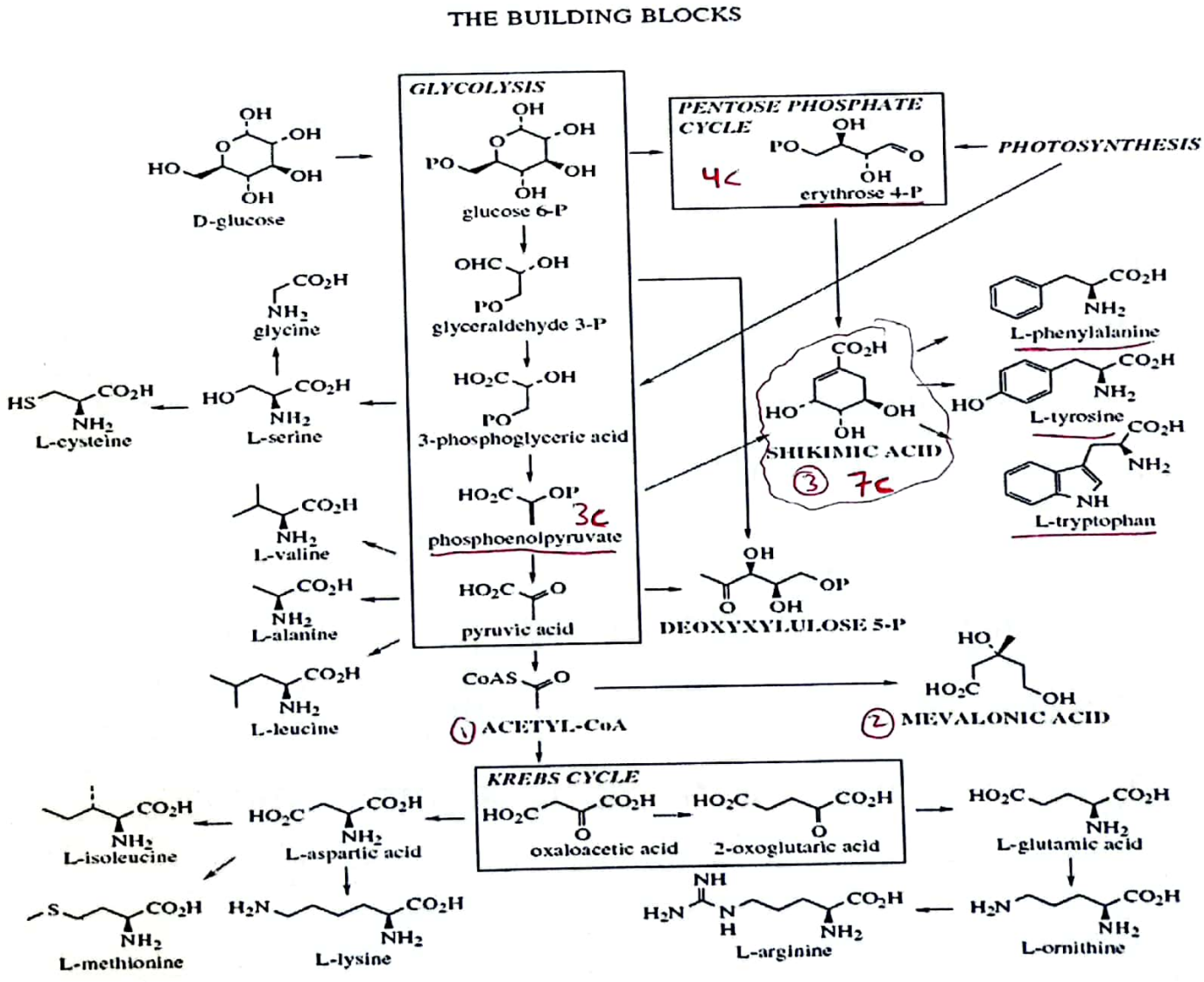
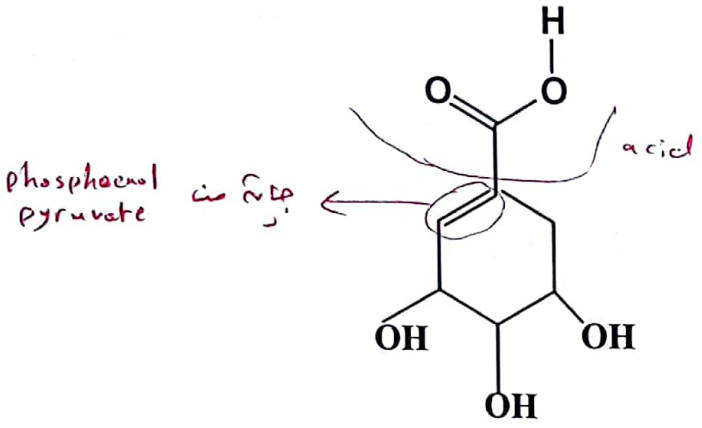
Shikimic acid pathway

Dr Dana Atoum



Shikimic Acid Pathway



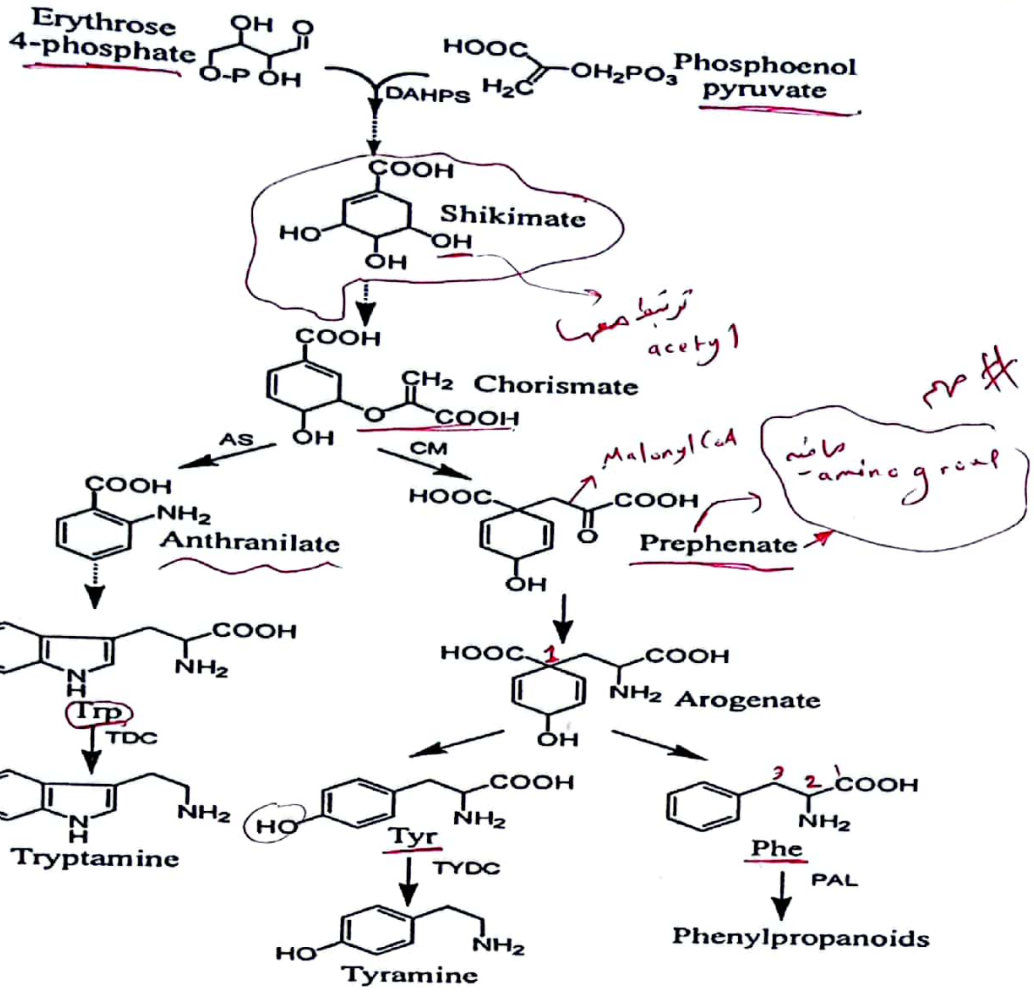
Shikimic Acid

Illicium anisatum **L. Illicinaceae {Shikimino-ki in Japanese} (1885)**

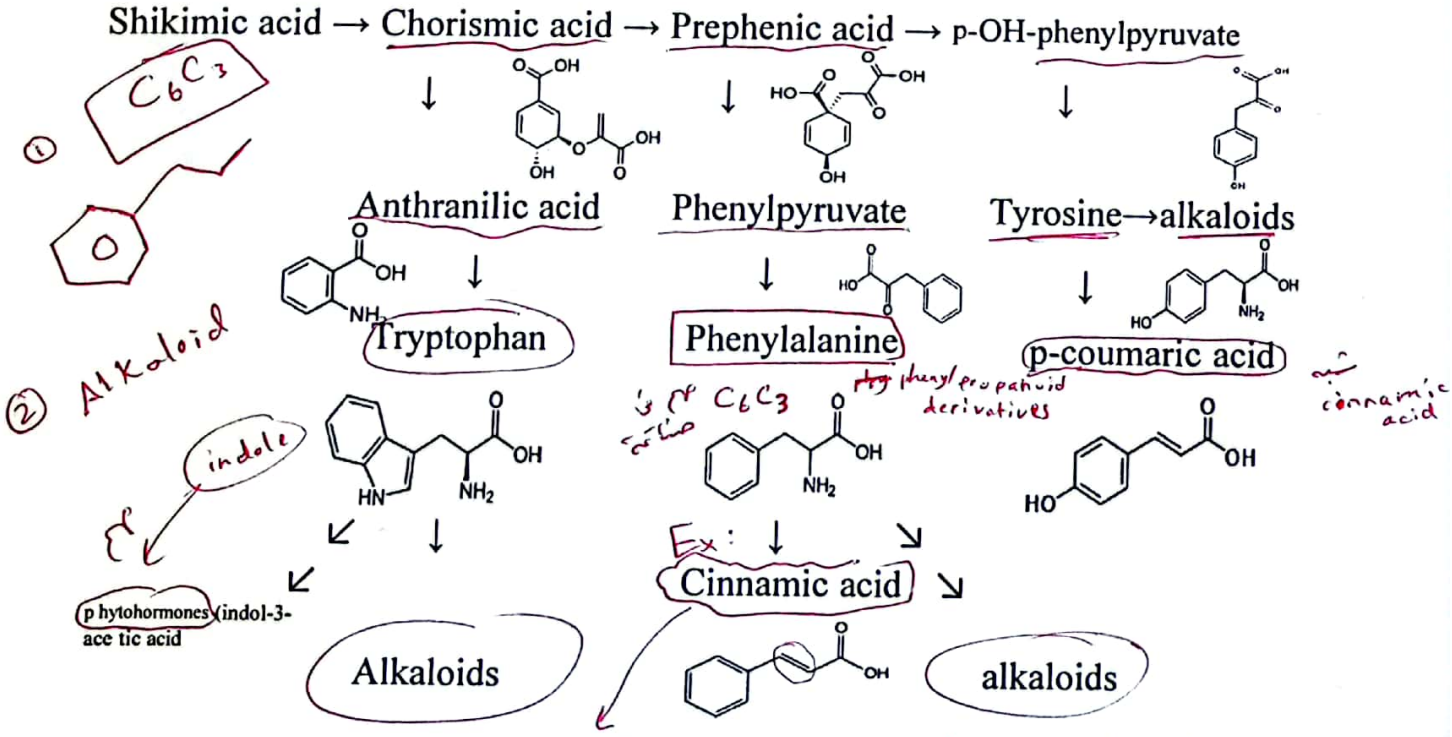
E. coli **mutant strains pathway intermediate**

Shikimic Acid Pathway

- Important for aromatic compounds
- Precursor of aromatic amino acids (Phe, Tyr, Try) biosynthesis in bacteria, fungi & higher plants. ANIMALS! HUMAN!
- Building block for other compounds: certain alkaloids, coumarins, phenyl propanes, vol. oils,..
- Starting point for tannins in all plants
- Provide protection of plants against micro-organisms

