اسم المقرر: علم الكيمياء النباتية
رمز المقرر: 473نبت

Phytochemistry 473 BOT

1

تعريف بعضو هيئة التدريس ومعلومات التواصل

اسم أستاذ المقرر للجزء النظري: د. فيصل الحربي
الكلية : كلية العلوم
القسم : النبات والأحياء الدقيقة
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خطة المقرر

وصف عام للمقرر:

مقدمة عن كيمياء النبات و أهميتها - المكونات الكيميائية للخلايا والأنسجة النباتية واستخداماتها – الصبغات – الأصماغ – القلويدات – اللبن النباتي - البروتينات – الكربوهيدرات – الدهون و الزيوت الطيارة والعطرية – استخلاص وفصل المركبات الكيميائية المختلفة في النبات.

قائمة الموضوعات
مقدمة عن كيمياء النبات و أهميتها
المكونات الكيميائية للخلايا والأنسجة النباتية واستخداماتها
الصبغات النباتية والاصماغ النباتية و اللبن النباتي
والكربوهيدرات والتعرف عليها وفائدتها في النبات
البروتينات والتعرف عليها وفائدتها في النبات
الدهون و الزيوت الطيارة والعطرية
استخلاص وفصل المركبات الكيميائية المختلفة في النبات.

3

قائمة - الكتب و المراجع :

Paul Haas (2010) an introduction to the chemistry of plant products. Nabu Press

Andrew Pengelly (2004) The constituents of medicinal plants : an introduction to the chemistry and therapeutics of herbal medicine [2nd ed]. Allen & Unwin

4

سياسة التقييم وتوزيع الدرجات

بناء على قرار مجلس الكلية:

يتم توزيع درجة المقرر الكلية (100 درجة) كالاتي:

1. درجة الأعمال الفصلية (60 درجة)

أ. الجزء النظري من المقرر (30 درجة) ويتم تدريسه من قبل د. فيصل الحربي.

ب. الجزء العملي من المقرر (30 درجة) ويتم تدريسه من قبل الأستاذ المسؤول عن العملي.

2. درجة الاختبار النهائي (40 درجة)

مواعيد الاختبارات الشهرية والنهائية:

1. الاختبار الفصلي للجزء النظري سوف يتم عقده في الفترة ما بين الأسبوع السابع الى التاسع من بداية الفصل الدراسي، وسوف يتم إعلام الطلاب بالموعد المحدد والمؤكد قبل الاختبار بأسبوع كامل.
2. الاختبار النهائي محدد موعده في البوابة الاكاديمية للطالب.

5

Introduction

Phytochemistry is the study of **phytochemicals**, which are chemicals derived from plants.

Phyto = Plants

chemistry

There are about 320,000 species of plants

Phytochemicals are chemical compounds produced by plants. (chemicals of plant origin)

- chemicals produced by plants through primary or secondary metabolism
- They generally have biological activity (Bioactive compound) in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators
- In total, there has been over 25,000 phytochemicals discovered
- Phytochemists study phytochemicals by first extracting and isolating compounds from the origin plant, followed by defining their structure or testing in laboratory model systems, such as cell cultures, in vitro experiments, or in vivo studies using laboratory animals.

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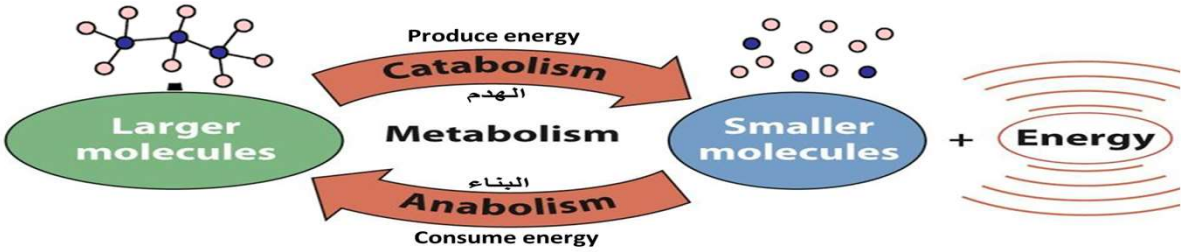

