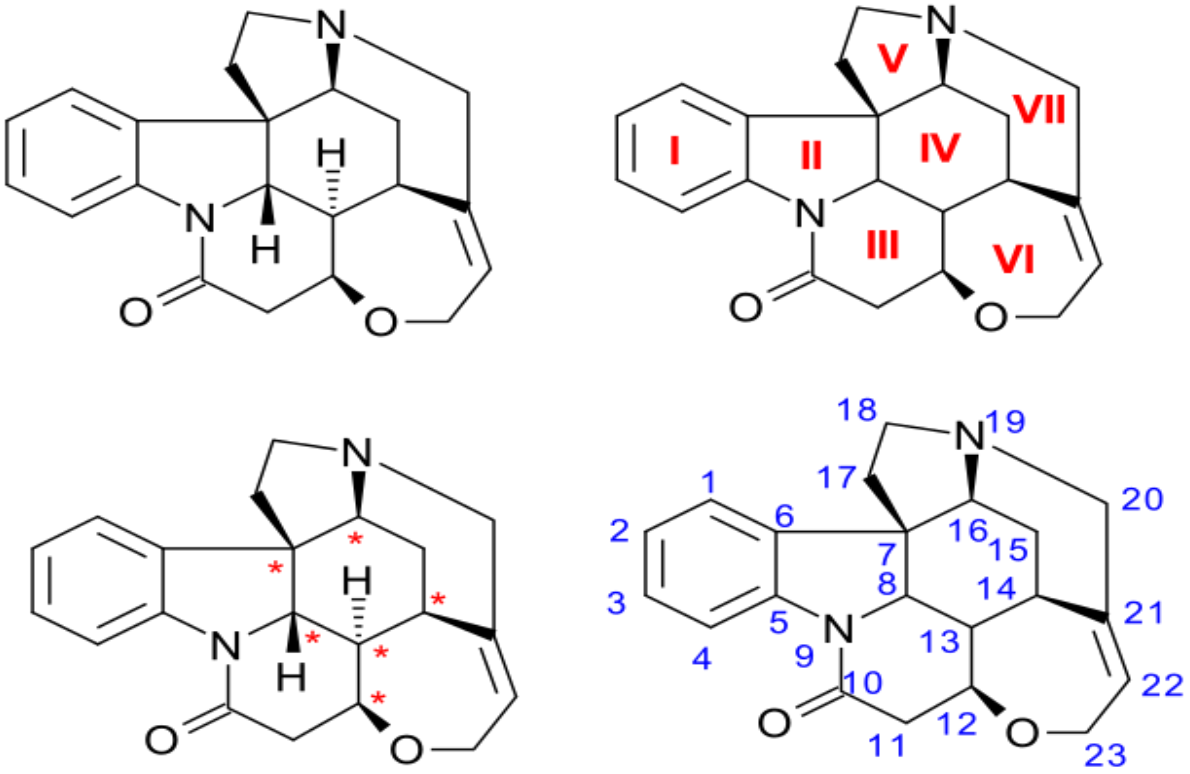


→The formation of β-collidine indicates the presence of 3, 4- disubstituted pyridine ring and amide type of Nitrogen is present in the indole nucleus.

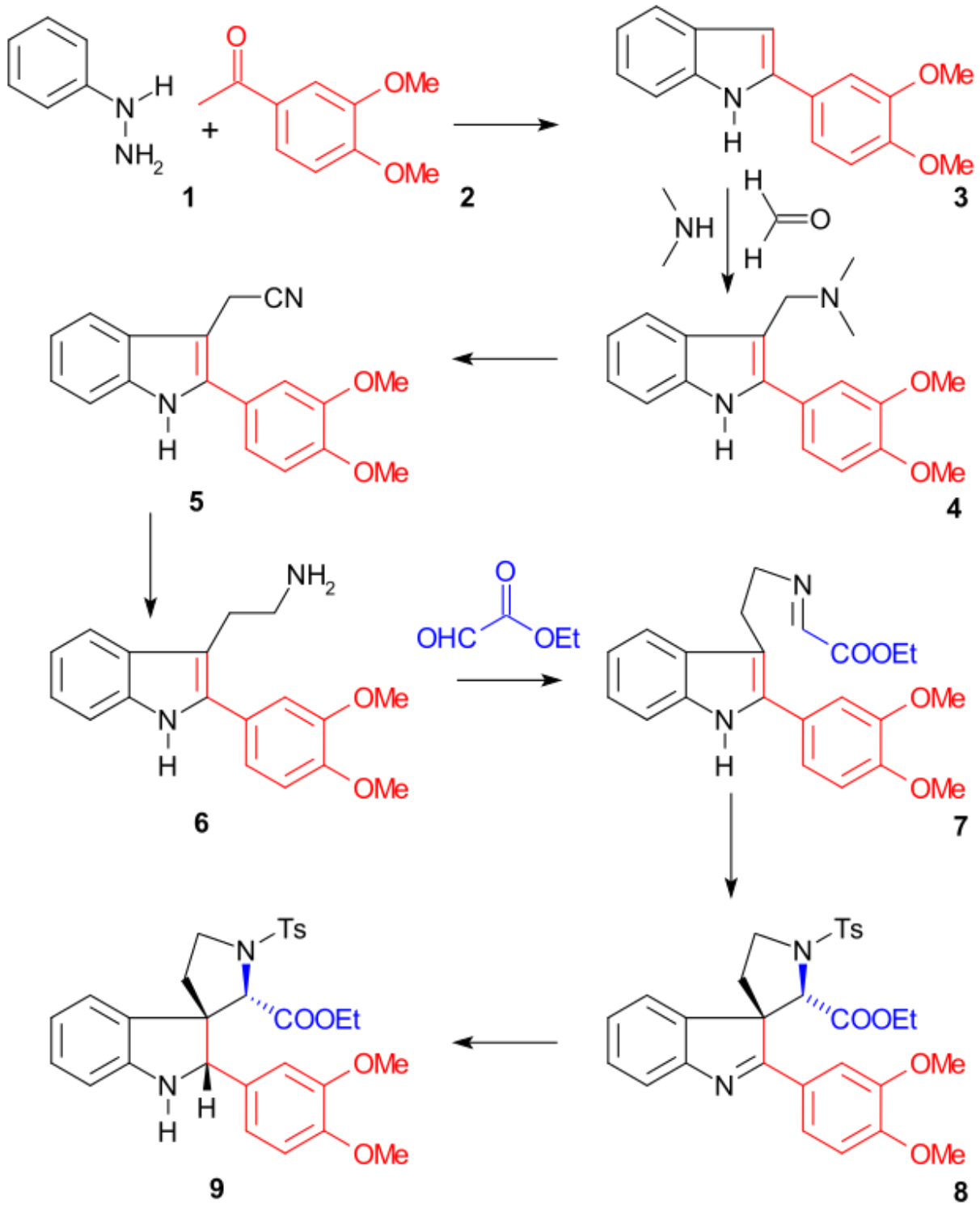
It means 3^0 basic Nitrogen present in the reduced pyridine nucleus.

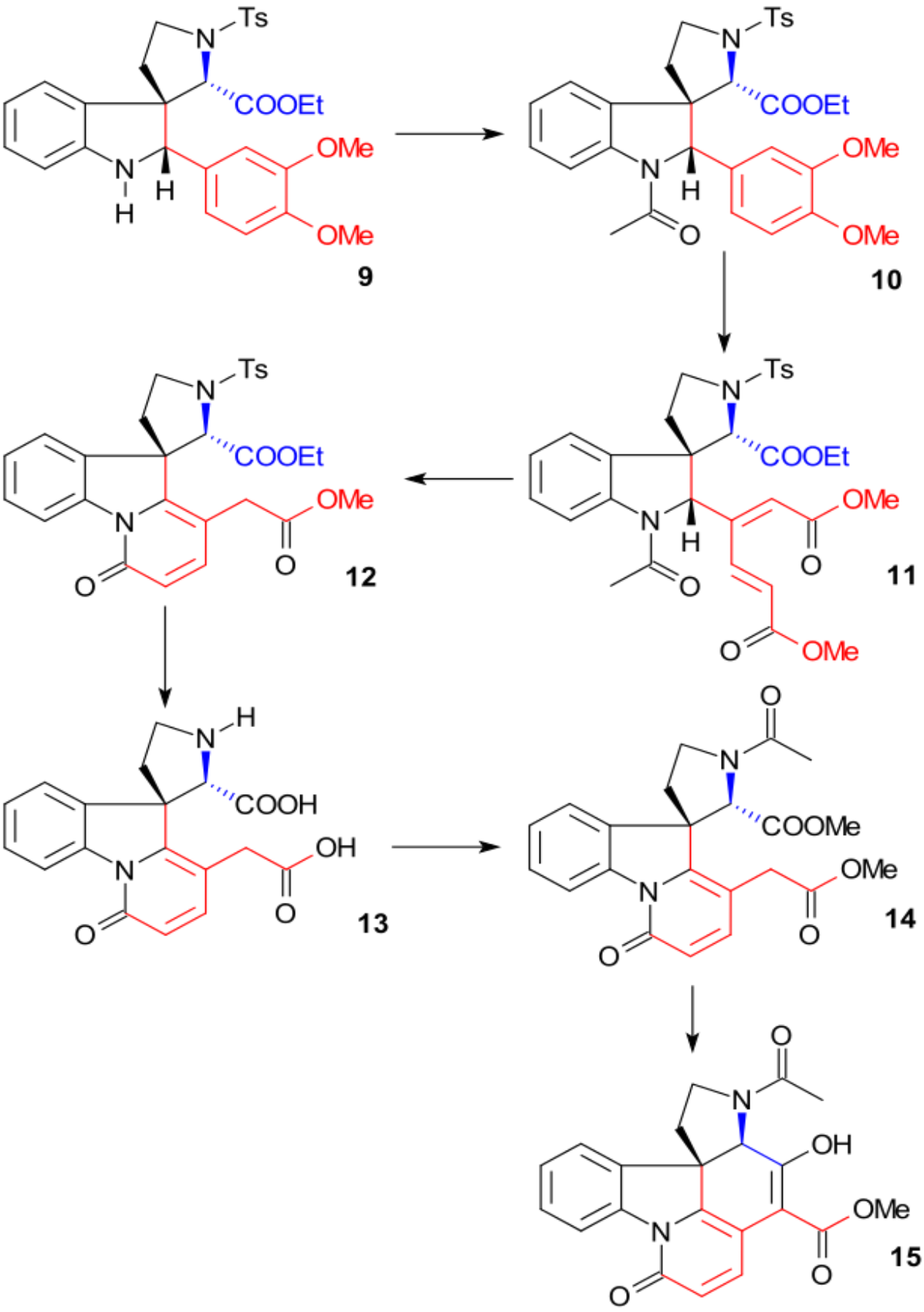
→The formation of Tryptamine indicates the remaining two carbon atoms are linked to β-position of indole nucleus.

→From the above information the strcture of strychnine is



Synthesis of Strychnine:

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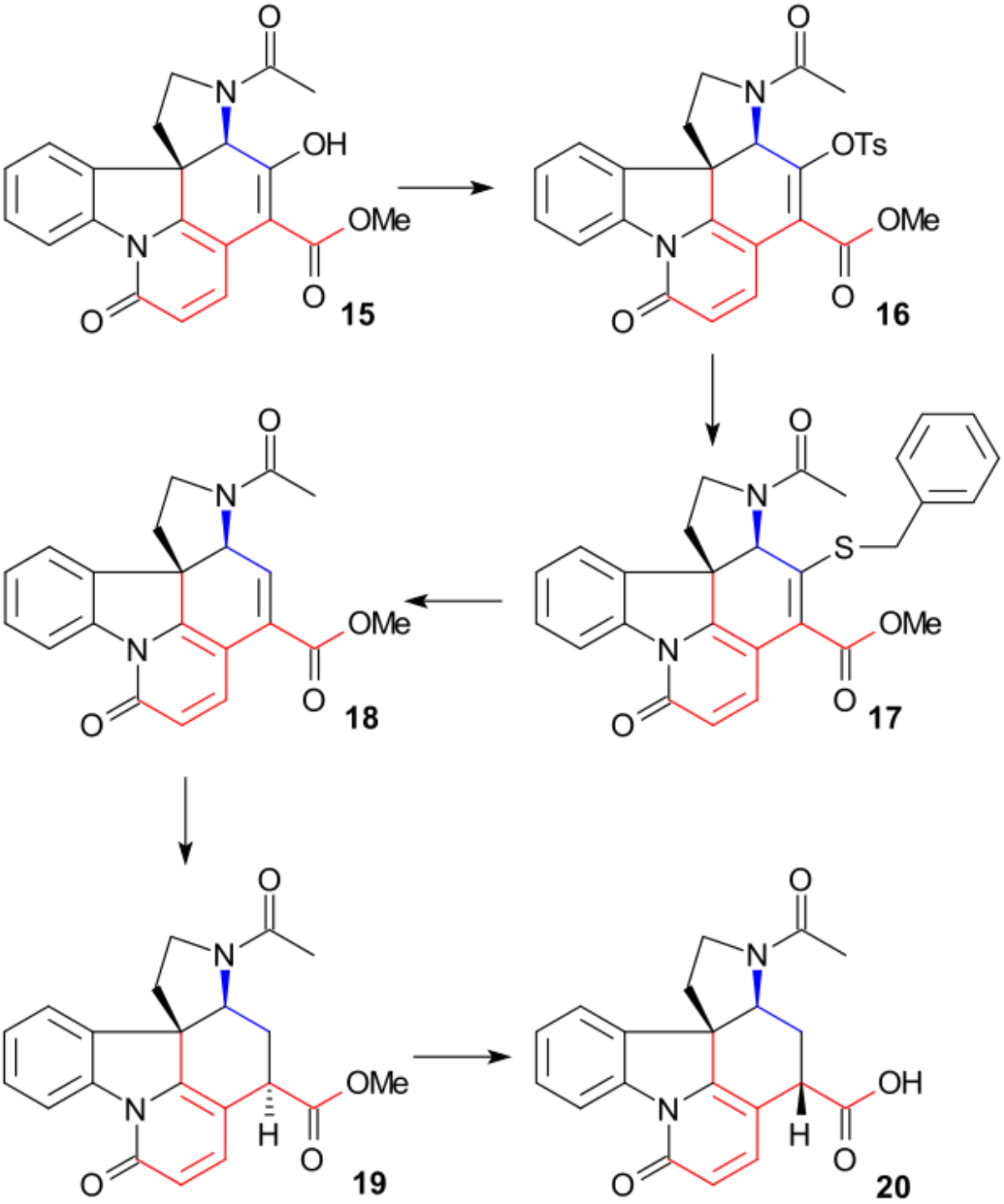
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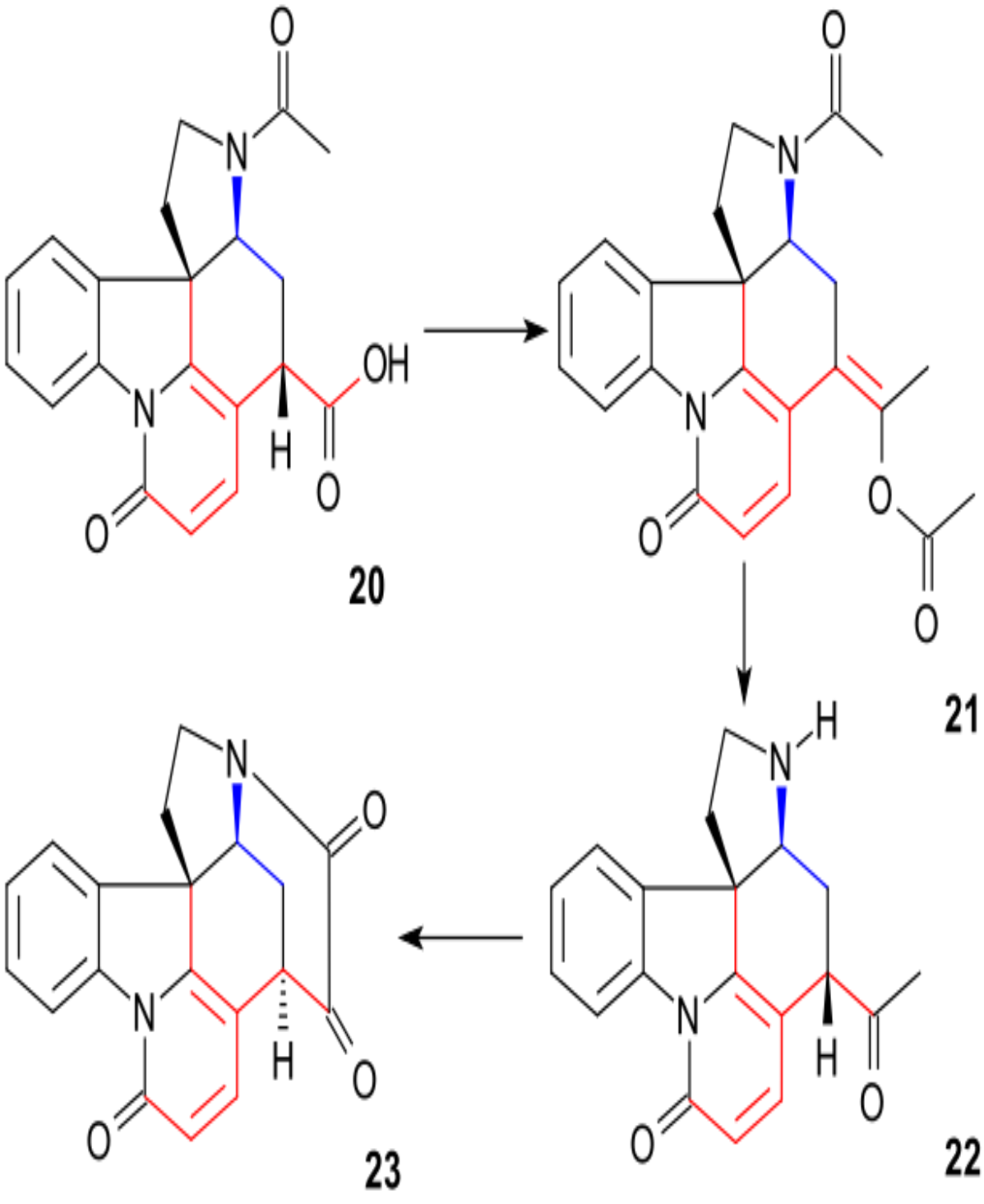
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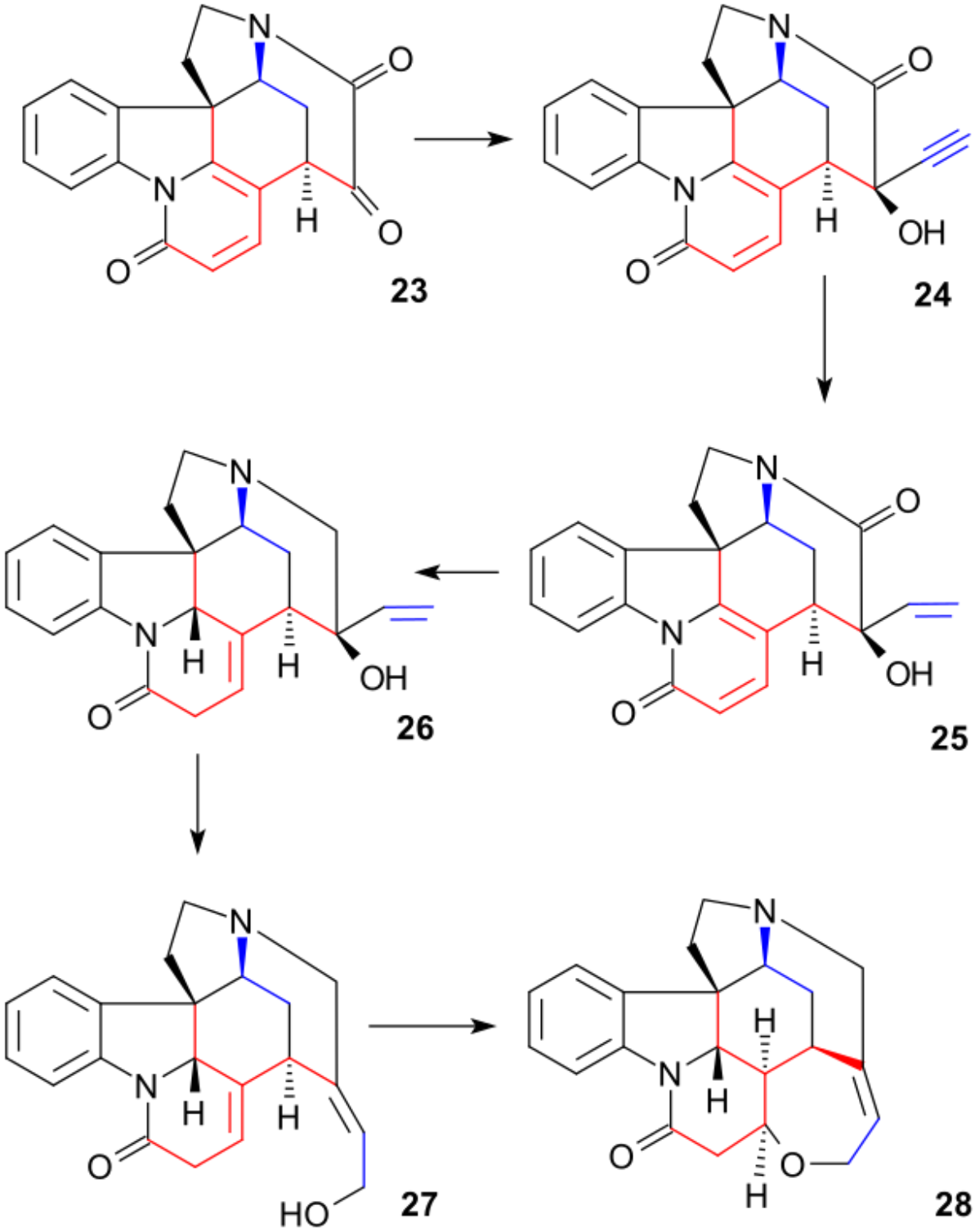
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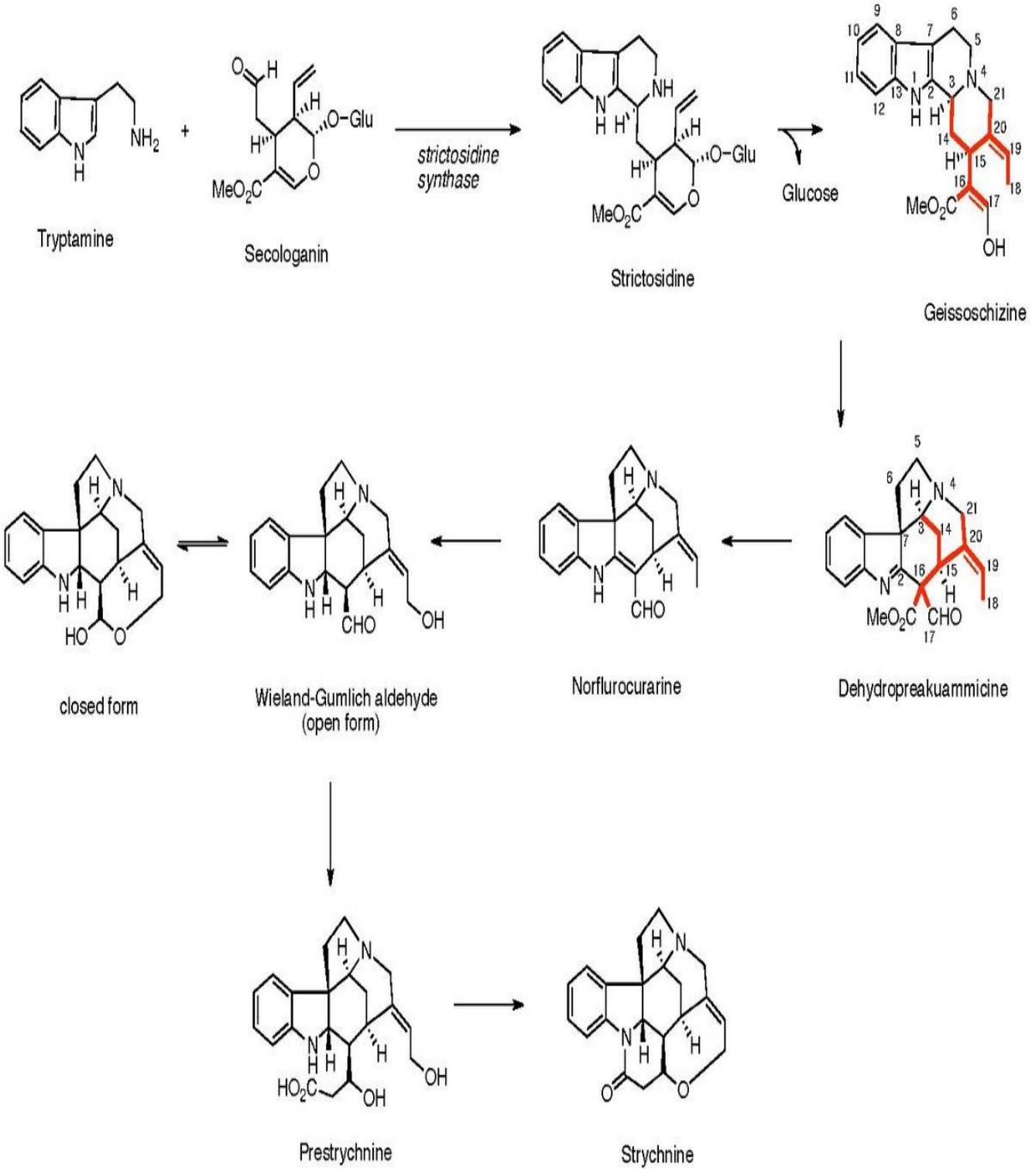
Strychnine

Bio synthesis:



4. Explain Structure Determination, Synthesis and Bio synthesis of Colchicine.

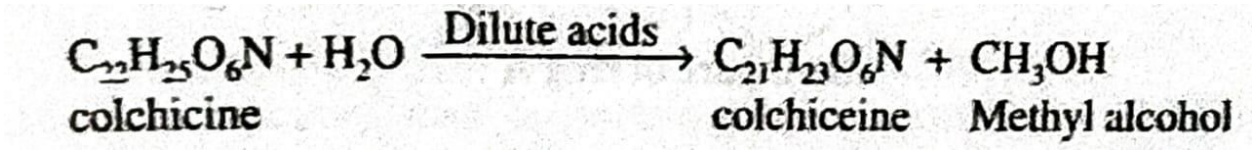
Structure Determination:

→From the analytical data molecular formula of Colchicine is $C_{22}H_{25}O_6N$.

→$D.B.E = 22 - \frac{25}{2} + \frac{1}{2} + 1$

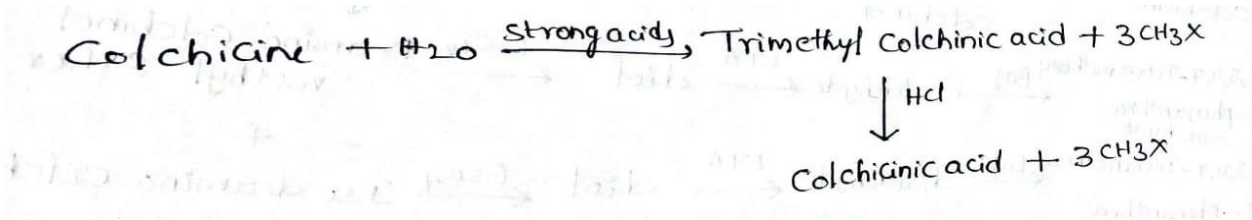
$= \frac{44-25+1+2}{2} = \frac{22}{2} = 11$ Double bonds.

Presence of enol methyl ether group:



The above reaction indicates the presence of enol methyl ether group.

Presence of acetamide group (-NH-CO-CH_3):



→It indicates the presence of 3 – OCH_3 groups in trimethyl colchicinic acid and hence in colchicines.

→In the above reaction 5 – oxygen atoms have been accounted. Out of 5 oxygen atoms 3 are – OCH_3 groups, one is present in enol methyl ether and another is in NH-CO-CH_3 group. The 6th oxygen does not react means indirectly it is presented as unreactive carbonyl group from the spectral data.

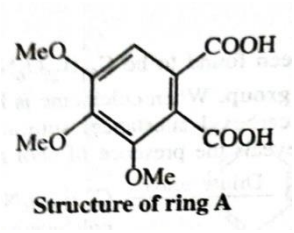
Tricyclic Nature of Colchicine:

→The saturated molecular formula of colchicines is $C_{16}H_{28}$. It is coincide with the general molecular formula C_nH_{2n-4}.

C_nH_{2n-4} indicates the tricyclic nature. So colchicine has tricyclic nature.

Size of Ring A:

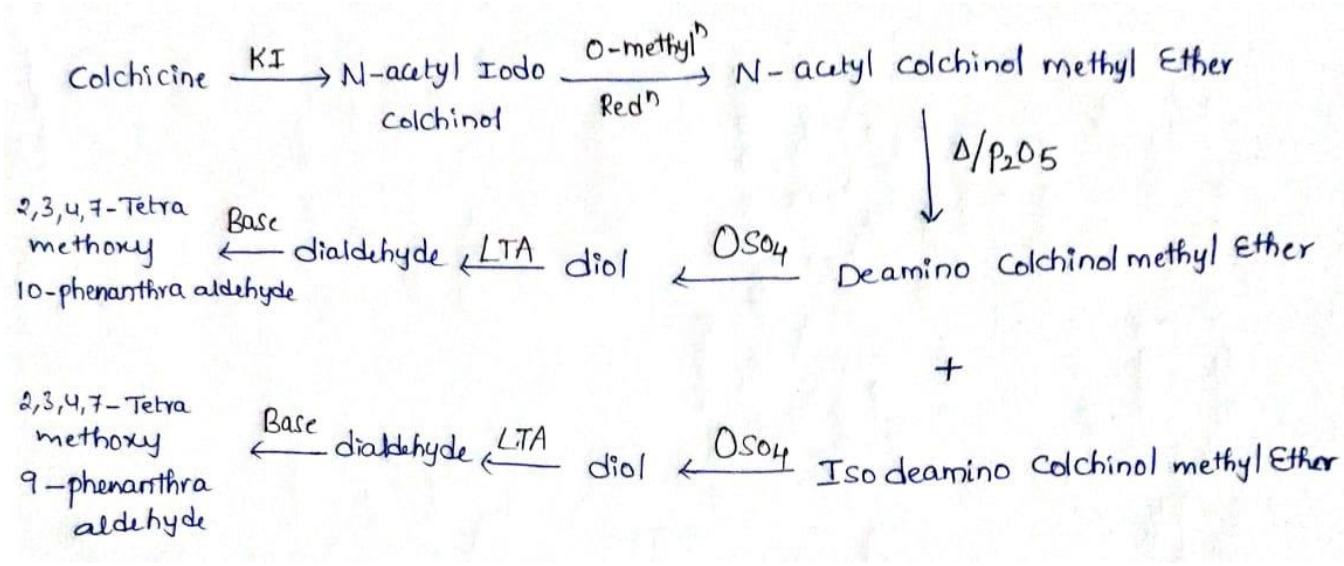
Colchicine + Oxidation ----------→



Structure of ring A

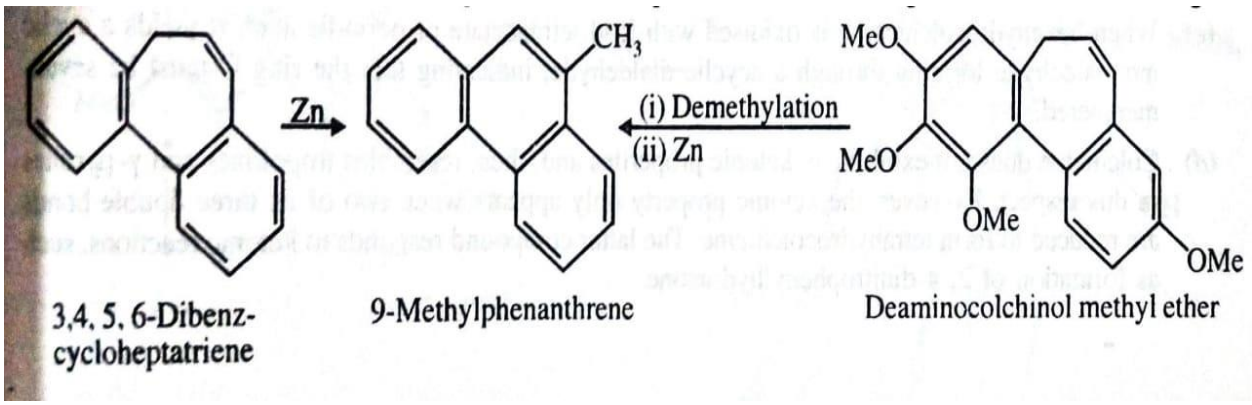
→It indicates the size of ring A is 6 numbered and having 3 – OCH_3 groups in benzenoid nucleus at adjacent carbons.