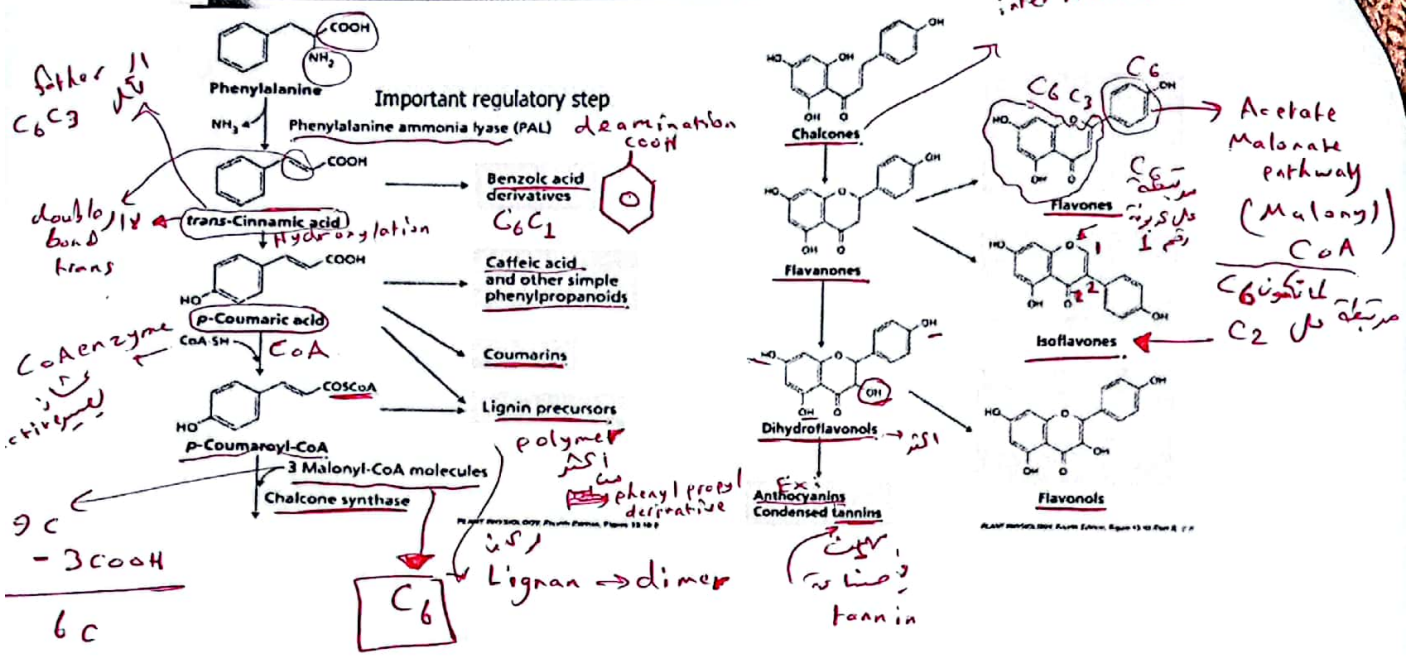


Scheme of the conversion of shikimic acid to cinnamic acid

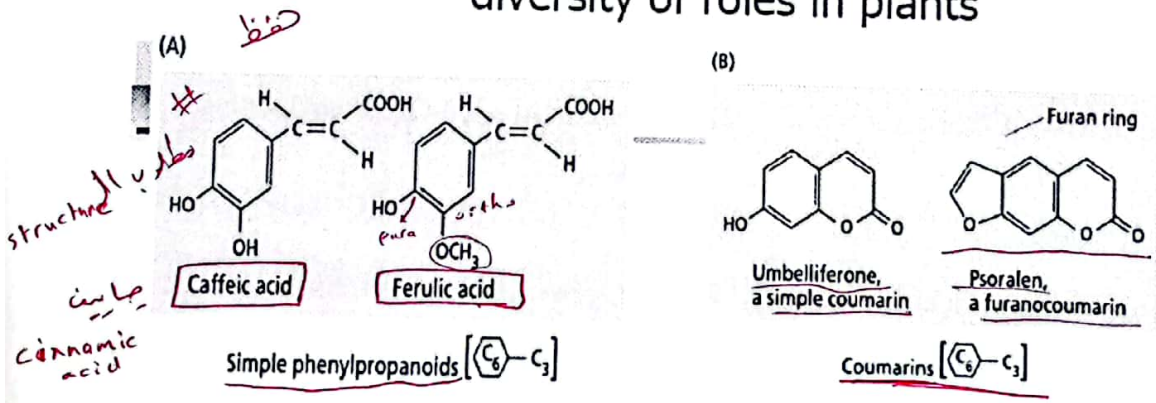


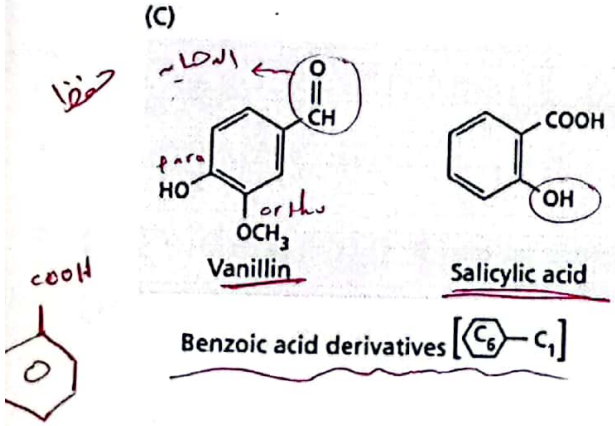
Phenolic biosynthesis from phenylalanine

-most abundant sources of plant phenolic compounds



Simple phenolic compounds play a great diversity of roles in plants





(A) Caffeic acid and ferulic acid may be released into the soil and inhibit the growth of neighboring plants----allelopathy.

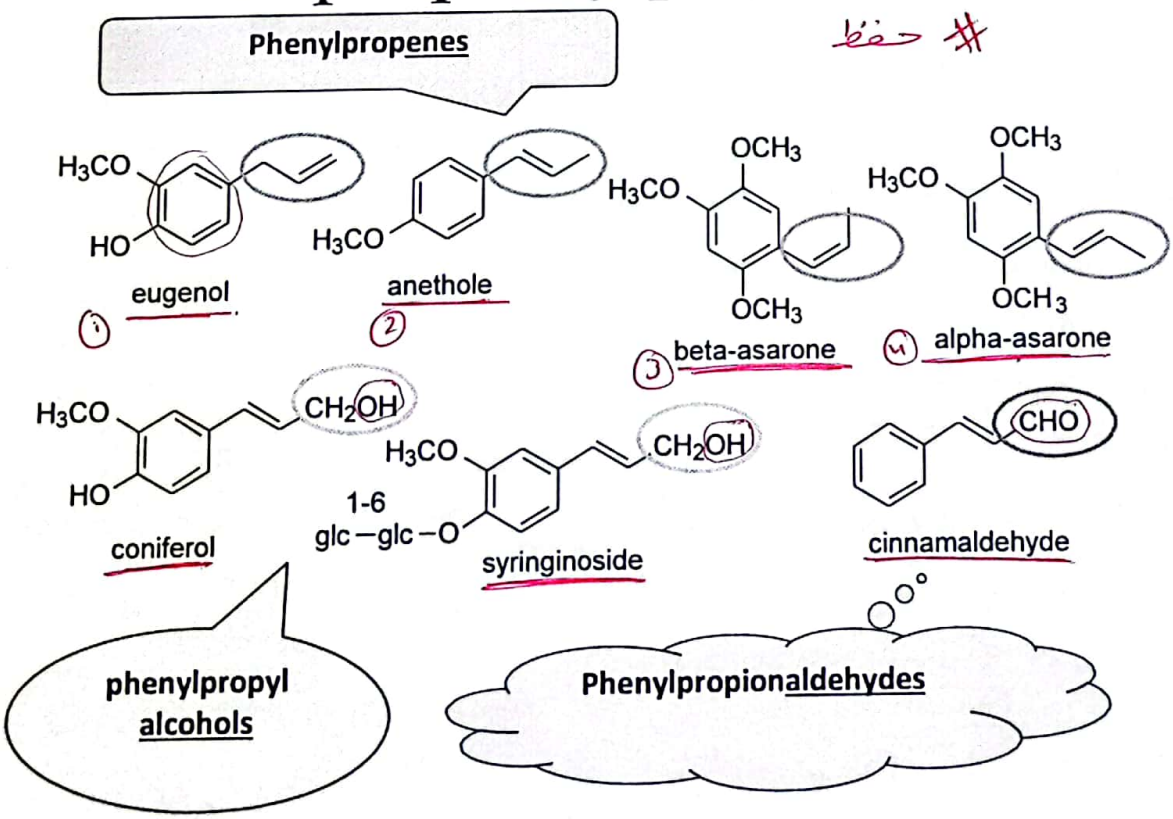
(B) Psoralen exhibits phototoxicity to insect herbivores after activation by UV-A.

(C) Salicylic acid is a plant growth regulator involved in systemic resistance to plant pathogens.

Phenylpropanoids (Cinnamates):

- Definition: Phenylpropanoids represent a large group of natural products containing a phenyl ring attached to a three-carbon propane side chain(C_6-C_3) in their structure.
- Biosynthesis: Derived from the aromatic amino acids phenylalanine and tyrosine or the intermediates of the shikimic acid biosynthetic pathway. The basic simplest nucleus of phenyl propanoids is **Cinnamic acid**.
- Categories: Simple Phenylpropanoids (phenylpropenes, phenylpropyl alcohols, phenylpropionaldehydes, phenylpropionic acids)

Simple phenylpropanoids



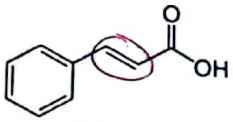
side chain يعني بتتأكسد

Possibilities of derivatives of cinnamic acid

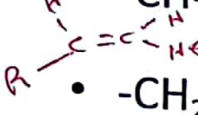
1 Substitution in aromatic ring

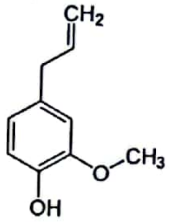
2 State of the oxidation of the side chain

- -CH=CH-COOH → Propenic acid, cinnamic acids

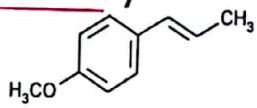


- -CH=CH-CH_2OH → Propenol, coniferylalcohols

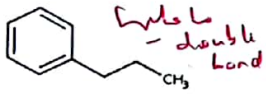




- -CH_2-CH=CH_2 → Allyl, e.g. Eugenol (in Clove Oil)
- -CH=CH-CH3 → Propenyl, e.g. Anethole (in Anise Oil)



