# Extraction, isolation, and structure elucidation of Glycosides, Flavanoids, Lignans and Purines.

By
Sana Raza
(Assistant professor)
Shambhunath Institute of Pharmacy

# GLYCOSIDES

**Definition**

- Glycosides are (usually) non-reducing compounds, on hydrolysis by reagents or enzymes yield one or more reducing sugars among the products of hydrolysis.

- Glycoside = sugar group + nonsugar group

  (glycone) (aglycone or genin)

- Glycone and aglycone are linked by glycosidic linkage.

- Sugar in glycosides is mostly beta-D glucose but other sugars like galactose, mannose, rhamnose, digitoxose can also be present.
- Glycosides can be in alpha or beta  form but plants contains only beta glycosides
- Therapeutic effect of glycosides is only due to aglycon part only and suger moiety facilitate absorption of glycoside, transportation of aglycone to site of action .
- So glycosides are hydrolysed to give aglycon part for action.

# Classification

Glycosides are classified on the following basis

**A. Type of aglycone in Glycoside**

**B. Type of sugar (glycone) part**

**C. Type of linkage between glycone and aglycone**

**D. based on there use**

# A. Type of aglycone in Glycoside

- **Anthraquinone or anthracene glycoside**
- **Sterols or cardiac glycoside**
- **Saponin glycoside**
- **Cyanogenic glycoside**
- **Isothiocynate glycoside**
- **Coumarins and furanocoumarins**
- **Aldehyde glycoside**
- **phenol glycoside**
- **Steroidal**
- **Miscellaneous glycosides**

# B. Type of sugar (glycone) part

- glucose- glucoside
- Rhamnose –rhamnoside
- Digitoxose-digitoxoside
- Glucose and rhamnose-glucorhamnoside
- Rhamnose and glucose-rhamnoglucoside

# C. Type of linkage between glycone and aglycone

- C-glycoside,-sugar linked to carbon atom of aglycone
- N-glycoside,- sugar linked to nitrogen atom of aglycone
- O-glycoside,- sugar linked to oxygen atom of aglycone
- S- glycoside- sugar linked to sulfur atom of aglycone

# D. based on there use

- Cathartics
- Cardio tonics
- Analgesics
- Anti-rheumatics
- Antiulcer

# CARDIAC GLYCOSIDES (Digoxin)

- Digoxin is a cardiac glycosides extracted from the species of D. lanata and D. lutea.
- The genins of all cardiac glycosides are steroidal in nature that acts as cardio tonic agents.
- They are characterized by their highly specific action on cardiac muscle, increasing tone, excitability and contractility of this muscle, thus allowing the weakened heart to function more efficiently.

# EXTRACTION AND ISOLATION OF DIGOXIN

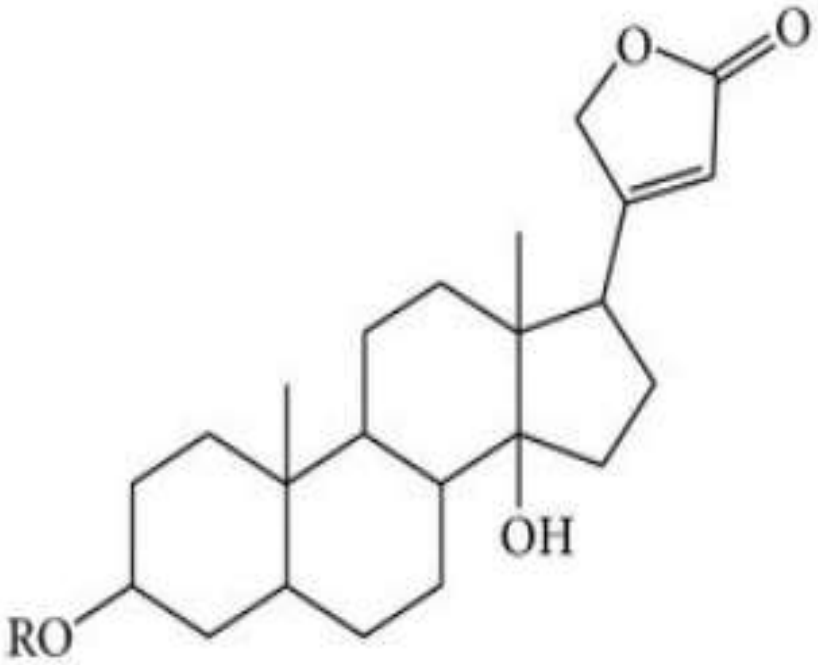
- The drug is pulverized and extracted with ethanol at low temperature.
- Followed by addition of lead acetate solution to remove the impurities.
- The ppt are removed by centrifugation , the cardiac glycosides present in the supernatant are extracted with chloroform
- The chloroform extract is evaporated under vacuum and the residue (cardiac glycoside) left behind is further purified by chromatography.