Size of Ring B:



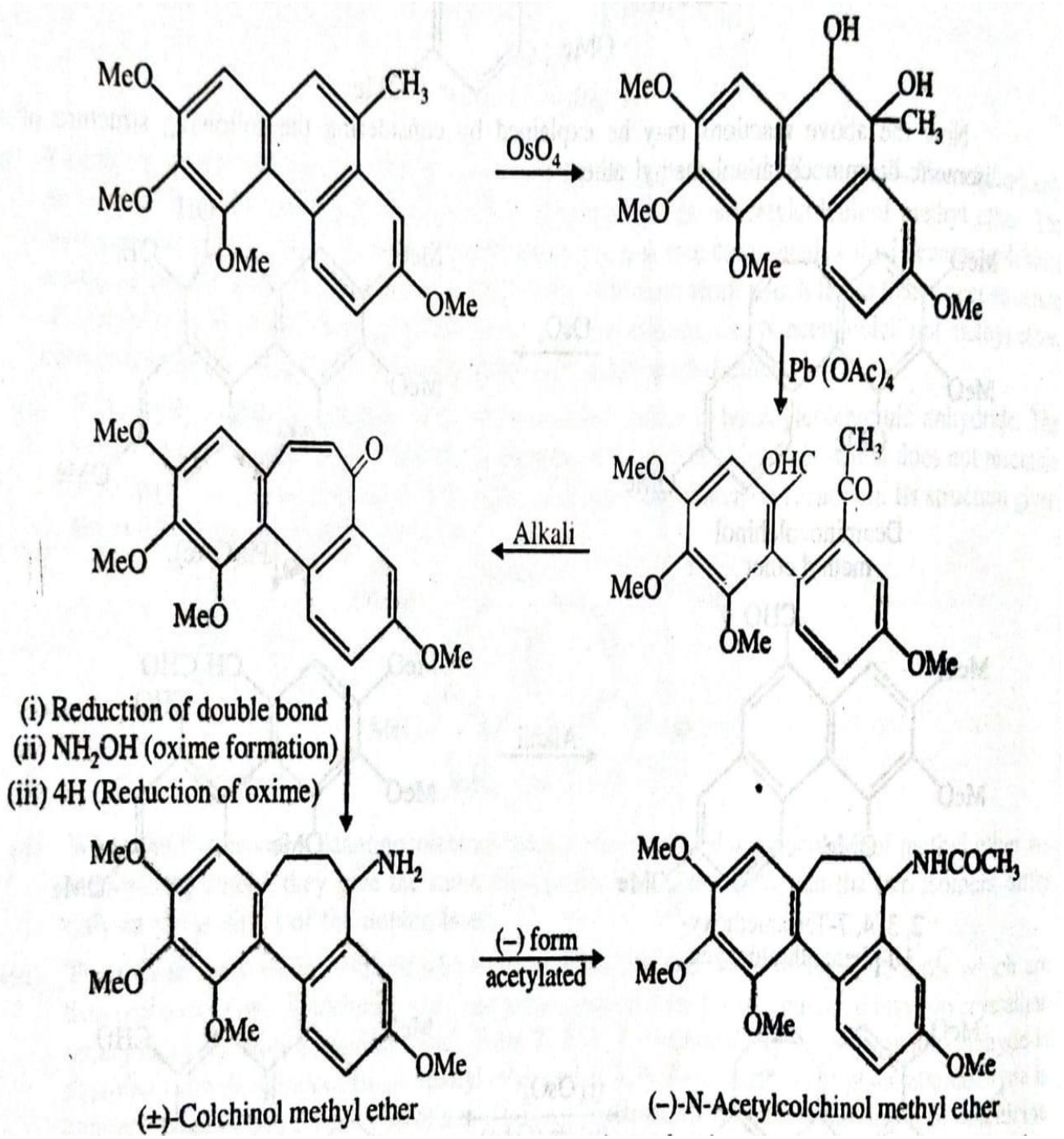
→The formation of 9-Phenantha aldehyde and 10- Phenantha aldehyde indicates the ring B may be 7 numbered ring and it was confirmed by the following reaction.

Windaus Reaction



From the above reaction size of ring B is 7 numbered ring.

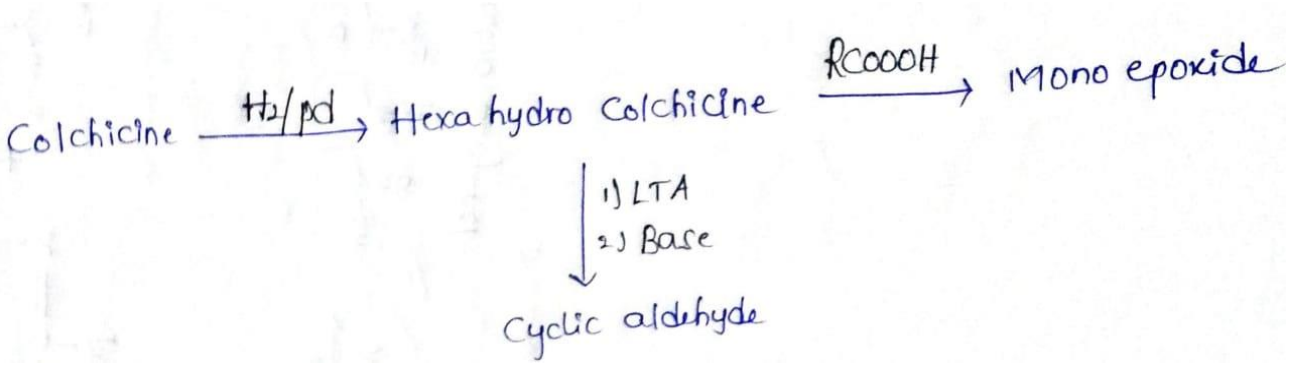
This is confirmed by the synthesis of N-acetyl colchinol methyl ether.



Size of Ring C:

In the above reactions the ring C has been showed to be 6 numbered ring but Diwar suggested that ring C has 7 numbered ring on the support of the following information.

1. Colchicine behaves as an ester.

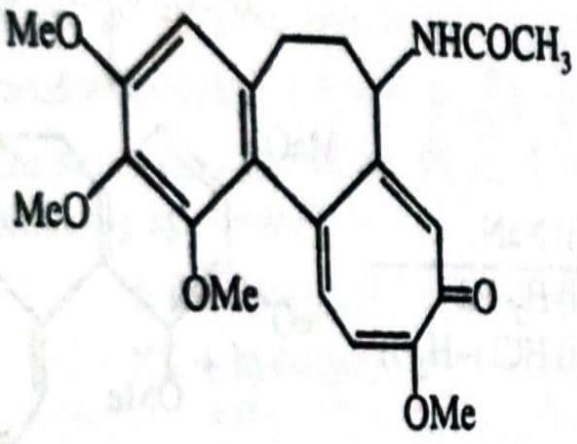


The formation of these products indicates the ring C having 3 double bonds and a ketonic group in same ring.

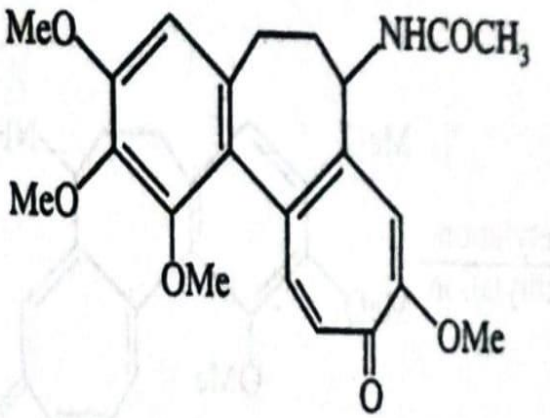
→Colchicine + HI ----------→ N-acetyl iodo colchinol.

The above reaction suggesting that the ring C of colchicines is a troplone ring.

→From all of the above information the structure of Colchicine may be

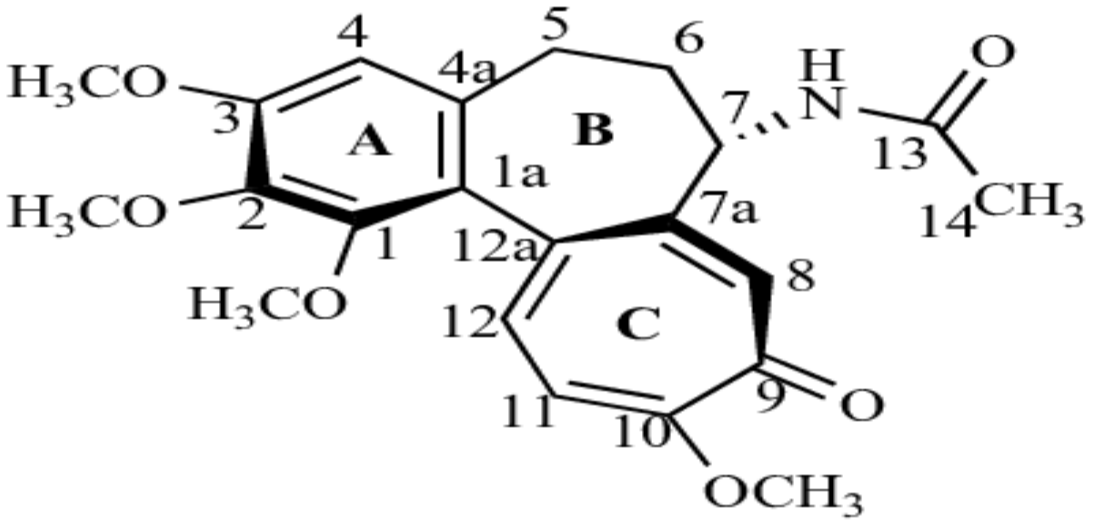


(I)

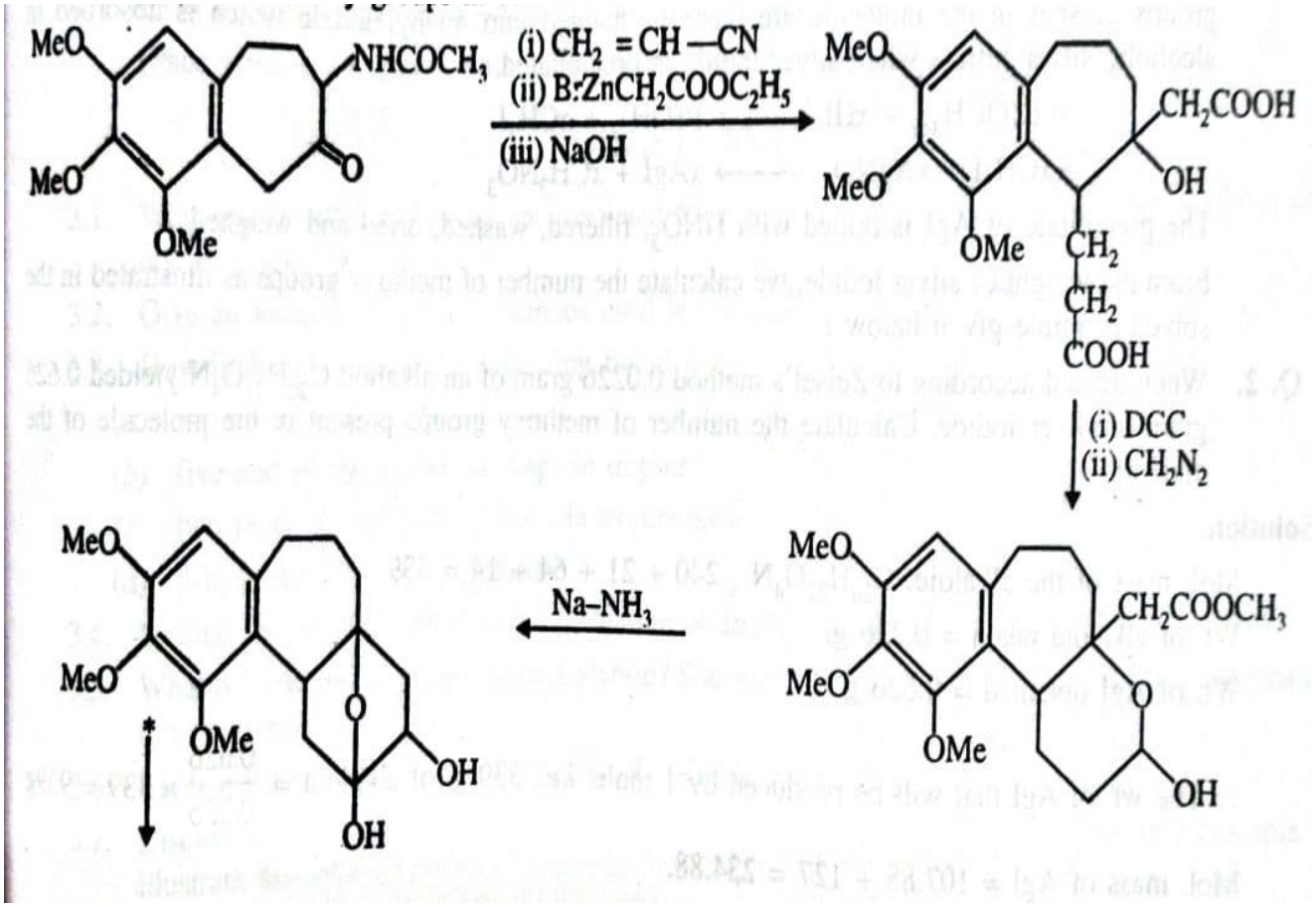


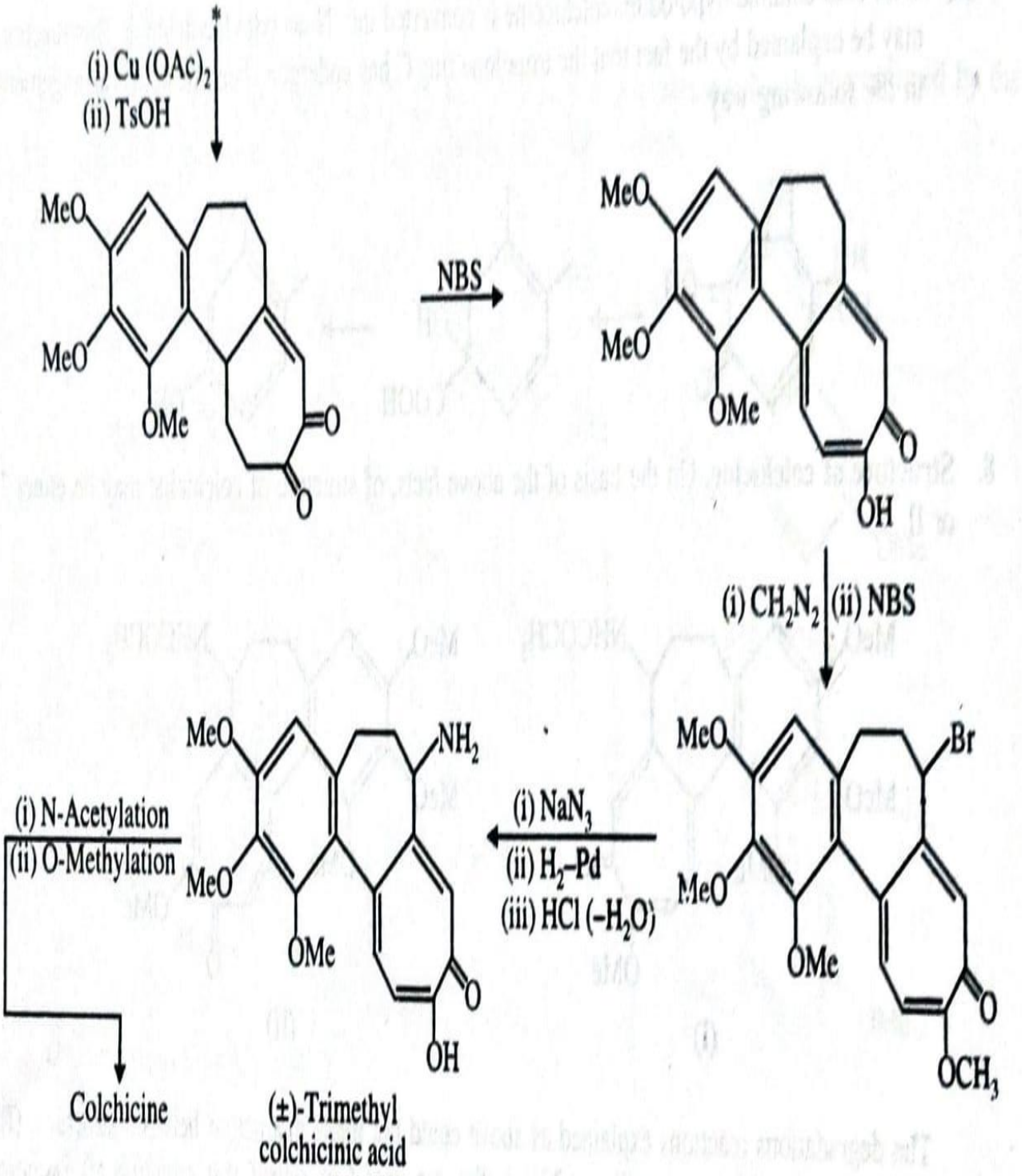
(II)

From the X-ray analysis and crystallographic methods structure (I) is correct structure for Colchicine.

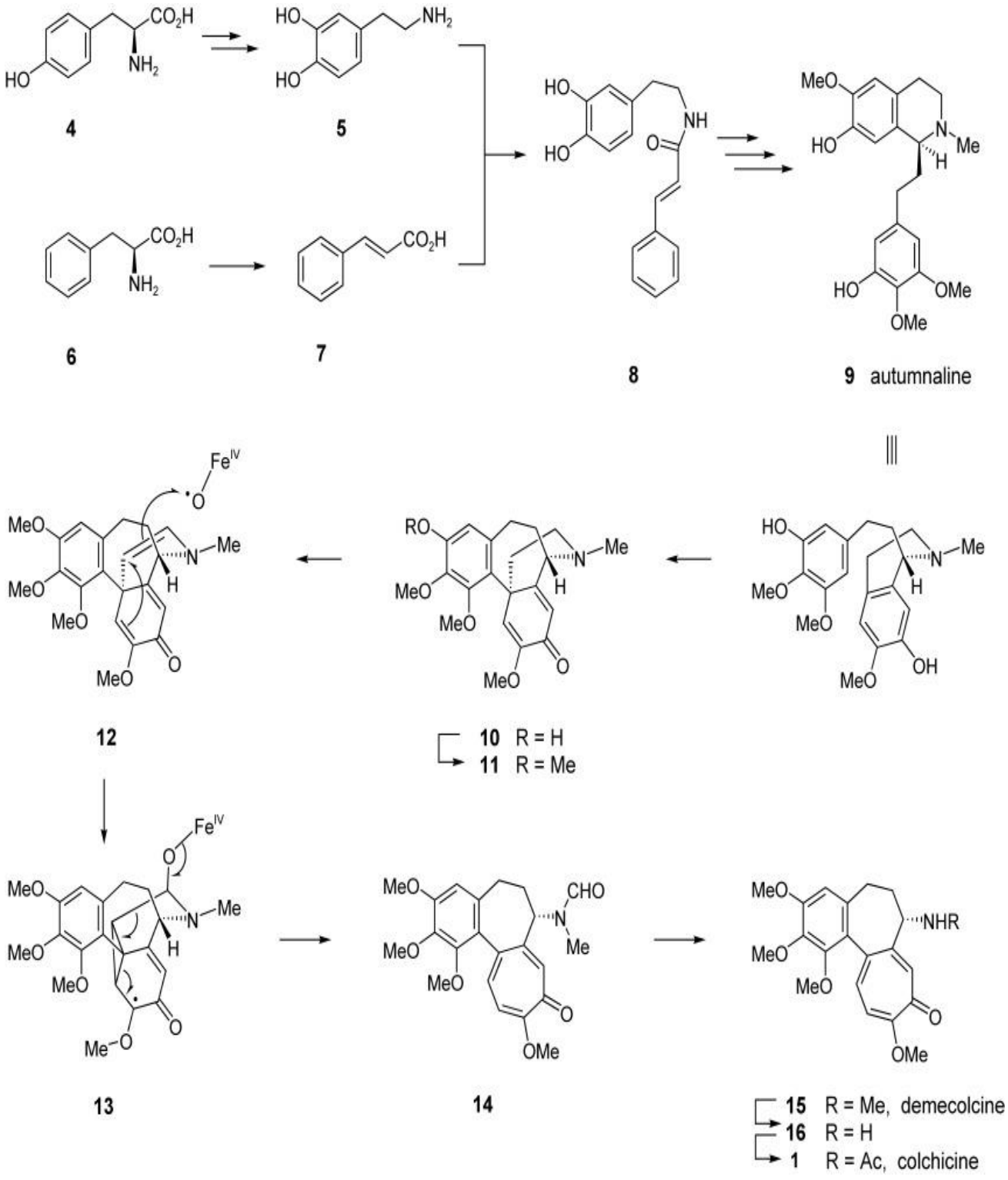


Synthesis of Colchicine:




(i) Cu (OAc)2
(ii) TsOH
MeO
MeO
OMe
O
O
NBS
MeO
MeO
OMe
O
OH
(i) CH2N2
(ii) NBS
MeO
Br
MeO
OMe
O
OCH3
(i) NaN3
(ii) H2–Pd
(iii) HCl (–H2O)
MeO
NH2
MeO
OMe
O
OH
(i) N-Acetylation
(ii) O-Methylation
Colchicine
(±)-Trimethyl
colchicinic acid

Bio synthesis of Colchicine:



UNIT-II
TERPENOIDS

TERPENOIDS :

There are many different classes of_naturally occurring compounds . Terpenoids also form a group of naturally occurring compounds majority of which occur in plants, a few of them have also been obtained from other sources. Terpenoids are volatile substances which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus.

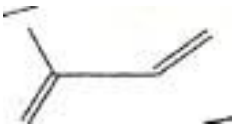
The term terpene' was given to the compounds isolated from terpentine, a volatile liquid isolated from pine trees. The simpler mono and sesqui terpenes are chief constituent of the essential oils obtained from sap and tissues of certain plants and trees. The di and tri terpenoids are not steam volatile. They are obtained from plant and tree gums and resins. Tetraterpenoids form a separate group of compounds called Carotenoids.

The term 'terpene was originally employed to describe a mixture of isomeric hydrocarbons of the molecular formula $C_{10}H_{16}$ occurring in the essential oils obtained from sap and tissue of plants, and-trees. But there is a tendency to use more general term terpenoids which include hydrocarbons and their Oxygenated derivatives. However the term terpene is being used these days by some authors to represent terpenoids.

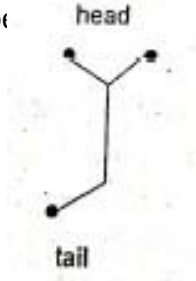
By the modern definition: "Terperioids are the hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives".

Isoprene rule: Thermal decomposition of terpenoids give isoprene as one of the product. Otto Wallach pointed out that terpenoids can be built up of isoprene unit.

Isoprene rule stats that the terpenoid molecules are constructed from two or more isoprene unit.

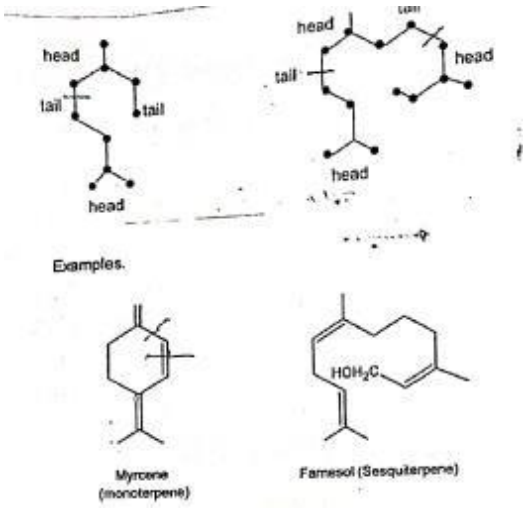


Further Ingold suggested that isoprene u[illegible] in the terpenoid via 'head to tail' fashion. Special isoprene rule states that the terpe[illegible]e are constructed of two or more isoprene units joined in [illegible]d to tail' fashion

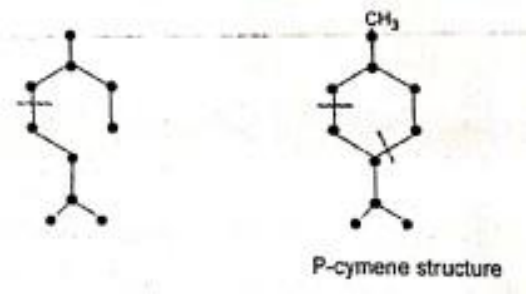


But this rule can only be used as guiding principle and not as a fixed rule. For example carotenoids are joined.tail to tail at their central and there are also some terpenoids whose carbon content is not a multiple of five.

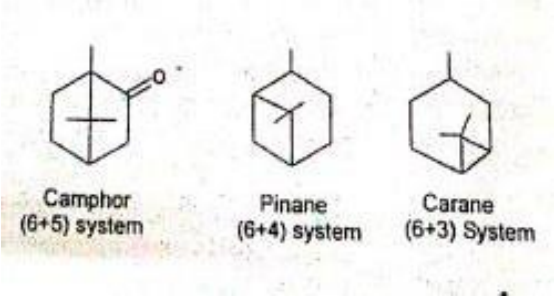
In applying isoprene rule we look only for the skeletal unit of carbon. The carbon skeletons of open chain mono terpenoids an sesqui terpenoids are,



Ingold (1921) pointed that a gem alkyl group affects the stability of terpenoids. He summarized these results in the form of a rule called `gem dialkyl rule' which may be stated as "Gem dialkyl group tends to render the cyclohexane ring unstable where as it stabilizes the three, four and five member rings."
This rule limits the number of possible structure in closing the open chain to ring structure. Thus the monoterpenoid open chain give rise to only one possibility for a monocyclic monoterpenoid i.e the p-cymene structure.



Bicyclic monoterpenodis contain a six member and a three member ring. Thus closure of the ten carbon open chain monoterpenoid gives three possible bicyclic structures.



Classification of Terpinoids

Most natural terpenoids hydrocarbon have the general formula $(C_5H_8)_n$. They can be classified on the basis of value of n or number of carbon atoms present in the structure.

Table-1: Classification of Terpenoids

S.No.	Number of carbon atoms	Value of n	Class
1.	10	2	Monoterpenoids($C_{10}H_{16}$)
2.	15	3	Sesquiterpenoinds($C_{15}H_{24}$)
3.	20	4	Diterpenoids($C_{20}H_{32}$)
4.	25	5	Sesterpenoids($C_{25}H_{40}$)
5.	30	6	Troterpenoids($C_{30}H_{48}$)
6.	40	8	Tetraterpenoids($C_{40}H_{64}$)
7.	>40	>8	Polyterpenoids$(C_5H_8)n$

Each class can be further subdivided into subclass according to the number of rings present in the structure.

i)**Acyclic Terpcnoids**: They contain open structure.

ii)**Monocyclic Terpenoids**: They contain one ring in the structure

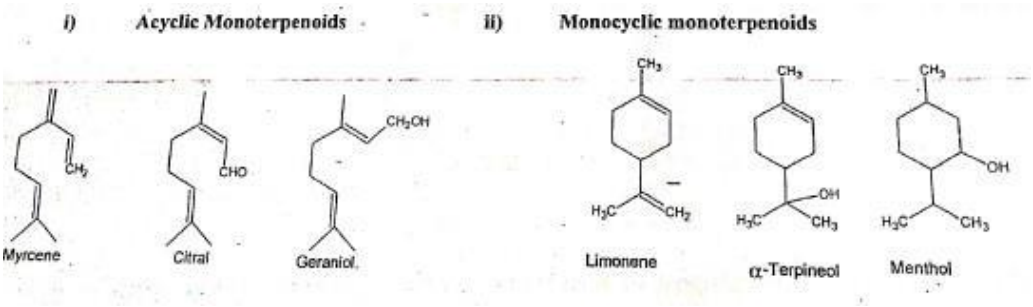
iii)**Bicyclic Terpenoids**: They contain two rings in the structure.

iv)**Tricyclic Terpcnoids**: They contain three rings in the structure.

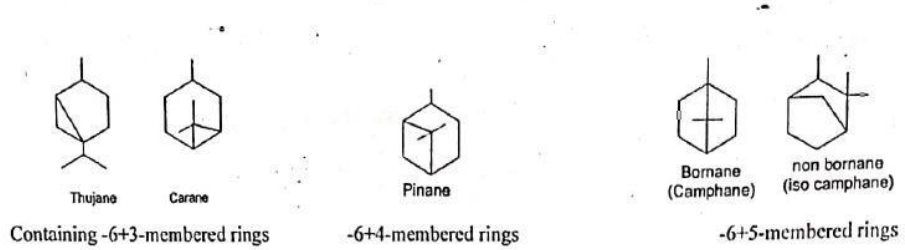
v)**Tetracyclic Terpenoids:** They contain four rings in the structure.

Some examples of mono, sesqui and di Terpenoids:

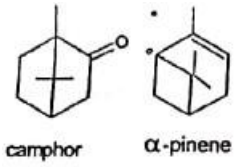
A) Mono terpenoids



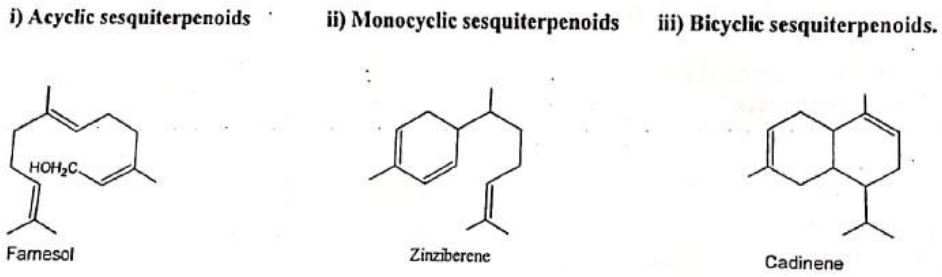
iii) **Bicyclic monoterpenoids**: These are further divided into three classes.
a)Containing -6+3-membered rings
b)Containing -6+4- membered rings.
c)Contining -6+5-membered rings



Some bicyclic monoterpenes are:

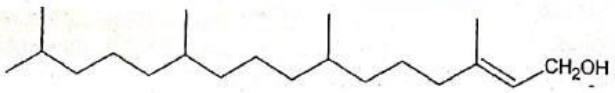


B) Sesquiterpenoids:



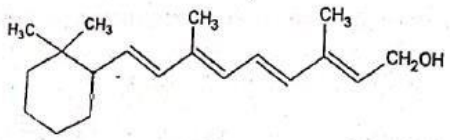
C) Diterpenoids:

i) **Acyclic diterpenoids**



Phytol

ii) ***Mono cyclic diterpenoids:***



Vitamin A

Isolation of mono and sesquiterpenoids

Both mono and sesquiterpenoids have common source i.e essential oils. Their isolation is carried out in two steps:

i)Isolation of essential oils from plant parts
ii)Separation of Terpenoids from essential oils

i) **Isolation of essentia1 oils from plant parts**: The plants having essential oils generally have the highest concentration at some particular time. Therefore better yield of essential oil plant material have to be collected at this particular time. e.g. From jasmine at sunset.there are four methods of extractions of oils.

a) Expression method
b) Steam distillation method
c) Extraction by means of volatile solvents
d) Adsorption in purified fats

Steam distillation is most widely used method. In this method macerated plant material is steam distilled to get essential oils into the distillate form these are extracted by using pure organic volatile solvents. If compound decomposes during steam distillation, it may be extracted with petrol at 50°C. After extraction solvent is removed under reduced pressure.

ii) **Separation of Terpenoids from essential oil**: A number of terpenoids are present in essential oil obtained from the extraction. Definite physical and chemical methods can be used for the separation of terpenoids. They are separated by fractional distillation. The terpenoid hydrocarbons distill over first followed by the oxygenated derivatives.

More recently different chromatographic techniques have been used both for isolation and separation of terpenoids.

General properties of Terpenoids

1. Most of the terpenoids are colourless, fragrant liquids which are lighter than water and volatile with steam. A few of them are solids e.g. camphor. All are soluble in organic solvent and usually insoluble in water. Most of them are optically active.

2. They are open chain or cyclic unsaturated compounds having one or more double bonds. Consequently they undergo addition reaction with hydrogen, halogen, acids, etc. A number of *addition* products *have* antiseptic properties.

3. They undergo polymerization and dehydrogenation.

4. They are easily oxidized nearly by all the oxidizing agents. On thermal decomposition, most of the terpenoids yields isoprene as one of the product.