- -CH_2-CH_2-CH_3 → n-propyl

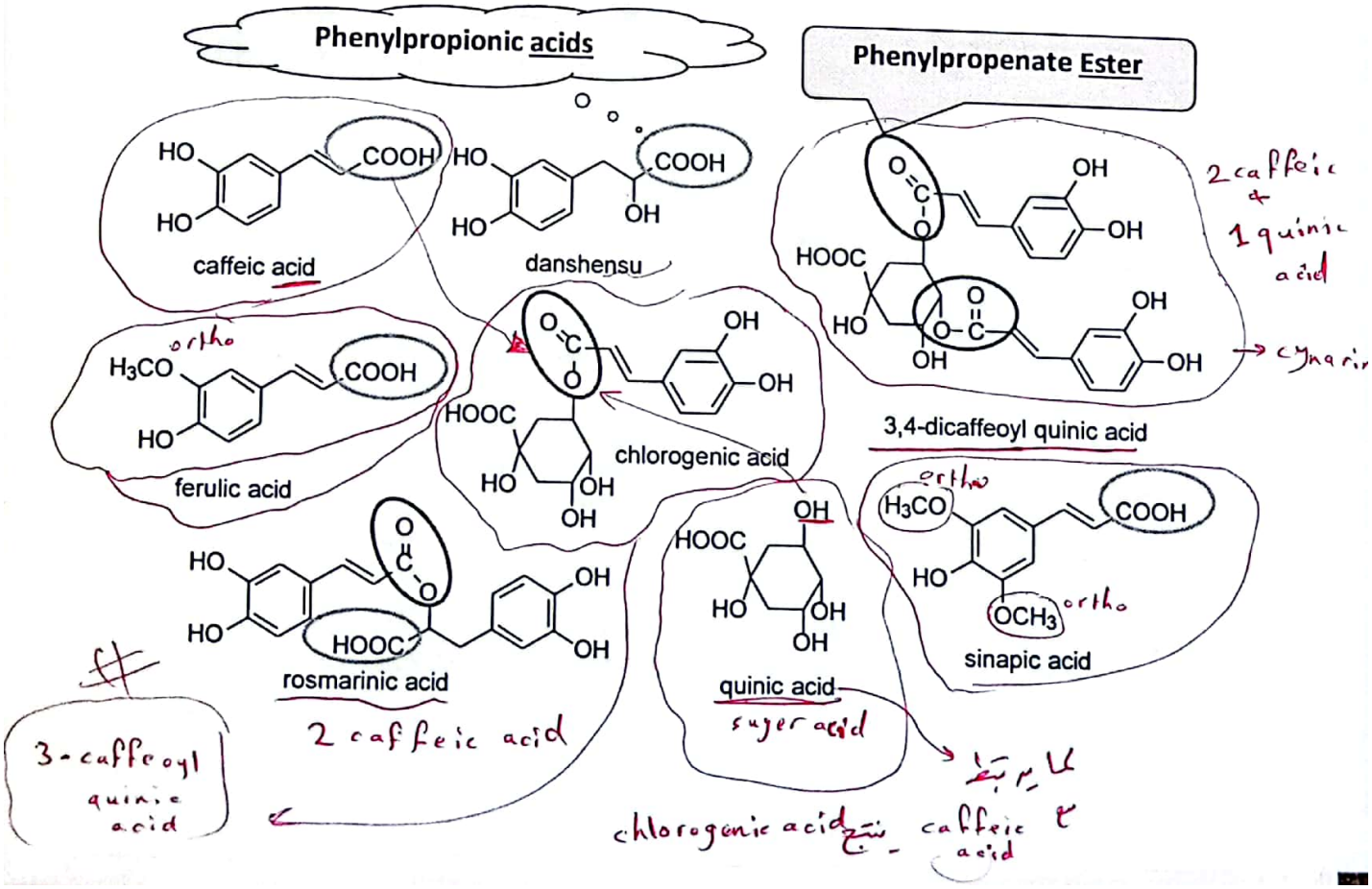


3- Shortening of the side chain

Formation of phenol carboxylic acids and simple phenols

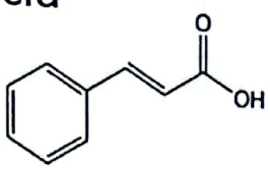
C6 benzoic acid C_6C_1

Simple phenylpropanoids

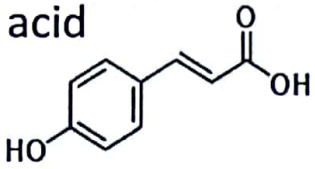


Cinnamic acids (Phenylpropionic acids)

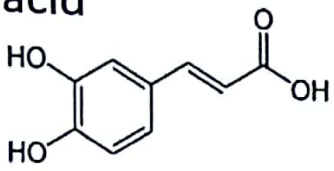
Cinnamic acid



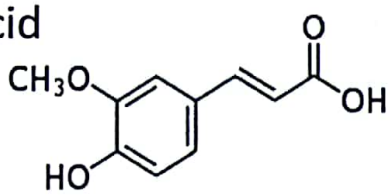
p-coumaric acid



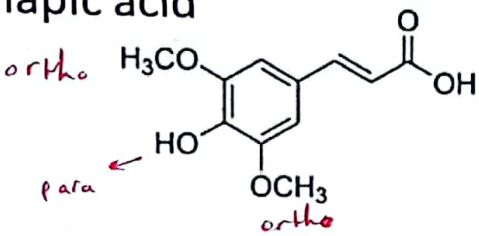
Caffeic acid



Ferulic acid



Sinapic acid



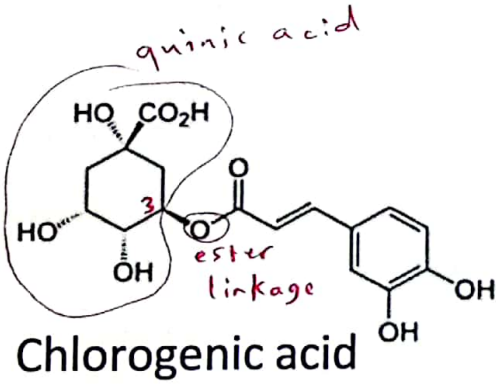
5-OH-ferulic acid

Tri-OH-cinnamic acid

Caffeic acid containing phenolics

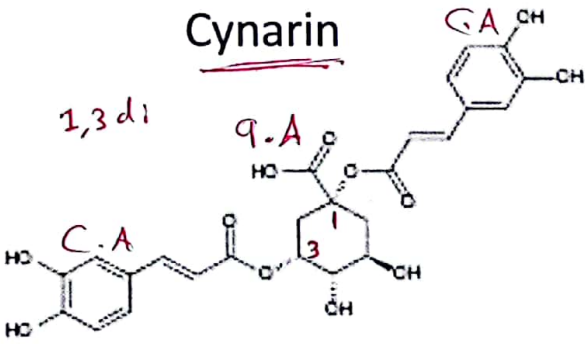
Chlorogenic acid (3-caffeoylester of quinic acid) and cynarin (1,~~4~~ 1,3 dicaffeoylester of quinic acid)

- C6-C3 acids may occur in the plants in free forms, as glycosides or as esters



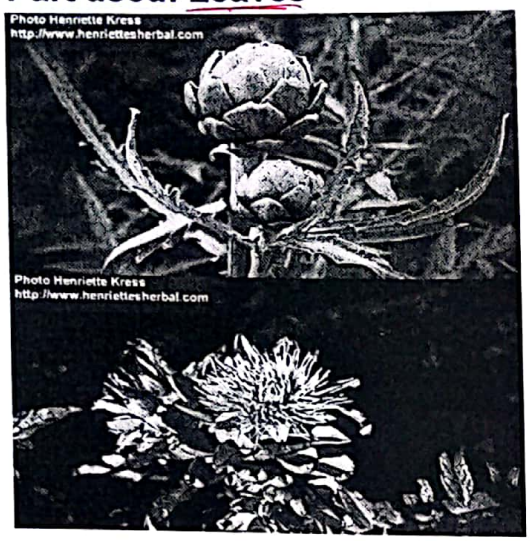
Chlorogenic acid

Cynarin

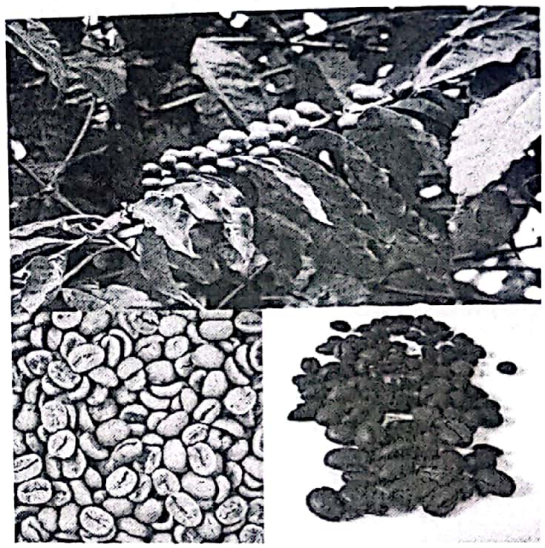


Caffeic acid containing phenolics

Cynara scolymus
(artichokeخرشوف)
Part used: Leaves



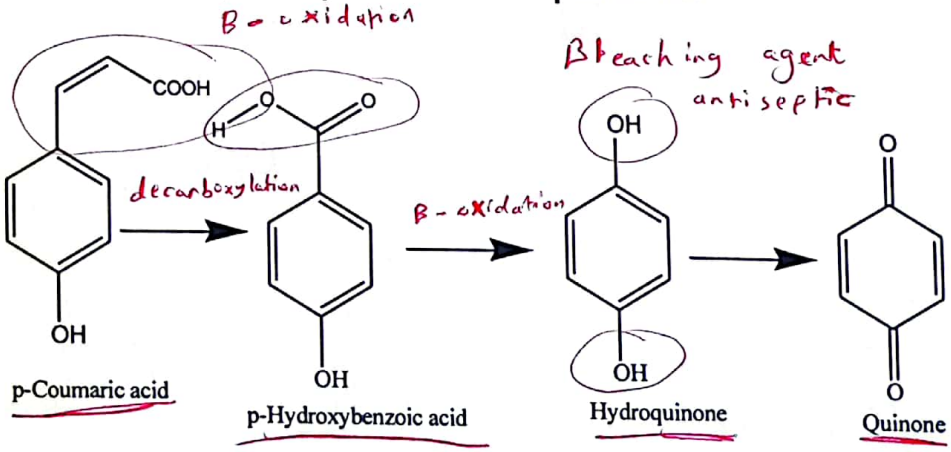
Coffea arabica (Coffee plant)
Part used: Beans



Both plants are source of Chlorogenic acid and Cynarin best known for their choleretic and lipid lowering effects (decrease cholesterol and triglycerides) and helpful in weight reduction regimens.

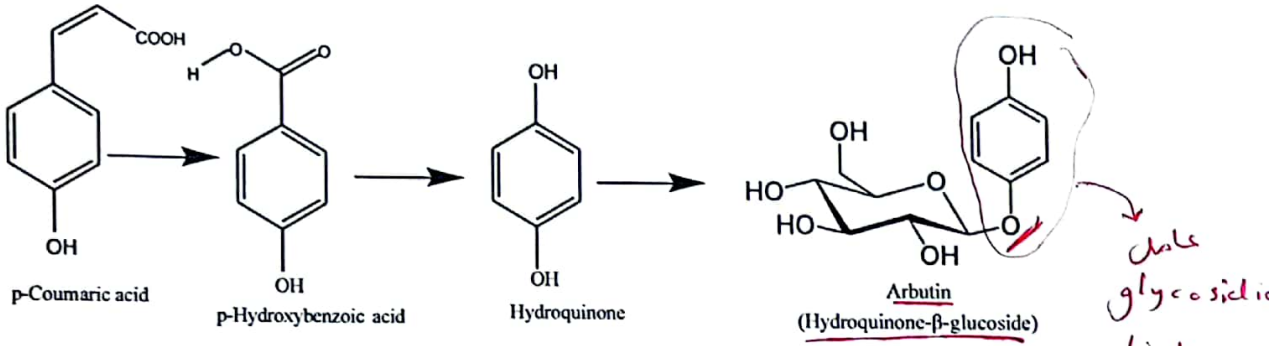
Formation of Phenylpropane Derivatives with Shorter Side Chain:

Shortening of side chain by β-oxidation and subsequent decarboxylation (simple phenols) which might be linked to oxidation→hydroquinone → quinone



Simple phenolics from SAP
C6 Phenols (hydroquinones)

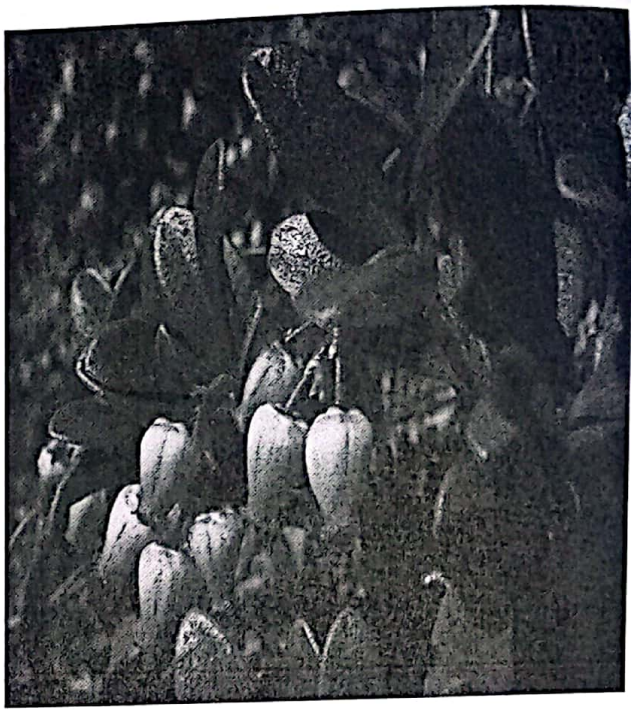
- Seldom occur naturally, but from benzoic acid decarboxylation (oxidative or not)
- Examples include Arbutin (in bearberry leaves): bioformed from 4-hydroxy benzoic acid:



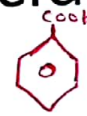
Simple phenolics from SAP
Bearberry (بدلا بنع)

- SN: *Arctostaphylos uva-ursi* L.
- Part used: Leaves
- Chemical composition: 6-10% phenolic glycosides: mainly arbutin and methyl-arbutin
- Pharmacology and uses:
  - Under alkaline urine, arbutin forms hydrquinone which is antiseptic.
  - Bearberry leaf extract is antiseptic and diuretic: traditionally used for Rx of benign UTIs and to enhance renal secretion.
  - Hydroquinone (and its methyl derivative: mequinol) is a topical skin bleaching agent (inhibits melanin synthesis): used for topical treatments of burn scars, chloasma, and freckles.