# CHEMICAL STRUCTURE

- These are composed of two structural features:
  1) The sugar(glycone) moiety.
  2) The non-sugar (aglycone - steroid) moieties
- The R group at the 17 position difines the class of cardiac glycoside. Two classes are:

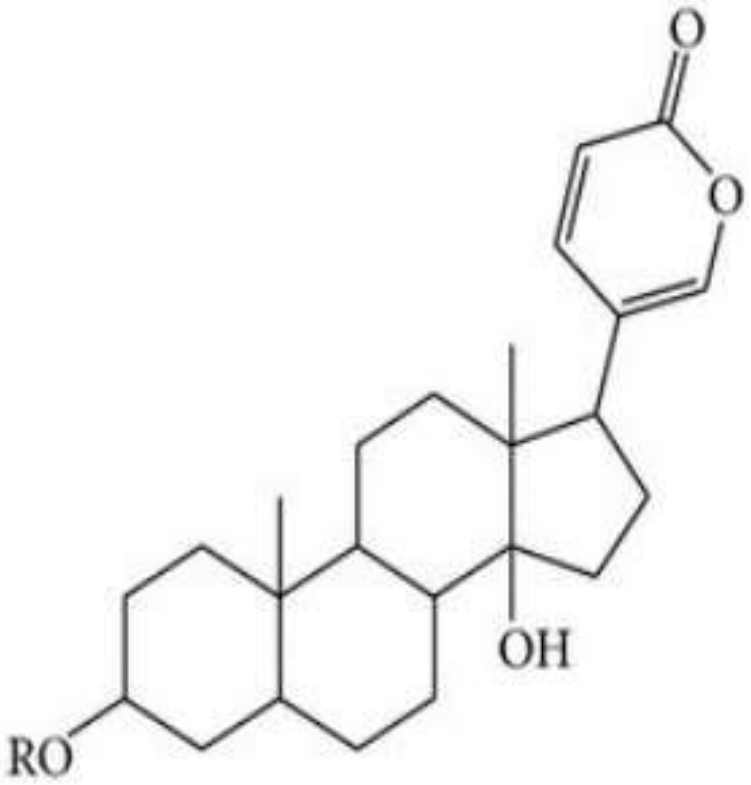
1. cardenolides
2. bufadienolides

- The cardenolides contains unstrutrated butyrolactone ring.
- The bufadienolides contains a pyrone ring.




Cardenolide

Digitoxin: R = digitoxose-digitoxose-digitoxose

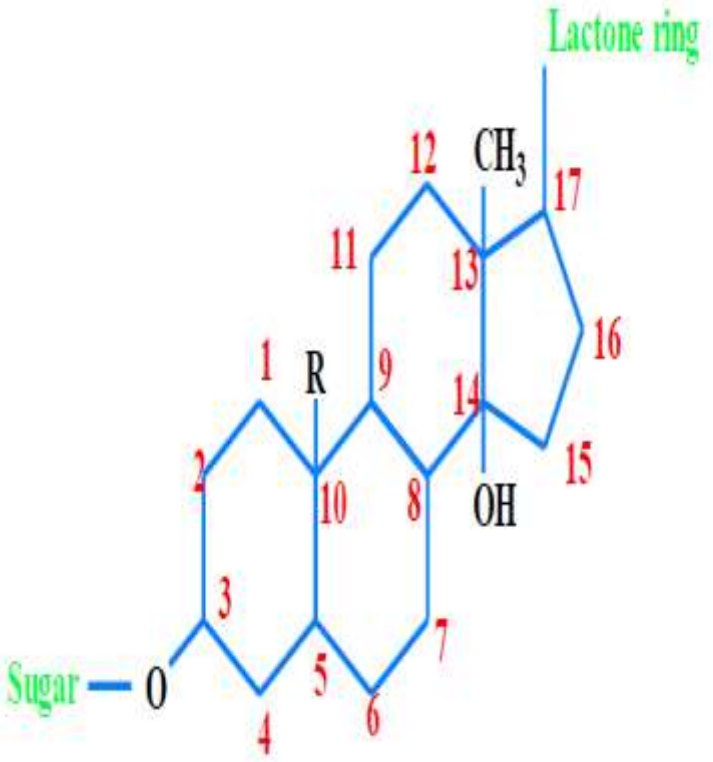



Bufadienolide

Bufalin: R = H

# STRUCTURE ELUCIDATION FOR DIGOXIN

- Its molecular formula is $C_{41}H_{64}O_{14}$
- The presence of β-OH at position C-3, which is always involved in a glycosidic linkage to a mono, di, tri, OR tetra saccharide.