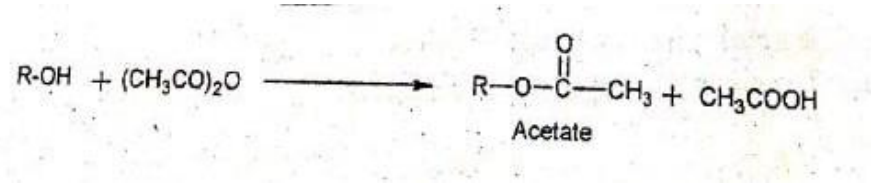
General Methods of structure elucidation

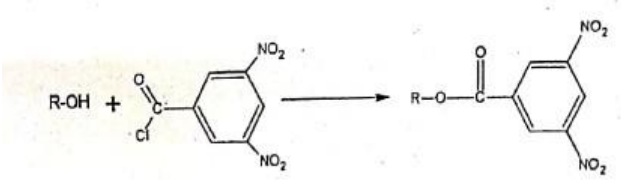
Terpenoids

1) Molecular formula: molecular formula is determined by usual quantitative analysis and mol.wt determination methods and by means of mass spectrometry. If terpenoid is optically active, its specific rotation can be measured.

2)Nature of oxygen atom present: If oxygen is present in terpenoids its functional nature is generally as alcohol aldehyde, ketone or carboxylic groups.

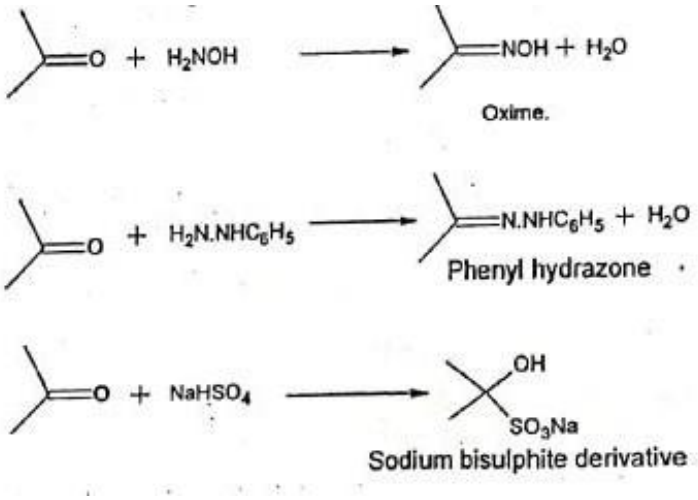
a) **Presence of oxygen atom present:** presence of —OH group can be determined by the formation of acetates with acetic anhydride and benzoyate with 3.5-dinitirobenzoyl chloride.





Primary alcoholic group undergo esterification more readily than secondary and tertiary alcohols.

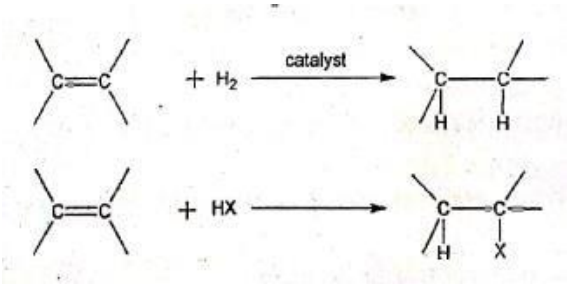
b) **Presence of >C=0 group**: Terpenoids containing carbonyl function form crystalline addition products like oxime, phenyl hydrazone and bisulphite etc.



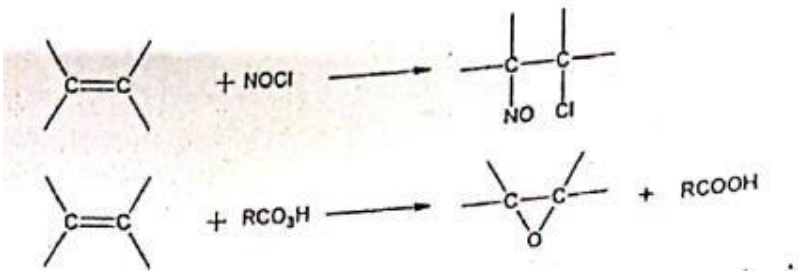
if carbonyl function is in the form of aldehyde it gives carboxylic acid on oxidation without loss of any carbon atom whereas the ketone on oxidation yields a mixture of lesser number of carbon atoms.

iii) **Unsaturation**: The presence of olefinic double bond is confirmed by means of bromine, and number of double bond determination by analysis of the bromide or by quantitative hydrogenation or by titration with monoperpthalic acid.

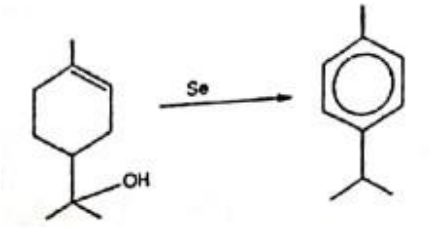
Presence of double bond also confirmed by means of catalytic hydrogenation or addition of halogen acids. Number of moles of HX absorbed by one molecule is equal to number of double bonds present.



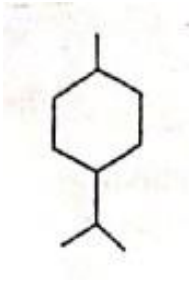
Addition of nitrosyl chloride(NOC1) **(Tilden's reagent)** and epoxide formation with peracid also gives idea about double bonds present in terpenoid molecule.



iv) **Dehydrogenation**: On dehydrogenation with sulphur, selenium, polonium or palladium terponoids converted to aromatic compounds. Examination of these products the skelton structure and position of side chain in the original terpenoids can be determined.
For example α-terpenol on Se-dehydrogenation_yields *p*-cymene.



Thus the carbon Skelton of terpenol is as follows.



v) **Oxidative degradation**: Oxidative degradation has been the parallel tool for elucidating the structure of terpenoids. Reagents for degradative oxidation are ozone, acid, neutral or alkaline potassium permanganate, chromic acid, sodium hypobromide, osmium tetroxide, nitric acid, lead tetra acetate and peroxy acids. Since oxidizing agents are selective, depending on a particular group to be oxidized, the oxidizing agent is chosen with the help of stfucture of degradation product.

vi) **Number of the rings present**: With the help of general formula of corresponding parent saturated hydrocarbon, number of rings present in that molecule can be determined.

vii) **Relation between general formula of compound and type of compounds**: Table 2

Table-2: Relation between general formula of compound and type of compounds

General formula of parent saturated Hydrocarbon	Type of structure
C_nH_{2n+2}	Acyclic
C_nH_{2n}	Monocyclic
C_nH_{2n-2}	Bicyclic
C_nH_{2n-4}	Tricyclic
C_nH_{2n-6}	Tetrayclic

For example limonene (mol. formula. $C_{10}H_{16}$)absorbs 2 moles of hydrogen to give tetrahydro Iimonene (mol. Formula $C_{10}H_{20}$) corresponding to the general formula. C_nH_{2n}. It means limonoene has monocyclic structure.

viii) **Spectroscopic studies:** All the spectroscopic methods are very helpful for the confirmation of structure of natural terpenoids and also structure of degradation products. The various methods for elucidating the structure of terpenoids are:

a) **UV Spectroscopy**: In terpenes containing conjugated dienes or α,β–unsaturated ketones, UV spectroscopy is very useful tool. The values of λ_{max} for various types of terpenoids have been calculated by applying Woodward's empirical rules. There is generally good agreement between calculation and observed values. Isolated double bonds, α,β-unsaturated esters , acids, lactones also have characteristic maxima.

b) **IR Spectroscopy**: IR spectroscopy is useful in detecting group such as hydroxyl group (-3400cm^{-1}) or an oxo group (saturated 1750-1700cm^{-1}). Isopropyl group, cis and trans also have characteristic absorption peaks in IR region.

c) **NMR Spectroscopy**: This technique is useful to detect and identify double bonds, to determine the nature of end group and also the number of rings present, and also to reveal the orientation of methyl group in the relative position of double bonds.

d) **Mass Spectroscopy**: It is now being widely used as a means of elucidating structure of terpenoids. Used for determining mol. Wt., Mol. Formula, nature of functional groups present and relative positions of double bonds.

ix) **X-ray analysis**: This is very helpful technique for elucidating structure and stereochemistry of terpenoids.

x) **Synthesis**: Proposed structure is finally confirmed by synthesis. In terpenoid chemistry,many of the synthesis are ambiguous and in such cases analytical evidences are used in conjunction with the synthesis.

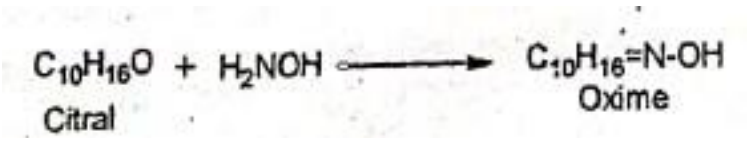
Citral

Citral is an acyclic monoterpenoid. It is a.major constituent of lemon grass oil in which it occurs to an extent of 60-80%. It is pale yellow liquid having strong lemon like odour and can be obtained by fractional distillation under reduced pressure from Lemongrass oil.

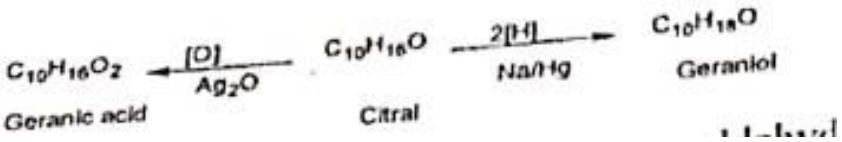
Constitution:

i) Mol. formula $C_{10}H_{16}O$, b.p-77°C

ii) Nature of Oxygen atom: Formation of oxime of citral indicates the presence of an oxo group in citral molecule.

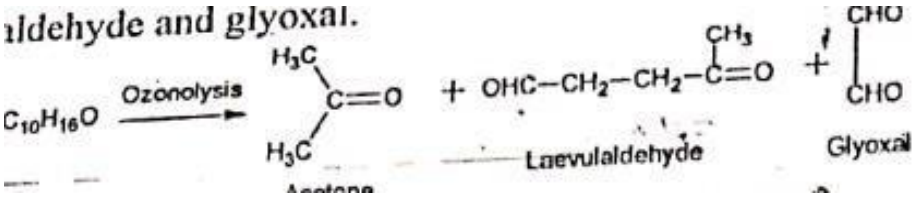


On reduction with Na/Hg it gives an alcohol called geraniol and on oxidation with silver oxide it give a monocarboxylic acid called Geranic acid without loss of any carbon atom.



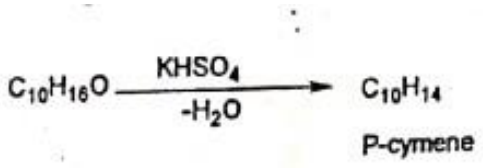
Both these reaction reveal that oxo group in citral is therefore an aldehyde group. Citral reduces Fehling's solution. further confirming the presence of aldehydic group.

iii)It adds on two molecule of Br_2 shows the presence of two double bonds. On ozonlysis, it gives acetone, laevulaldehyde and glyoxal.

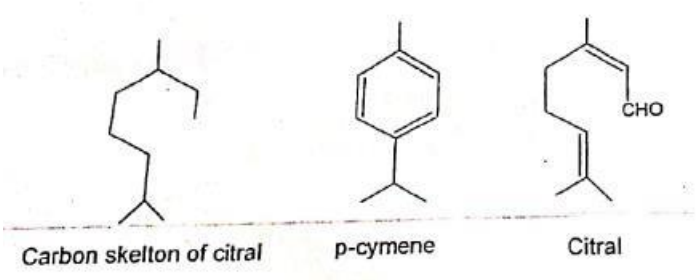


Formation of above products shows that citral is an acyclic compound containing two double bonds. Corresponding saturated hydrocarbon of citral (mol. Formula $C_{10}H_{22}$) corresponds to the general formula C_nH_{2n+2} for acyclic compounds, indicating that citral must be an acyclic compound.

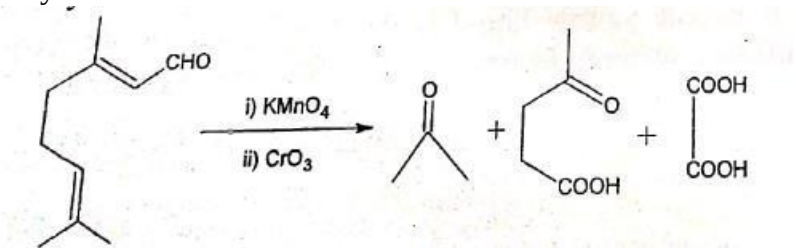
iv) Formation of *p*-cymcne and product obtained from the ozonolysis reveals that citral is formed by the joining of two isoprene units in the head to tail fashion



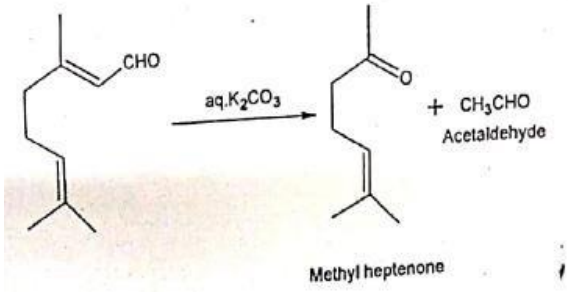
v) On the basis of above facts following structure was proposed for citral.



vi) Above structure was further supported by the degradation of citral on treatment with alkaline $KMnO_4$ followed by chromic acid.



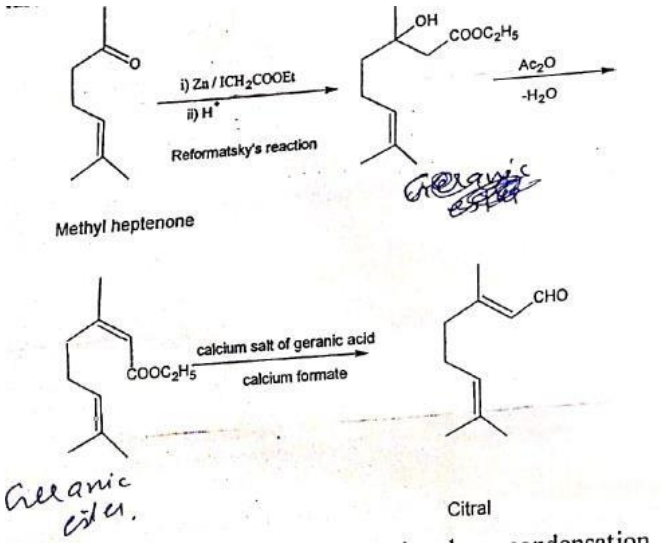
Verley found that citral on boiling with aqueous potassium carbonate yielded 6-methyl hept-5- ene-2-one and acetaldehyde. The formation of these can only be explained on the basis of proposed structure.



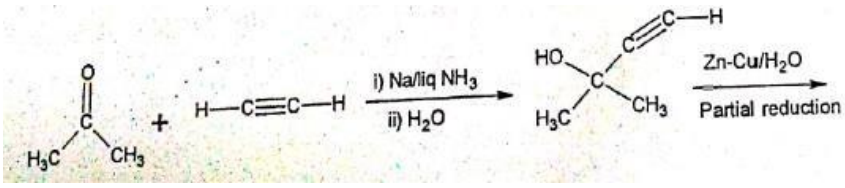
It Appears that citral is product of aldol condensation of these two.

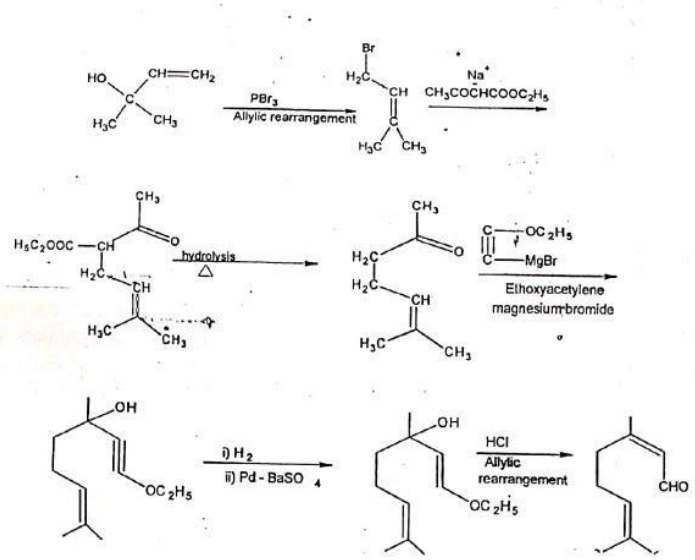
Synthesis: Finally the structure of citral was confirmed by its synthesis.

a) **Barbier-Bouveault-Tiemann's synthesis**: In this synthesis methyl heptenone is converted to geranic ester by using Reformatsky's reaction. Geranic ester is then converted to citral by distilling a mixture of calcium salts of geranic and formic acids.

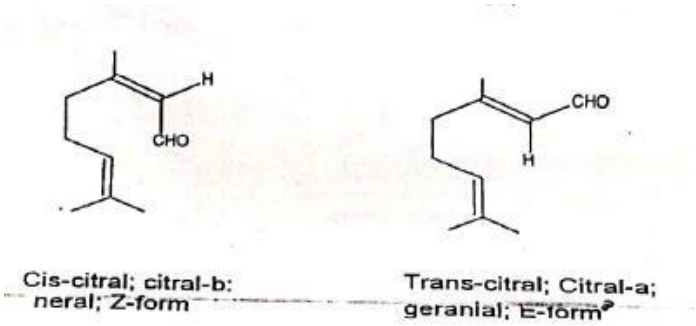


b)**Arens-Van Drop's Synthesis**: This synthesis involves condensation of acetone with acetylene in the presence of liquid ammonia. Condensation product is then reduced and treated with PBr_3, allylic rearrangement takes place. The rearranged product so obtained is treated with sodium salt of acetoacetic ester and then hydrolysed to yield methyl heptenone. The latter compound on condensation with ethoxy acetylene magnesium bromide, followed by the partial reduction and acidification yields citral by allylic rearrangement.





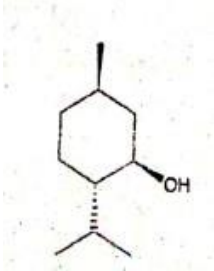
Isomerism of citral: two geometrical isomers occur in nature.



The existence of the two isomeric Citrals in natural citral has been confirmed chemically by the formation of two different semicarbazones and formation of geraniol and nerol on reduction.

Menthol

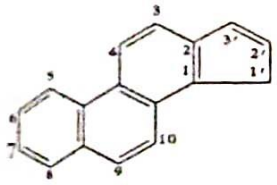
Menthol is the major constituent of Mentha Piperi. It is used as an antiseptic and anesthetic. Menthol (also called peppermint camphor or mint camphor) is the major constituent of peppermint oil and is responsible for its odour and taste and the cooling sensation when applied to the skin. It is ingredient in cold balms. Menthol is optically active compound with mol. formula $C_{10}H_{20}O$.



11 Steroids

§1. Introduction

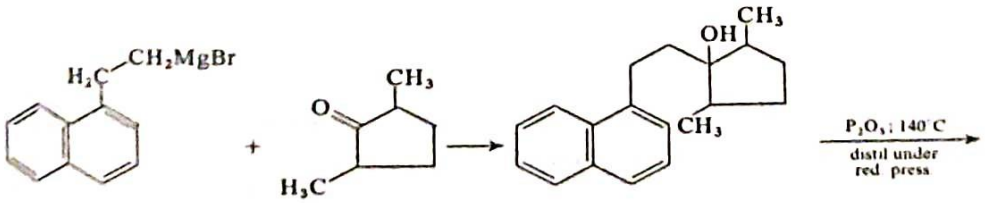
The steroids form a group of structurally related compounds which are widely distributed in animals and plants. Included in the steroids are the sterols (from which the name *steroid* is derived), vitamin D, the bile acids, a number of sex hormones, the adrenal cortex hormones, some carcinogenic hydrocarbons, certain sapogenins, etc. The structures of the steroids are based on the 1,2-cyclopentenophenanthrene skeleton (Rosenheim and King, 1932; Wieland and Dane, 1932). All the steroids give, among other products, Diels' hydrocarbon on dehydrogenation with selenium at 360°C (Diels, 1927). In fact, a steroid could be defined as any compound which gives Diels' hydrocarbon when distilled with selenium. When the distillation with selenium is carried out at 420°C, the steroids give mainly chrysene (**10** §4b) and a small amount of picene (**10** §4c).

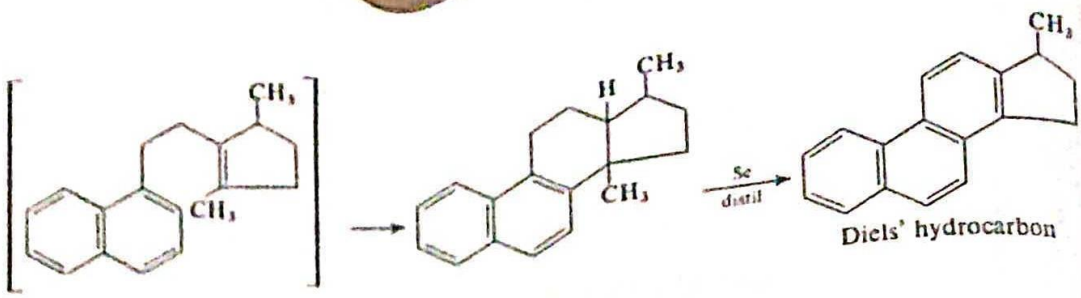


1,2-cyclopentenophenanthrene

In the earlier work, the various steroids were designated by trivial names, but the tendency now is to discard these in favour of systematic names, which may be applied when the structure is known (see §7).

Diels' hydrocarbon is a solid, m.p. 126–127°C. Its molecular formula is $C_{18}H_{16}$, and the results of oxidation experiments, X-ray crystal analysis and absorption spectrum measurements showed that the hydrocarbon is probably 3′-methyl-1,2-cyclopentenophenanthrene. This structure was definitely established by synthesis, *e.g.*, that of Harper, Kon and Ruzicka (1934), who used the Bogert–Cook method [**10** §2vi], starting from 2-(1-naphthyl)-ethylmagnesium bromide and 2,5-dimethylcyclopentanone.





Sterols

§2

Sterols occur in animal and plant oils and fats. They are crystalline compounds, and contain an alcoholic group; they occur free or as esters of the higher fatty acids, and are isolated from the unsaponifiable portion of oils and fats. Cholesterol, 5α-cholestan-3β-ol (cholestanol) and 5β-cholestan-3β-ol (coprostanol) are the animal sterols; ergosterol and stigmasterol are the principal plant sterols. The sterols that are obtained from animal sources are often referred to as the *zoosterols*, and those obtained from plant sources as the *phytosterols*. A third group of sterols, which are obtained from yeast and fungi, are referred to as the *mycosterols*. This classification, however, is not rigid, since some sterols are obtained from more than one of these groups.

§3. **Cholesterol,** $C_{27}H_{46}O$, m.p. 149°C.

This is the sterol of the higher animals, occurring free or as fatty esters in all animal cells, particularly in the brain and spinal cord. Cholesterol was first isolated from human gallstones (these consist almost entirely of cholesterol). The main sources of cholesterol are the fish-liver oils, and the brain and spinal cord of cattle. Lanoline, the fat from wool, is a mixture of cholesteryl palmitate, stearate and oleate.

Cholesterol is a white crystalline solid which is optically active, ($[\alpha]_D$ 39°). Cholesterol (and other sterols) gives many colour reactions, *e.g.*,

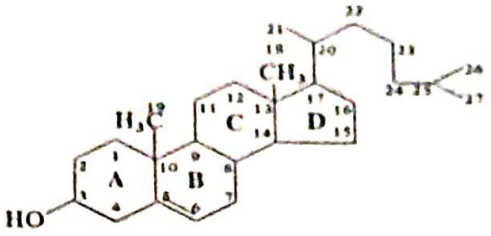
(i) *The Salkowski reaction* (1908). When concentrated sulphuric acid is added to a solution of cholesterol in chloroform, a red colour is produced in the chloroform layer.

(ii) *The Liebermann–Burchard reaction* (1885, 1890). A greenish colour is developed when a solution of cholesterol in chloroform is treated with concentrated sulphuric acid and acetic anhydride.

When an ethanolic solution of cholesterol is treated with an ethanolic solution of digitonin (a saponin; see §32), a large white precipitate of cholesterol digitonide is formed. This is a molecular complex containing one molecule of cholesterol and one of digitonin, from which the components may be recovered by dissolving the complex in pyridine (which brings about complete dissociation) and then adding ether (the cholesterol remains in solution and the digitonin is precipitated). An alternative method is to dissolve the digitonide in dimethyl sulphoxide and heat on a steam bath. Dissociation occurs, and on cooling only the sterol is precipitated (Issidorides *et al.*, 1962). Digitonide formation is used for the estimation of cholesterol. An interesting point in this connection is that 3β-hydroxysteroids usually form complexes with digitonin, whereas the corresponding 3α-compounds do not (see §5 for the meaning of α and β).

The structure of cholesterol was elucidated only after a tremendous amount of work was done, particularly by Wieland, Windaus and their coworkers (1903–1932). Only a very bare outline is

given here, and in order to appreciate the evidence that is going to be described, it is necessary to have the established structure of cholesterol at the beginning of our discussion. (I) is the structure of



(I)

cholesterol, and shows the method of numbering. The molecule consists of a *side-chain* and a *nucleus* which is composed of four rings; these rings are usually designated A, B, C and D (or (I), (II), (III) and (IV)), beginning from the six-membered ring on the left (see also (iii) below). It should be noted that the nucleus contains two angular methyl groups, one at C-10 and the other at C-13.

(i) **Structure of the ring system.** Under this heading we shall deal with the nature of the ring system present in cholesterol; the problem of the angular methyl groups is dealt with later [see (iv)].

The usual tests for functional groups showed that cholesterol contains one double bond and one hydroxyl group. Now let us consider the following set of reactions.

$$\underset{C_{27}H_{46}O\ (I)}{\text{Cholesterol}} \xrightarrow{H_2—Pt} \underset{C_{27}H_{48}O\ (II)}{\text{Cholestanol}} \xrightarrow{CrO_3} \underset{C_{27}H_{46}O\ (III)}{\text{Cholestanone}} \xrightarrow[HCl]{Zn—Hg} \underset{C_{27}H_{48}\ (IV)}{\text{Cholestane}}$$

The conversion of cholesterol into cholestanol (II) shows the presence of one double bond in (I) and the oxidation of (II) to the ketone cholestanone (III) shows that cholesterol is a secondary alcohol. Cholestane (IV) is a saturated hydrocarbon, and corresponds to the general formula C_nH_{2n-6}, and consequently is tetracyclic; thus cholesterol is tetracyclic. [D.B.E. of cholestane is $27 + 1 - 48/2 = 4$.]

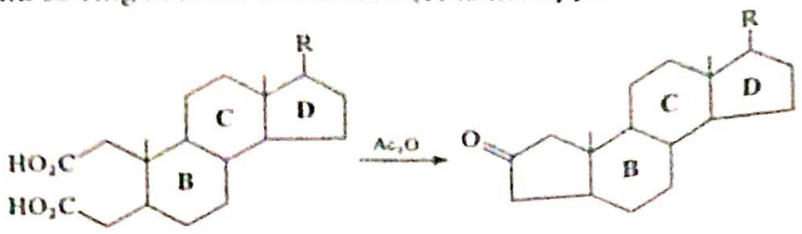
When cholesterol is distilled with selenium at 360°C, Diels' hydrocarbon is obtained (see §1). The formation of this compound could be explained by assuming that this nucleus is present in cholesterol. The yield of this hydrocarbon, however, is always poor, and other products are always formed at the same time, particularly chrysene (see §1). Thus, on the basis of this dehydrogenation, the presence of the cyclopentenophenanthrene nucleus must be accepted with reserve. Rosenheim and King (1932) thought that chrysene was the normal product of the selenium dehydrogenation, and so proposed (on this basis and also on some information obtained from X-ray analysis work of Bernal, 1932; see §5) that the steroids contained the chrysene skeleton. Within a few months, however, Rosenheim and King (1932) modified this suggestion, as did also Wieland and Dane (1932). These two groups of workers proposed that the cyclopentenophenanthrene nucleus is the one present in cholesterol (*i.e.*, in steroids in general). This structure fits far better all the evidence that has been obtained from a detailed investigation of the oxidation products of the sterols and bile acids, and has now been confirmed by the synthesis of cholesterol (see §9).

(*a*) The nature of the *nucleus* in sterols and bile acids was shown to be the same, since 5β-cholanic acid (cholanic acid) or 5α-cholanic acid (allocholanic acid) is one of the oxidation products (see §5).

(*b*) The oxidation of the bile acids led to the formation of products in which various rings were opened. The examination of these products showed that the positions of the hydroxyl groups were limited mainly to three positions 3, 7 and 12, and further work showed that the hydroxyl groups behaved differently towards a given reagent (see also §5).

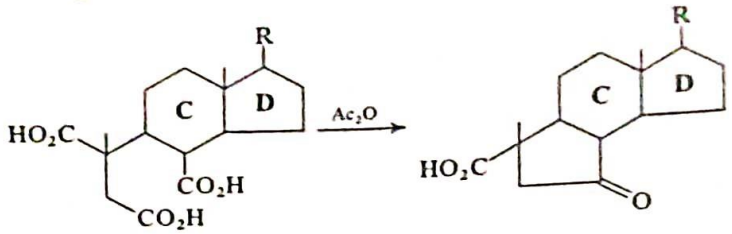
(c) The rings in the steroid nucleus were opened to give a dicarboxylic acid and the relative positions of the two carboxyl groups with respect to each other were determined by the application of **Blanc's rule**: On heating with acetic anhydride, 1,5-dicarboxylic acids form cyclic anhydrides, and 1,6-dicarboxylic acids form cyclopentanones with elimination of carbon dioxide (see also Vol. I).

Ring A. Cholesterol and the cholic acids were converted into the dicarboxylic acid (A) which gave a cyclopentanone, and so ring A is six-membered (R is the appropriate side-chain).



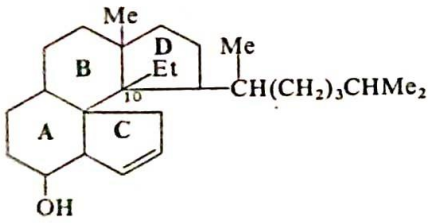
(A)

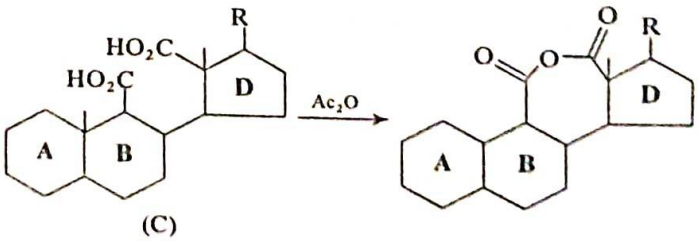
Ring B. Cholesterol was converted into the tricarboxylic acid (B) which gave the cyclopentanone derivative shown. Hence ring B is six-membered.



(B)

Ring C. Deoxycholic acid was converted into a dicarboxylic acid which gave a cyclic anhydride. It was therefore assumed that ring C was five-membered, and this led Windaus and Wieland (1928) to propose the following formula for cholesterol, and the uncertain point (at that time) was the nature of the two extra carbon atoms. These were *assumed* to be present as an ethyl group at position 10, but Wieland *et al.* (1930) finally proved that there was no ethyl group at this position. These two 'homeless' carbon atoms were not placed until Rosenheim and King first proposed that steroids contained the chrysene nucleus and then proposed the cyclopentenophenanthrene nucleus (see above). Bernal (1932) also showed, from the X-ray analysis of cholesterol, ergosterol, etc., that the molecule was thin, whereas the above structure for the steroid nucleus would be rather thick.

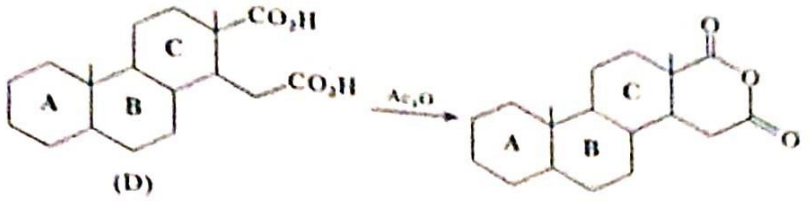




(C)

If we use the correct structure of cholesterol, the cyclisation reaction results in the formation of a *seven-membered cyclic anhydride*. Thus, in this case (and in some others), the Blanc rule fails and leads to erroneous conclusions.

Ring D. 5β-Cholestane (Coprostane) was converted into etiobilianic acid (see (iii), below), and this gave a cyclic anhydride. Hence ring D is five-membered.