Arctostaphylos uva-ursi
Bearberry



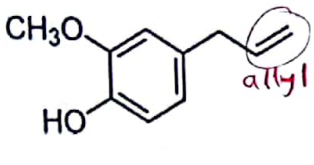
Benzoic acids (C6-C1) related to cinnamic acids C_6C_3



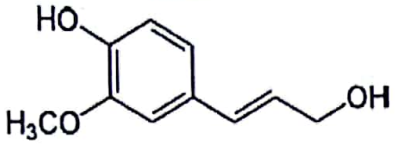
Cinnamic acid	Benzoic acid
p-coumaric acid.....	*p*-OH-benzoic acid
Caffeic acid.....	Protocatechuic acid
Ferulic acid.....	Vanillic acid
Sinapic acid.....	Syringic acid
Tri-OH-cinnamic acid.....	Gallic acid

Vanilla planifolia (Orchidaceae)

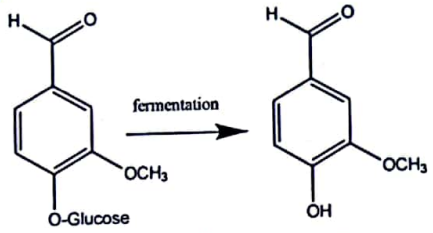
- Vanillin produced from eugenol (in plants) or lignin is a degradation by-product of paper from wood (large scale preparation of vanillin)
- Fermentation under blankets in the sun



Eugenol

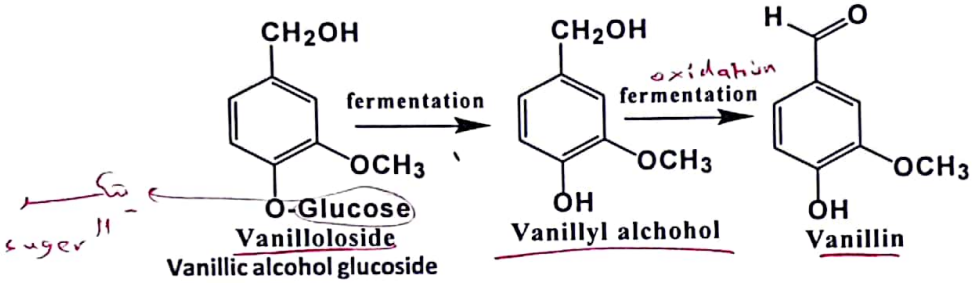


Coniferylalcohol (lignin monomer)



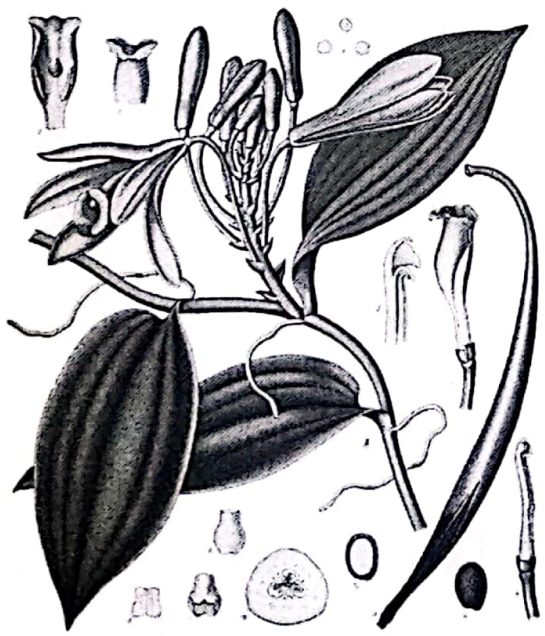
Vanillin glucoside

Vanilloside (odourless) is liberated during fermentation & oxidized to vanillin

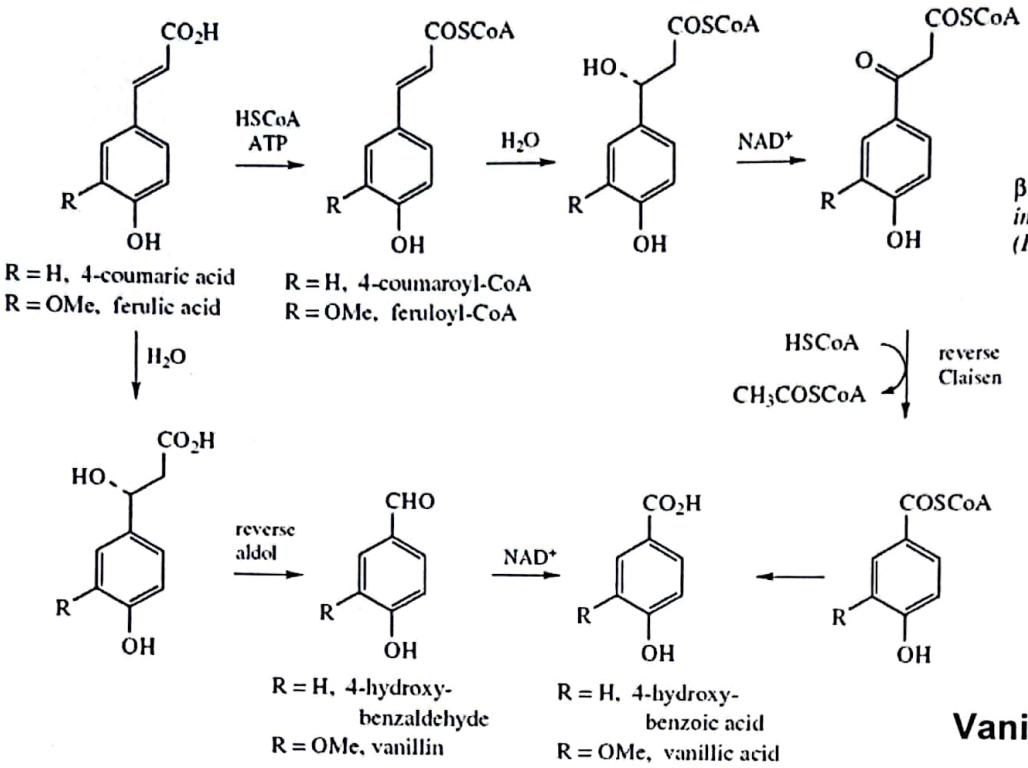


- Fine aroma and taste is due to other compounds better than pure vanillin

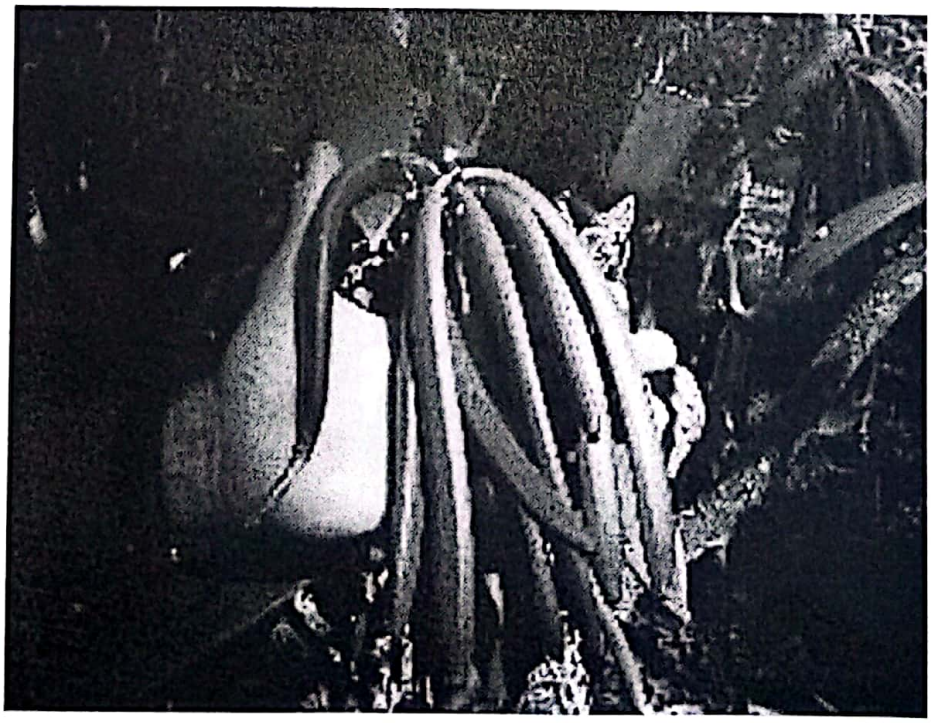
Vanilla planifolia

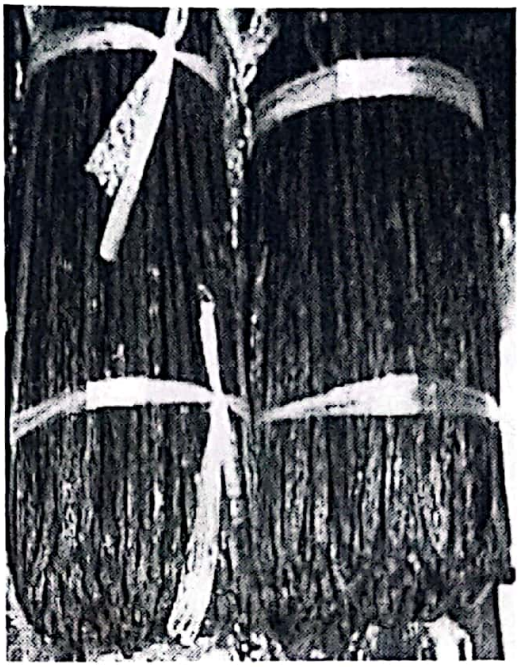


Benzoic Acids From C_6C_3 Compounds

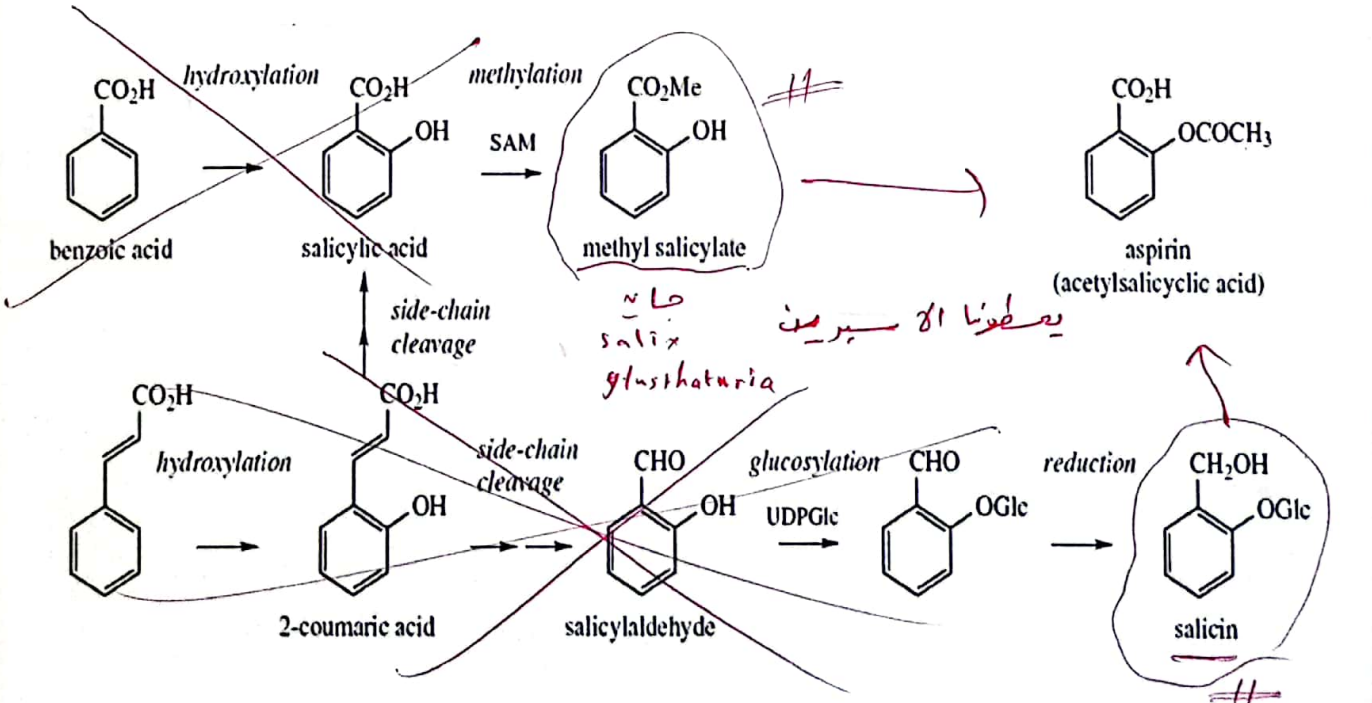


Vanilla pods (*Vanilla planifolia*)