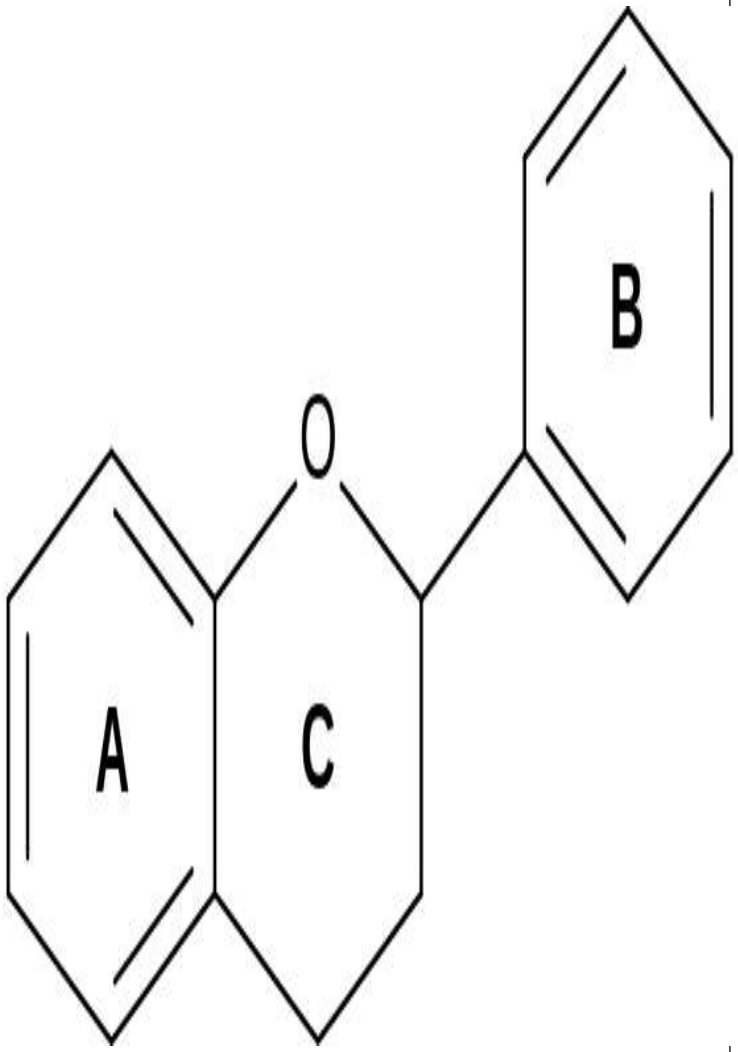


- The presence of another *β-OH group at C-14.*
- The presence of unsaturated 5 or 6- membered lactone ring at position C-17, also in the *β configuration.*
- The A/B ring junction is usually (cis), while the B/C ring junction is always (Trans) and the C/D ring junction is in all cases (cis).
- Additional OH groups may be present at C-5, C-11 and C-16.
- Cardiac glycosides that α-β unsaturated5-membered lactose ring in position C-17 are known as cardenolides. These are represented by the digitalis and straphanthus group.

- Digitalis glycosides contain angular methyl group at C-10, while strophanthus glycoside are characterized by presence of either an aldehydic (CHO) or primary alcoholic (C`H2OH) group at C-10.
- Cardiac agents that have doubly unsaturated 6-membered lactone ring in position C-17 are referred to as Bufadienolides. This group includes the squill glycosides and the toad venom, Bufotoxin
- With the exception of D-glucose and L-rhamnose, all the other sugars that are found in cardiac glycosides are uncommon deoxy-sugars e.g., Digitoxose, Cymarose, Thevetose.