(D)

(ii) **Positions of the hydroxyl group and double bond.** Let us consider the following reactions:

$$\underset{C_{27}H_{46}O\,(III)}{\text{Cholestanone}} \xrightarrow{HNO_3} \underset{C_{27}H_{46}O_4\,(V)}{\text{Dicarboxylic acid}} \xrightarrow{300^\circ C} \underset{C_{26}H_{44}O\,(VI)}{\text{Ketone}}$$

Since the dicarboxylic acid (V) contains the same number of carbon atoms as the ketone (III) from which it is derived, the keto group in (III) must therefore be in a ring. Also, since pyrolysis of the dicarboxylic acid (V) produces a ketone with the loss of one carbon atom, it therefore follows from Blanc's rule that (V) is either a 1,6- or 1,7-dicarboxylic acid. Now we have seen that the nucleus contains three six-membered rings and one five-membered ring. Thus the dicarboxylic acid (V) must be obtained by the opening of ring A, B or C, and consequently it follows that the hydroxyl group in cholesterol (which was converted into the keto group in cholestanone; see (i) above) is in ring A, B or C.

Actually *two* isomeric dicarboxylic acids are obtained when cholestanone is oxidised. The formation of these two acids indicates that the keto group in cholestanone is flanked on either side by a methylene group, *i.e.*, the grouping $-CH_2COCH_2-$ is present in cholestanone. Examination of the reference structure (I) of cholesterol shows that such an arrangement is possible only if the hydroxyl group is in ring A.

Now let us consider the further set of reactions:

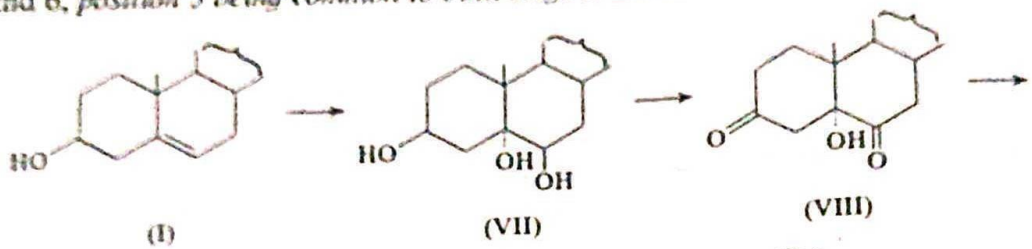
$$\underset{C_{27}H_{46}O\,(I)}{\text{Cholesterol}} \xrightarrow[CH_3CO_2H]{H_2O_2} \underset{C_{27}H_{48}O_3\,(VII)}{\text{Cholestanetriol}} \xrightarrow{CrO_3} \underset{C_{27}H_{44}O_3\,(VIII)}{\text{Hydroxycholestanedione}} \xrightarrow[(ii)\ Zn-CH_3CO_2H]{(i)\ -H_2O}$$

$$\underset{C_{27}H_{44}O_2\,(IX)}{\text{Cholestanedione}} \xrightarrow{CrO_3} \underset{C_{27}H_{44}O_8\,(X)}{\text{Tetracarboxylic acid}}$$

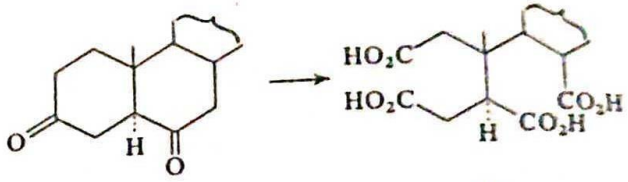
In the conversion of (I) into (VII), the double bond in (I) is hydroxylated. Since only two of the three hydroxyl groups in (VII) are oxidised to produce (VIII), these two groups are secondary alcoholic groups (one of these being the secondary alcoholic group in cholesterol), and the third, being resistant to oxidation, is probably a tertiary alcoholic group. Dehydration of (VIII) (by heating *in vacuo*) and subsequent reduction of the double bond forms (IX), and this, on oxidation, gives a tetracarboxylic acid *without loss of carbon atoms*. Thus the two keto groups in (IX) must be in *different* rings; had they been in the *same* ring, then carbon would have been lost and (X) not obtained. It therefore follows that the hydroxyl group and double bond in cholesterol must be in *different* rings. Furthermore, since (IX) forms a pyridazine derivative with hydrazine, (IX) is a γ-diketone. Since we have already tentatively placed the hydroxyl group in ring A, the above reactions can be readily explained if we place the hydroxyl group at position 3, and the double bond between 5 and 6. In the following equations only rings A and B are drawn; this is an accepted convention of focusing attention on any part of the steroid molecule that is under consideration (also note that full lines represent groups lying above the plane, and broken lines groups lying below the plane; see also §5). Noller

(1939) has shown that the pyridazine derivative is a polymer, and so the interpretation that (IX) is a γ-diketone is rendered uncertain. Supporting evidence, however, for the above interpretation is afforded by the fact that when cholesterol is heated with copper oxide at 290° C, cholestenone (XI) is produced, and this on oxidation with permanganate forms a keto-acid (XII) with the loss of one carbon atom. The formation of (XII) indicates that the keto group and the double bond in cholestenone are in the *same* ring. The ultraviolet absorption spectrum of cholestenone, $\lambda_{max.}$ 240 nm, shows that the keto group and the double bond are conjugated (Menschick *et al.*, 1932). These results can be explained if we assume that the double bond in cholesterol migrates in the formation of cholestenone, the simplest explanation being that the hydroxyl group is in position 3 and the double bond between 5 and 6, *position 5 being common to both rings A and B*. Thus:

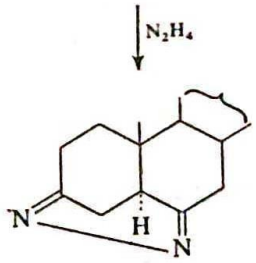
(i)



(I) (VII) (VIII)

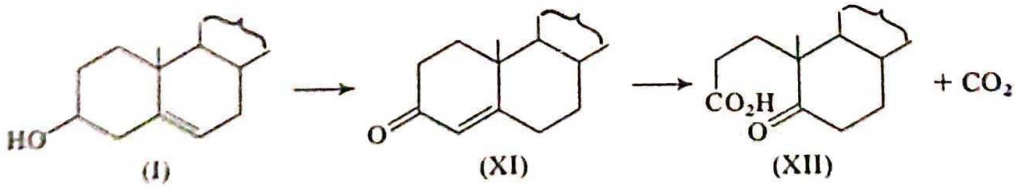


(IX) (X)



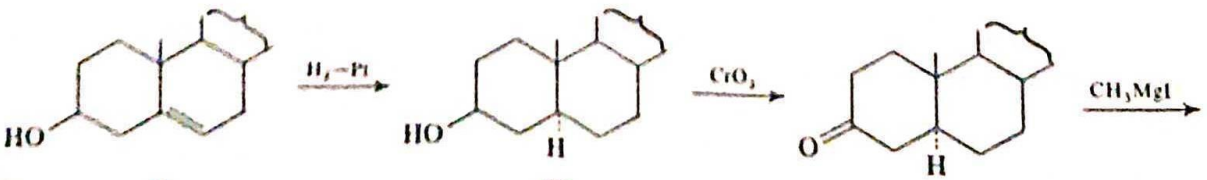
pyridazine derivative

(ii)



(I) (XI) (XII)

The position of the hydroxyl group at position 3 is definitely proved by the experiments of Kon *et al.* (1937, 1939). These authors reduced cholesterol (I) to cholestanol (II), oxidised this to cholestanone (III), treated this with methylmagnesium iodide and dehydrogenated the product, a tertiary alcohol (XIII), to 3',7-dimethylcyclopentenophenanthrene (XIV) by means of selenium. The structure of (XIV) was proved by synthesis, and so the reactions may be formulated as follows, with the hydroxyl at position 3.

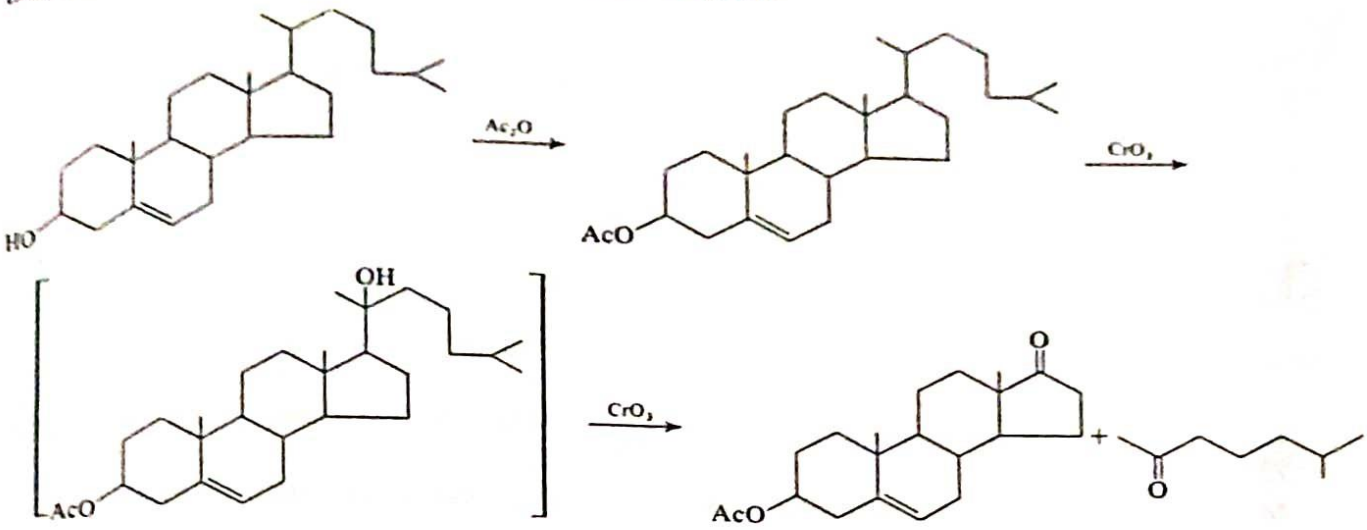


(I) (II) (III)

(XIII) (XIV)

The stereochemistry of the various reactions given above is discussed in §§5 and 8.

(iii) **Nature and position of the side-chain.** Acetylation of cholesterol produces cholesteryl acetate and this, on oxidation with chromium trioxide, forms a steam-volatile ketone and the acetate of a hydroxyketone (which is not steam volatile). The ketone was shown to be isohexyl methyl ketone, $CH_3CO(CH_2)_3CH(CH_3)_2$. Thus this ketone is the side-chain of cholesterol, the point of attachment of the side-chain being at the carbon of the keto group. These results do not show where the side-chain is attached to the nucleus of cholesterol, but if we accept that the position is at 17, then we may formulate the reactions as follows:



The nature of the side-chain has also been shown by the application of the Barbier–Wieland degradation. Since this method also leads to evidence that shows *which ring* of the nucleus is attached to the side-chain, we shall consider the problem of the nature of the side-chain again.

The Barbier–Wieland degradation offers a means of 'stepping down' an acid one carbon atom at a time as follows:

$$RCH_2CO_2H \xrightarrow[HCl]{CH_3OH} RCH_2CO_2CH_3 \xrightarrow{2C_6H_5MgBr} RCH_2C(OH)(C_6H_5)_2 \xrightarrow{-H_2O} RCH{=}C(C_6H_5)_2 \xrightarrow{CrO_3} RCO_2H + (C_6H_5)_2CO$$

Methylmagnesium bromide may be used instead of phenylmagnesium bromide, and the alcohol so obtained may be directly oxidised:

$$RCH_2C(OH)(CH_3)_2 \xrightarrow{CrO_3} RCO_2H + (CH_3)_2CO$$

In the following account, only phenylmagnesium bromide will be used to demonstrate the application of the method to the steroids.

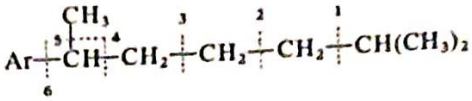
Cholesterol was first converted into 5β-cholestane (coprostane). If we represent the nucleus of 5β-cholestane as Ar, and the side-chain as C_n, then we may formulate the degradation of 5β-cholestane as follows (B–W represents a Barbier–Wieland degradation):

$$5\beta\text{-Cholestane} \xrightarrow{CrO_3} CH_3COCH_3 + 5\beta\text{-Cholanic acid} \xrightarrow{B-W} (C_6H_5)_2CO + \text{Nor-}5\beta\text{-cholanic acid} \xrightarrow{B-W}$$
$$Ar{-}C_8 \qquad\qquad Ar{-}C_{8-3} \qquad\qquad Ar{-}C_{8-4}$$

$$(C_6H_5)_2CO + \text{Bisnor-}5\beta\text{-cholanic acid} \xrightarrow{B-W} (C_6H_5)_2CO + \text{Etiocholyl methyl ketone} \xrightarrow{CrO_3} 5\beta\text{-Etianic acid}$$
$$Ar{-}C_{8-5} \qquad\qquad Ar{-}C_{8-6} \qquad\qquad Ar{-}C_{8-7}$$

The formation of acetone from 5β-cholestane indicates that the side-chain terminates in an isopropyl group. The conversion of bisnor-5β-cholanic acid into a ketone shows that there is an alkyl group on the α-carbon atom in the former compound. Furthermore, since the ketone is oxidised to 5β-etianic acid (formerly known as aetiocholanic acid) with the loss of one carbon atom, the ketone must be a methyl ketone, and so the alkyl group on the α-carbon atom in bisnor-5β-cholanic acid is a methyl group.

Now the carboxyl group in etianic acid is directly attached to the nucleus; this is shown by the following fact. When etianic acid is subjected to one more Barbier–Wieland degradation, a ketone, etiocholanone, is obtained and this, on oxidation with nitric acid, gives a dicarboxylic acid, etiobilianic acid, *without loss of any carbon atoms*. Thus etiocholanone must be a *cyclic* ketone, and so it follows that there are *eight* carbon atoms in the side-chain, which must have the following structure in order to account for the foregoing degradations (see also the end of this section (iii)):



In addition to the Barbier–Wieland degradation, there are also other methods for degrading the side-chain:

(i) Gallagher *et al.* (1946) have introduced a method to eliminate *two* carbon atoms at a time:

$$ArCHMeCH_2CH_2CO_2H \xrightarrow[(ii)\ CH_2N_2]{(i)\ SOCl_2} ArCHMeCH_2CH_2COCHN_2 \xrightarrow{HCl} ArCHMeCH_2CH_2COCH_2Cl \xrightarrow[AcOH]{Zn}$$
$$ArCHMeCH_2CH_2COCH_3 \xrightarrow[(ii)\ -HBr]{(i)\ Br_2} ArCHMeCH{=}CHCOCH_3 \xrightarrow{CrO_3} ArCHMeCO_2H$$

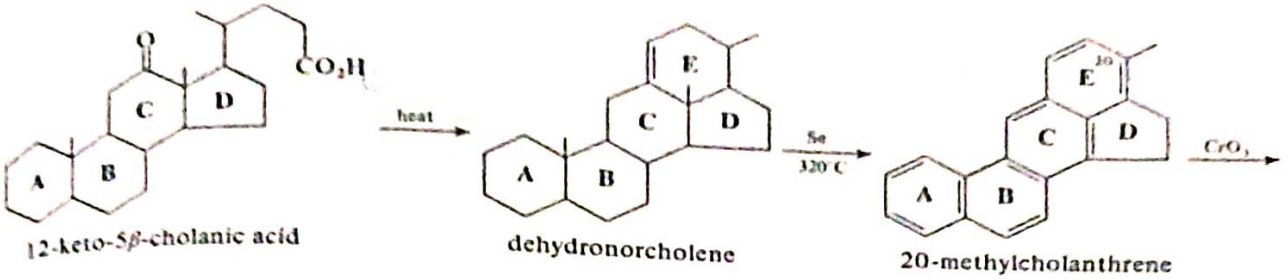
(ii) Miescher *et al.* (1944) have introduced a method to eliminate *three* carbon atoms at a time:

$$ArCHMeCH_2CH_2CO_2Me \xrightarrow{2PhMgBr} ArCHMeCH_2CH_2C(OH)Ph_2 \xrightarrow{-H_2O} ArCHMeCH_2CH{=}CPh_2 \xrightarrow[succinimide]{N\text{-bromo-}}$$
$$ArCHMeCHBrCH{=}CPh_2 \xrightarrow{-HBr} ArCMe{=}CHCH{=}CPh_2 \xrightarrow{CrO_3} ArCOMe$$

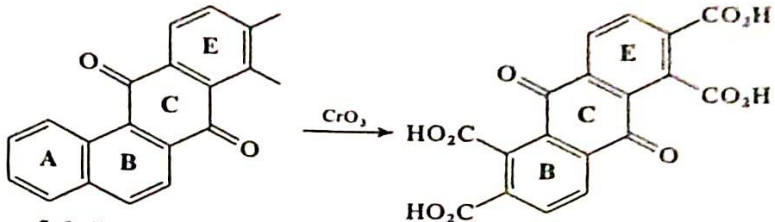
(iii) Jones *et al.* (1958) have carried out the fission of a steroid side-chain with an acid catalyst and have then subjected the volatile products to chromatography. This method has been used with as little as 30 mg of material.

The problem now is: Where is the position of this side-chain? This is partly answered by the following observation. The dicarboxylic acid, etiobilianic acid, forms an anhydride when heated with acetic anhydride. Thus the ketone (etiocholanone) is probably a five-membered ring ketone (in accordance with Blanc's rule), and therefore the side-chain is attached to the five-membered ring D. The actual point of attachment to this ring, however, is not shown by this work. The formation of Diels' hydrocarbon (§1) from cholesterol suggests that the side-chain is at position 17, since selenium dehydrogenations may degrade a side-chain to a methyl group (see **10** §2vii). Position 17 is also supported by evidence obtained from X-ray photographs and surface film measurements. Finally, the following chemical evidence may be cited to show that the position of the side-chain is 17. As we have seen above, 5β-cholanic acid may be obtained by the oxidation of 5β-cholestane. 5β-Cholanic acid may also be obtained by the oxidation of deoxycholic acid (a bile acid; see §14) followed by a Clemmensen reduction. Thus the side-chains in cholesterol and deoxycholic acid are in the same

position. Now deoxycholic acid can also be converted into 12-keto-5β-cholanic acid which, on heating to 320°C, loses water and carbon dioxide to form dehydronorcholene (Wieland *et al.*, 1930). This, when distilled with selenium, forms 20-methylcholanthrene, the structure of which is indicated by its oxidation to 5,6-dimethyl-1,2-benzanthraquinone which, in turn, gives on further oxidation, anthraquinone-1,2,5,6-tetracarboxylic acid (Cook, 1933). Finally, the structure of 20-methylcholanthrene has been confirmed by synthesis (see 10§5b). The foregoing facts can be explained only if the side-chain in cholesterol is in position 17; thus:



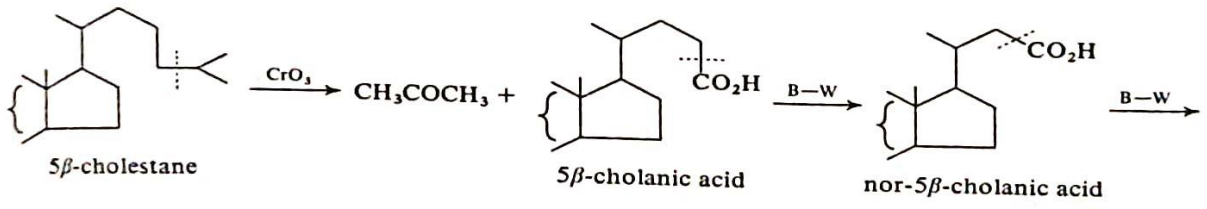
12-keto-5β-cholanic acid — dehydronorcholene — 20-methylcholanthrene



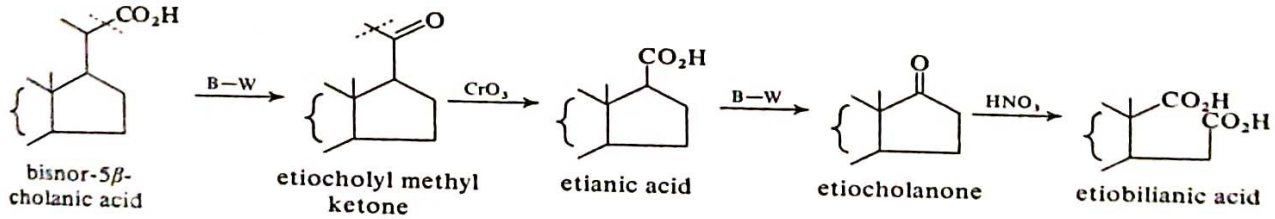
5,6-dimethyl-1,2-benzanthraquinone — anthraquinone-1,2,5,6-tetracarboxylic acid

It should be noted that the isolation of methylcholanthrene affords additional evidence for the presence of the cyclopentenophenanthrene nucleus in cholesterol.

Thus, now that we know the nature and position of the side-chain, we can formulate the conversion of 5β-cholestane into etiobilianic acid as follows:

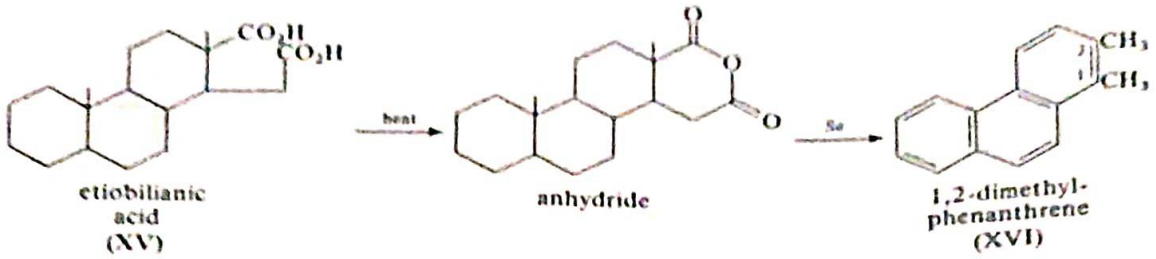


5β-cholestane — 5β-cholanic acid — nor-5β-cholanic acid



bisnor-5β-cholanic acid — etiocholyl methyl ketone — etianic acid — etiocholanone — etiobilianic acid

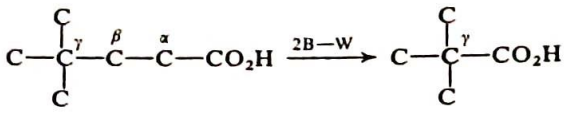
A point of interest in this connection is that when the anhydride of etiobilianic acid is distilled with selenium, 1,2-dimethylphenanthrene is obtained (Butenandt *et al.*, 1933). This also provides proof for the presence of the phenanthrene nucleus in cholesterol, and also evidence for the position of the C-13 angular methyl group (see (iv)).



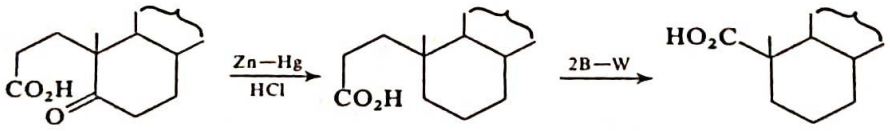
etiobilianic acid (XV) — anhydride — 1,2-dimethylphenanthrene (XVI)

(iv) Positions of the two angular methyl groups. The cyclopentenophenanthrene nucleus of cholesterol accounts for seventeen carbon atoms, and the side-chain for eight. Thus twenty-five carbon atoms in all have been accounted for, but since the molecular formula of cholesterol is $C_{27}H_{46}O$, two more carbon atoms must be fitted into the structure. These two carbon atoms have been shown to be angular methyl groups.

In elucidating the positions of the hydroxyl group and double bond, one of the compounds obtained was the keto-acid (XII). This compound, when subjected to the Clemmensen reduction and followed by two Barbier–Wieland degradations, gives an acid which is very difficult to esterify, and evolves carbon monoxide when warmed with concentrated sulphuric acid (Tschesche, 1932). Since these reactions are characteristic of an acid containing a carboxyl group attached to a tertiary carbon atom (*cf.* abietic acid, **8** §32), the side-chain in (XII) must be of the type

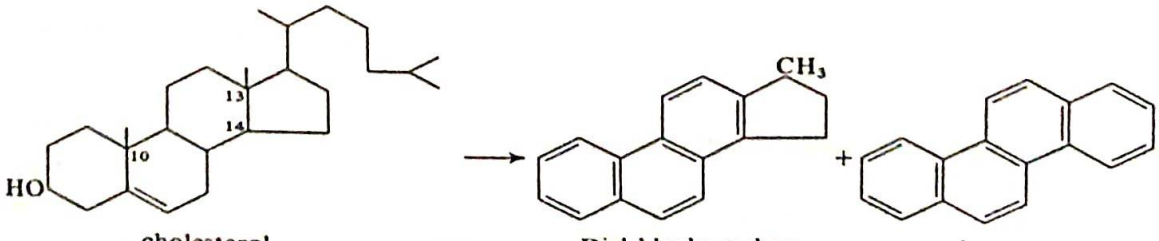


Thus there must be an alkyl group at position 10 in (XII). This could be an ethyl group (as originally believed by Windaus and Wieland) or a methyl group, provided that in the latter case the second 'missing' carbon atom can be accounted for. As we shall see later, there is also a methyl group at position 13, and so the alkyl group at position 10 must be a methyl group. On this basis, the degradation of (XII) may be formulated:



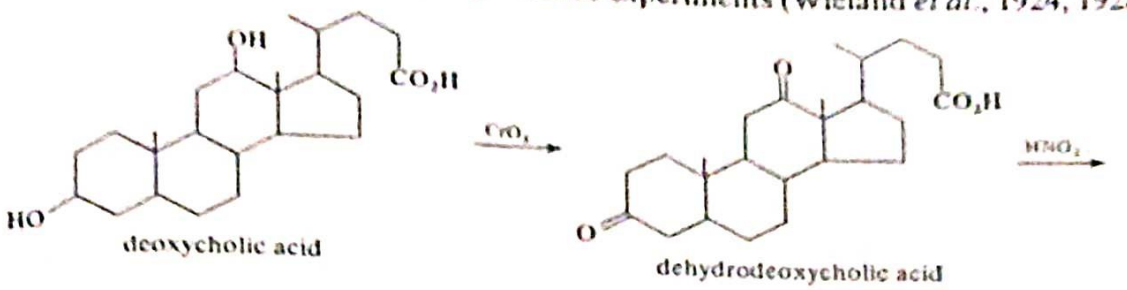
(XII)

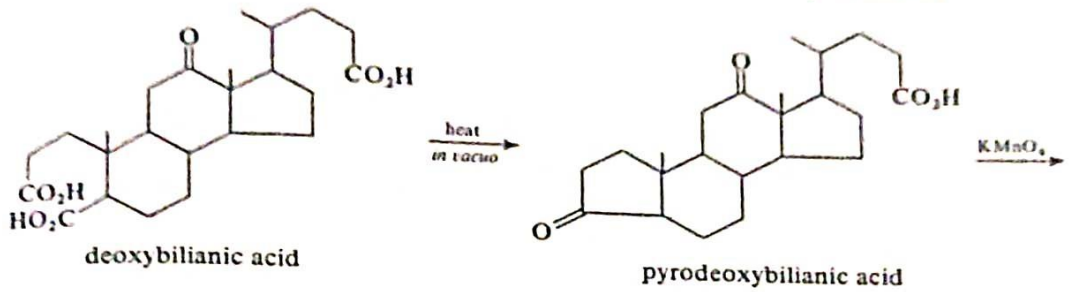
The position of the other angular methyl group is indicated by the following evidence. When cholesterol is distilled with selenium, chrysene is obtained as well as Diels' hydrocarbon (see §1). How, then, is the former produced if the latter is the ring skeleton of cholesterol? One possible explanation is that there is an angular methyl group at position 13, and on selenium dehydrogenation, this methyl group enters the five-membered ring D to form a six-membered ring; thus:

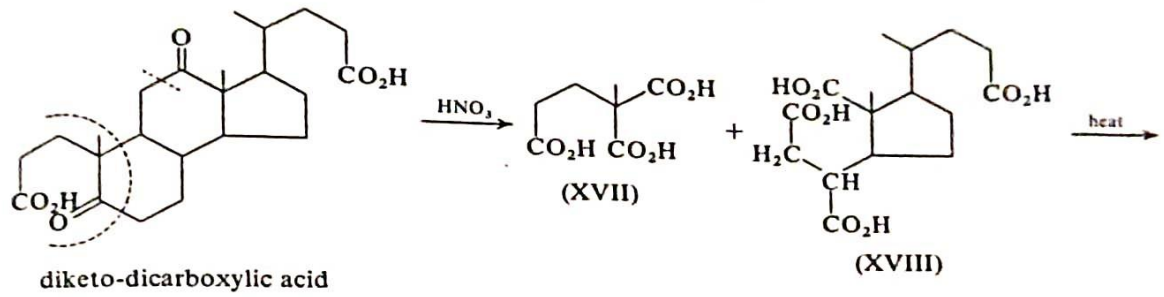


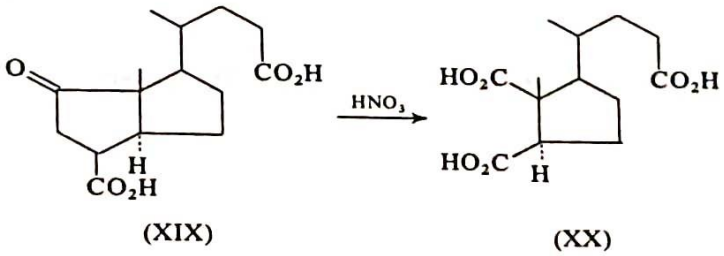
cholesterol — Diels' hydrocarbon — chrysene

This evidence, however, is not conclusive, since ring expansion could have taken place had the angular methyl group been at position 14. Further support for the positions of the two angular methyl groups is given by the following degradative experiments (Wieland *et al.*, 1924, 1928, 1933):









(XVII) was shown to be butane-2,2,4-tricarboxylic acid; thus there is a methyl group at position 10. (XVIII) was shown to be a tetracarboxylic acid containing a cyclopentane ring with a side-chain

$$—CH(CH_3)CH_2CH_2CO_2H.$$

Thus this compound is derived from ring D. (XX) was also shown to be a tricarboxylic acid containing a cyclopentane ring. Furthermore, one carboxyl group in (XX) was shown to be attached to a tertiary carbon atom, and so it follows that there is a methyl group at 13 or 14. (XX) was then shown to have the *trans* configuration, *i.e.*, the two carboxyl groups are *trans*. Thus its precursor (XIX) must have its two rings in the *trans* configuration (the methyl group and hydrogen atom at the junction of the rings are thus *trans*). Theoretical considerations of the strain involved in the *cis*- and *trans*-forms of (XIX) suggest that the *cis*-form of (XIX) would have been obtained had the methyl group been at position 14. Thus the position of this angular methyl group appears (from this evidence)

to be at 13, and this is supported by the fact that etiobilianic acid ((XV), section (iii)) gives 1,2-dimethylphenanthrene (XVI) on dehydrogenation with selenium. Had the angular methyl group been at position 14, 1-methylphenanthrene would most likely have been obtained.

§9. Synthesis of cholesterol

Before describing the synthesis of cholesterol, we shall discuss the problem of the synthesis of complex molecules in general. Many examples of these syntheses have already been described (see Ch. 8, Terpenoids. Ch. 9, Carotenoids). Two difficulties of the classical chemists were the isolation of pure compounds from natural sources and the separation of isomers (usually geometrical and optical) formed in the various steps of a synthesis. Modern methods of separation, particularly chromatography, have overcome these problems. Also, recent syntheses have been more successful and more elegant due to the increased knowledge of reaction mechanisms and to the introduction of selective reagents.

An interesting development in the presentation of recent syntheses is the *discussion* of the reasons that led to the adoption of the sequence of steps for carrying out the synthesis. Classical chemists obviously also had their reasons for carrying out their syntheses in a particular way, but these are not often described or are only briefly mentioned in their publications.

A characteristic feature of recent syntheses is the use of control elements. These may be divided into two types: **regiospecific** or **regioselective control elements**, and **stereospecific** or **stereoselective control elements**. The terms 'specific' and 'selective' are used in the sense described in 4 §5k. Regiospecific control elements are groups which have been deliberately introduced to cause reactions to occur at a specific site in a molecule and, if necessary, can be readily removed without affecting the rest of the molecule. Stereospecific control elements are those which cause a reaction to proceed in such manner that the product has one particular type of geometry rather than another. Control elements were used by the classical chemists, but many more of these elements have now been introduced. Some examples of their application have already been described, *e.g.*, regiospecific: protecting groups, activating of a methylene group by an adjacent oxo group; stereospecific: asymmetric synthesis (more correctly this is an example of stereoselectivity), stereochemical control by steric effects, addition and elimination reactions.