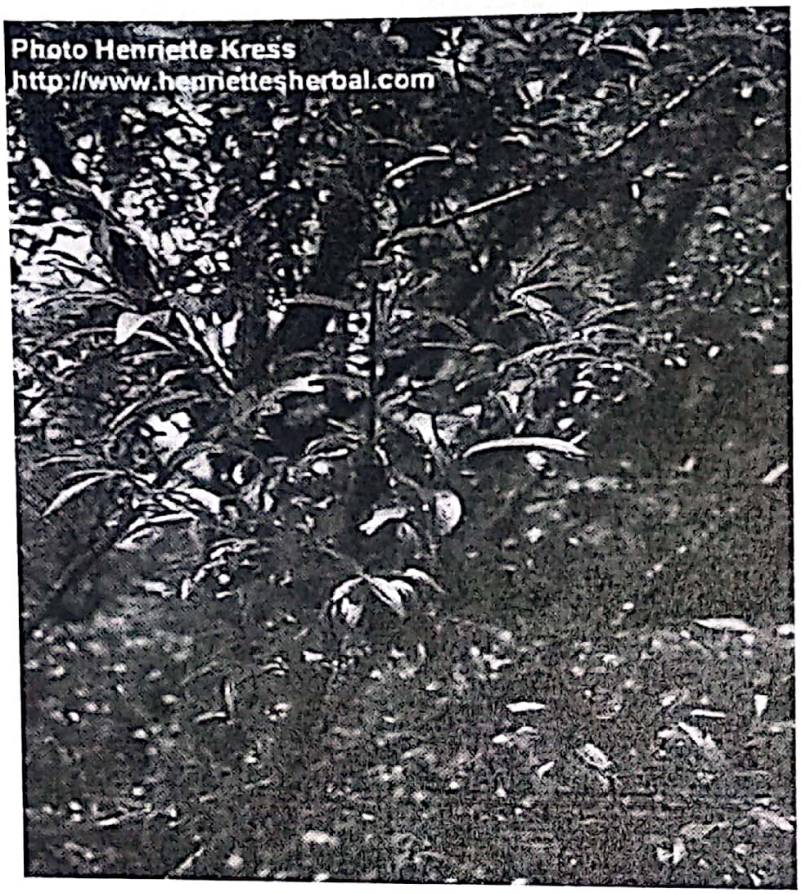


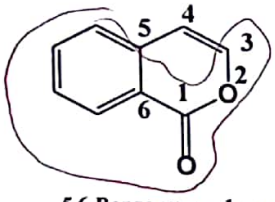


Salicylates



• *Salix alba* (Willow)

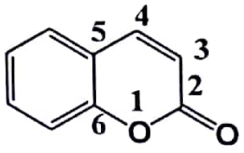




5,6-Benzopyran-1-one
isocoumarin

Coumarins

C_6C_3

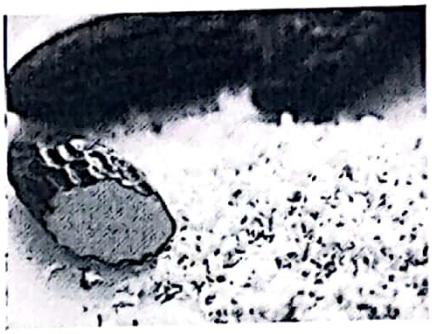


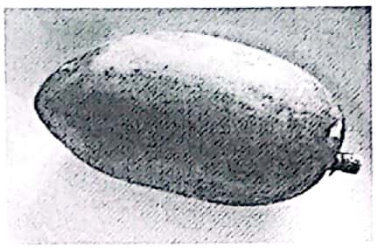
5,6-Benzopyran-2-one
coumarin

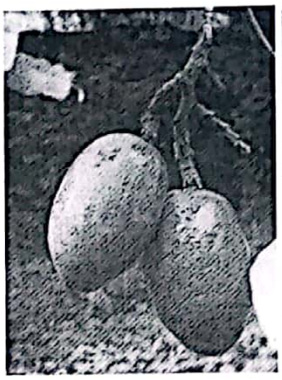
chromophore (يعطي UV light ↓ tanning)

- Name derived from **Coumarouna** (local Guyana name) for the seeds of *Dipteryx odorata* (Fabaceae), common named for Tonka bean
- Seeds as spirits spice (not used now, hepatotoxic & carcinogenic)
- Characteristic **new mown hay smell**
- Commonly used as sun **UV protector**
- **Absorb** short wave UV **230-315** nm & **transmit** long-wave UV **315 - 400** nm resulting in brown sun tan

Dipteryx odorata (Tonka beans)

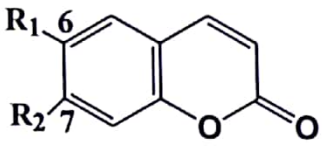




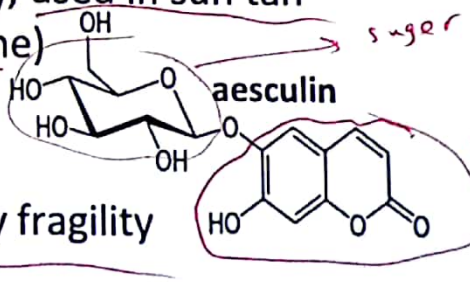


Characters of Coumarins

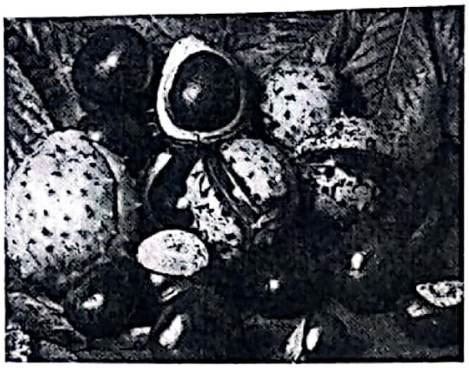
- Free coumarins are **organic solvents-soluble**
- Derivatives of α-chromone, differ in benzene ring substitutions (OH, OCH_3, CH_3)
- Common in **Apiaceae, Asteraceae, Fabaceae, Lamiaceae, Poaceae, Moraceae, Rutaceaea & Solanaceae**
- Characteristic UV (blue, yellow & purple) enhanced by ammonia
- **Umbelliferone** R_1=H, R_2=OH
- **Aesculetin** R_1=R_2= OH
- **Scopoletin** R_1=OCH_2, R_2= OH



- *Aesculus hippocastanum* (horse-chestnut) (Hippocastanaceae) leaves & bark rich in aesculin (6β-D-glucosyloxyl-7-OH-coumarin, or 6β-D-glucosyl-Aesculetin); used in sun tan preparations (both glycoside & aglycone)



- Symptomatic Rx of cutaneous capillary fragility



- Leaves & bark of Horse chest nuts

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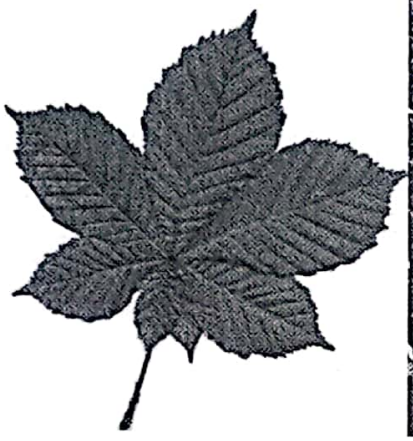
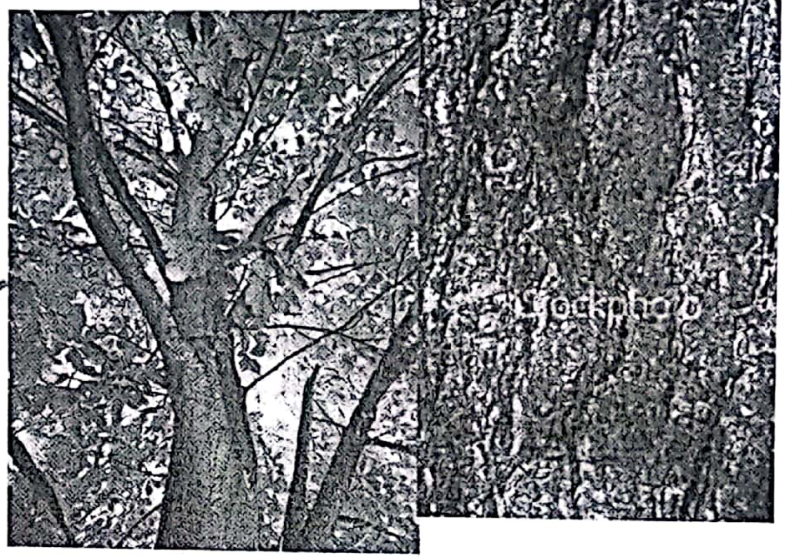


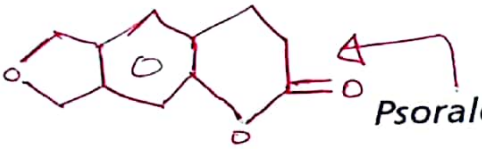


Dicoumarol and Warfarin

The cause of fatal haemorrhages in animals fed spoiled sweet clover (*Melilotus officinalis*; Leguminosae/Fabaceae) was traced to dicoumarol (bishydroxycoumarin) (Figure 4.31). This agent interferes with the effects of vitamin K in blood coagulation (see page 163), the blood loses its ability to clot, and thus minor injuries can lead to severe internal bleeding. Synthetic dicoumarol has been used as an oral blood anticoagulant in the treatment of thrombosis, where the risk of blood clots becomes life threatening. It has been superseded by salts of **warfarin** and **acenocoumarol** (**nicoumalone**) (Figure 4.32), which are synthetic developments from the natural product. An overdose of warfarin may be countered by injection of vitamin K_1.

Warfarin was initially developed as a rodenticide, and has been widely employed for many years as the first choice agent, particularly for destruction of rats. After consumption of warfarin-treated bait, rats die from internal haemorrhage. Other coumarin derivatives employed as rodenticides include **coumachlor** and **coumatetralyl** (Figure 4.32). In an increasing number of cases, rodents are becoming resistant to warfarin, an ability which has been traced to elevated production of vitamin K by their intestinal microflora. Modified structures **defenacoum** and **brodifenacoum** have been found to be more potent than warfarin, and are also effective against rodents that have become resistant to warfarin.