# FLAVONOIDS (QUERCETIN)

- These are mostly yellow pigmet in plant.
- The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain having the carbon skeleton C6 - C3 - C6 and they are the plant pigments and they are having polar nature and is solouble in methanol and water.



- Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognised as flower pigments in most angiosperm families (flowering plants).
- However, their occurence is not restricted to flowers but include all parts of the plant.
- They are secondary metabolite and effective in CNS disorders.
- Flavonoids have been known to have antiviral, antiallergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities

# QUERCETIN

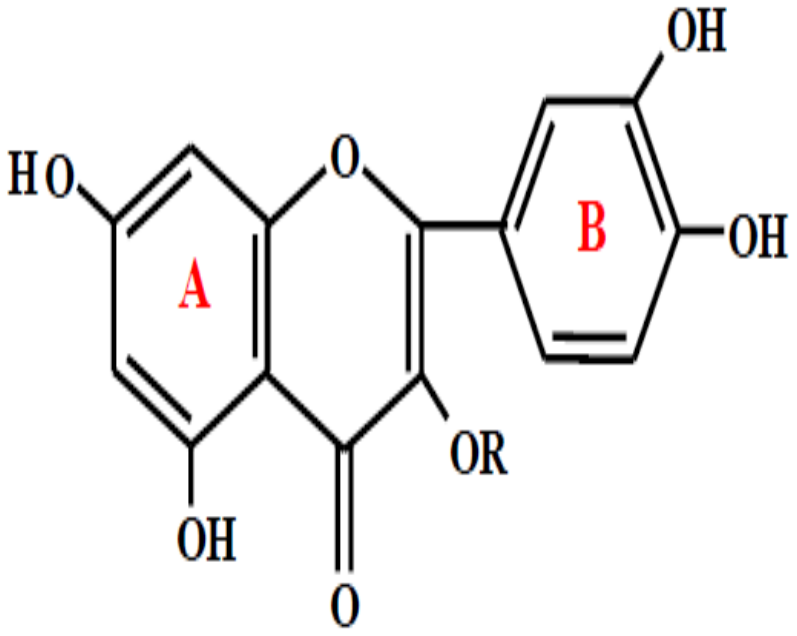
- Source-It obtained from the bark of Quercus tinctoria.
- Quercitrin is quercitin 3-O-rhamnoside.
- Quercitrin on acid hydrolysis yields rhamnose and quercetin.
- The aglycone quercetin occurs in bearberry leaves (Uva Ursi) and has a diuretic action of the leaves.

# EXTRACTION AND ISOLATION.

- Plant parts are dried. Finely ground plant material extract with methanol.
- The extract is concentrated to dryness in vacuum at 40°C to remove methanol.
- The extract is treated with a mixture of hexane, DCM, EtOAc, BuOH.
- The combined organic layer of each partition is evaporated to dryness in vacuum at 40°C using rotatory evaporator.
- The extract flavonoids can be isolated by column chromatography.

# STRUCTURE ELUCIDATION

- Molecular formula is $C_{15}H_{10}O_7$
- It contains 5 hydroxyl groups.
- Quercetin is fused with KOH to produced phloroglucinol and protocatechuic acid.




Quercetin: R=H

Quercetrin: R= rhamnosyl

Rutin: R=rutinosyl

- Quercetin is methylated and produces pentamethylquercetin, boiled with alkali ethanol gives 6-hydroxy, 2, 4, trimethoxyacetaphenone and vatic acid. Indicate that quecrectin is 3,3,4,5,7 pentahydroxy flavones.