- Based on the biological requirements in plants, the naturally occurring phytochemicals can be broadly classified into primary metabolites and secondary metabolites

- Plant metabolism
  - Primary
    - directly involved in normal growth, development, and reproduction of plant species
  - Secondary
    - not directly involved in those processes, but usually has important ecological functions like defences against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes
    - Eg. coloring agents, attractive smells, etc

  Both: famous for their beneficial effects on human health

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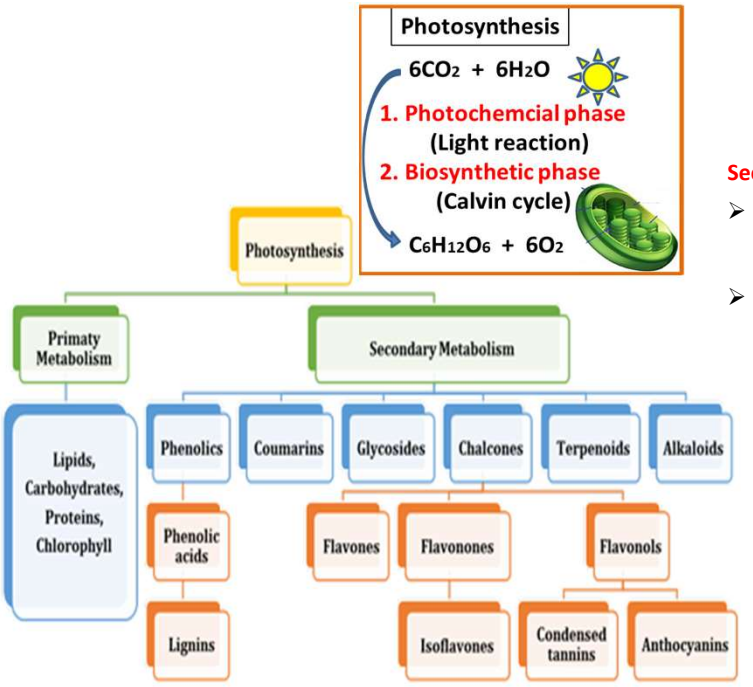
Primary

- Includes:
1. Lipids.
2. Proteins.
3. Carbohydrates.
4. Chlorophyll.

- directly involved in normal growth, development, and reproduction of plant species.



Secondary

- Includes: the rest of phytochemicals.
- not directly involved in those processes, but usually has important ecological functions like defenses against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes

8

So, from the plant point views (perspectives)

وعليه، فإن فوائد هذه المواد الكيميائية النباتية بالنسبة للنبات هي كالاتي

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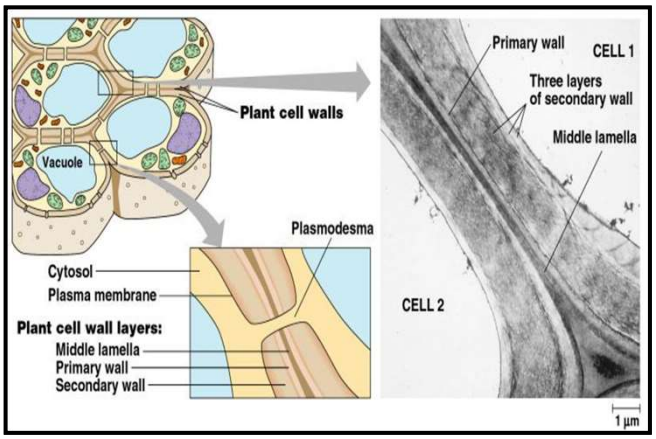
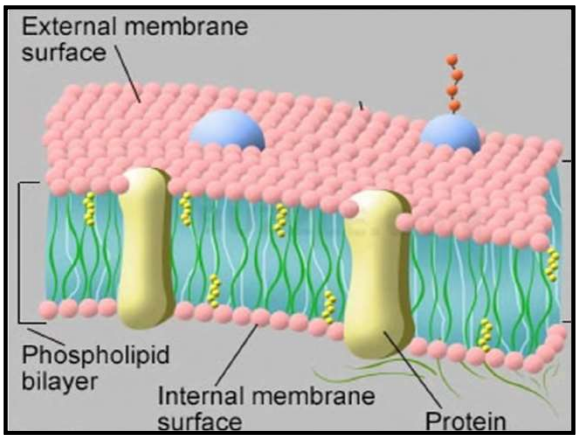
Primary metabolites are directly involved in normal growth & development