A simple molecule may be described as one which is small and whose total synthesis requires a relatively small number of steps. Very often, such a synthesis may be readily achieved by 'working backwards'. On the other hand, a complex molecule may be described as a large molecule whose total synthesis requires a large number of steps. Furthermore, the synthesis of a complex molecule usually involves problems of stereochemistry. It is important to note, however, that success in achieving a synthesis, be it of a simple or a complex molecule, ultimately depends on a very good knowledge of organic reactions and their application.

Some points that may be noted for the general approach to the synthesis of complex molecules are (see the appropriate reading references):

(i) The recognition of structural units within the molecule which can be formed and/or assembled by known chemical methods. Starting materials should be readily accessible. The first objective is assisted by examination of the molecule (to be synthesised) for any type of symmetry. Recognition of symmetry will lead to a shorter route. Structural units within a molecule are termed 'synthons', and their recognition may suggest routes for the synthesis. Furthermore, recognition of a relationship of the molecule to some other *known* compound may permit the use of a complicated synthon if the known compound is readily available.

(ii) The necessity of obtaining the best yields of the products is of paramount importance, and to achieve this may require the use of control elements.

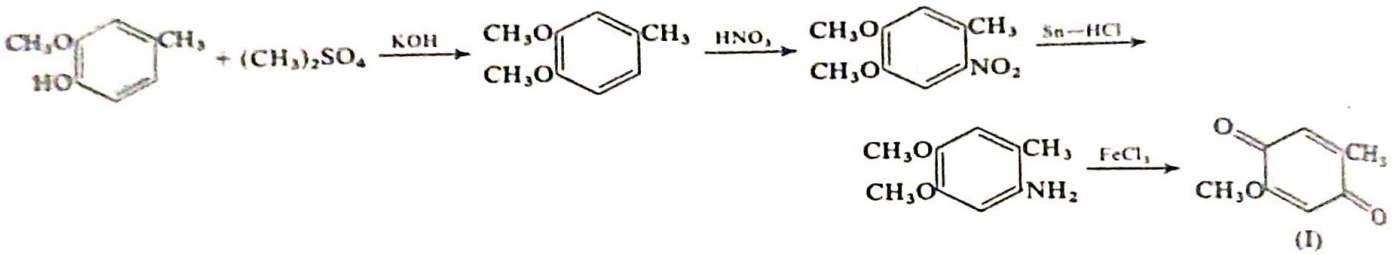
(iii) The relative positions of chiral centres (when present) may give information on the type of control elements required to give the desired configurations.

(iv) The presence of reactive functional groups which can give rise to neighbouring group participation may suggest steps that lead to a desired intermediate, *e.g.*, by temporary cyclisation and so controlling the stereochemical course of the reaction.

We shall now discuss the synthesis of cholesterol and consider it in the light of the above discussion. Basically, the synthesis of steroids involves the construction of the steroid nucleus in the form of the required conformation. The early methods started with ring A or rings A/B, and the other rings were then built up as follows: A → AB → ABC → ABCD. However, as the number of selective reagents increased, different starting points and different orders of fusion were developed, *e.g.*, (i) AB → ABCD; (ii) AC → ABC → ABCD; (iii) AD → ABCD; (iv) BC → BCD → ABCD; (v) CD → ACD → ABCD; (vi) CD → BCD → ABCD.

Two groups of workers, *viz.*, Robinson *et al.* (1951) and Woodward *et al.* (1951), have synthesised cholesterol. One of the outstanding difficulties in the synthesis of steroids is the stereochemical problem. The cholesterol nucleus contains eight chiral centres and so 256 optical isomers are possible (see also §4 for further details). Thus every step in the synthesis which produced a new chiral centre had to result in the formation of some (the more the better) of the desired stereoisomer, and at the same time resolution of racemic modifications also had to be practicable. Another difficulty was attacking a particular point in the molecule without affecting the other parts. This problem led to the development of specific reagents. The following is an outline of the Woodward synthesis. Some steps are not stereospecific or even stereoselective. Later syntheses of various steroids are superior in this respect (see, *e.g.*, aldosterone, §28b). The synthesis of cholesterol described here is of the type: C → CD → BCD → ABCD.

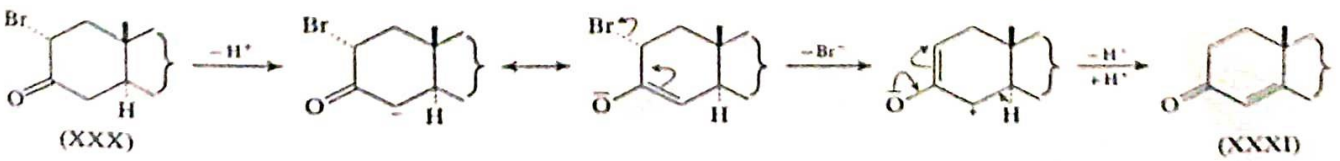
4-Methoxy-2,5-toluquinone (I) was prepared from 2-methoxy-*p*-cresol as follows:



(I)

(I) was condensed with butadiene (Diels–Alder reaction) to give (II). This has the *cis* configuration and was isomerised (quantitatively) to the *trans*-isomer (III) by dissolving in aqueous alkali, adding a seed crystal of the *trans*-isomer and then acidifying. Isomerisation occurs *via* the enolate to give the more stable *trans*-isomer (see also §5; configuration of the nucleus). (III), on reduction with lithium aluminium hydride, gave (IV). (IV) is a vinyl ether of a glycol which, on treatment with aqueous acid, undergoes hydrolysis (demethylation) to give a β-hydroxyketone which is readily dehydrated to (V) in acid solution. Conversion of (V) to (VI) by removal of the hydroxyl group was carried out by a new technique: (V) was acetylated and the product, the ketol acetate, was heated with zinc in acetic anhydride to give (VI) [reduction with metal and acid usually reduces α,β-unsaturated bonds in ketones]. (VI), on treatment with ethyl formate in the presence of sodium methoxide, gave the hydroxymethylene ketone (VII) [Claisen condensation]. When this was treated with ethyl vinyl ketone in the presence of potassium t-butoxide, (VIII) was formed (Michael condensation). The object of the double bond in the ketone ring in (VI) is to prevent formylation occurring on that side of the keto group, and the purpose of the formyl group is to produce an active methylene group (this is now flanked on *both* sides by carbonyl groups). The necessity for this 'activation' lies in the fact that ethyl vinyl ketone tends to self-condense, and consequently decrease the yield of (VIII). Both operations are examples of the introduction of regiospecific control elements. (VIII) was now cyclised quantitatively by means of potassium hydroxide in aqueous dioxan to the single product (IX). This is the desired compound; the other possible isomer ((IX) with the two hydrogens *cis* instead of *trans* as shown) is not formed since the *cis*-isomer is less stable than the *trans* due to greater steric interactions in the former, *i.e.*, the cyclisation is stereospecific (steric effect control). Also, the cyclisation

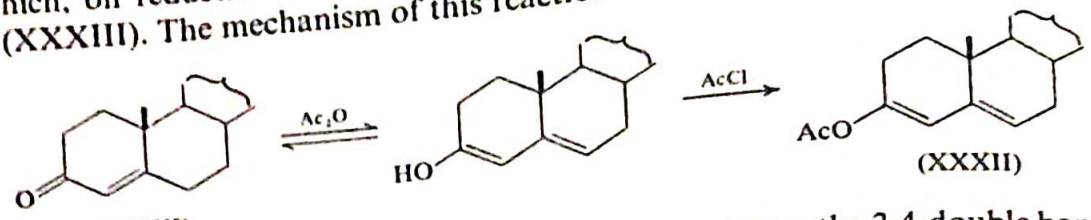
occurs by an intramolecular aldol condensation followed by dehydration. (IX) was then treated with osmium tetroxide to give two *cis*-glycols of structure (X) [one is *cis* with respect to the angular methyl group and the other is *trans*]. Glycol formation occurs readily at the isolated double bond (the other two double bonds are conjugated and so have less double bond character than an isolated double bond; the reaction with osmium tetroxide is very sensitive to this change). These glycols were separated and the desired isomer (the one insoluble in benzene) was treated with acetone in the presence of anhydrous copper sulphate to give the isopropylidene derivative (XI). This, on catalytic reduction (H_2—Pd/$SrCO_3$) gave (XII) which was condensed with ethyl formate in the presence of sodium methoxide to give (XIII), and this was then converted into (XIV) by means of methylaniline. The purpose of this treatment was to block undesired condensation reactions on this side of the keto group (at this position 3); this is another example of a regiospecific control element. When (XIV) was condensed with vinyl cyanide (cyanoethylation) and the product hydrolysed with alkali, the product was a mixture of two keto acids. These were separated and the stereoisomer (XV) [methyl group in front and propionic acid group behind the plane of the rings] was converted into the enol lactone (XVI) which, on treatment with methylmagnesium bromide, gave (XVII), and this, on ring closure by means of alkali, gave (XVIII). When this was oxidised with periodic acid in aqueous dioxan, the dialdehyde (XIX) was obtained (*via* hydrolysis of the diol), and this, when heated in benzene solution in the presence of a small amount of piperidine acetate, gave (XX) [and a small amount of an isomer]. This cyclisation occurs by an intramolecular aldol condensation under the influence of the base, piperidine acetate. Since either aldehyde group can be involved in the condensation, two products are possible. In (XIX), the upper methylene group is *cis* to the hydrogen atom at C-14, whereas the lower methylene group is *cis* to the 18-methyl group. Hence, the upper methylene group experiences less steric hindrance than the lower one and consequently it is the former that loses a proton to form the carbanion. Therefore (XX) is the predominant isomer. (XX) was oxidised to the corresponding acid which was then converted into the methyl ester (XXI) with diazomethane. (XXI), a racemate, was resolved by reduction of the keto group with sodium borohydride to the hydroxy esters [(±)-3α- and (±)-3β-]. The (+)-form of the 3β-alcohol was preferentially precipitated by digitonin, and this stereoisomer was now oxidised (Oppenauer oxidation) to give the desired stereoisomer (+)-(XXI). This was catalytically reduced (H_2—Pt) to (XXII), which was then oxidised to (XXIII) which was now a mixture of stereoisomers (from the mixture of (XXII); H at 17 behind and in front). These were separated, reduced (sodium borohydride), and hydrolysed. The β-isomer, (XXIV), was converted into the methyl ketone by first acetylating, then treating with thionyl chloride and finally with dimethylcadmium. This acetylated hydroxyketone, (XXV), on treatment with isohexylmagnesium bromide, gave (XXVI). This was a mixture of isomers (a new chiral centre has been introduced at position 20). (XXVI), on dehydration, gave one product, (XXVII), and this, on catalytic hydrogenation (H_2—Pt), gave a mixture of 5α-cholestanyl acetates (the chiral C-20 has been re-introduced). These acetates were separated and the desired isomer, on hydrolysis, gave 5α-cholestan-3β-ol, (XXVIII), which was identical with natural cholestanol. The conversion of cholestanol into cholesterol (XXXIII) is then carried out by a series of reactions introduced by various workers. Bromination of (XXIX) in acetic acid in the presence of hydrogen bromide (as catalyst) gives the 2α-bromo-derivative ((XXX); see §8). (XXX), on treatment with pyridine, gives (XXXI). The mechanism of this elimination is uncertain. A possibility is that because the *equatorial* bromine is difficult to remove by the E2 mechanism, a 1,4-elimination occurs by removal of a proton



(XXX) (XXXI)

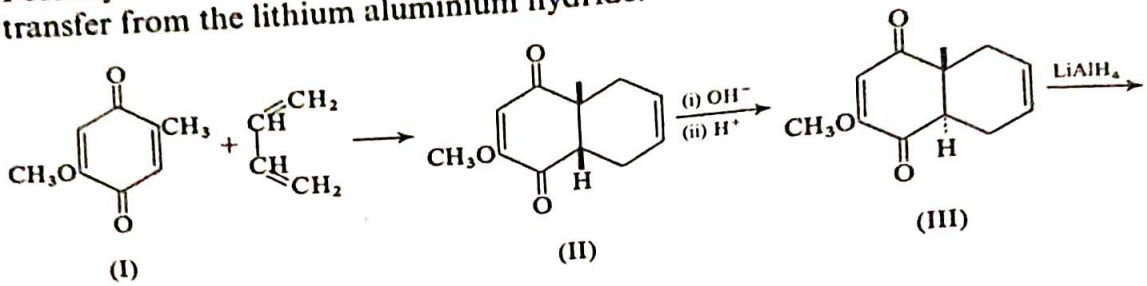
from position 4 by the base (the methylene group in this position is activated by the adjacent oxo group; *cf.* however, the bromination of acetone).

Heating (XXXI) with acetyl chloride in the presence of acetic anhydride gives the enol acetate (XXXII) which, on reduction with lithium aluminium hydride followed by acidification, gives cholesterol (XXXIII). The mechanism of this reaction is uncertain.

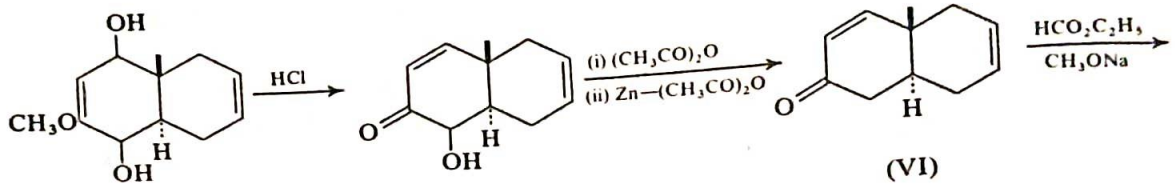


(XXXI) (XXXII)

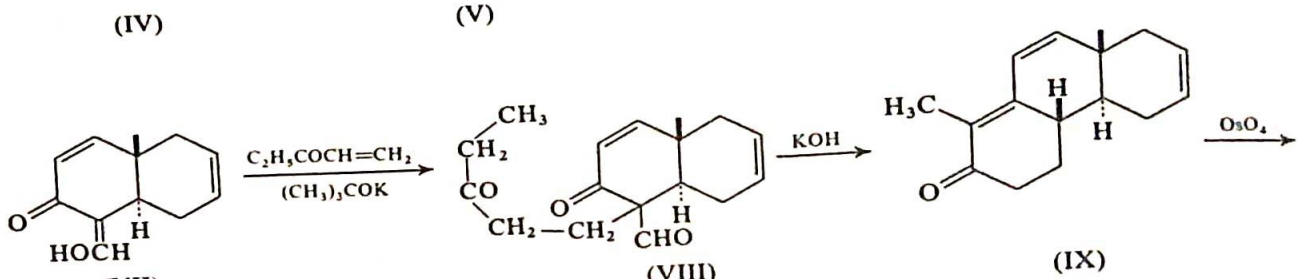
Possibly the electron-attracting effect of the acetoxy-group activates the 3,4-double bond to hydride transfer from the lithium aluminium hydride.



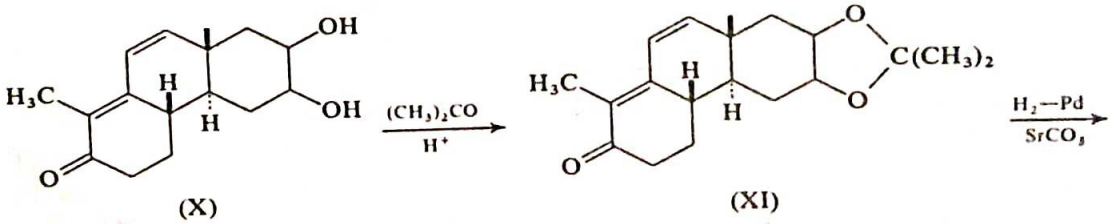
(I) (II) (III)



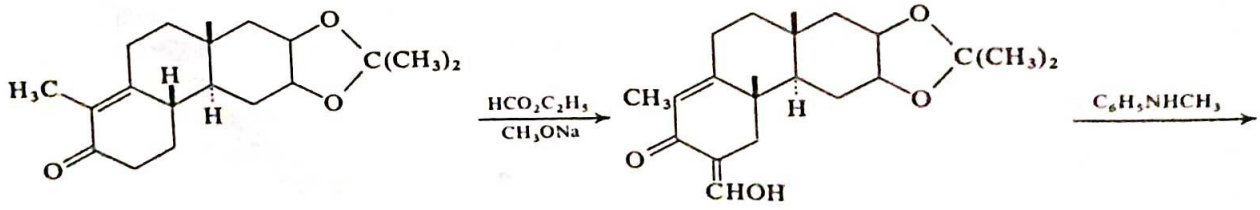
(IV) (V) (VI)



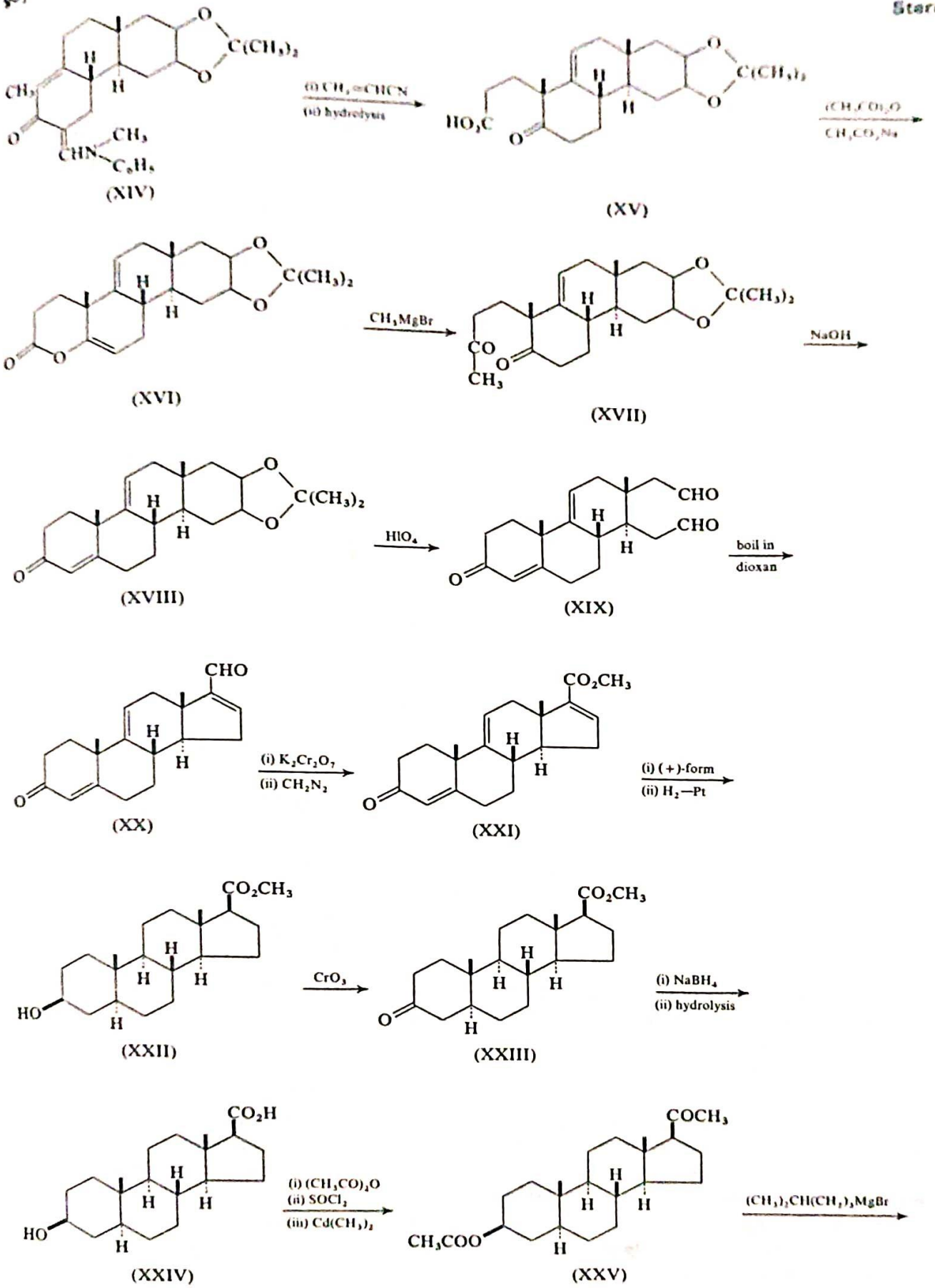
(VII) (VIII) (IX)



(X) (XI)



(XII) (XIII)


(XIV)
(XV)
(XVI)
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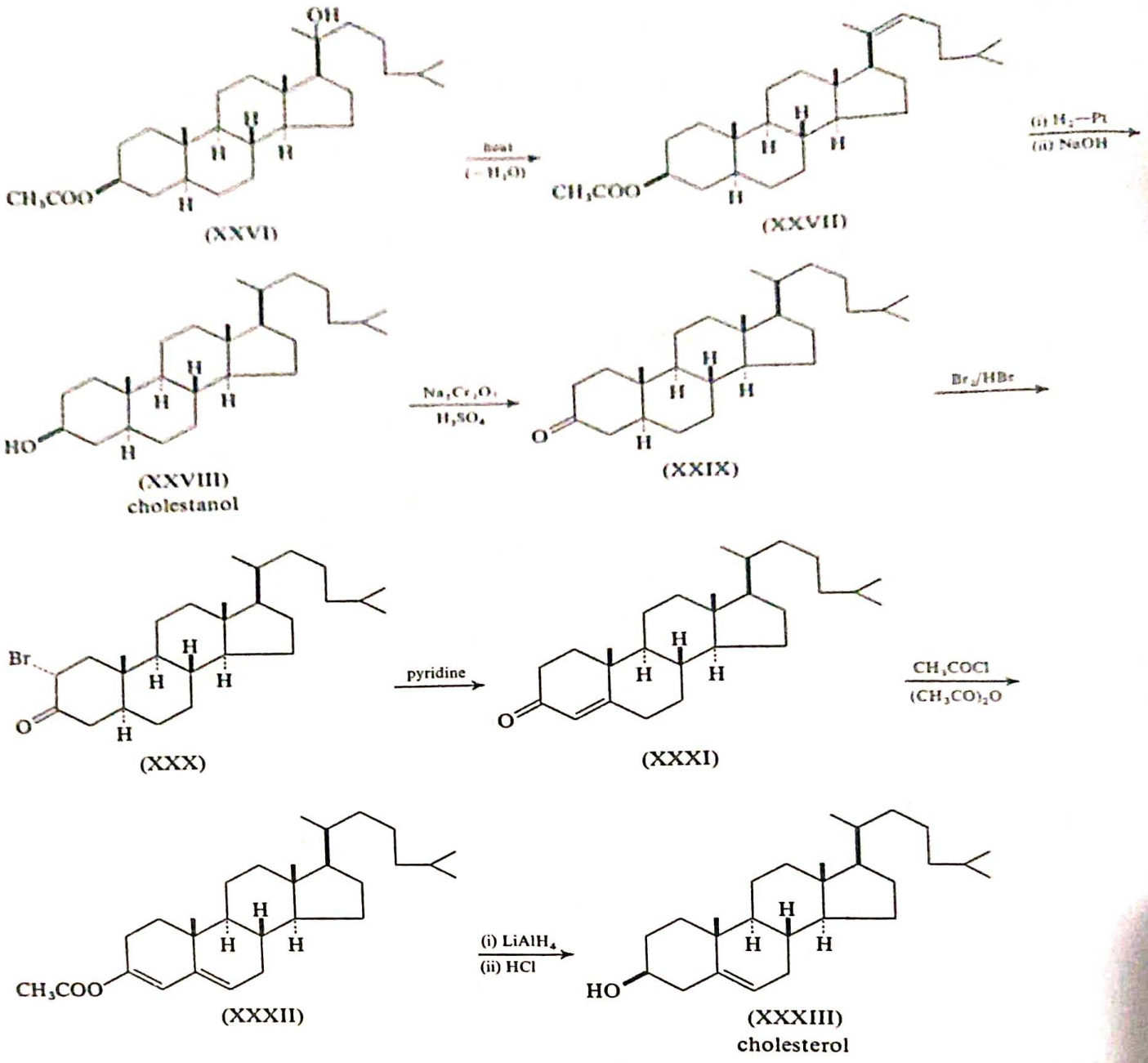
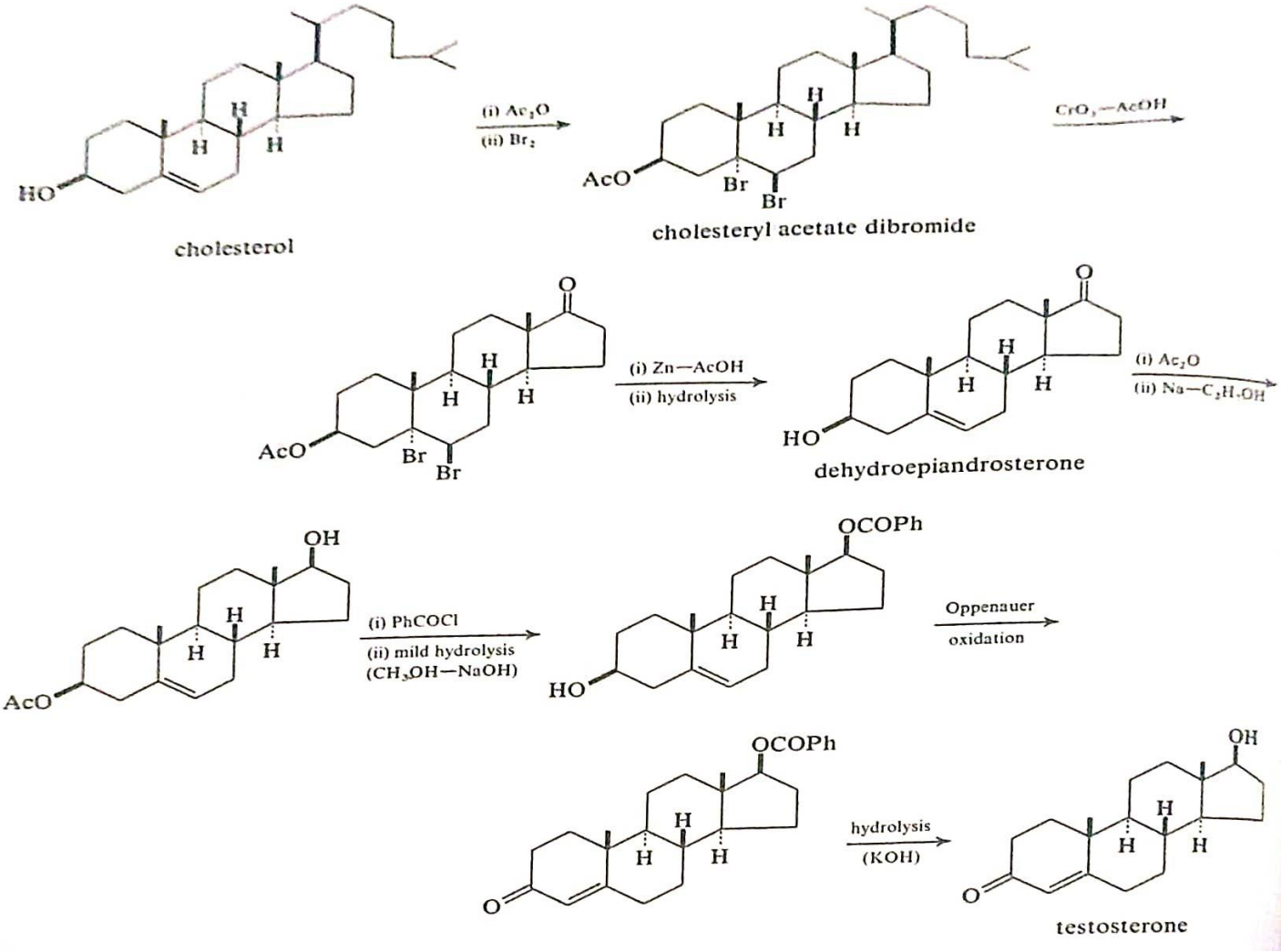
(XXI)
(XXII)
(XXIII)
(XXIV)
(XXV)



An important point to note is that this total synthesis has involved a very large number of steps, and in most cases of this type the overall yield is very small. It may vary from about 4 to about 0·0005 per cent, depending on the number of steps involved. Thus, these syntheses cannot be expected to be a commercial source of these compounds. However, once a total synthesis has been accomplished, other syntheses of the compound may be carried out by starting from any particular intermediate prepared in the sequence. Such a compound may actually occur naturally or be a degradation product of the desired final product. In many cases, the starting material may be a natural compound that can be efficiently converted into the desired product. In such cases, the synthesis of the desired product is referred to as a **partial synthesis**. In general, most complex molecules have been prepared by partial syntheses before total syntheses have been achieved. Partial syntheses can be commercially important, but complete confirmation of structure is always necessary, and this is usually achieved by total synthesis.

This preparation of testosterone establishes the structure of this hormone which had been shown to contain one hydroxyl group and an α,β-unsaturated ketone group.