Melilotus officinalis البرسيم الحلو



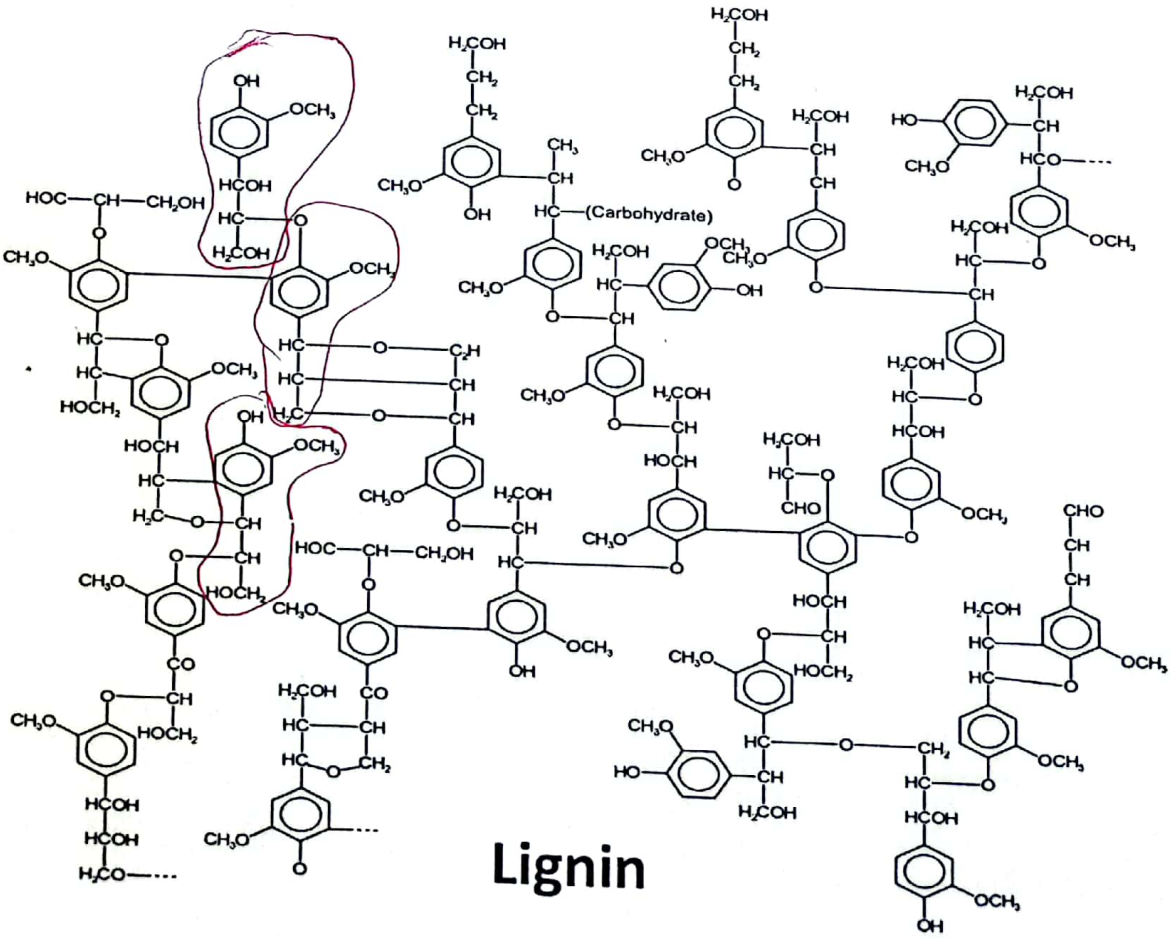
Psoralens

Psoralens are linear furocoumarins which are widely distributed in plants, but are particularly abundant in the Umbelliferae/Apiaceae and Rutaceae. The most common examples are psoralen, bergapten, xanthotoxin, and isopimpinellin (Figure 4.33). Plants containing psoralens have been used internally and externally to promote skin pigmentation and sun-tanning. Bergamot oil obtained from the peel of *Citrus aurantium* ssp. *bergamia* (Rutaceae) (see page 179) can contain up to 5% bergapten, and is frequently used in external suntan preparations. The psoralen, because of its extended chromophore, absorbs in the near UV and allows this radiation to stimulate formation of melanin pigments (see page 129).

Methoxsalen (xanthotoxin; 8-methoxypsoralen) (Figure 4.36), a constituent of the fruits of *Ammi majus* (Umbelliferae/Apiaceae), is used medically to facilitate skin repigmentation where severe blemishes exist (vitiligo). An oral dose of methoxsalen is followed by long wave UV irradiation, though such treatments must be very carefully regulated to minimize the risk of burning, cataract formation, and the possibility of causing skin cancer. The treatment is often referred to as PUVA (psoralen + UV-A). PUVA is also of value in the treatment of psoriasis, a widespread condition characterized by proliferation of skin cells. Similarly, methoxsalen is taken orally, prior to UV treatment. Reaction with psoralens inhibits DNA replication and reduces the rate of cell division. Because of their planar nature, psoralens intercalate into DNA, and this enables a UV-initiated cycloaddition reaction between pyrimidine bases (primarily thymine) in DNA and the furan ring of psoralens (Figure 4.36). In some cases, di-adducts can form involving further cycloaddition via the pyrone ring, thus cross-linking the nucleic acid.

Lignin & Lignans

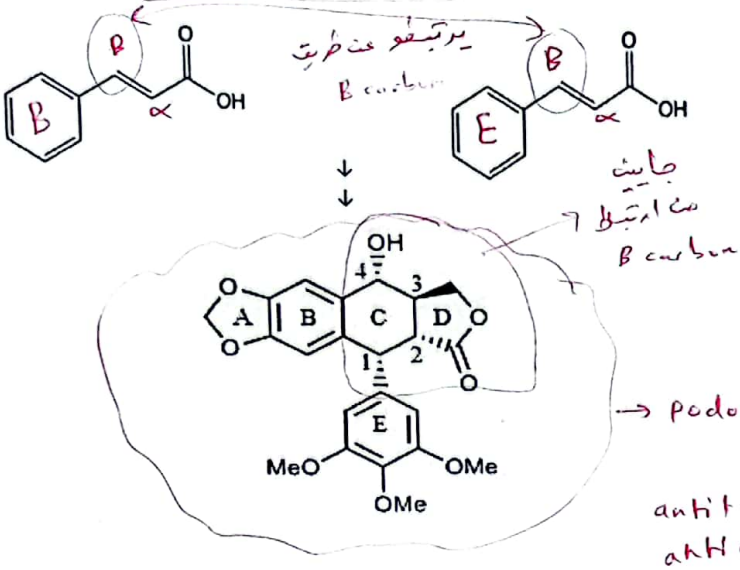
- Lignin is plant polymer acting as strengthening material for plant cell wall & matrix for cellulose micro-fibrils
- Represent a large no. of aromatic material based on C_6C_3 building unit
- Lignins formed by oxidative coupling of hyroxycinnamyl alcohol monomers by peroxidase enzymes [p-coumaryl-, coniferyl- and sinapyl-alcohol]
- Lignans are dimeric phenylpropanes (C-18) coupled at the central carbon of the side chain [via their ß-carbon of the side chain]



Lignin

Formation of the lignans

Dimers of cinnamic acid linked via their ß-carbons and further modifications; i.e. Podophyllotoxin



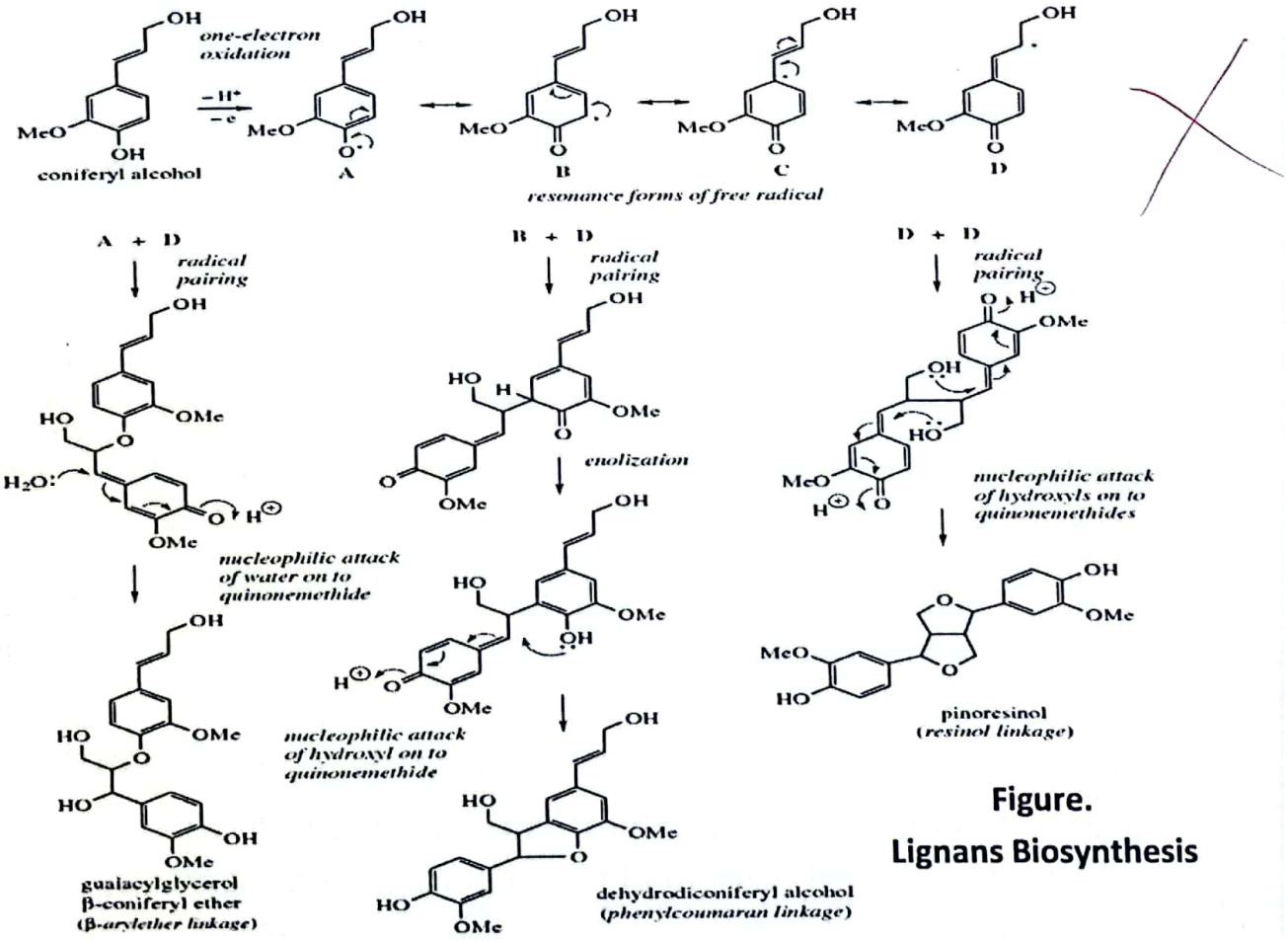
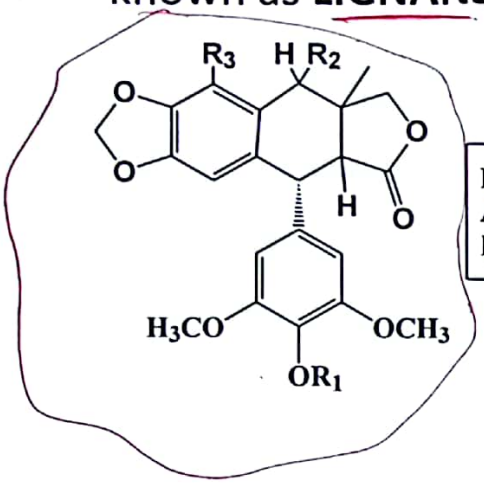


Figure.
Lignans Biosynthesis

Podophyllotoxin & Peltatins

- Lignans formed by oxidative coupling of 2 cinnamic acid residues (dimeric phenylpropane derivatives) known as **LIGNANS**

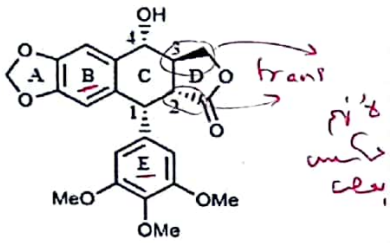


Podophyllotoxin	$R_1=CH_3$	$R_2=OH$	$R_3=H$
Alpha-Pelatin	$R_1=H$	$R_2=H$	$R_3=OH$
Beta- Pelatin	$R_1=CH_3$	$R_2=H$	$R_3=OH$

inactive isomer يكون المركب out side inside

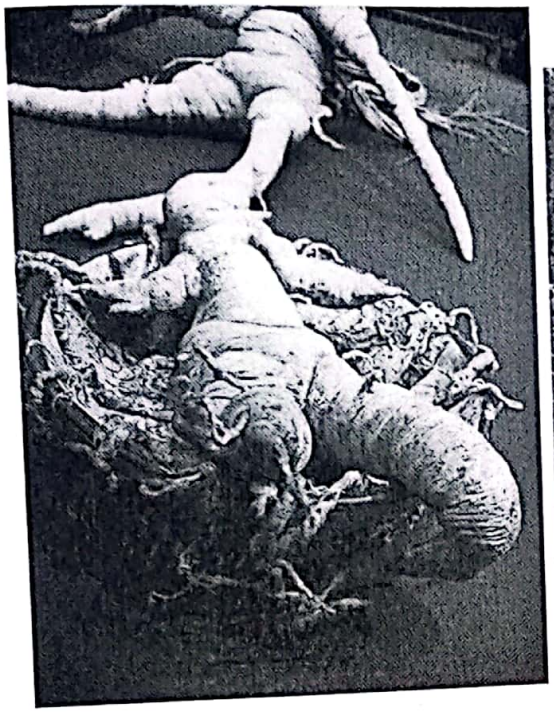
Podophyllotoxin: 2 C_6C_3 [Coniferylalcohol]