- Cellulose & Hemicellulose (carbohydrates)
- Proteins

} Cell wall

- Phospholipid
- Proteins

} Plasma membrane

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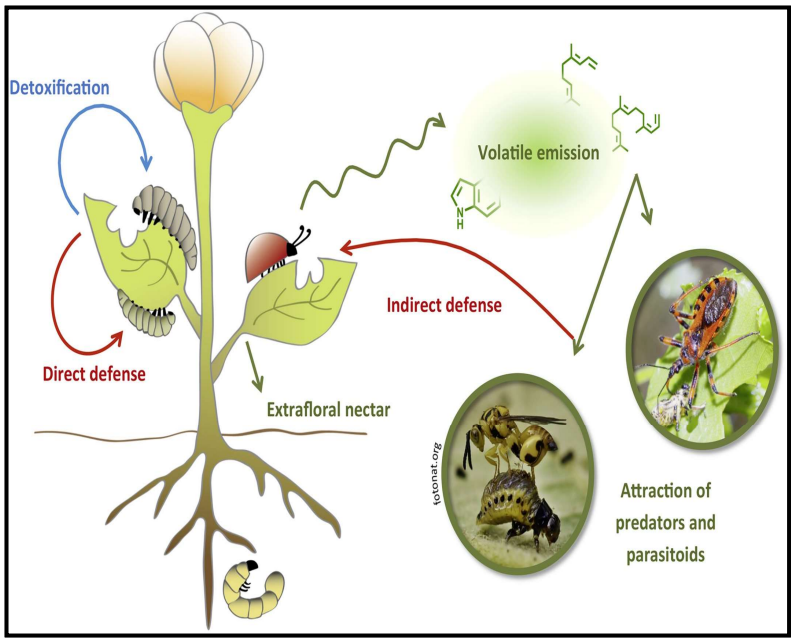
Examples of ecological functions like defenses against predators, parasites



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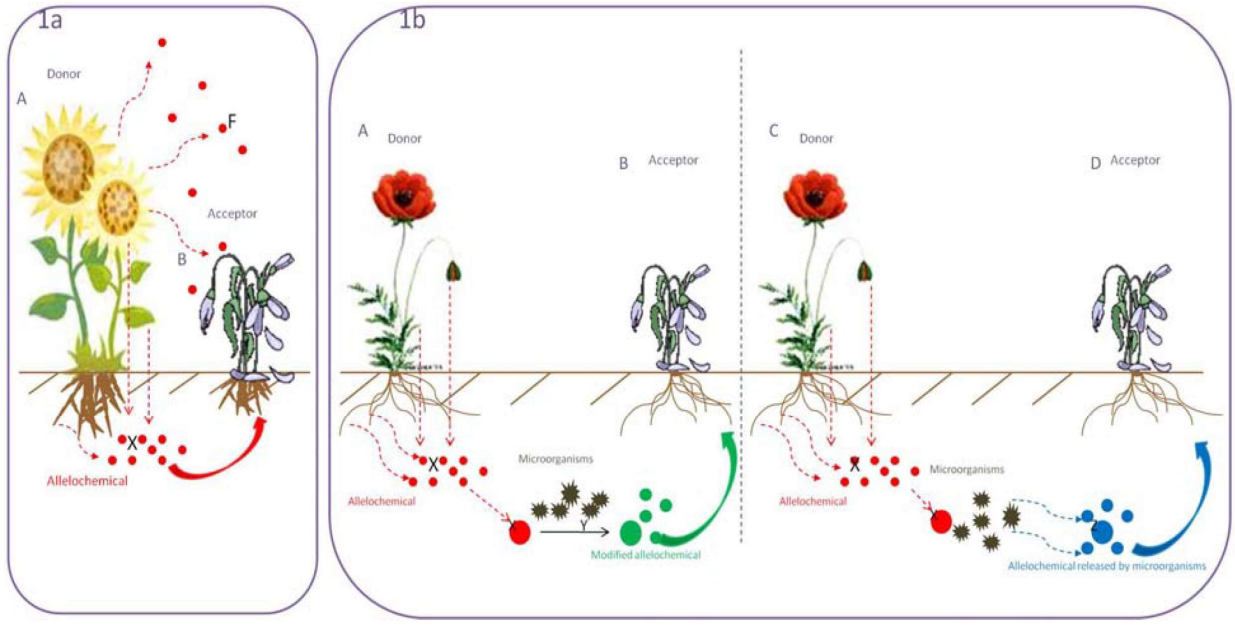
Examples of ecological functions like interspecies competition (allelopathy)