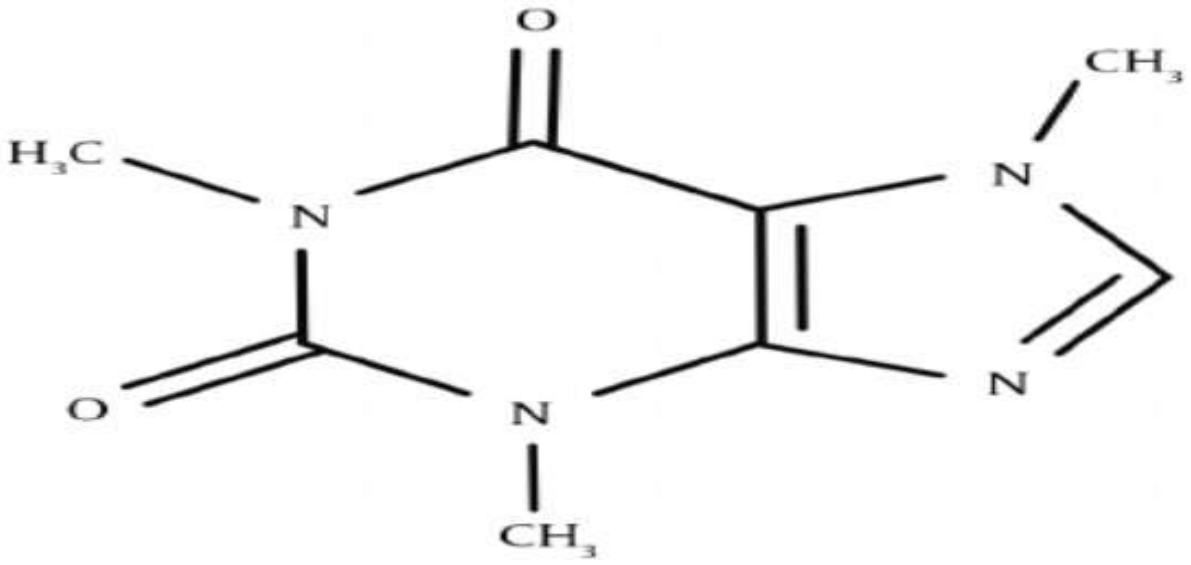
USES OF FLAVONOIDS:

- Increase capillary resistance and decrease vitamins C & P deficiency.
- They are recommended in the treatment of thrombopenia (blood coagulation).
- They are reported of value in the treatment of influenza, when given with ascorbic acid.

# PURINE (CAFFEINE)

- A purine is a heterocyclic aromatic organic compound that consists of a pyrimidine ring fused to an imidazole ring.
- A moderate amount of purine is also contained in beef, pork, poultry, fish and seafood, asparagus, cauliflower, spinach, mushrooms, lentils, beans, oatmeal, wheat bran, wheat germ, and haws.
- Caffeine is chemically 1,3,7-trimethylxanthine ($C_8H_{10}N_4O_2$).
- It is a central nervous system (CNS) stimulant of the methylxanthine class of psychoactive drugs.

- The most well known source of caffeine is the seed (commonly incorrectly referred to as the "bean") of COFFEE plants. Beverages containing caffeine are ingested to relieve or prevent drowsiness and to increase one's energy level.
- Caffeine is extracted from the plant part containing it for making beverages by steeping it in water, a process called infusion.



# EXTRACTION AND ISOLATION OF CAFFEINE

Tea bags, calcium carbonate and water were obtained.

The mixture was boiled gently with continuous swiriling.

The mixture was filtred by using suction filtration.

The mixture was cooled down in ice water bath.

Sodium chloride was added and the solution was extracted with dichloromethane two times.

The organic layer was collected and dried with magnesium sulphate anhydrous.

The solvent was filtered and evaporated by using rota-vapor.

The compound was weighed and was purified.

The pure compound was characterized by using IR spectroscopy and GC-MS.

# Structure Elucidation

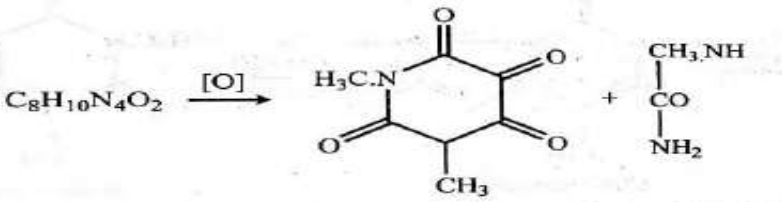
The structural elucidation of caffeine is described below:

1) Its molecular composition is $C_8H_{10}N_4O_2$.

2) On heating with hydriodic acid at about 200°, caffeine gives three moles of methyl iodide [Herzig method] suggesting the presence of three N—$CH_3$ groups.

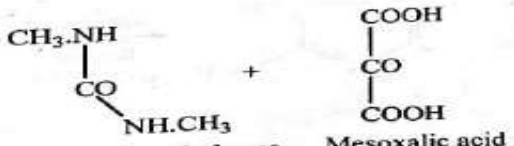
$$—N.CH_3 \xrightarrow[200°]{HI} —NH + CH_3I \xrightarrow{AgNO_3} AgI\downarrow$$

3) Caffeine on oxidation with potassium chlorate in hydrochloric acid solution gives 1, 3-dimethylalloxan and monomethyl urea. 1, 3-Dimethylalloxan is identified as mesoxalyl-*sym*-dimethyl urea since on hydrolysis, it gives *sym*-dimethyl urea and mesoxalic acid.