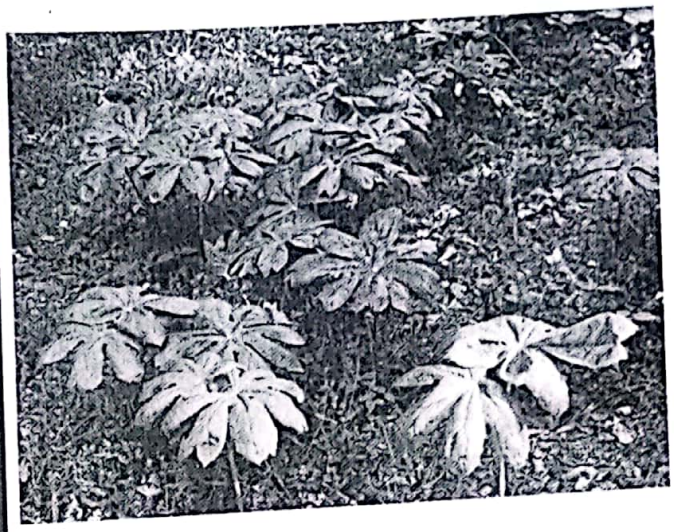
via several intermediates



- Dried root & rhizhome of *Podophyllum peltatum* [May apple, mandrake] (Berberidaceae) USA & Canada
- Structure elucidated in 1930's; planar structure with 4 chiral centers
- **C_2H_5OH extract = Podophyllin** (20% podophyllotoxin, 10% β-peltatin, 5% α-peltatin) ethanol
- **Traditionally as cathartic, purgative, antiviral, warts remedy**
- Trans lactone ring is essential for anti-tumor action; aromatization of ring C↓activity. Too toxic to be used clinically!
- [OH^-] converts into **inactive isomer** (e.g. ***epi***-podophyllatoxin)
- Classified as microtubule inhibitor [inhibits polymerization of tubulin and stop cell division at the beginning of metaphase]

Root & Rhizhome of *Podophyllum peltatum*





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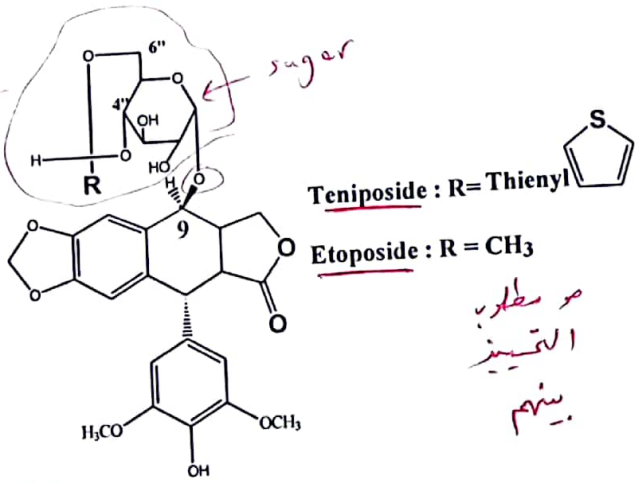
Podophyllotoxin Derivatives

Mitotic Spindle Poisons

- *epi*-podophyllotoxin glycoside is used to prepare 2 clinically useful compounds for malignant diseases
- **Mechanism of action:** inhibit polymerization of tubulin and stop cell division at the beginning of metaphase
- **Tiniposide:** Rx bladder cancer
- **Etoposide:** Rx small cell lung cancer, leukemia & Hodgkin's disease



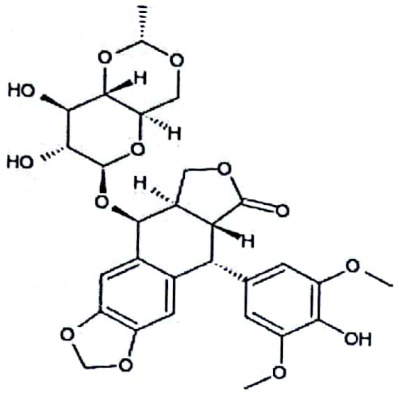
Teniposide : R= Thienyl

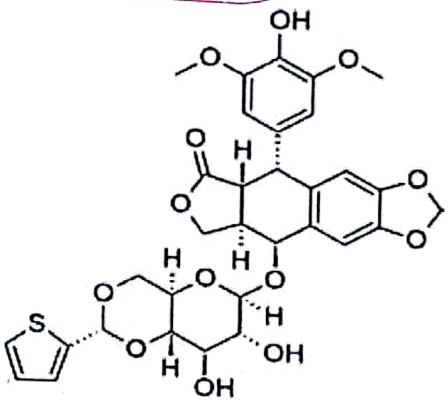
Etoposide : R = CH_3

***epi*-Podophyllotoxin**

Etoposide and teniposide

- As semisynthetic anticancer agents developed from 4'-demethylepipodophyllotoxin
- They act as Topo II inhibitors (prevention of DNA synthesis and replication)
- **Etoposide** : small cell lung cancer, breast cancer, leukemia & Hodgkin's disease,... **Teniposide** : brain and bladder cancer,...
- **Glycosides < active than genins < side effects**





Podophyllum

dophyllum consists of the dried rhizome and roots of *Podophyllum hexandrum* (*P. emodi*) *P. peltatum* (Berberidaceae). *Podophyllum hexandrum* is found in India, China, and Himalayas and yields Indian podophyllum, whilst *P. peltatum* (May apple or American ndrake) comes from North America and is the source of American podophyllum. Plants are ected from the wild. Both plants are large-leafed perennial herbs with edible fruits, though er parts of the plant are toxic. The roots contain cytotoxic lignans and their glucosides, *exandrum* containing about 5%, and *P. peltatum* about 1%. A concentrated form of the ve principles is obtained by pouring an ethanolic extract of the root into water, and drying precipitated podophyllum resin or 'podophyllin'. Indian podophyllum yields about 6–12% esin containing 50–60% lignans, and American podophyllum 2–8% of resin containing 18% lignans.

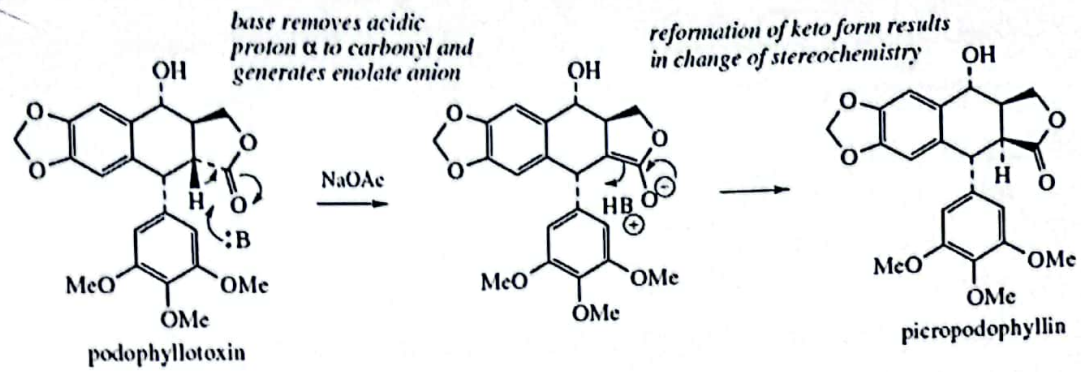


Figure 4.22

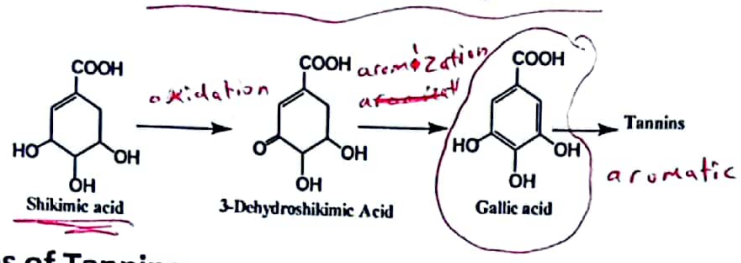
4′-demethylpodophyllotoxin glucoside. Attempted synthesis of the glucoside inverted the stereochemistry at the sugar–aglycone linkage, and these agents are thus derivatives of 4′-demethylepipodophyllotoxin (Figure 4.21). **Etoposide** is a very effective anticancer agent, and is used in the treatment of small cell lung cancer, testicular cancer and lymphomas, usually in combination therapies with other anticancer drugs. It may be given orally or intravenously. The water-soluble pro-drug **etopophos** (etoposide 4′-phosphate) is also available. **Teniposide** has similar anticancer properties, and, though not as widely used as etoposide, has value in paediatric neuroblastoma.

Remarkably, the 4′-demethylepipodophyllotoxin series of lignans do not act via a tubulin-binding mechanism as does podophyllotoxin. Instead, these drugs inhibit the enzyme topoisomerase II, thus preventing DNA synthesis and replication. Topoisomerases are responsible for cleavage and resealing of the DNA strands during the replication process, and are classified as type I or II according to their ability to cleave one or both strands. Camptothecin (see page 365) is an inhibitor of topoisomerase I. Etoposide is believed to inhibit strand-rejoining ability by stabilizing the topoisomerase II–DNA complex in a cleavage state, leading to double-strand breaks and cell death. Development of other topoisomerase inhibitors based on podophyllotoxin-related lignans is an active area of research. Biological activity in this series of compounds is very dependent on the presence of the *trans*-fused five-membered lactone ring, this type of fusion producing a highly-strained system. Ring strain is markedly reduced in the corresponding *cis*-fused system, and the natural compounds are easily and rapidly converted into these *cis*-fused lactones by treatment with very mild bases, via enol tautomers or enolate anions (Figure 4.22). Picropodophyllin is almost devoid of cytotoxic properties.

Podophyllotoxin is also found in significant amounts in the roots of other *Podophyllum* species, and in closely related genera such as *Diphylleia* (Berberidaceae).

Tannins & Gallic acid

- Gallic acid is the building block of many tannins

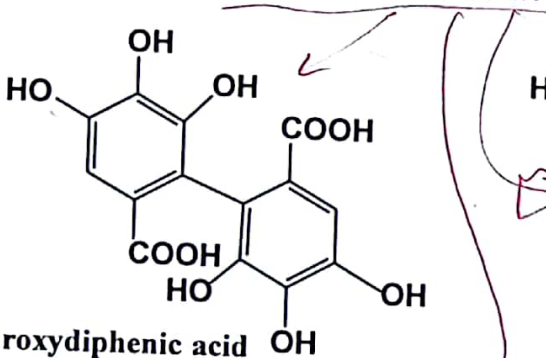


Properties of Tannins:

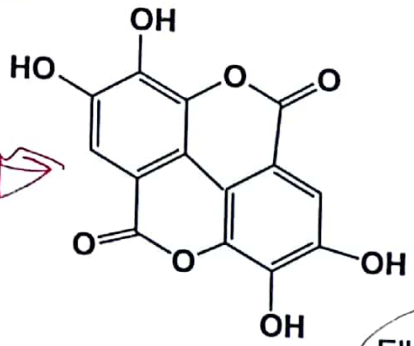
- H_2O solubility !! (colloidal aq. Solution in A` PH)
- Binds proteins to form indigestible complex (leather tanning)
- Amorphous
- Astringent taste (unripe fruits, in all organs or organ-specific)
- Fe^{+2} salts binding producing dark blue or greenish black sol. Complexes (ink)
- Ppt with metals e.g. Cu, Pb (acetate salts to separate tannins from extracts); ppt gelatin and alkaloids;
- Large MW (1000-5000)
- Pseudotannins (MW~500)

Hydrolysable tannins

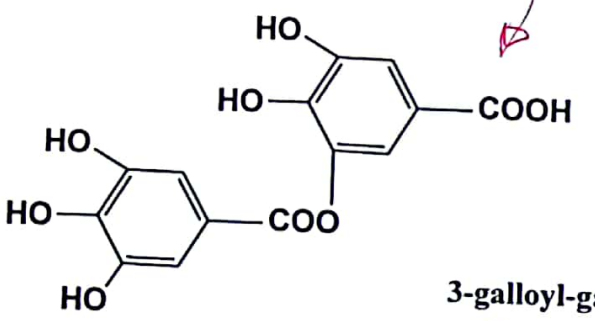
- Possibilities of condensation of the gallic acid