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Examples of facilitating the reproductive processes

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The interactions between humans and phytochemicals

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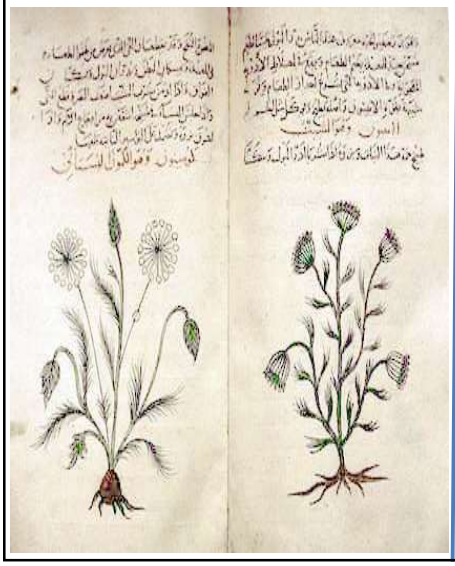
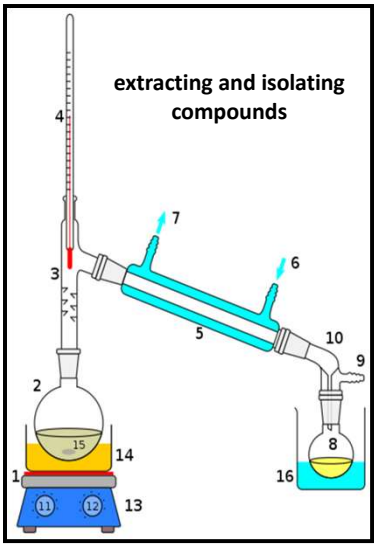
- plants have provided the necessities of life such as food, shelter, clothing, and medicine to humanity from the very beginning of human civilization



- Today, phytochemists study phytochemicals by first extracting and isolating compounds from the origin plant, followed by defining their structure or testing in laboratory model systems, such as cell cultures, in vitro experiments, or in vivo studies using laboratory animals.



cell cultures, in vitro experiments

in vivo studies using laboratory animals

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In last lecture في المحاضرة السابقة تحدثنا عن

- Phytochemicals → Phyto = Plants
- Over 25,000 phytochemicals discovered
- Bioactive compound (have biological activities: growth, defense, attraction, competition)
- Based on the **biological requirements in plants** →

1) Phytochemical primary metabolites (Lipids, Proteins, Carbohydrates, & Chlorophyll).
2) Phytochemical secondary metabolites (the rest of phytochemicals).

- Based on: **Biosynthetic origin, Solubility properties, Presence of certain key functional groups** → Phytochemicals classes (phytochemists Classification)
- **Phytochemical Secondary metabolites** do not fall into the category of essential nutrients.
  - They aren't absolutely needed by the body – in other words, they are not *nutritionally essential* ,But some have beneficial effects on human health.
  - Not vitamin.