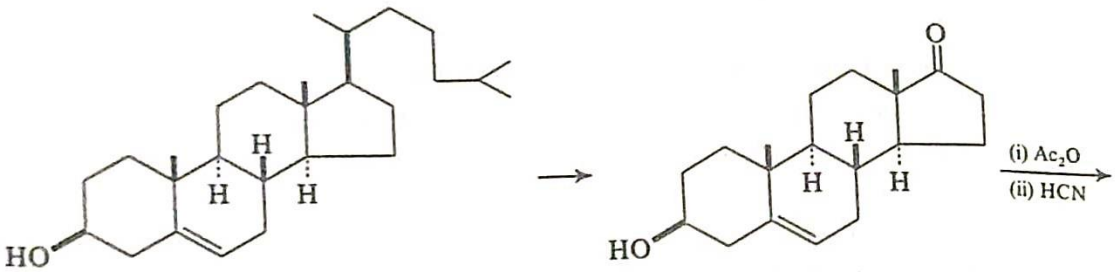
cholesterol

cholesteryl acetate dibromide

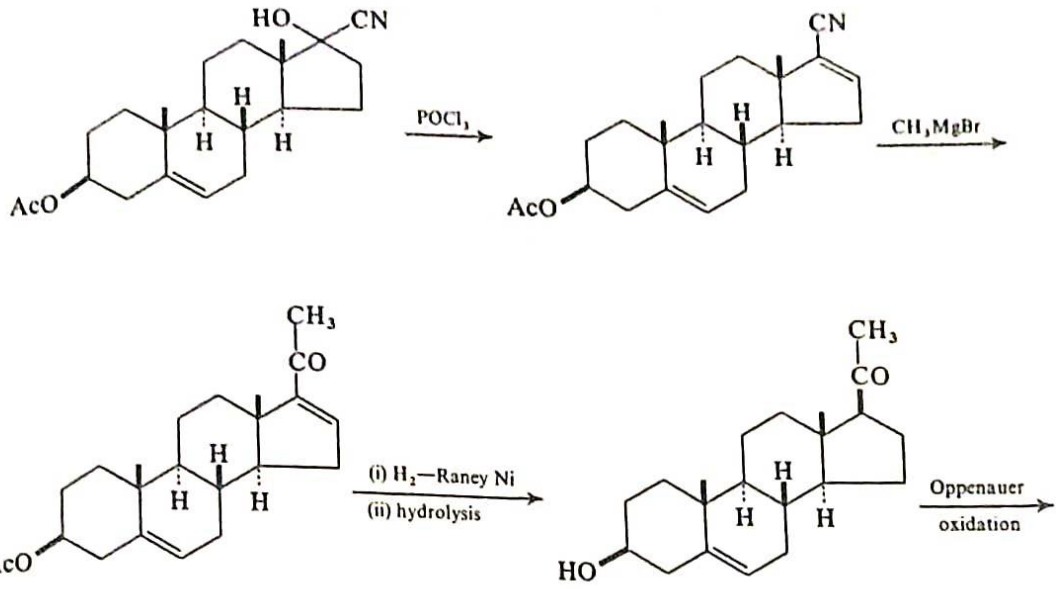
dehydroepiandrosterone

testosterone

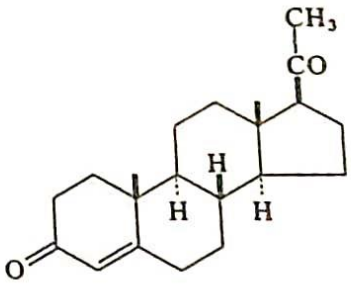
(ii) **Progesterone from cholesterol** (Butenandt *et al.*, 1939). Cholesterol is first converted into dehydroepiandrosterone (see §19), and then as follows:



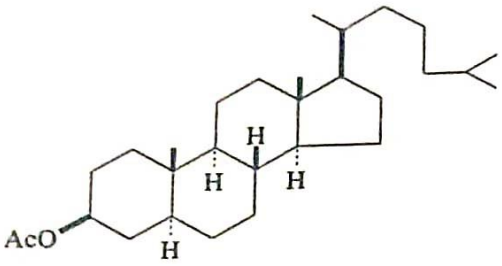
cholesterol dehydroepiandrosterone



pregnenolone

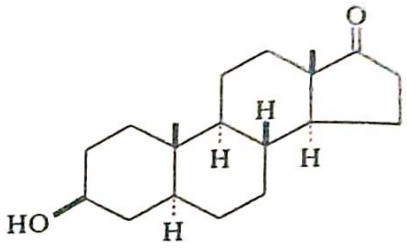


progesterone

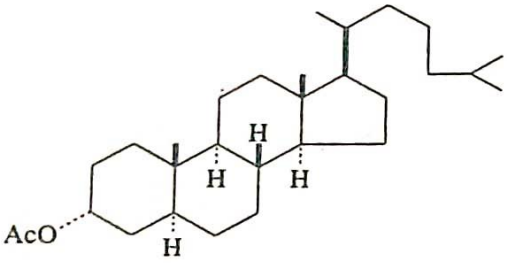


5α-cholestanyl 3β-acetate

(i) CrO_3
(ii) hydrolysis

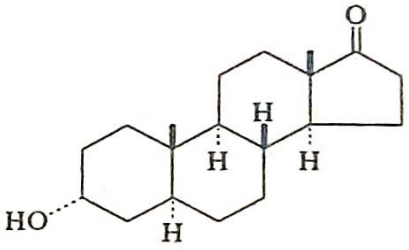


epiandrosterone



5α-cholestanyl 3α-acetate

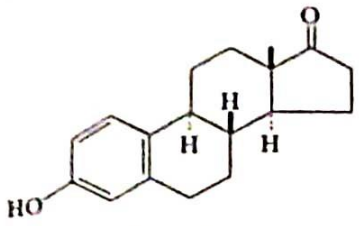
(i) CrO_3
(ii) hydrolysis



androsterone

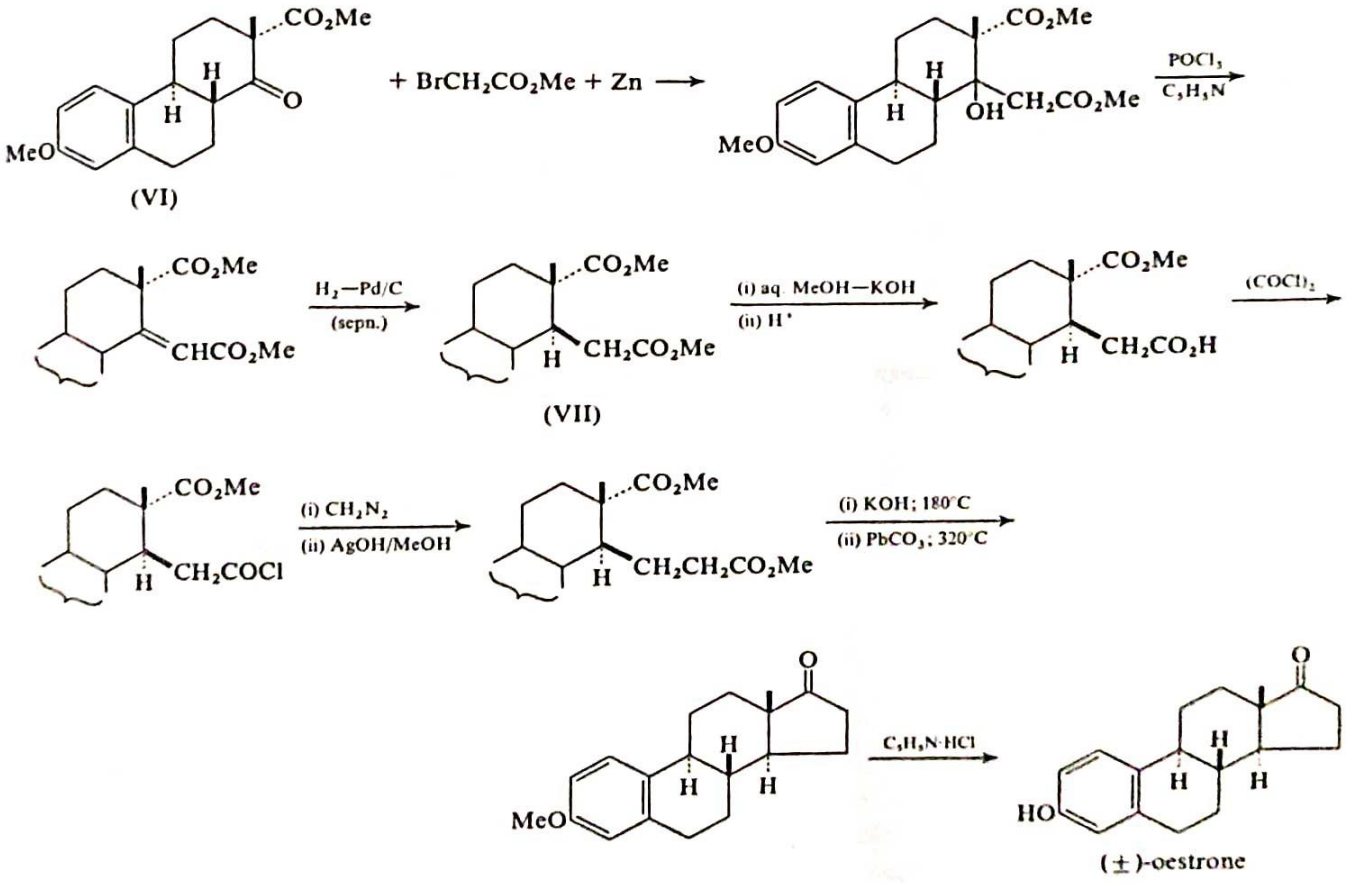
The structure of (V) has been confirmed by synthesis (Cook *et al.*, 1935). Thus the structure of oestrone is as shown (see also below).

This has been confirmed by the total synthesis of Anner and Miescher (1948). These authors started with the phenanthrene derivative (VI) which had been prepared previously by Robinson *et al.* (1938), and by Bachmann *et al.* (1942). The first step of the Anner–Miescher synthesis involves the Reformatsky reaction, and a later one the Arndt–Eistert synthesis.



oestrone

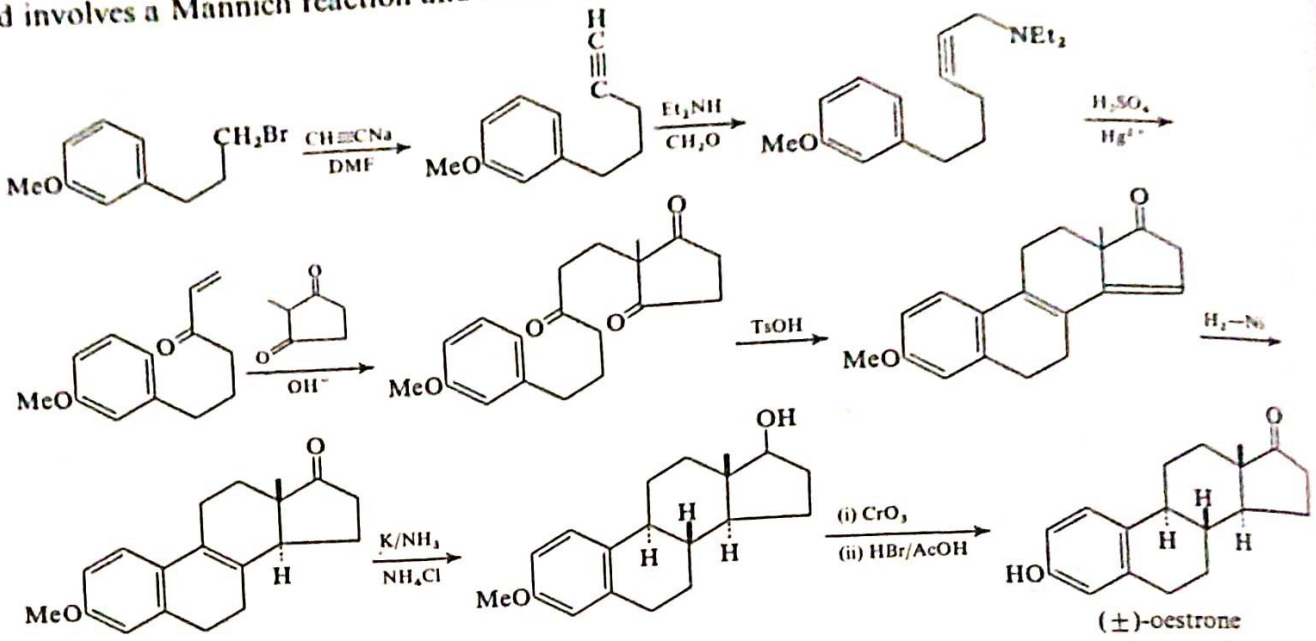
The stereochemical problems involved in the synthesis of oestrone are not so complicated as in cholesterol, since only four chiral centres are present in the hormone (*cf.* §5). (VI) contains 3 chiral centres and so four racemates are possible. Three have been isolated by Anner and Miescher, and one of these was converted into (±)-oestrone (C/D *trans*) and the stereoisomer (C/D *cis*), (±)-iso-oestrone. These were separated and the (±)-oestrone resolved with (−)-menthoxyacetic acid. The (+)-enantiomer that was obtained was shown to be identical with the natural compound. The *trans*-B/C fusion of the racemate used (for the oestrone synthesis) was deduced from other synthetic work, and the



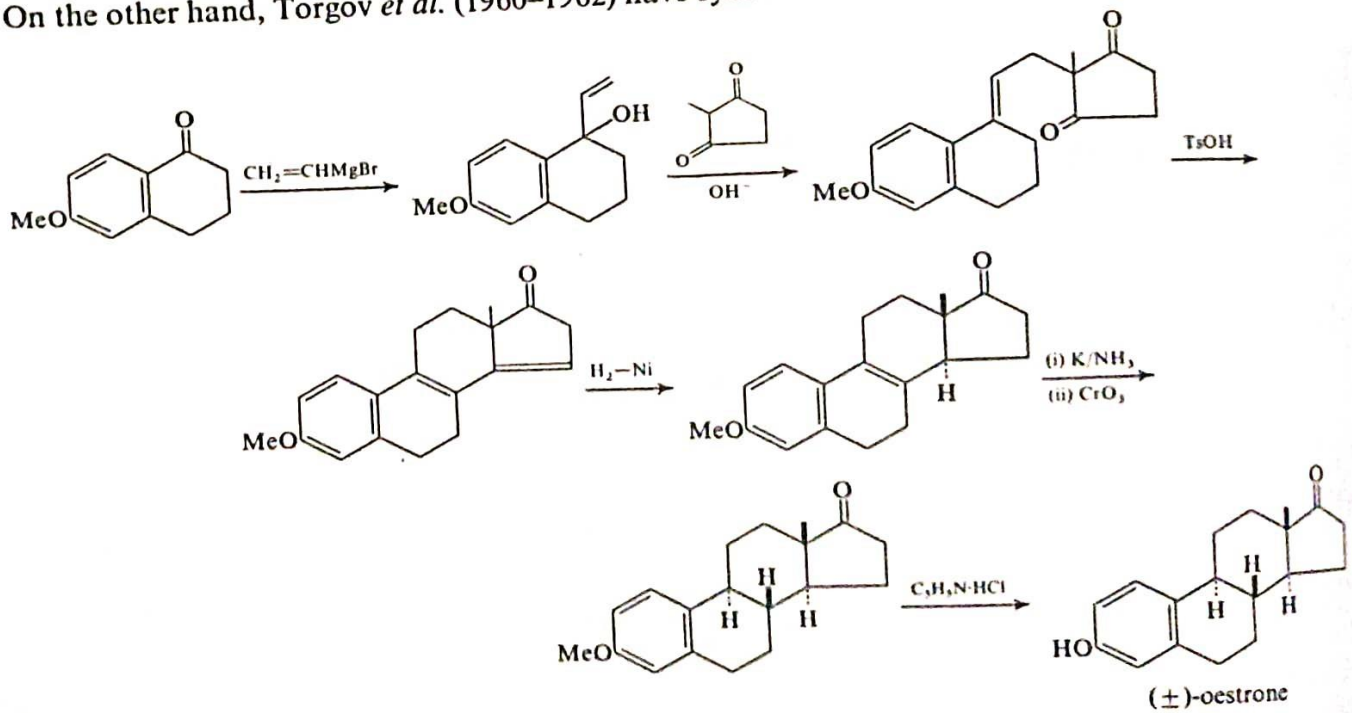
β-configuration of the CH_3-18 had already been established (see above). The catalytic reduction step produced a mixture of stereoisomers (dimethyl esters). These were separated by fractional crystallisation and the one chosen for the oestrone synthesis, (VII), was that which was identical with the methyl ether dimethyl ester of 'natural' (+)-*trans*-marrianolic acid (see formula II, §21).

Miescher and Anner have also prepared various isomers of oestrone by using other stereoisomers of (VI) and (VII), *e.g.*, (±)-iso-oestrone (C/D *cis*).

Johnson *et al.* (1958, 1962) have also carried out a total synthesis of oestrone; each step in their synthesis was stereospecific, but Hughes *et al.* (1960) have described total syntheses of oestrone which appear to be simpler than any previous method and just as efficient. The better method is as follows and involves a Mannich reaction and a Michael condensation (see Vol. I).

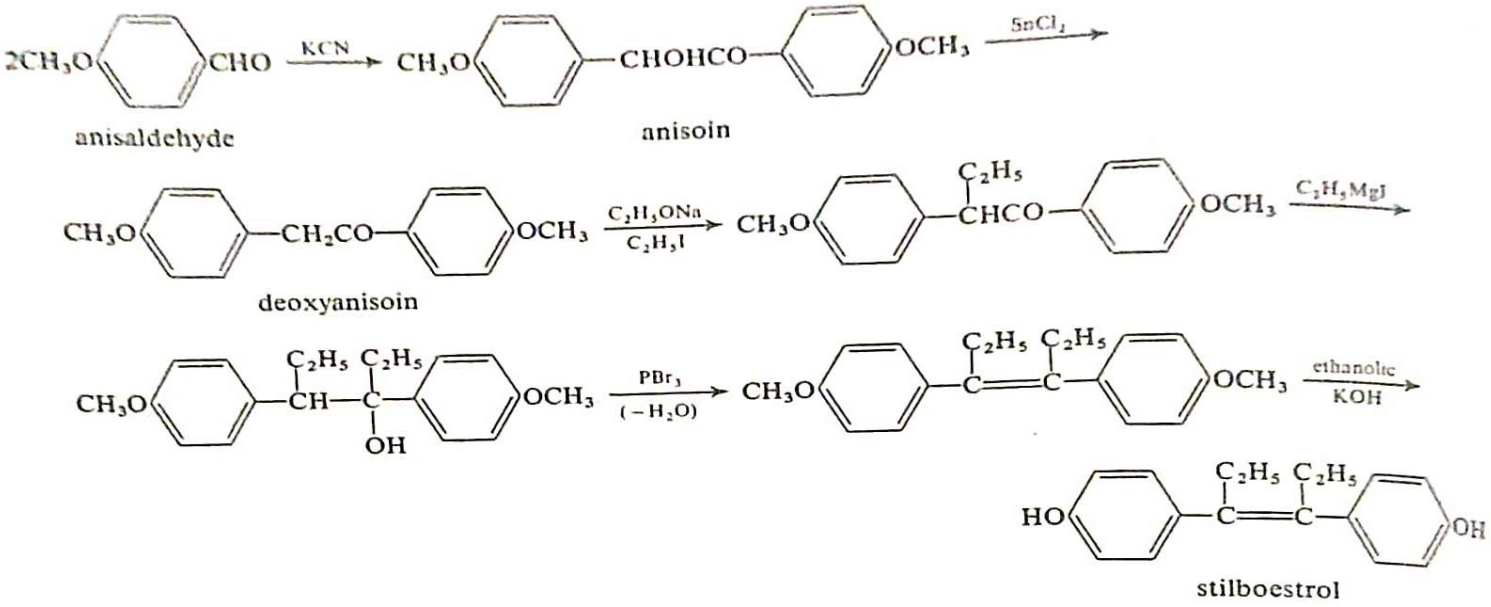


On the other hand, Torgov *et al.* (1960–1962) have synthesised oestrone as follows:

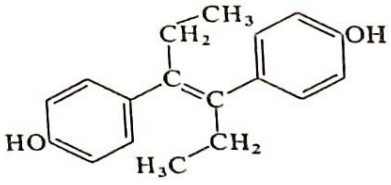


§24. Artificial hormones

Many compounds with oestrogenic activity but not of steroid structure have been prepared synthetically. **Stilboestrol** (4,4'-dihydroxydiethylstilbene) was prepared by Dodds *et al.* (1939) as follows:



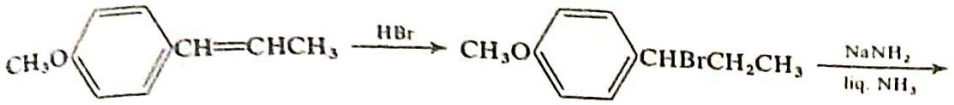
The above structure of stilboestrol can exist in two geometrical isomeric forms; it is the *trans*-form which is the active substance, and this configuration has been confirmed by X-ray analysis (Crowfoot *et al.*, 1941).



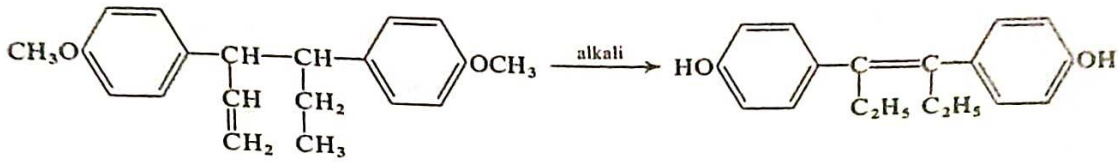
trans-stilboestrol

Kharasch *et al.* (1943) have introduced a simpler synthesis of stilboestrol. Anethole is treated with hydrobromic acid and the product, anethole hydrobromide, is then treated with sodamide in liquid ammonia. The

resulting compound (I) gives stilboestrol on demethylation and isomerisation in the presence of alkali. The structure of (I) is uncertain, but it is believed to be the one given.

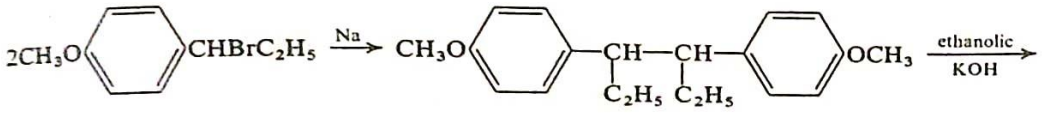


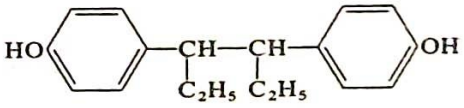
anethole



(I)

Stilboestrol is more active than oestrone when administered subcutaneously, and it can also be given orally. **Hexoestrol** (dihydrostilboestrol) may be prepared from anethole hydrobromide as follows:





hexoestrol

The active form is the *meso*-isomer (as shown by X-ray crystallography by Crowfoot *et al.*, 1941).

UNIT-IV

Flavonoids

INTRODUCTION

Flavonoids in the broad sense of the term are virtually universal plant pigments. Almost always water-soluble, they are responsible for the color of flowers, fruits, and sometimes leaves. Examples are yellow flavonoids (chalcones, aurones, and yellow flovonols) and red, blue, or purple anthocyanins. When they are not directly visible, they contribute to the color by acting as copigments : for example, colorless flavone and flavonol copigments protect anthocyanins.

All flavonoids – approximately 4000- have a common biosynthetic origin, and therefore possess the same basic structural element, namely the 2-phenylchromane skleton. We can classify them :

2-phenylbenxopyriliums, i.e., anthocyanins

2-phenylchromones

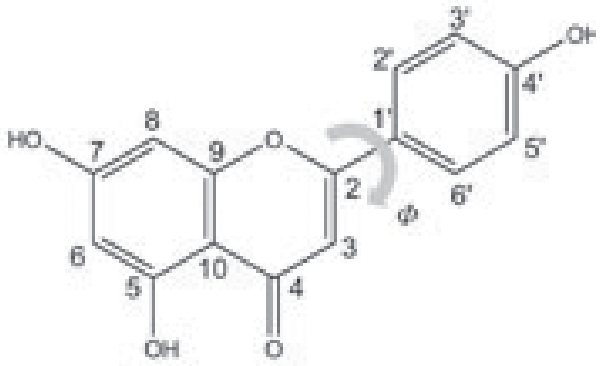
- flavones, flavonols, and their dimers
- flavanones, and dihydroflavonols
- isoflavones, isoflavanones

2-pheylchromane

- flavans
- flavan-3-ols, flavan-3,4-diols

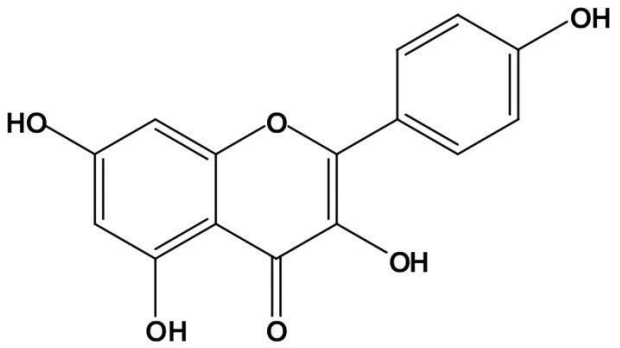
Chalcones and dihydrochalcones (the pyran ring opens)

2-benzylidene coumaranones (= aurones)



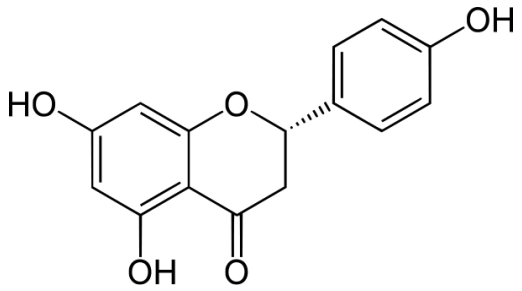
Apigenin

FLAVONE



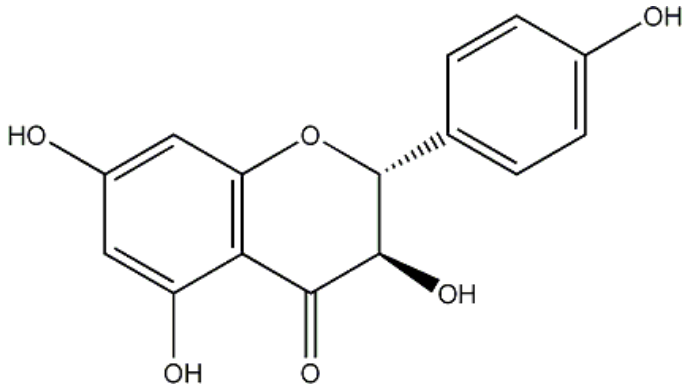
Kaempferol

FLAVONOL



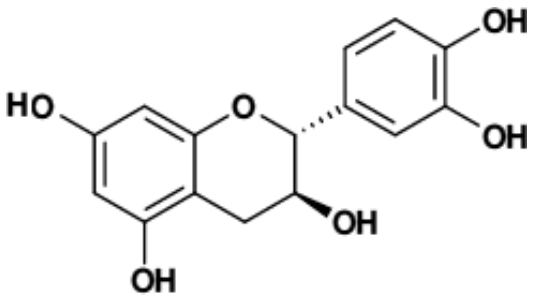
Naringenin

FLAVANONE



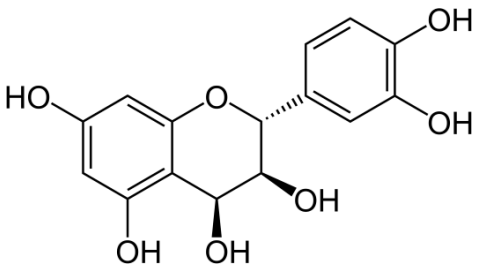
Dihydrokaempferol

DIHYDROFLAVONOL



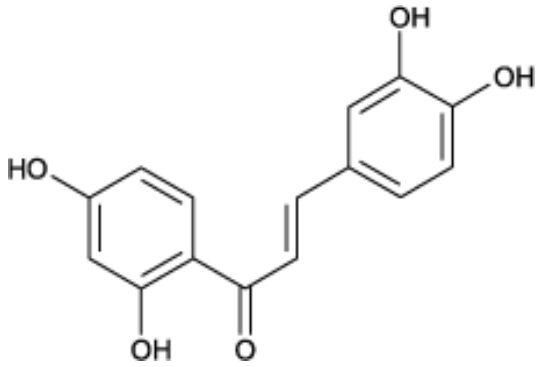
Catechin

FLAVAN-3-OL



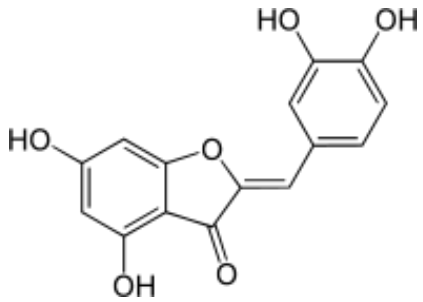
Leukocyanidin

FLAVAN 3,4-OL



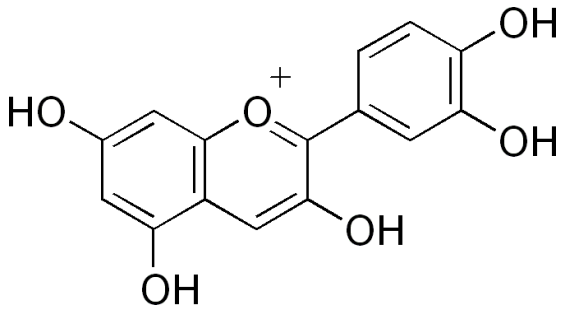
Butein

CHALCONE



Hispidol

AURONE



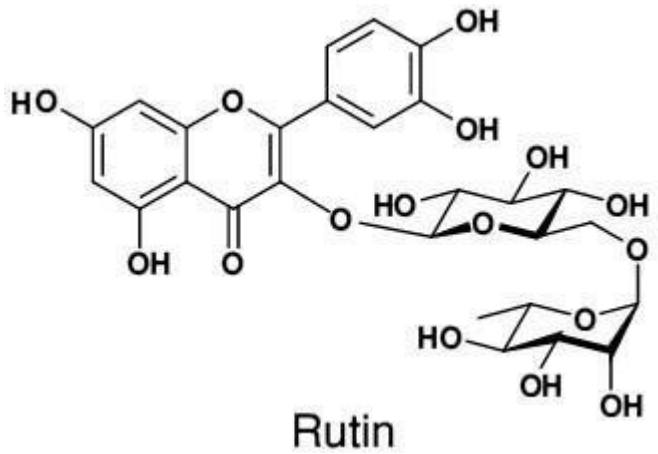
Cyanidin

ANTHOCYANIDIN

Except algae, flavonoids are wide distributed in the plant kingdom. They occur free or as their O- and C-glycosides and O-uronic derivatives. The glycosidic forms of flavonoids are water-soluble, accumulate in vacuoles, and depending on the species, either concentrate in the epiderm of the leaves or spread in both the epiderm and the mesophyl. In flowers, they are concentrated in epidermal cells.

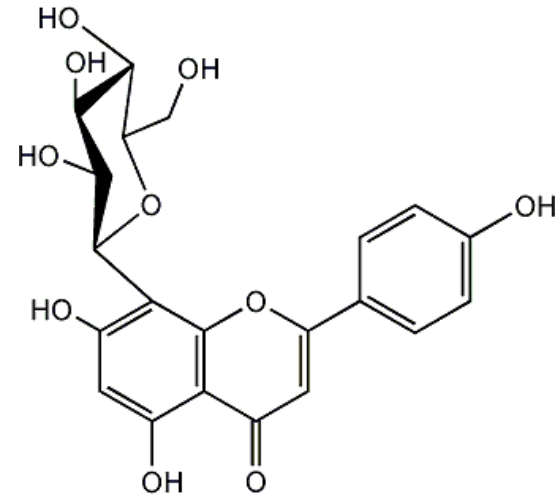
CHEMICAL STRUCTURE AND CLASSIFICATION

All flavonids contain mostly a OH, OCH_3, or O-gl at the 5,7 and 4' positions. A flavonol always contains a substituent on the 3. position. Flavanones are characterized by the absence of 2,3 double bond. C-Glycosylflavonoids are not rare. The bond is established between the asymmetric carbon on the sugar, and the C-6 or C-8 of the aglicone.



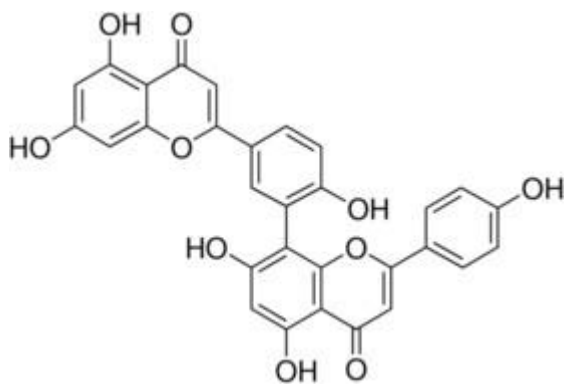
Rutin

Flavon 3-O-glycosid (flavonol)



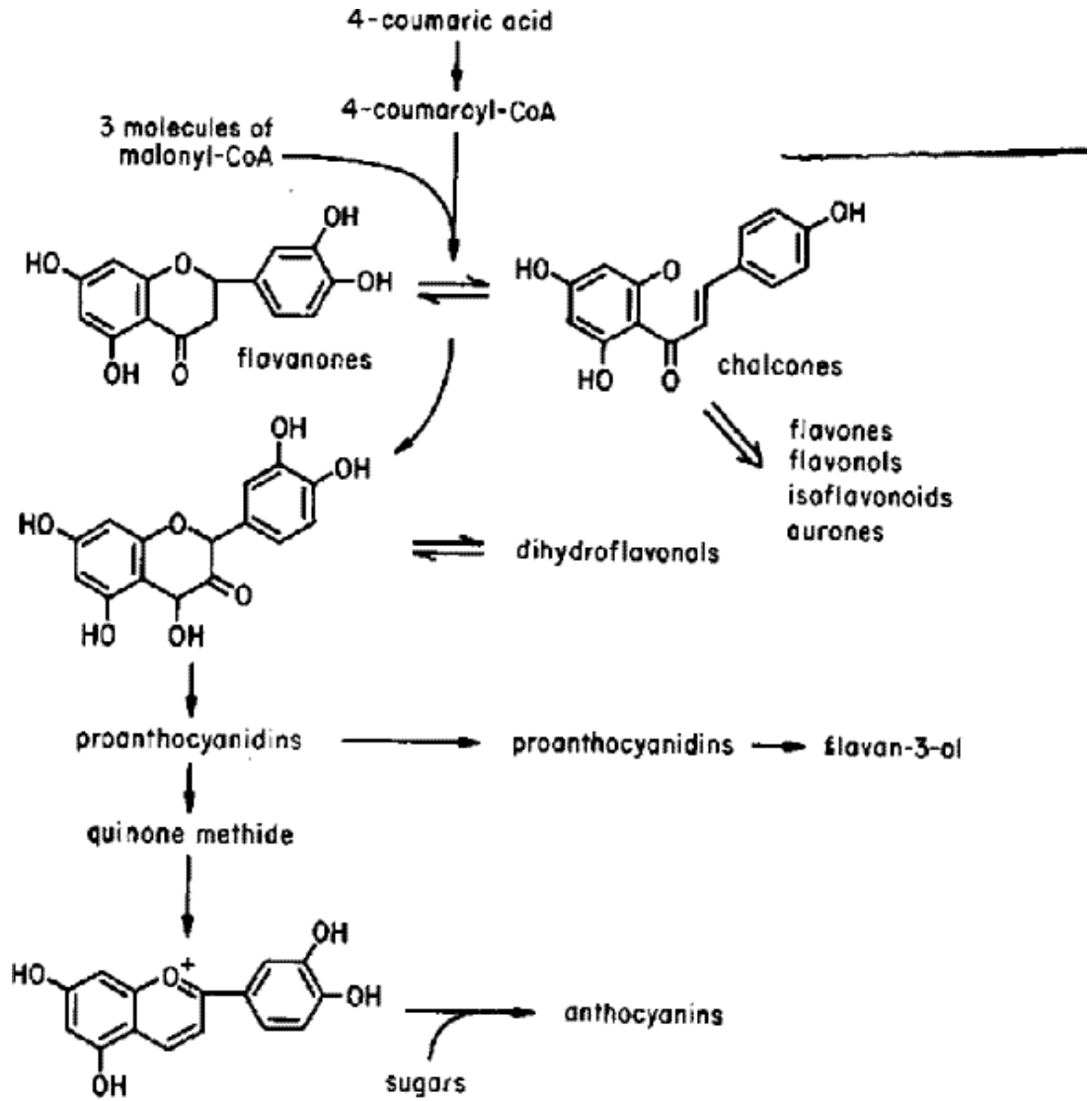
Vitexin

Flavon 8-C-glycosid



Amentoflavone (biflavonoide)

BIOSYNTHETIC ORIGIN



PHISYCO-CHEMICAL PROPERTIES, EXTRACTION, CHARACTERIZATION, AND QUANTITATION

Although,as general rule, glycosides are water-soluble and soluble in alcohols. Aglycones are, for the most part, soluble in apolar organic solvents; when they have at least one free phenolic group, they dissolve in alkaline solutions. Lipophilic flavonoids are directly extracted by solvents of medium polarity (e.g., dichloromethane); next they must be separated from the waxes and fats extracted simultaneously (of course, a preliminary hexane wash is possible; but the selectivity of this solvent is not absolute). The glycosides can be extracted, most often at high temperature, by acetone or by alcohols (ethanol, methanol) mixed with water.