Hexahydroxydiphenic acid



Ellagic Acid



3-galloyl-gallic acid

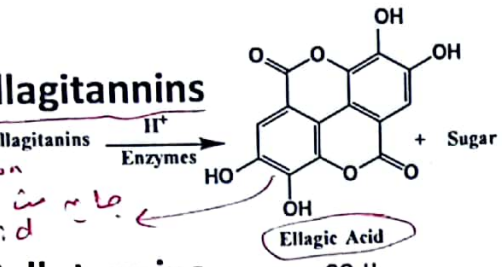
Classification of Tannins

Hydrolysable

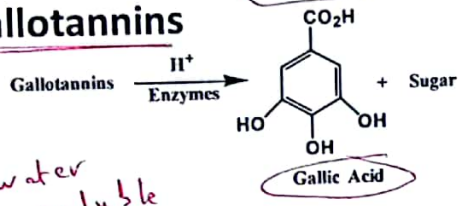
H^+ or enzymes Rx produce simple molecules

Simple, hydrolysable, Galloyl esters of sugars, phenolic moiety is shikimate-derived

A) Ellagitannins

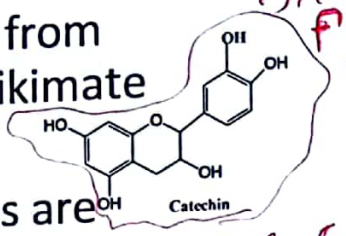


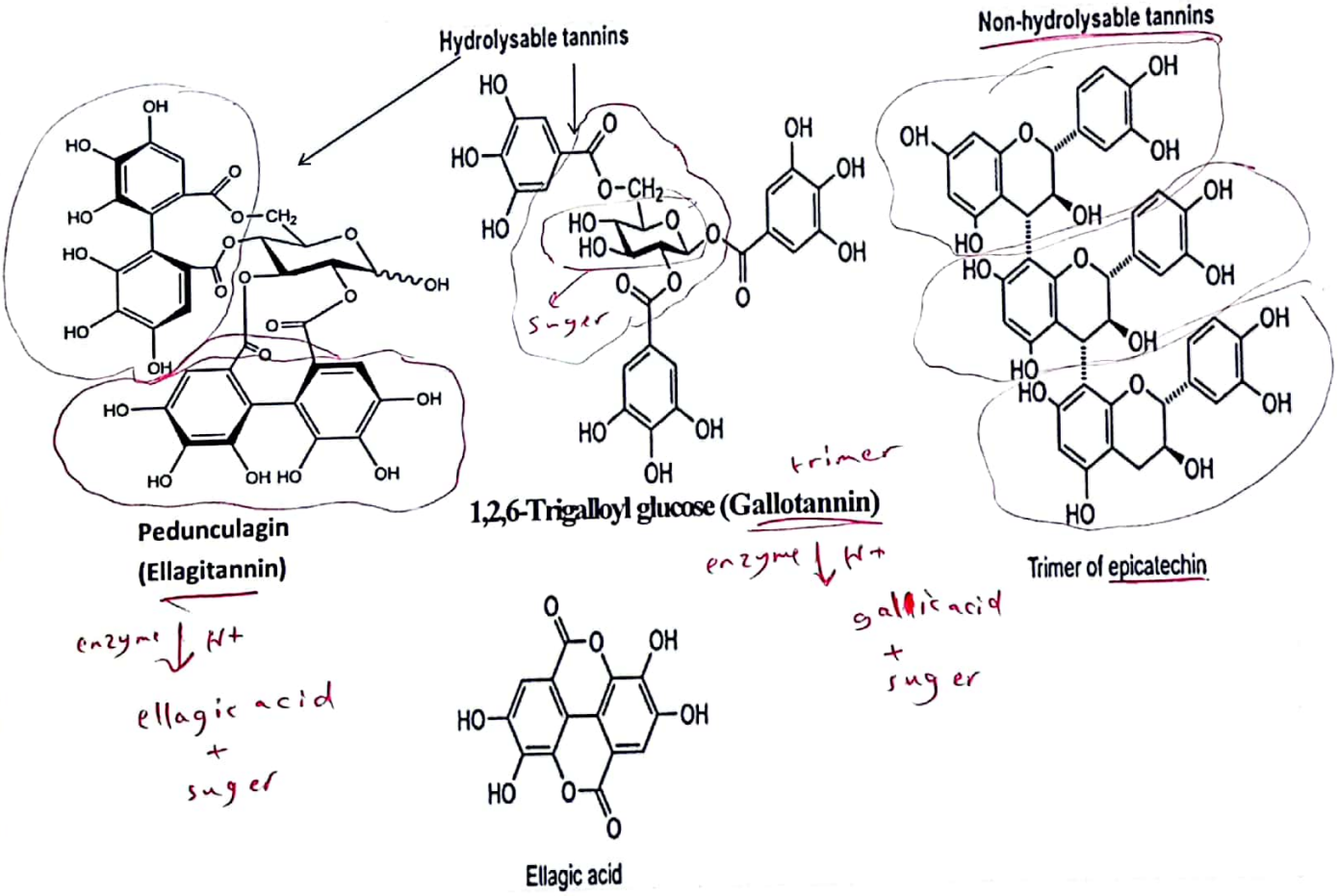
B) Gallotannins



Condensed

- H^+ or Enzymes Rx produce complex insoluble compounds
- Complex polymers
- Biosynthesis from acetate & shikimate pathway
- Building units are catechins + flavonoids $C_6C_3C_6$ estrefied with gallic acid



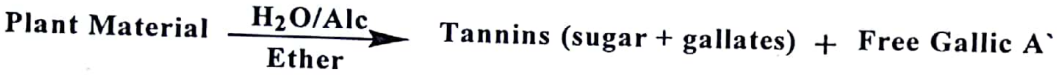


Medicinal Value of Tannins

Limited Application:

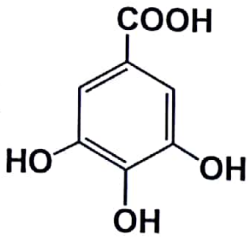
Based on ability to bind proteins to form indigestible complex

1. Diarrhea
2. Bleeding gums
3. Skin injuries preparations



Hamamelis (witch-hazel)

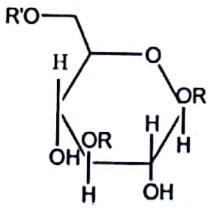
- Dried leaves of *Hamamelis virginiana* L. (Hamamelidaceae)
- Sources: North America & Canada
- Rich in gallotannins → Hydrolysable tannin



Gallic acid

Uses:

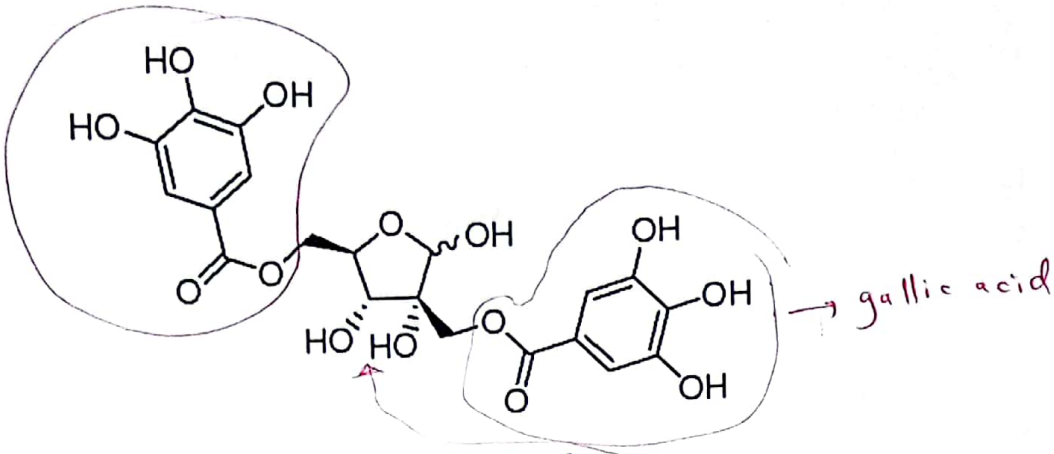
1. Infusions & extracts in hemorrhoids (topical pharmaceuticals)
2. Topically skin inflammation
3. Face lotions as astringent



R= GALLIC ACID

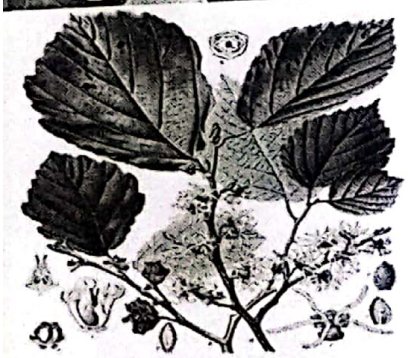
R' = TRIGALLIC ACID

Hamamelis virginiana (Witch Hazel) Tannins



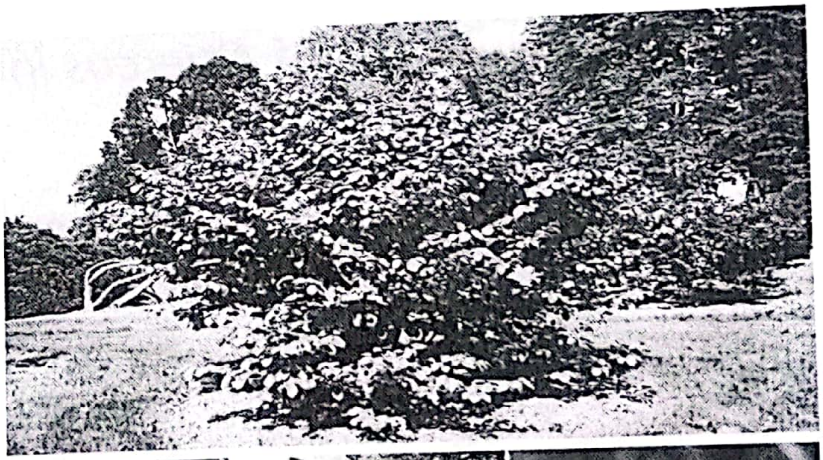
Hamamelitannin

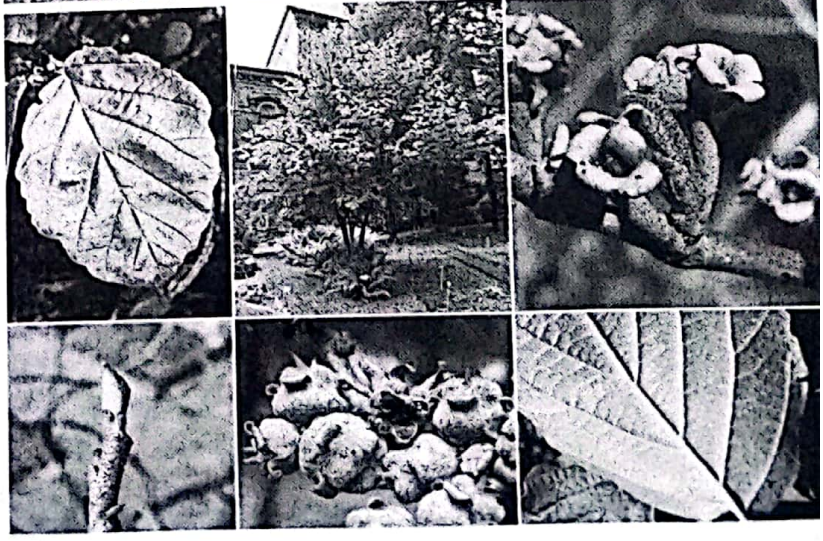
2-C-(hydroxymethyl)-D-ribofuranose 2',5-digallate



Hamamelis virginiana

(witch-hazel)



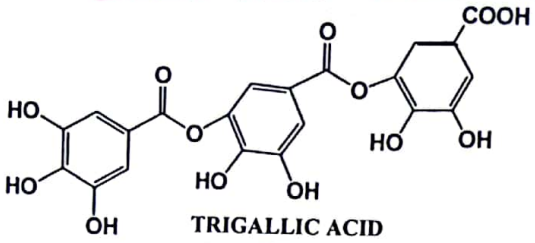


Galls (Nutgalls)

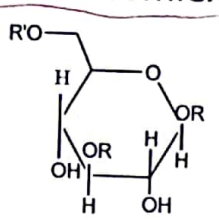
- Vegetable growths as a result of insect infections of the leaves & twigs of *Quercus infetoria* (Fagaceae), Oak (طويلم)
- Contains 70-80% tannins (Sources: Turkey, Syria, Greece & Iran)

Uses: الاستعمالات التقليدية

1. Rx of catarrh & infection
2. Stop bleeding locally
3. Alkaloid poisoning remedy (insoluble comlex)
4. Ink preparation
5. Textiles dyeing esp. leather



TRIGALLIC ACID



R= GALLIC ACID

R' = TRIGALLIC ACID

Galls (Nutgalls) of *Quercus infectoria* (Oak)

البلوط

- Caused by insects