1

- Based on the biological requirements in plants: phytochemical primary metabolites (Lipids, Proteins, Carbohydrates, & Chlorophyll) / phytochemical secondary metabolites (the rest of phytochemicals).

phytochemical primary metabolites

- directly involved in normal growth, development, and reproduction of plant species

phytochemical secondary metabolites

- not directly involved in those processes, but usually has important ecological functions like
  1) defenses against predators, parasites and diseases.
  2) interspecies competition.
  3) to facilitate the reproductive processes. Eg. coloring agents, attractive smells, etc

- Based on:
  1) Biosynthetic origin المسار الحيوي التصنيعي
  2) Solubility properties خصائص الذوبان
  3) Presence of certain key functional groups وجود مجموعات كيميائية معينة

Phytochemicals classes (phytochemists Classification)

Phytochemical classes

- Phenolic compounds — المركبات الفينولية
- Terpenoids compounds — المركبات التيربنويدز
- Organic acids, lipid and related compounds — الأحماض العضوية، الدهون، واشباهها
- Nitrogen compounds — المركبات النيتروجينية
- Sugar and their derivatives — السكر و مشتقاته
- Macromolecules — الجزيئات الكبرى

2

Biosynthetic origin ? **ماذا نقصد بالمسار الحيوي التصنيعي**

Let's review two basic processes in plants

Photosynthesis

$6CO_2 + 6H_2O$

1. Photochemcial phase (Light reaction)
2. Biosynthetic phase (Calvin cycle)

$C_6H_{12}O_6 + 6O_2$

Photosynthesis, which builds sugars out of smaller molecules, is an anabolic pathway.

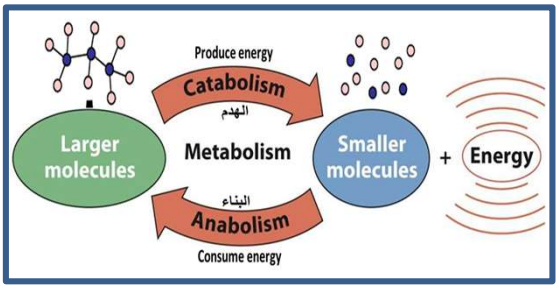
Cellular respiration, which breaks sugar down into smaller molecules, is a catabolic pathway.

Respiration

$C_6H_{12}O_6 + 6O_2$

1. Glycolysis
2. Krebs cycle (TCA) cycle

$6CO_2 + 6H_2O + ATP(Energy)$



The energy released from Catabolic reactions generally has been stored in high-energy bonds of an intermediate energy carrier molecule such as ATP

(**ATP**) —phosphate bond is split releasing Energy→ (**ADP**)

adenosine triphosphate — adenosine diphosphate

This released energy is needed & used for reactions التفاعلات

3

The reaction of photosynthesis

carbon dioxide + water —energy from light, chlorophyll in leaves→ glucose + oxygen

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$$

The 6-carbon sugar glucose, a product of photosynthesis, is

- mostly translocated in the form of sucrose (a 12-carbon sugar) to nourish non photosynthesizing parts of the plant,
- or it may be polymerized into starch for storage.

Notice:

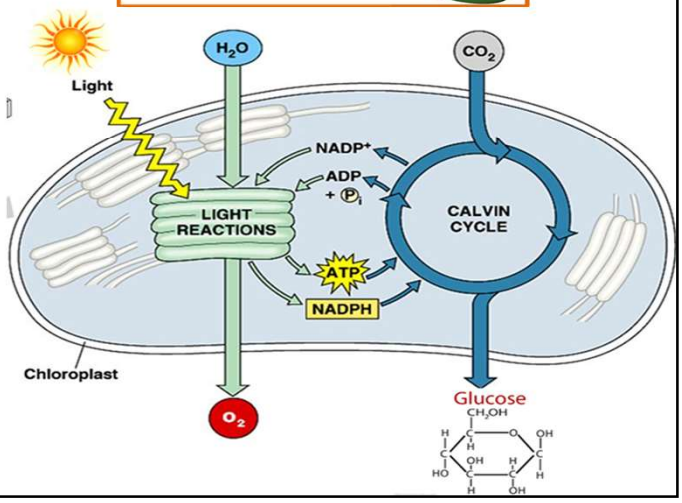
- (ATP) breaks down into (ADP) in Calvin Cycle, and this released energy helps in fixing CO_2 into glucose.
- In (light reaction), the water molecular breakdown into Hydrogen and Oxygen, & the released energy helps in the conversion of (ADP) into (ATP)