Solvent evaporation under vacuum can be the next followed, when only the aqueous phase is left, by a series of liquid-liquid extractions by nonmiscible solvents : petroleum ether which eliminates lipids, toluene which eliminates chlorophyl, chloroform or diethyl ether which extract free aglycones; and ethyl acetate which dissolves the majority of glycosides. The free saccharides remain in the aqueous phase with the most polar glycosides when these are present.

The separation and purification of the different flavonoids is based on the usual chromatographic techniques (on polyamide, cellulose, or sephadex gel). They also can be isolated by HPLC.

Characterization

Although several color reactions allow the characterization of aglycones and glycosides in crude extracts, preliminary work on these extracts is conventionally dominated by TLC analysis.

- directly, since chalcones and aurones are usually visible, and turn orange and red respectively, in the presence of ammonia vapors;
- by examination under UV light before and after spraying with aluminium trichloride, or before and after exposure to ammonia vapors;
- after spraying with a 1% solution of the ester of 2-aminoethanol and diphenylboric acid, in other words the «Naturstoff Reagenz A (NA)»by examination under UV light;

• after spraying with ferric chloride, anisaldehyde, diazotized sulfanilic acid or other general reagents for phenols;

• by utilizing more or less specific reactions or properties, such as :

- Reaction known as cyanidin (or Shibata) reaction, with magnesium powder, or with zinc (Shinoda) both in the presence of hydrochloric acid.

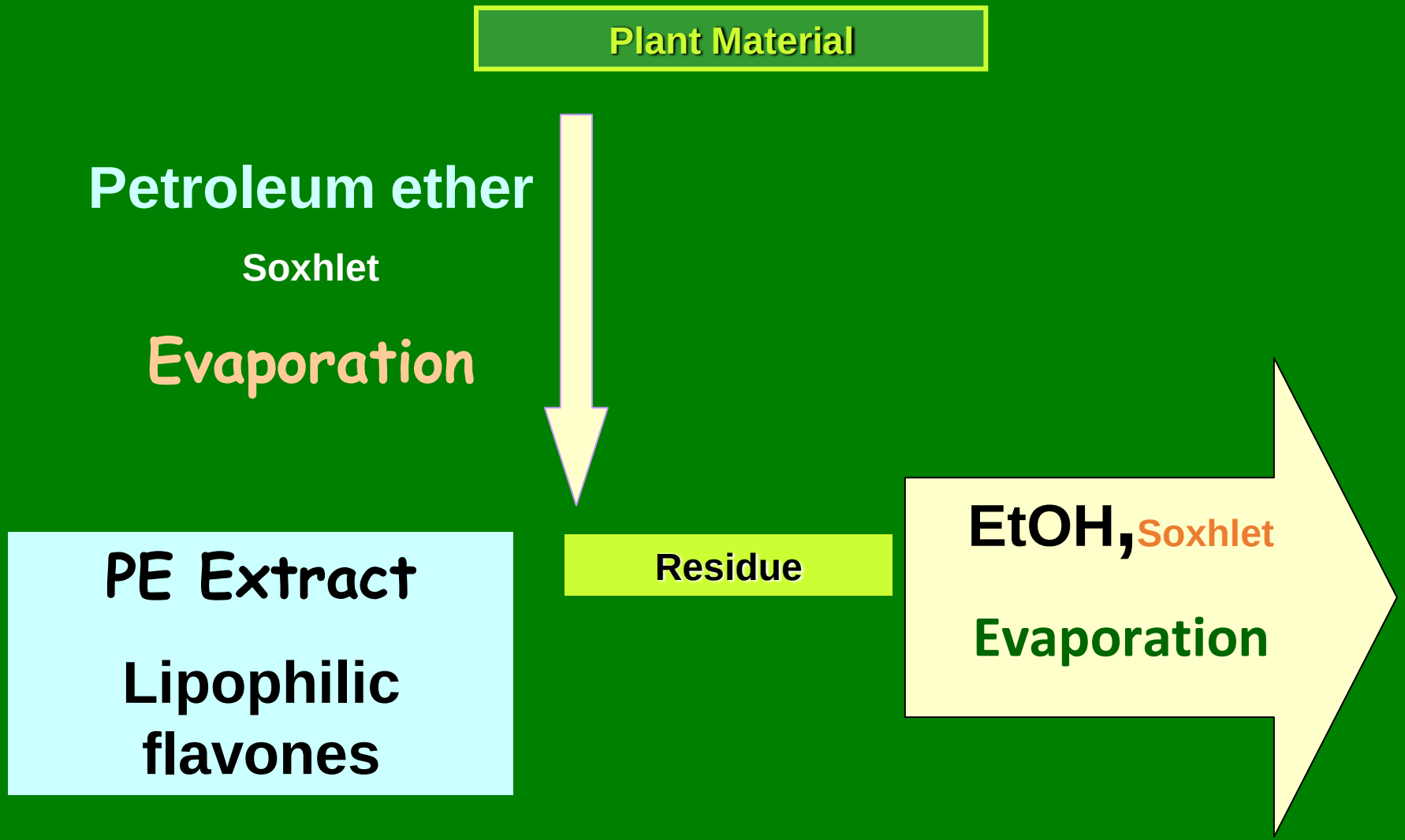
Flavones → orange

Flavonols → red ,

Flavanones → purple colors.

Structure elucidation : especially UV and NMR techniques are very useful.

ISOLATION PROCEDURE



EtOH Extract

+ H_2O

Shaking with toluene

Toluene phase

chlorophyll+side compounds

Aqueous phase

chloroform

Aqueous phase

Separating funnel (EtOAc)

Chloroform phase

AGLYCONES

EtOAc phase

GLYCOSIDES

$CHCl_3$ phase

AGLYCONES

EtOAc phase

GLYCOSIDES

Evaporation under vacuo

Column chromatography

Pure flavones

Identification and reactives:

Shibata reaktion : Drog: Extraction with EtOH, adding Shibata reactive (EtOH + H_2O + conc. HCl 1: 1 : 1) and **Mg** . **After oxydoreduction**

FLAVONES

FLAVONOLS

FLAVANONES

Isoflavones, chalcones and aurones do not react.

$FeCl_3$ reactive :

Mostly green, sometimes dark blue

(side by side trihydroxy groups)

Flavanones Clared red

KOH reactive : **Yellow - orange**

Special reactives : NA reactive

BIOLOGICAL PROPERTIES

The main property that is recognized for flavonoids is «venoactivity», in other words their ability to decrease capillary permeability and fragility. Because of this property they were referred as «**vitamin P**». Vitamin P is more active together with vitamin C.

Flavonoids and Free Radicals

Many properties, shown in vitro, could explain the actions of flavonoids. Initially, it was postulated that they act on the reduction of dehydroacorbic acid via glutathione by acting as hydrogen donors. The more reducing the flavonoid, the greater is the ascorbic acid sparing.

It is now more generally accepted that the phenols that flavonoids scavenge free radicals formed under different circumstances :

- anoxia
- inflammation
- lipidic autoxidation

Biochemically, free radicals are thought to be responsible for nucleic acid alterations, mutations, initiations and promotion of carcinogenesis, and cellular damage, because of their ability to react with membrane phospholipids, among other reason. The antagonist effect towards free radical production can be studied experimentally. This has spurred research, including epidemiplogic studies, on the potential role of antioxidants (i.e., free radical scavengers), such as flavonoids, some lignans, and other metabolites found in the daily diet, in preventative therapy.

Other Properties

Flavonoids have antispasmodic, diuretic, anti-inflammatory properties. They are also enzyme inhibitors in vitro.

USES OF FLAVONOID-CONTAINING DRUGS

Some crude drugs are used for the industrial extraction of flavonoids, for example total citroflavonoids, diosmin, hesperidin, rutin (diosmin occurs in *Citrus* but is obtained by semisynthesis). Others, the activity of which is due to several active principles, are used as titrated extracts (*Ginkgo*). Flavonoids are also always found in herbal teas.

CHIEF FLAVONOIDS ON THE MARKET
Citroflavonoids (Bioflavonoids)
Flavonoids from the Fruits of Various *Citrus species*
(Citri pericarpium)

Citrus aurantium **var. *amara* - turunç (bitter orange)**
Citrus aurantium **var. *dulcis* - portakal (orange)**
Citrus aurantium **var. *bergamiae* – bergamot (bergamot**
Citrus limonum **– limon (lemon)**
Citrus reticulata **– mandalina (mandarin)**
Citrus paradisi **– greyfurt (grapefruit)**

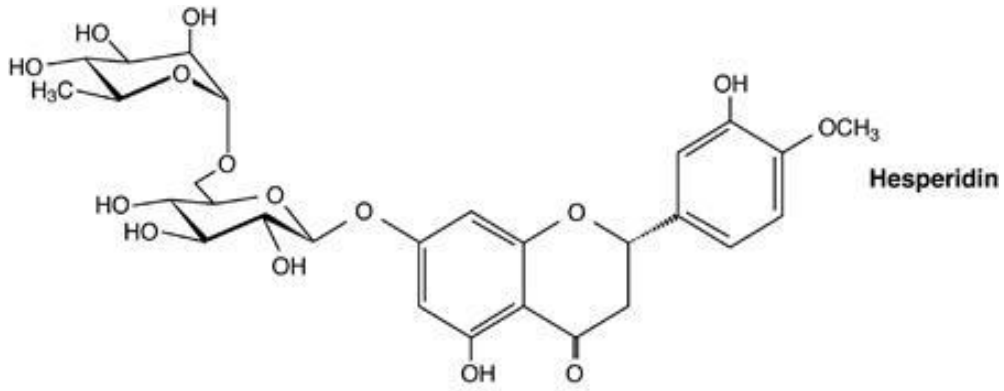
Citrus species, widely used for their essential oil, they are also a source of pectins and flavonoids. These are very abundant in the pericarp, and are mainly flavanone glycosides (hesperidin or hesperetin 7-O-rutinoside, neohesperidin, naringin, eriodyctin, eriocitrin). The pericarps also contain flavone glycoside (diosmin). Neohesperidin and naringin are found in bitter orange, hesperidin, in sweet orange, and grapefruit is rich in naringin. Citroflavonoids are extracted from pericarps and pulps with water, and isolated using different procedures. Currently the pharmaceutical industry uses:

A mixture of total citroflavonoids

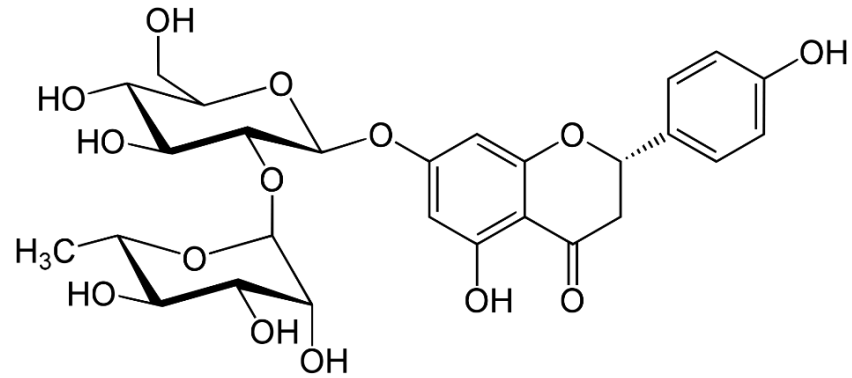
Glycosides of pure flavanones : hesperidin, naringin

Semisynthetic derivatives such as hesperidin methyl chalcone

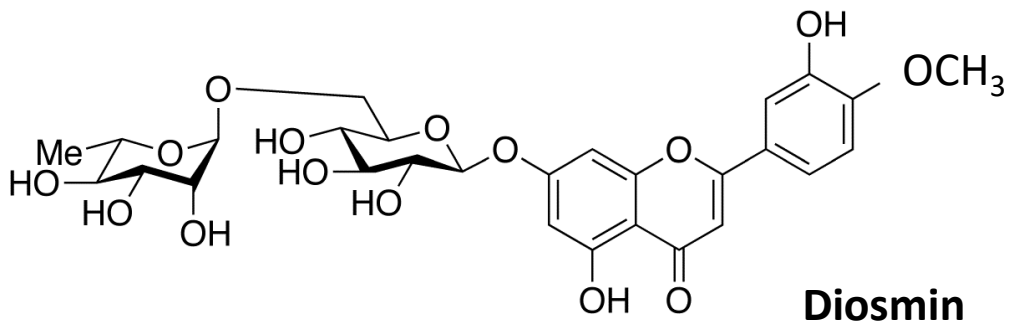
A flavone glycoside obtained by semisynthesis :diosmin



Hesperidin



Naringin



Diosmin

All of these flavonoids are used pure (diosmin, naringin) or in combination (with ascorbic acid, aesculetin, ruscosides, and more).

The accepted indications for preparations containing high doses of citroflavonoids are to improve the symptoms of venous and lymphatic vessel insuffiency, for the adjunctive treatment of the functional signs of capillary fragility, and to treat the functional symptoms of the acute attack of piles.

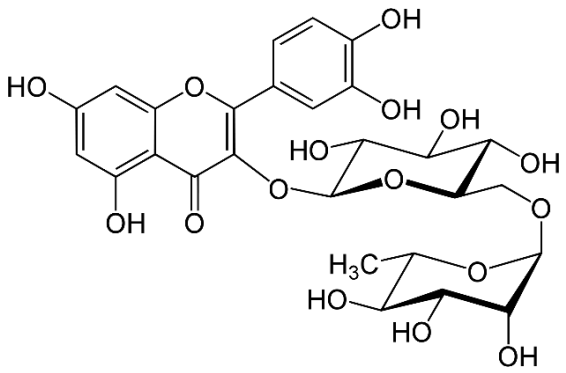
DRUGS RICH IN RUTIN

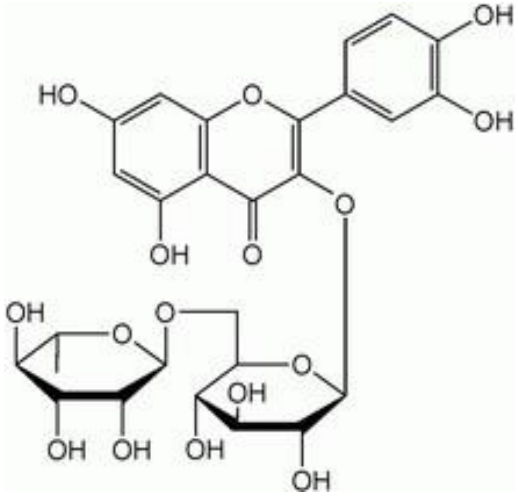
Sophorae flos Japanese pagoda tree sofora

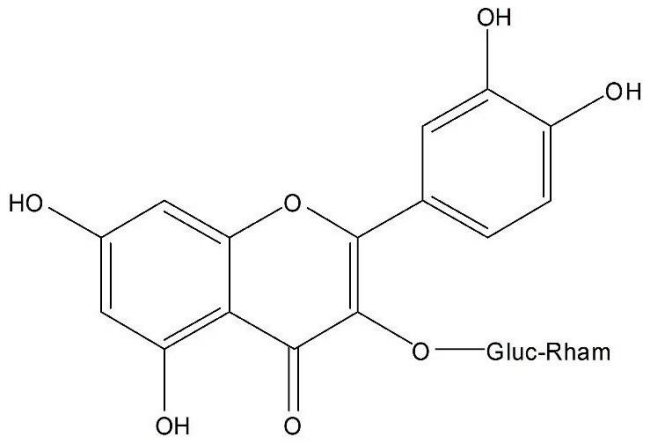
Sophora japonica **Fabaceae**

Rutin :Qercetin 3-O-rutinoside

Sources of rutin : Although rutin is reletively abundant in plants, only a small number of drugs contain quantities sufficient for industrial extraction.







(**rutin**, quercetin-3-rutinoside)

RUTIN

Fagopyri folium **Buckwheat** **karabuğday yaprağı**

Fagopyrum esculentum **Polygonaceae**

Other sources :

Eucalyptus macrorrhyncha (folium)

Dimorphandra spec. (fructus)

Rutin extraction from Sophorae flos does not present any special difficulties : extraction by boiling water and crystallization upon cooling, recrystallization from ethanol.

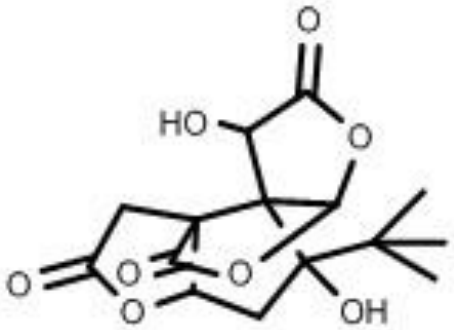
Rutin alone or in combination (with citroflavonoids,ascorbic acid, aesculetin, ruscosides, and more) is promoted for the symptoms of venous and lymphatic vessel insuffiency, for the adjunctive treatment of the signs of capillary fragility, and to treat the functional symptoms of the acute attack of piles.

DRUGS FOR WHICH PART OF THE ACTIVITY MAY BE DUE TO FLAVONOIDS

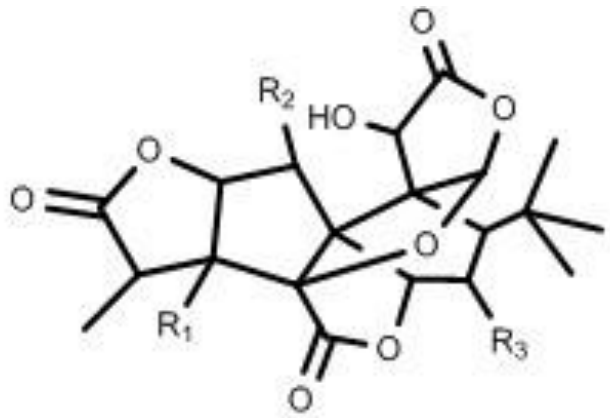
Ginkgo folium maidenhair tree gingko, mabet ağacı yaprağı

Ginkgo biloba **Ginkgoaceae**

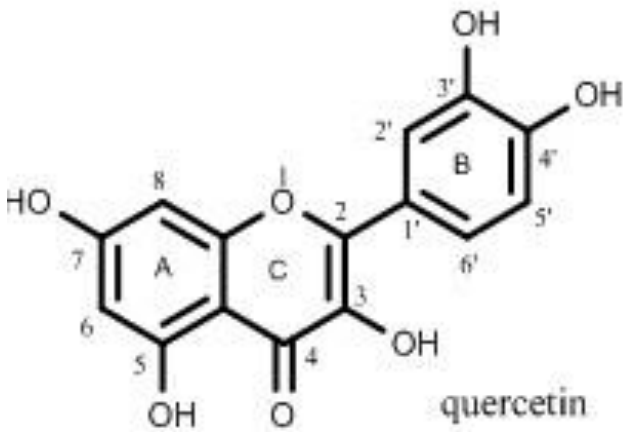
The main principles of Ginkgo folium are flavonoids (0.5-1%), and terpenoid lactones (diterpenoids : ginkgolides A-M (up to 0.5%), and a sesquiterpenoid : bilobalide (0.1%). The flavonoids are represented by about twenty flavonol glycosides, namely O-glycosides, quercetin and kaempferol 3-O-rhamnosides and 3-rutinosides. The ginkgo leaf also contains flavan-3-ols, proanthocyanidins, and biflavonoids which are 3'→8'' biflavones (amentoflavone, bilobetol, ginkgetin sciadopitysin). Known as ginkgolides A, B, C, J (and M in the roots), ginkgo diterpenes have a very specific hexacyclic structure, and three lactone rings.



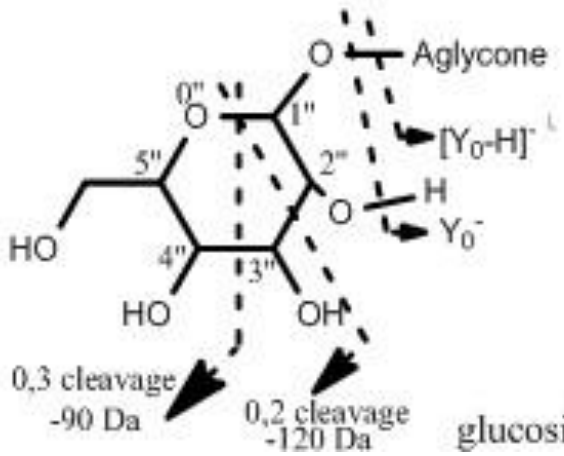
bilobalide



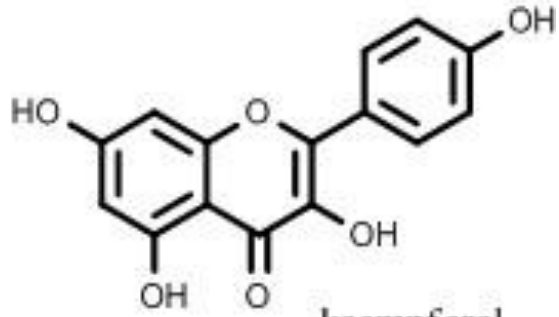
	R_1	R_2	R_3
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide J	OH	H	OH



quercetin

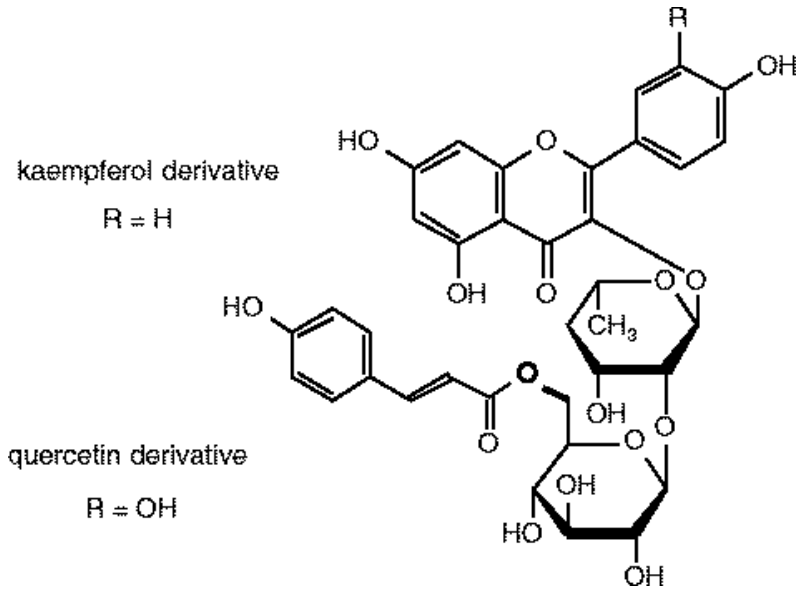


glucoside

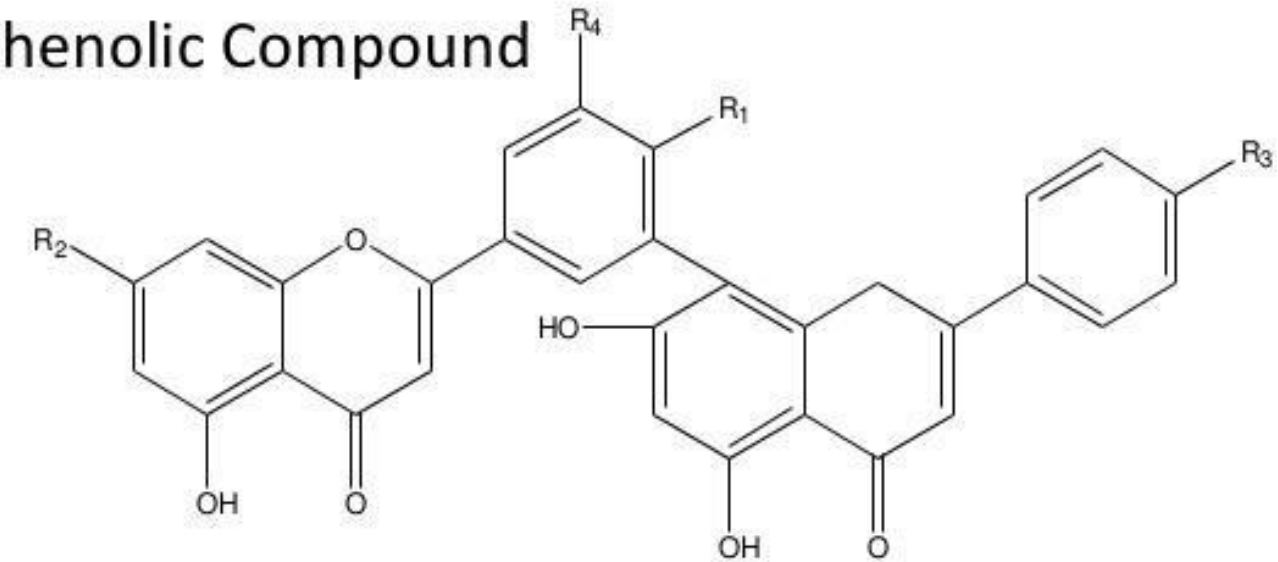


kaempferol

Structures



- Phenolic Compound



Amentoflavone:	$R_1=R_2=R_3=R_4=H$
Bilobetin:	$R_1=OCH3$, $R_2=R_3=OH$, $R_4=H$
Ginkgetin:	$R_1=R_2=OCH3$, $R_3=OH$, $R_4=H$
Isoginkgetin:	$R_1=R_3=OCH3$, $R_2=OH$, $R_4=H$
5'-methyloxybilobetin:	$R_1=R_4=OCH3$, $R_2=R_3=OH$
Sciadopitysin:	$R_1=R_2=R_3=OCH3$, $R_4=H$

Pharmacological activity : Ginkgolide B is an inhibitor of the platelet activating factor (= PAF). This anti-PAF activity and the activities of flavonoids, particularly as free radical scavengers, may explain the numerous properties of ginkgo extract that have been observed. This extract is said to be a vasoregulating agent (an arterial vasodilator and a venous vasoconstrictor able to decrease capillary fragility), an inhibitor of cyclo-oxygenase and lipoxygenase, and an inhibitor of platelet and erythrocyte aggregation. It decreases capillary hyper-permeability, improves irrigation, and activates cell metabolism, particularly in the cortex (by increasing glucose and oxygen uptake). The terpene-containing fractions prolong the survival of hypoxic rats; they protect neurons and astrocytes from damage by transient ischemia.

Uses : **Gingko leaves are used to produce an extract titrated 24% flavonoids and 6% ginkgolides-bilabolide**. This extract has undergone several dozen human clinical tirials, especially to assess its efficacy for «cerebral insuffiency».

Main pharmacological properties of ginkgo

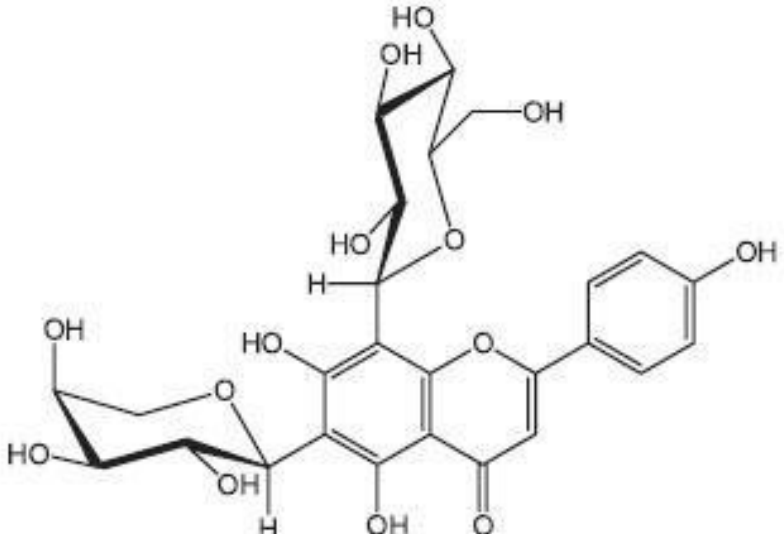
Antioxidant properties
Anti platelet activating factor (anti-PAF) activity
Anti-Ischemic properties
Dementia
Alzheimer's Disease
Tinnitus
Interminent claudication
Macular degeneration

Passiflorae herba **passion flower** **çarkıfelek**

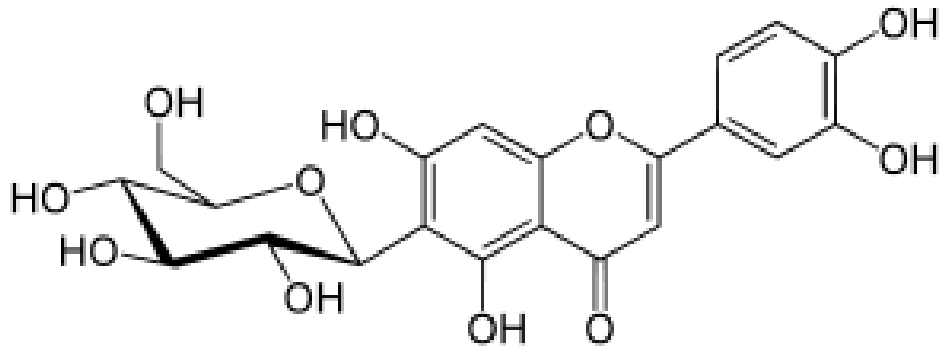
Passiflora incarnata **Passifloraceae**

Chemical Classifictation : Next to phenolic acids, coumarins, phytosterols, and traces of indole alkaloids (harman, harmol, harmine), the drug can contain up to 2.5% flavonoids. The major ones are flavone di-C-glycosides : schaftoside and isoschaftoside (apigenin C-glucosyl-C-arabinosides, 8,6 and 6,8 isomers) together with other flavone C-glycosides (isovitexin, isoorientin, vicenin…)

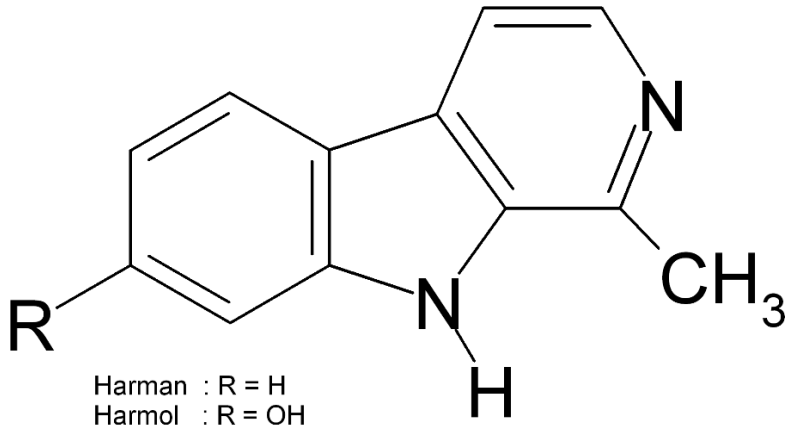
Pharmaceutical activity and Uses : Tradition attributes to the drug sedative, antispasmodic, and tranquilizing properties. All the compounds are together responsible for the acivities (flavonoids, alkaloids, even in minor amounts, and other compounds). The drug (in infusions), its galenical preparations (powders, extract, tincture), and the phytopharmaceuticals containing it are traditionally used by the oral route to treat abnormalities of the cardiac rhythm in the adult (normal heart) and to treat the symptoms of nervousness in adults and children, particularly minor sleeplessness. In Germany (Commission E),: the drug can be used for nervous restlessness, and for mild sleeping difficulties and gastrointestinal signs of nervous origin.