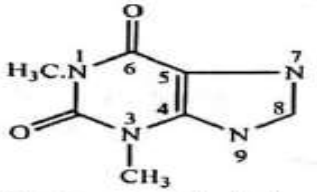

Caffeine — 1,3-Dimethylalloxan + Monomethyl urea

Hydrolysis

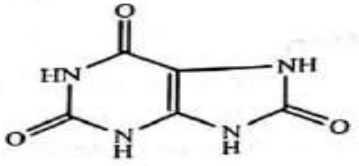



*sym*-Dimethyl urea + Mesoxalic acid

The oxidation of caffeine to 1, 3-dimethylalloxan and monomethyl urea suggests beyond doubt that the caffeine has uric acid skeleton which on oxidation gives alloxan and urea. Moreover, the formation of 1, 3-dimethylalloxan also establishes the position of two of the three methyl groups. Hence the part structure of caffeine may be written as below:

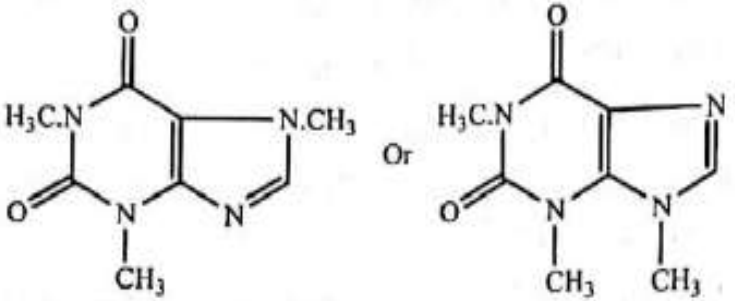



Part structure of caffeine



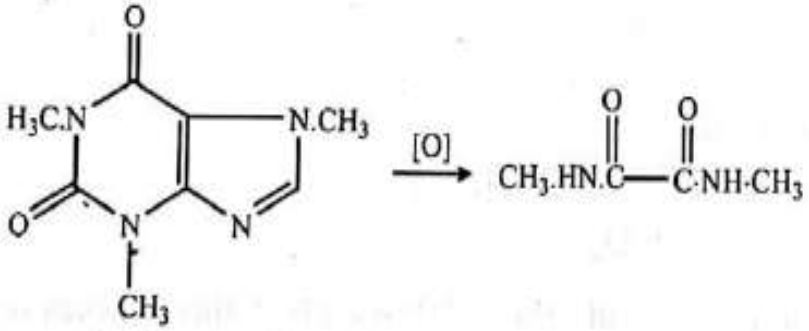

Uric acid

4) Now the only problem is to assign the position of the third N-methyl group. From the above part structure of caffeine, it is obvious that the third N-methyl group may either attach to $N_7$ or $N_9$ and theoretically the following two structures are possible for caffeine.