Photosynthesis

$6CO_2 + 6H_2O$

1. Photochemcial phase (Light reaction)
2. Biosynthetic phase (Calvin cycle)

$C_6H_{12}O_6 + 6O_2$



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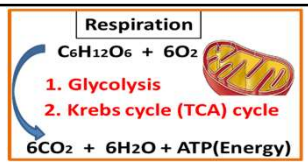
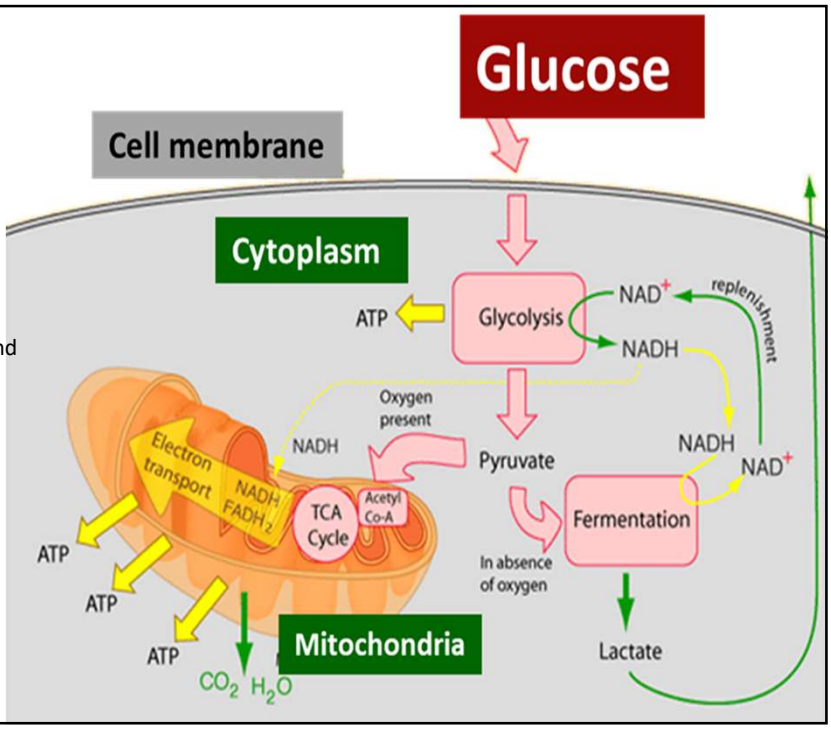


Glycolysis, or glycolytic pathway

- sequence of 10 chemical reactions that breaks down glucose.
- One molecule of glucose makes two molecules of pyruvate (or pyruvic acid) and two molecules of ATP.
- Thus, glycolysis produces both

1) ATP for cellular energy requirements.
2) building blocks for synthesis of other cellular products.

- The pyruvate enters the tricarboxylic acid cycle (TCA cycle) if enough oxygen is present or is fermented into lactic acid or ethanol if not.