Isoschaftoside



Isoorientin



Harman : R = H
Harmol : R = OH
Harmin : R = O-Methyl

***Helichrysum* spec.**
everlasting

Asteraceae

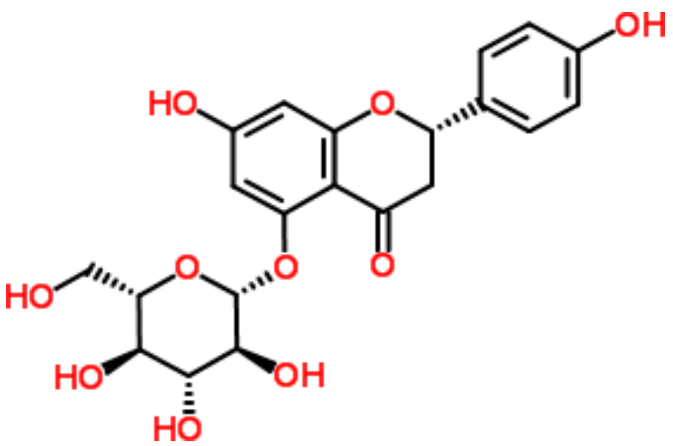
Helichrysi flos
ölmez çiçek

Helichrysum plicatum

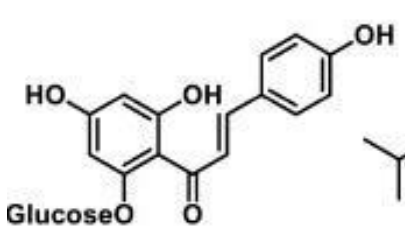
H. graveolens

H. orientale

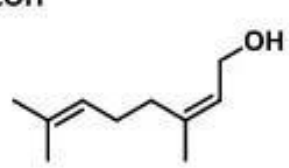
Helichrysum species have been used traditionally to treat urinary stones, especially in Anatolia , in the form of herbal teas (infusions). The capitula (flowers) are rich in flavonoids (helichrysin A and B, isosalipurposide, astragalin, naringenin, apigenin, apigenin 7-glucoside). Especially *Helichrysum plicatum*, *Helichrysum orientale* and *Helichrysum graveolens* are very rich in flavonoids (more than 5%). No specific side-effects have been reported.



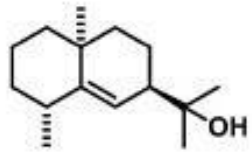
Helichrysin A (-)-naringenin-5-O-glucoside
Helichrysin B (±)-naringenin-5-O-glucoside



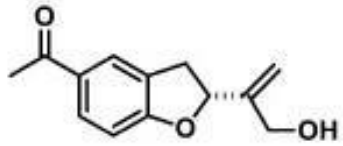
Isosalipurposide



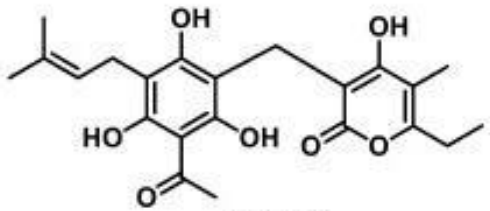
Nerol



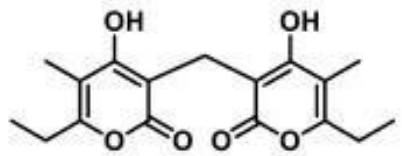
Rosifoliol



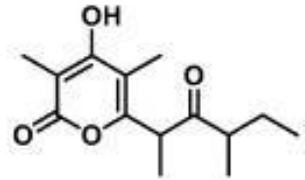
10-Hydroxytremetone



Arzanol



Helipyrone



Mycropyrone

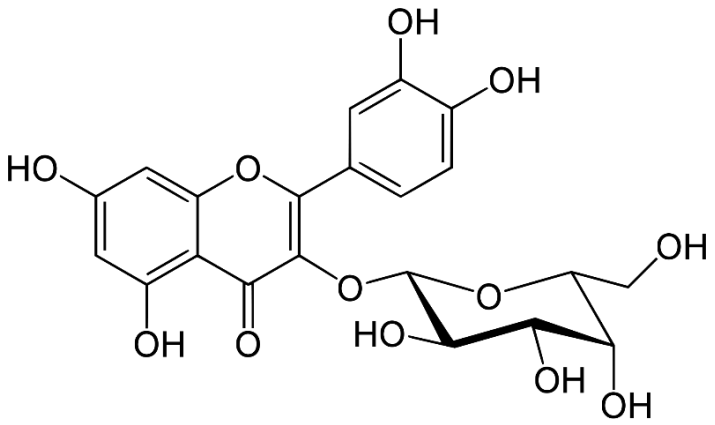
Helichrysum conglobatum
grows in Cyprus

H. sanguineum
rich in anthocyanins

Betulae folium **birch** **huş ağacı yaprağı**

Betula alba, B. pendula **Betulaceae**

The drug contains about 2-3% flavonoids (especially hyperoside = quercetin-3-O-galactoside), and saponins. It is used for its diuretic activity as urinary tract cleanser. The drug is not recommended for heart and kidney diseases.



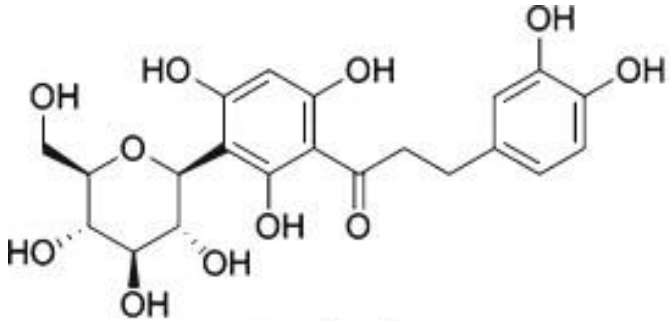
hyperoside

Aspalathi folium **rooibos tea** **roybos çayı**

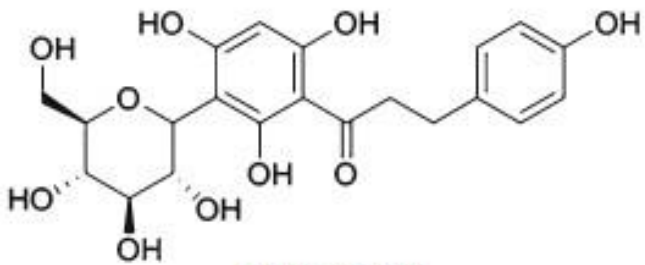
Aspalathus linearis **Fabaceae**

The young leaves, fermented, and dried are used as an alternative to tea, particularly in South Africa. They are reputed to be sedative and to promote digestion and sleeping, it is also used in weight-loss programs. The consumption of this tea is currently spreading, including in Europe, especially because some believe that it has antioxidant properties.

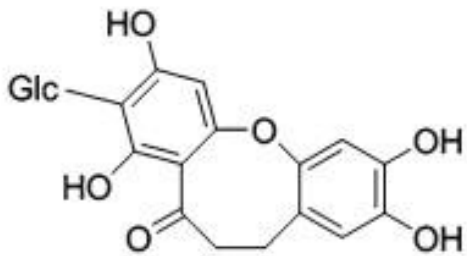
The stems and leaves do not contain caffeine, but they contain ascorbic acid, phenolic acids, and flavonoids (C-glycosides : aspalathin, orientin, isoorientin). Aspalathin is a dihydroxychalcone C-glycoside. Characteristic of the fresh plant, it disappears completely during fermentation. The fermented product is rich in quercetin. The literature contains no reports of toxicity or side effects.



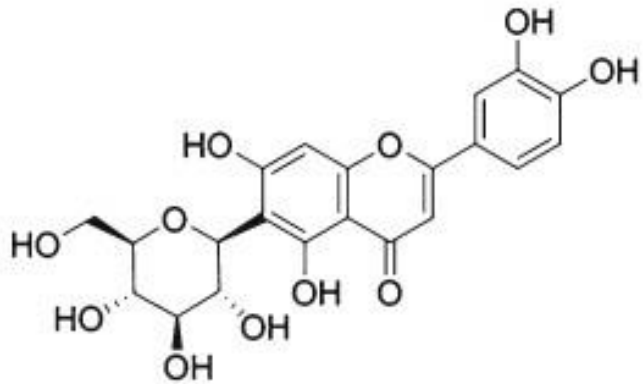
Aspalathin



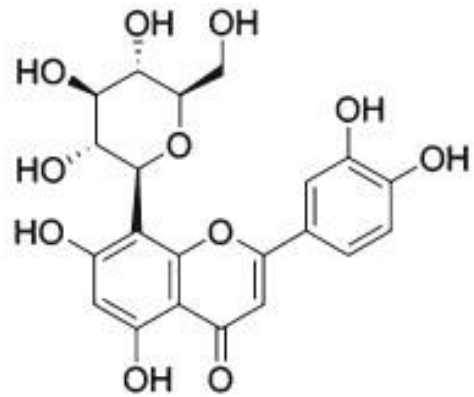
Nothofagin



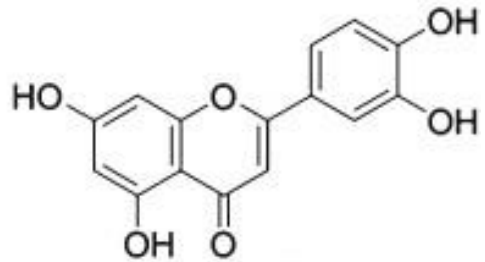
Aspalalinin



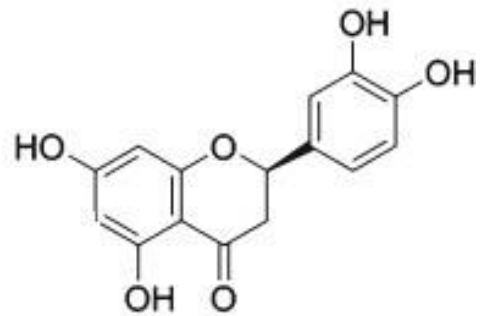
Isoorientin



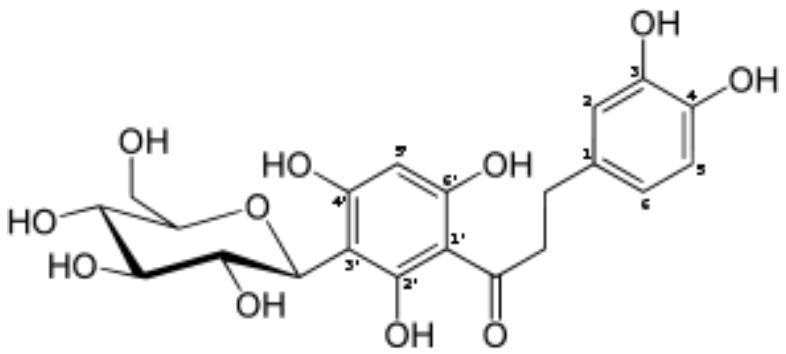
Orientin



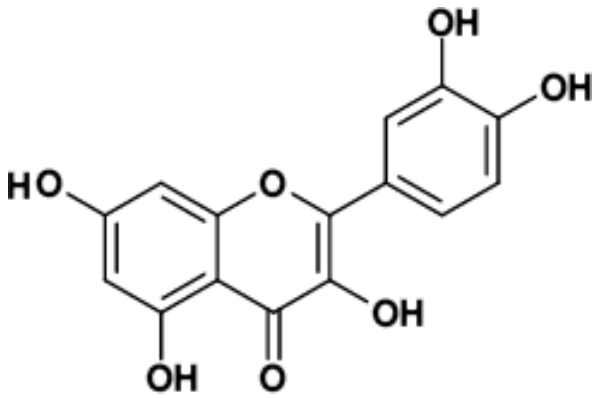
Luteolin



Eriodictyol



Aspalathin



Quercetin

Other plants/Drugs rich in flavonoids

Thymus vulgaris kekik (thyme) herba spasmolytic

Chamaemelum nobile roman papatyası (roman chamomile) flos anti-inflammatory

Achillea millefolium civan perçemi (yarrow) flos spasmolitic

Equisetum arvense at kuyruğu (horsetail) herba diuretic

Isoflavonoids

Isoflavonoids are characterized, like flavonoids, by a C_{15} skleton of the Ar-C_3-Ar type, but one which is now rearranged to be a 1,2-diphenylpropane : all molecules in this group can be related to the skleton of 3-phenylchromane. The distribution of these flavonoids is rather limited : they are in fact almost specific to the Fabaceae. The most common compounds are isoflavones, which occur in the free state, or, less commonly, as glycosides (O-glycosides, or exceptionally C-glycosides).

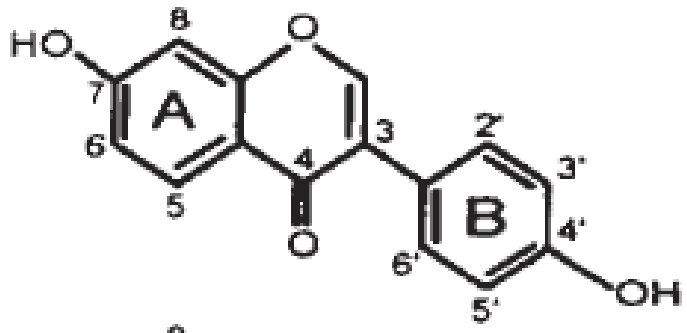
BIOLOGICAL ACTIVITY : In plants a good number of isoflavonoid structures are phytoalexins, in other words substances produced by the plant in response to an infection by a pathogenic agent most often fungal in nature. Estrogenic properties are also known, the other activity is the insecticide activity of rotenoids.

Sojae semen **soybean** **soya fasulyesi tohumu**

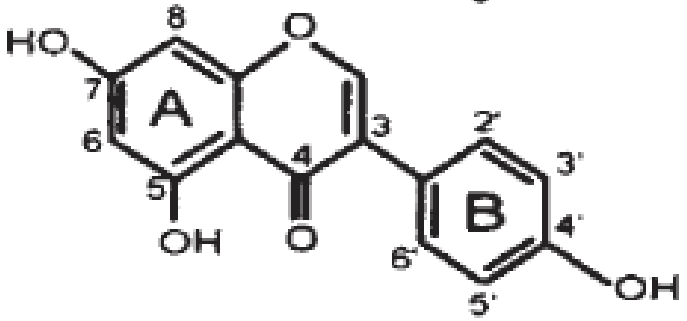
Glycine max **Fabaceae**

Estrogenic activity of isoflavonoids : The occurence of isoflavonoids raises the question of their potential impact on human health. In the soybean, the concentration of daidzein (7,4'-dihydroxyisoflavone), genistein (5,7,4'-tri hydroxyisoflavone), and their glycosyl derivatives can reach 3 g/kg. These isoflavones bind to estrogen receptors, and most, they have a weak estrogenic activity. They are also tyrosine-kinase inhibitors which may have a role in the transformation and cell polyferation phenomena. Pure genistein is also an anticarcinogen.

Several recent studies suggest that isoflavones and soybean decreases the symptoms of menopause (hot flashes and others) and reduce the risk of osteoporosis.

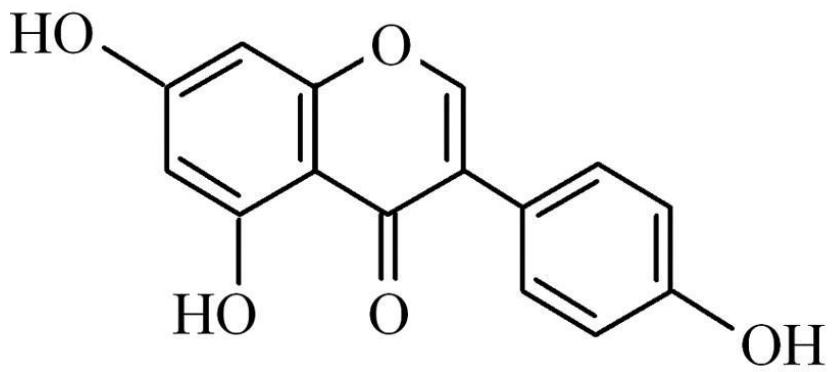


Daidzein
(4',7-dihydroxyisoflavone)

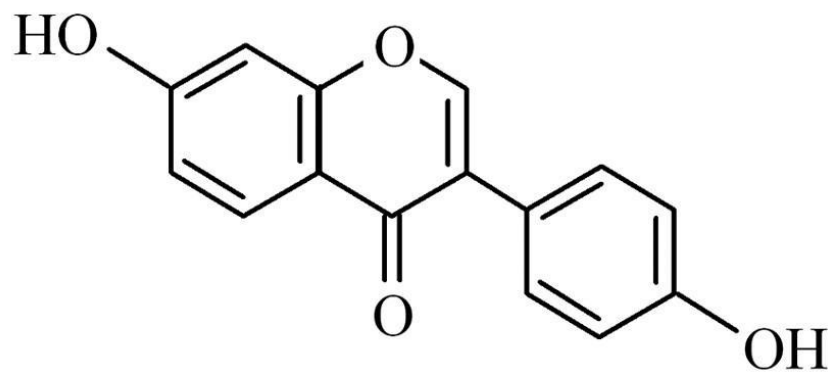


Genistein
(4',5,7-trihydroxyisoflavone)

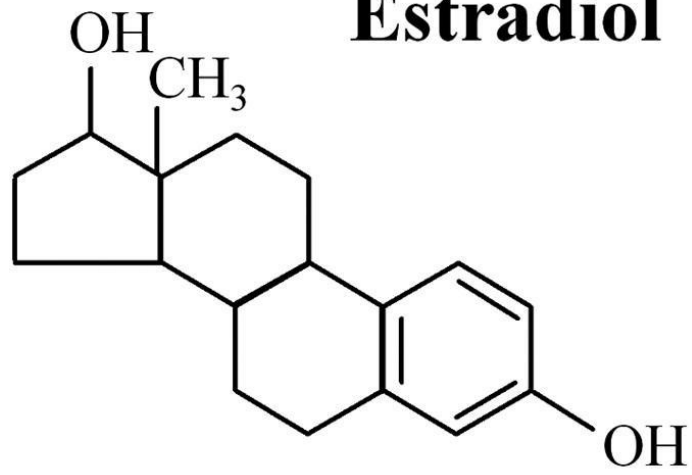
Genistein



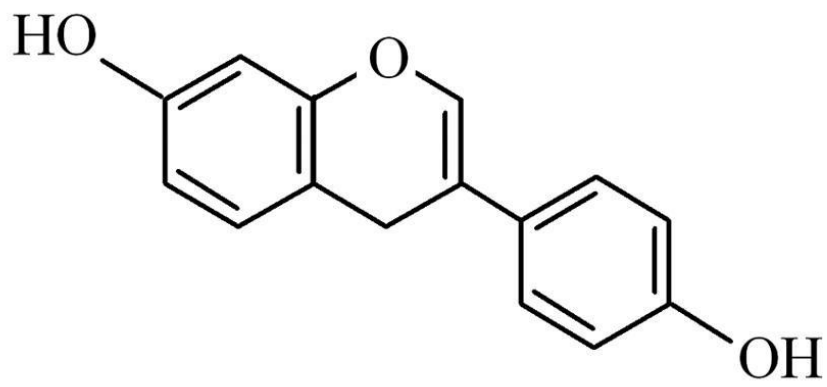
Daidzein



Estradiol



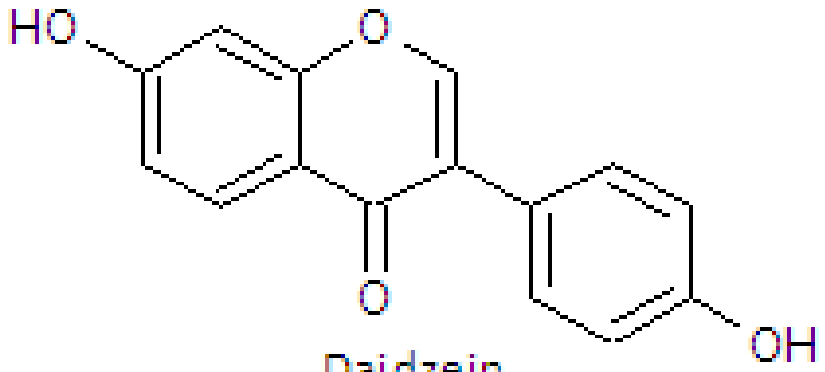
Equol



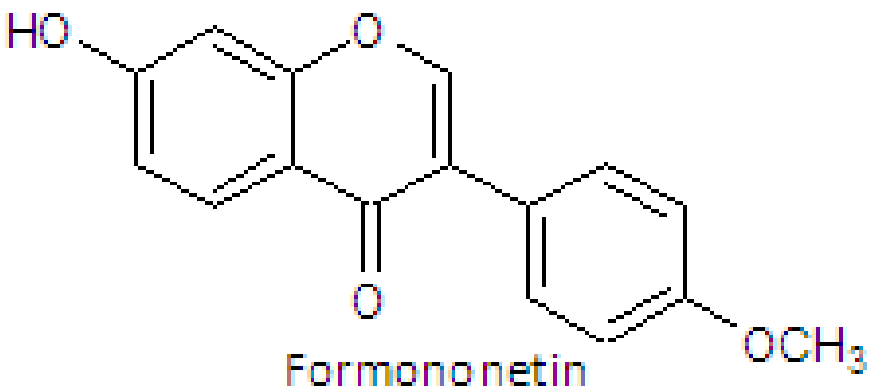
Trifolii pratensi herba **red clover** **kırmızı yonca**

Trifolium pratense **Fabaceae**

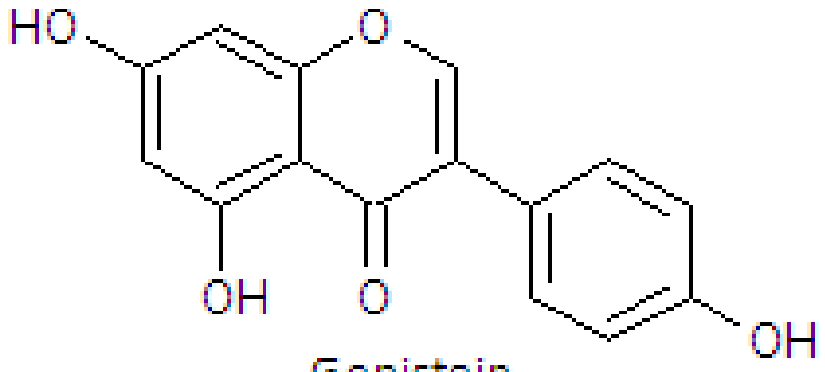
Like soybean, this drug is also rich in isoflavonoids (daidzein, genistein, formononetin, biochanin A..) and is used for the same purposes. By using of this drug, one should be careful, because it also contains some coumarins with anticoagulan activity.



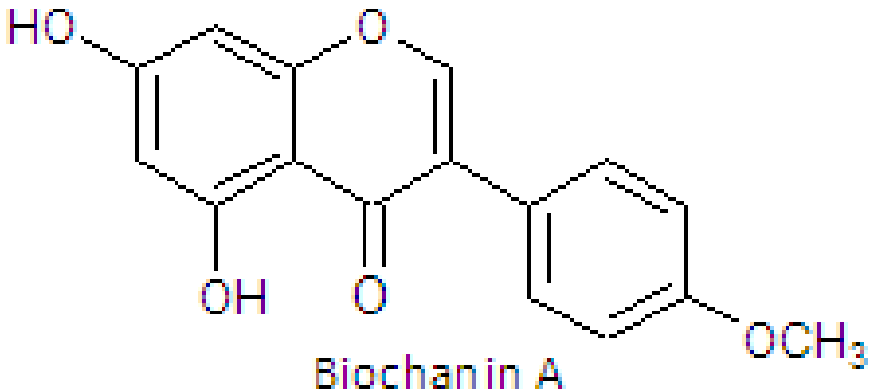
Daidzein



Formononetin



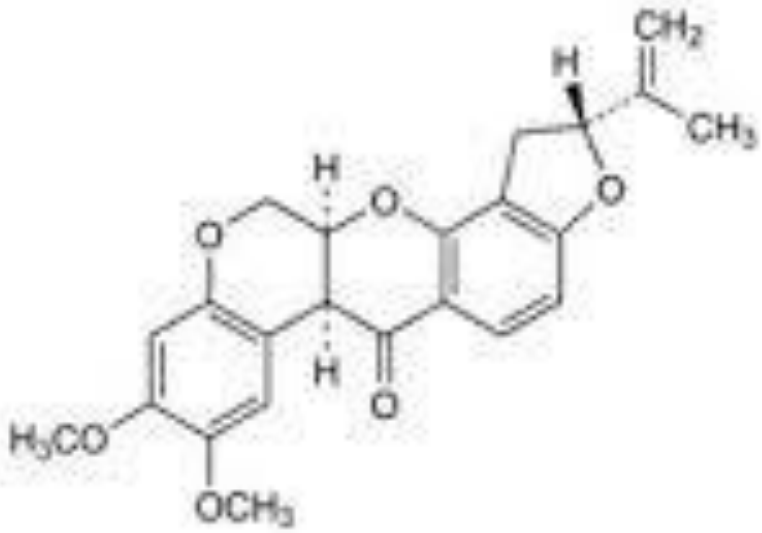
Genistein



Biochanin A

Rotenoids : These compounds, biogenetically related to isoflavonoids, have in common a four-ring structure: a chromanochromanon. The chief representative of the group is rotenone, the major active principle in the roots of various tropical Fabaceae.

Rotenone



Derridis radix **derris**

Derris elliptica **Fabaceae**

Derris are vines growing in southeast Asia. In their area of origin, the roots of these vines are traditionally used as insecticidal and ichthyotoxic agents. The drug consists of the root, and on the market, an extract is frequently found which is enriched and titrated to contain about 30% rotenone.

Rotenone is responsible for the insecticidal peoperties. The main market outlet for rotenoid-containing Fabaceae (powder, extracts, rotenone) is phytopharmacy (treatment of house plants, and, sometimes, of vegetable gardens) and the extermination of ectoparasites of domestic animals

Flavonolignans are addition products of a phenylpropanoid alcohol, onto a flavonoid. These compounds are very rare compounds. The only pharmaceutical drug containing these type of compouds is :

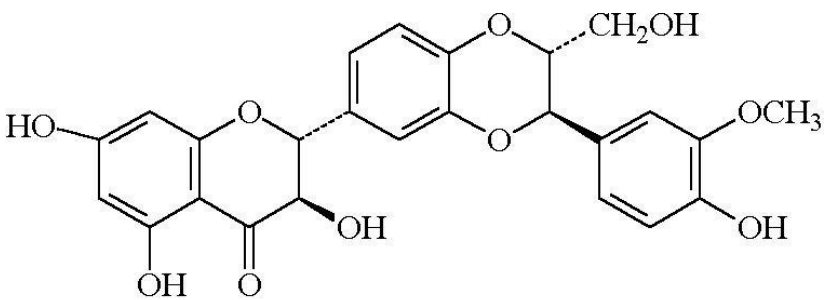
Silybi mariae fructus = Cardui mariae fructus.

Silybi mariae fructus = Cardui mariae fructus **St. Mary thistle**

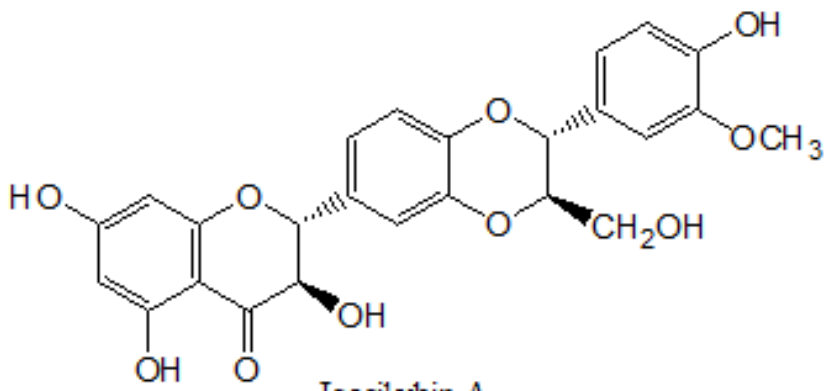
deve dikeni, meryemana dikeni

***Silybum marianum* (*Carduus marianus*)** **Asteraceae**

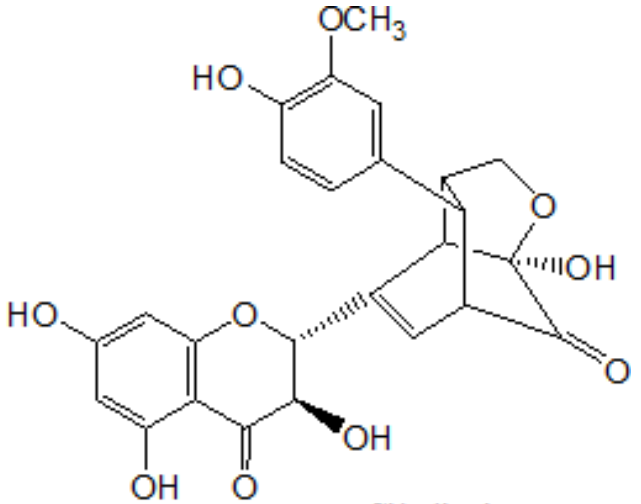
Chemical Composition : The drug contains 20 to 30% lipids, proteins, sugars, flavonoids (taxifolin, quercetin). The constituents responsible for the activity are **flavonolignans** initially isolated as a mixture of a phenylpropanoid alcohol (coniferyl alcohol), onto a 2,3-dihydroflavonol (taxifolin). **This mixture, commonly known as silymarin**, represents 1.5 to 3% of the weigth of the drug. Silybin (silibinin) is the major constituent of this mixture. The other constituents of silymarin are silydianin, silychristin and the simple flavonoid taxifolin.



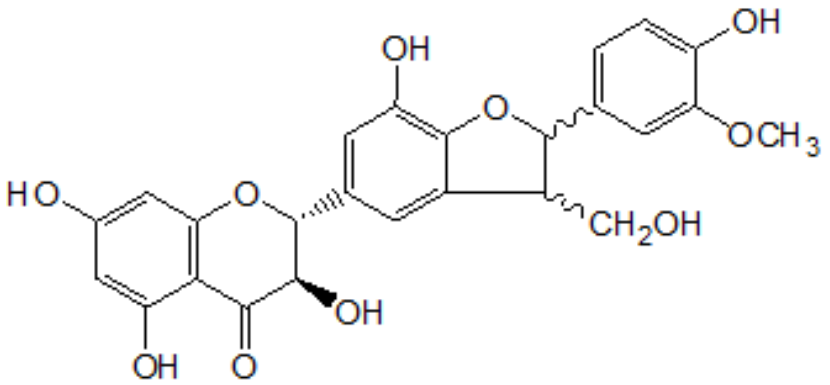
Silybin



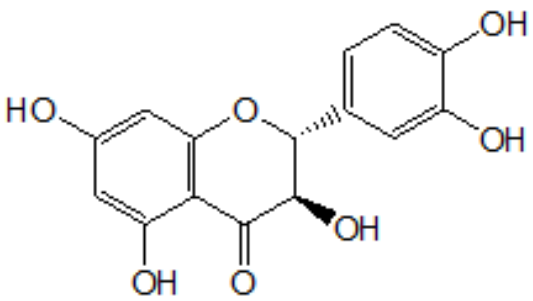
Isosilybin A



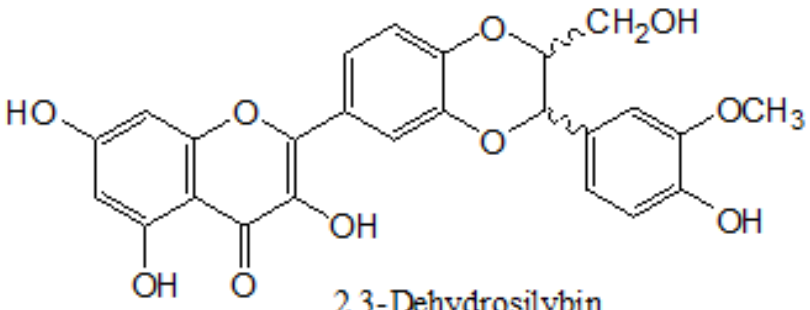
Silydianin



Silychristin



(+)-Taxifolin



2,3-Dehydrosilybin

Pharmacological Activity : Multiple experimental studies tend to demonstrate the **antihepatotoxic** activity of silymarin : prevention of the toxic effects of carbon tetrachloride, galactosamine, and other toxins at the level of the hepatic parenchima, **protection against the harmful effect of phalloidin administered parenterally**. Silymarin inhibits membrane lipid peroxidation, acts as a free radical scavenger, and inhibits the formation of leukotriene. It is thought to have a stabilizing effect on mebranes, and in the case of the *Amanita* toxin, it may compete for binding sites. It is devoid of acute or chronic toxicity and has practically no side effects.

Uses : In Germany and in other European countries, silymarin, or extracts titrated for silymarin are promoted as a treatment, per os for liver damage from poisoning and as an adjunctive treatment for chronic liver disease and cirrhosis; **an injectable form is used to treat *Amanita phalloides* poisoning**. The phytomedicines, containing the extracts of the achenes (fructus), are tradionally used orally for the symptomatic treatment of functional digestive signs thought to have a hepatic origin. The very low water solubility of the flavonolignans makes it unlikely that (very rare used) herbal tea forms have an antihepatotoxic activity.