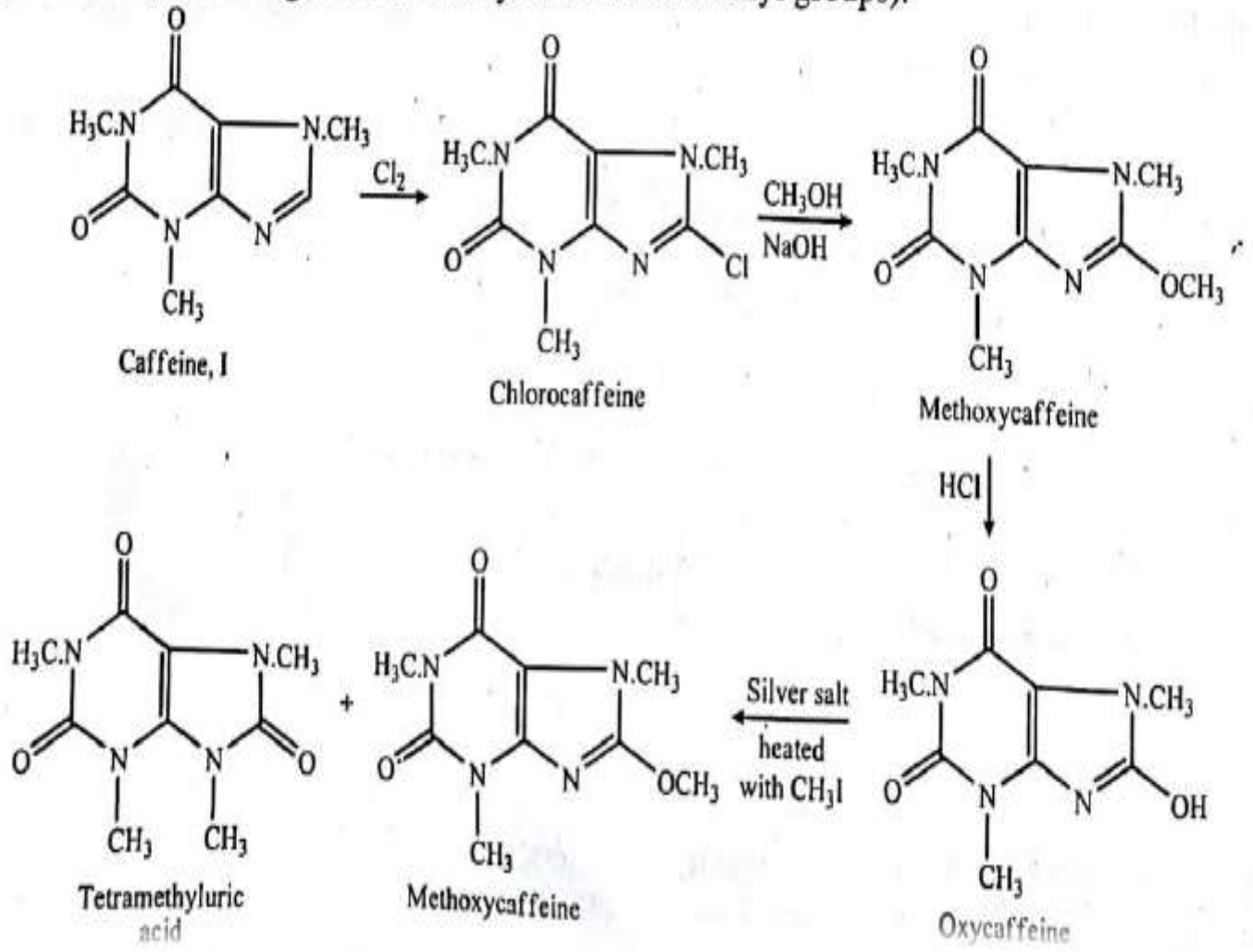

I II

5) On vigorous oxidation, caffeine gives *sym*-dimethyloxamide ($CH_3NH.CO.CO.NHCH_3$) as one of the products which indicates the presence of the grouping $CH_3.N—C—C—N{\cdot}CH_3$. From the observation of the above two structures of caffeine, it is clear that this grouping is present only in structure I.




Caffeine, I

6) The structure I for caffeine also explains **Fischer** observation that on treatment with $CH_3I$, the silver salt of oxycaffeine, obtained by the consecutive treatment of caffeine with chlorine, methanolic-alkali and hydrogen chloride, gives a mixture of tetramethyl uric acid (containing four N-methyl groups) and methoxycaffeine (containing three N-methyl and one O-methyl groups).



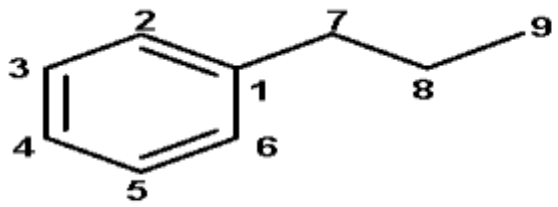
# LIGNAN

- The term “Lignan” is a group of dimeric

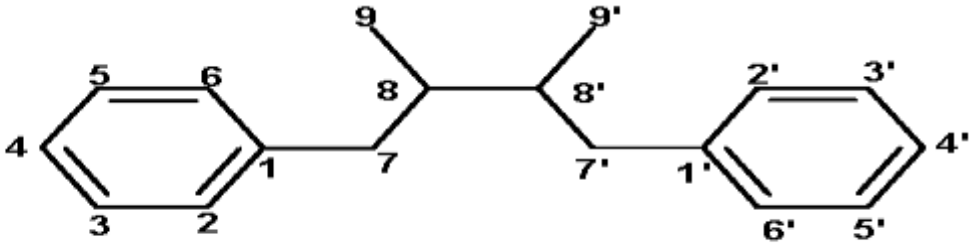
phenylpropanoids where two phenylpropanoid molecules are attached by its central carbon (C8).