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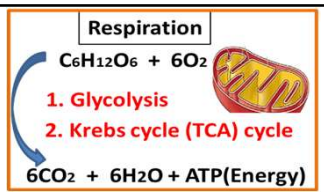
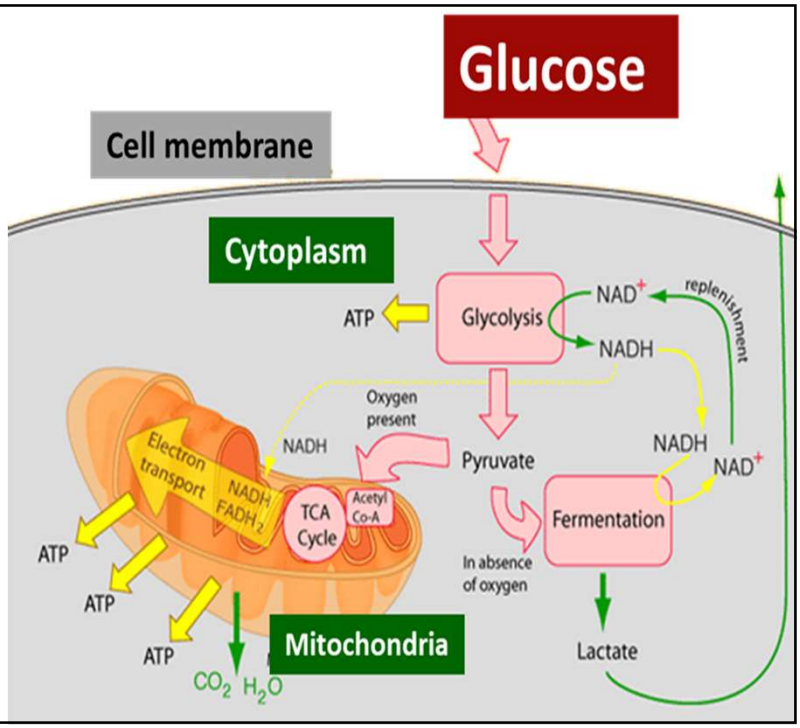


Tricarboxylic acid cycle, (TCA cycle), also called Krebs cycle and citric acid cycle,

- Before the pyruvate enter the TCA cycle, they must be degraded into a two-carbon compound called acetyl coenzyme A (acetyl CoA).
- Then acetyl CoA is converted into carbon dioxide and energy (ATP) in the mitochondria.



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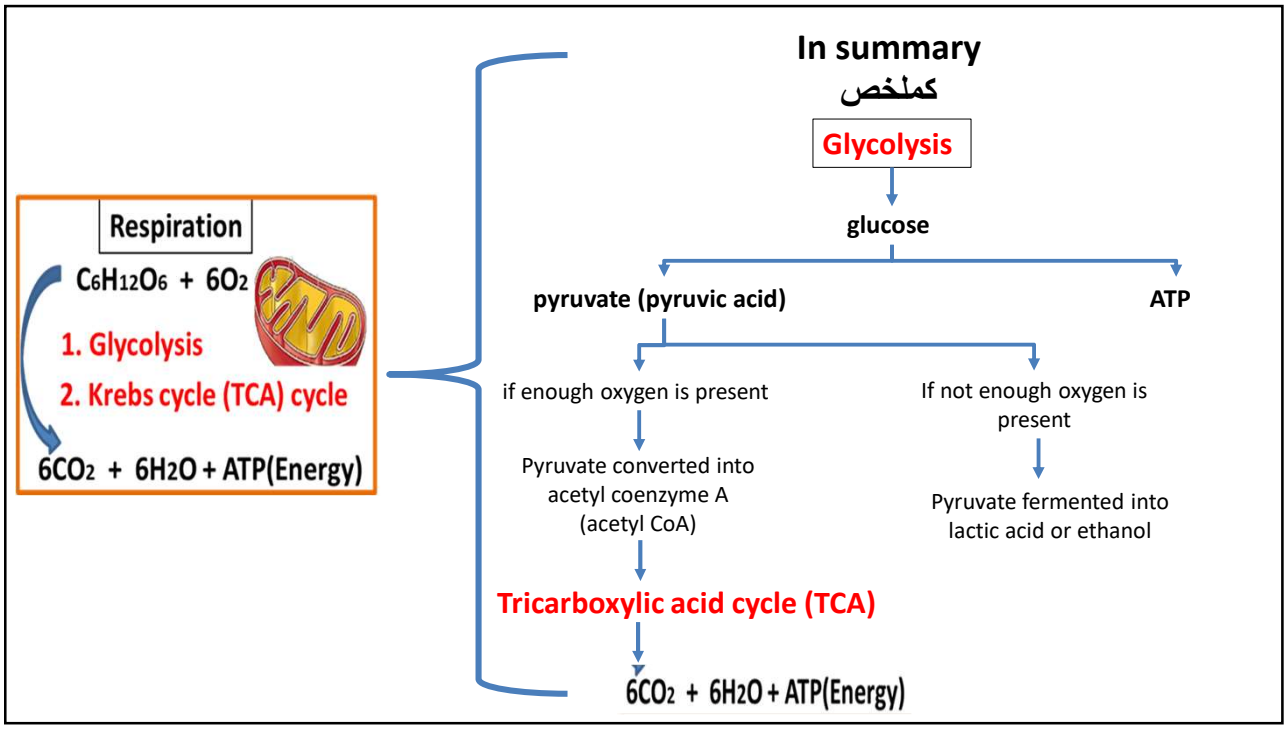