**Lignans are a subgroup of non-flavonoid polyphenols.**

- They are widely distributed in the plant kingdom, being present in more than 55 plant families, where they act as **antioxidants and defence molecules against pathogenic fungi** and **bacteria.**
- Flowing structure contains
- a) Phenylpropanoid and b) Lignan




a




b

- **Biological activity of Lignans are** Antiviral ,Anticancer ,Cancer prevention, Anti-inflammatory, antimicrobial ,antioxidant , immunosuppressive, Hepatoprotective, Osteoporosis prevention.
- The richest dietary source is **flaxseed (linseed), that contains mainly** secoisolariciresinol, but also lariciresinol, pinoresinol and matairesinol in good quantity . They are also found in sesame seeds, **whole grains.**

# PODOPHYLOTOXIN

- It is a non-alkaloid toxin lignan extracted from the roots and rhizomes of Podophyllum
- It is a natural product isolated from podophyllum peltatum and podophyllum emodi.
- Etoposide, a podophyllotoxin used in the treatment of cancer.
- Podophyllotoxin bears four consecutive chiral centers. The molecule also contains four almost planar fused rings. The podophyllotoxin molecule includes a number of oxygen containing functional groups: an alcohol, a lactone, three methoxy groups, and an acetal

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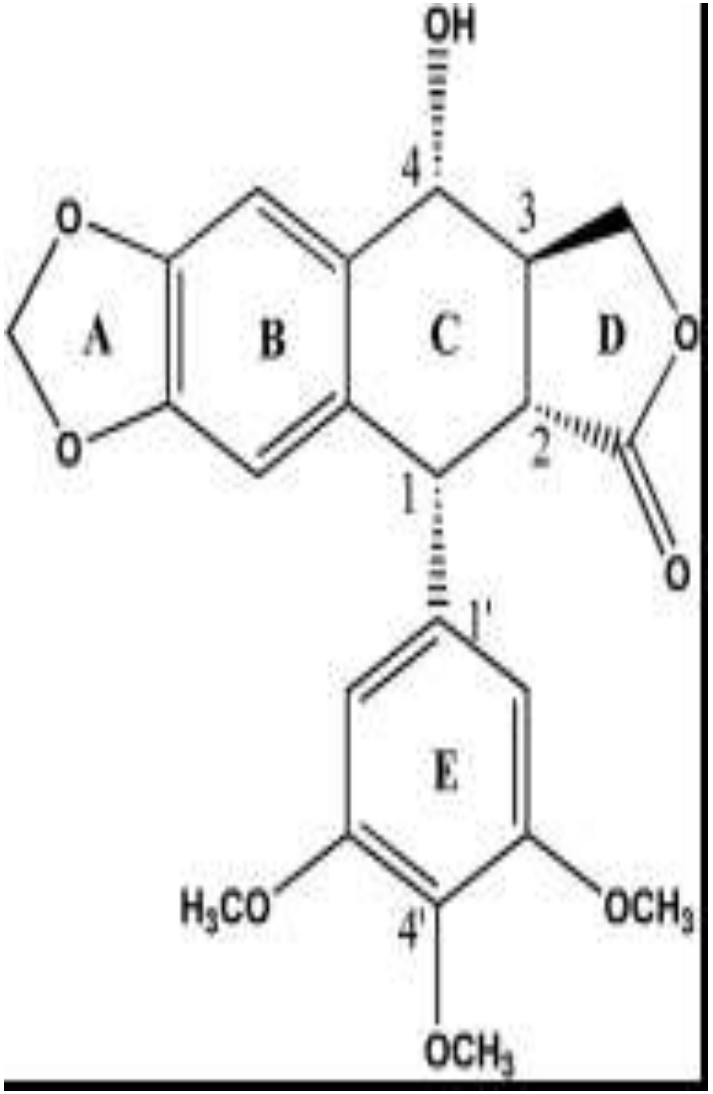

# Extraction and Isolation

- Podophyllotoxin has traditionally been isolated from podophyllin, resin of Podophyllum rhizome.
- The resin (50 g) of P. peltatum was extracted with hot CHCl3 over a day.
- The soluble fraction was chromatographed on neutral alumina , and eluted with CHCl3 to yield 20.4 g (40.8 %) of podophyllotoxin.

# THANK YOU