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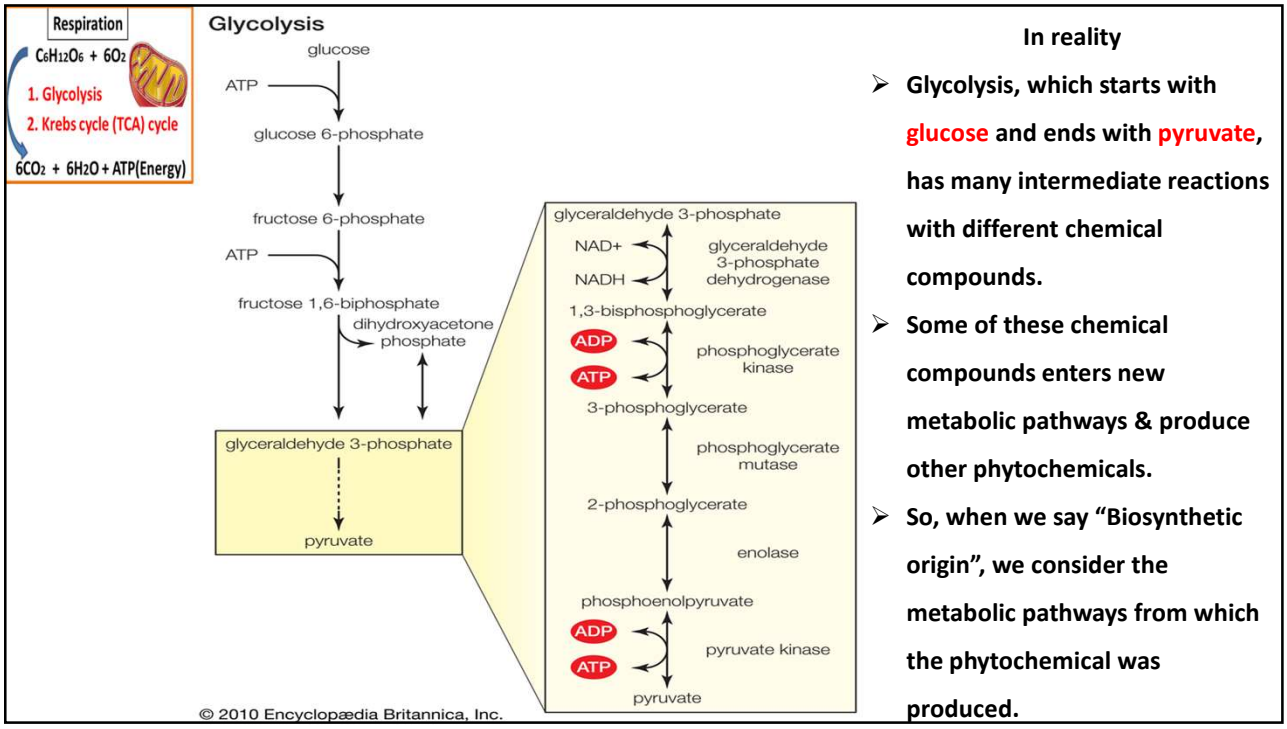

In reality

- **Glycolysis, which starts with glucose and ends with pyruvate, has many intermediate reactions with different chemical compounds.**
- **Some of these chemical compounds enters new metabolic pathways & produce other phytochemicals.**
- **So, when we say "Biosynthetic origin", we consider the metabolic pathways from which the phytochemical was produced.**

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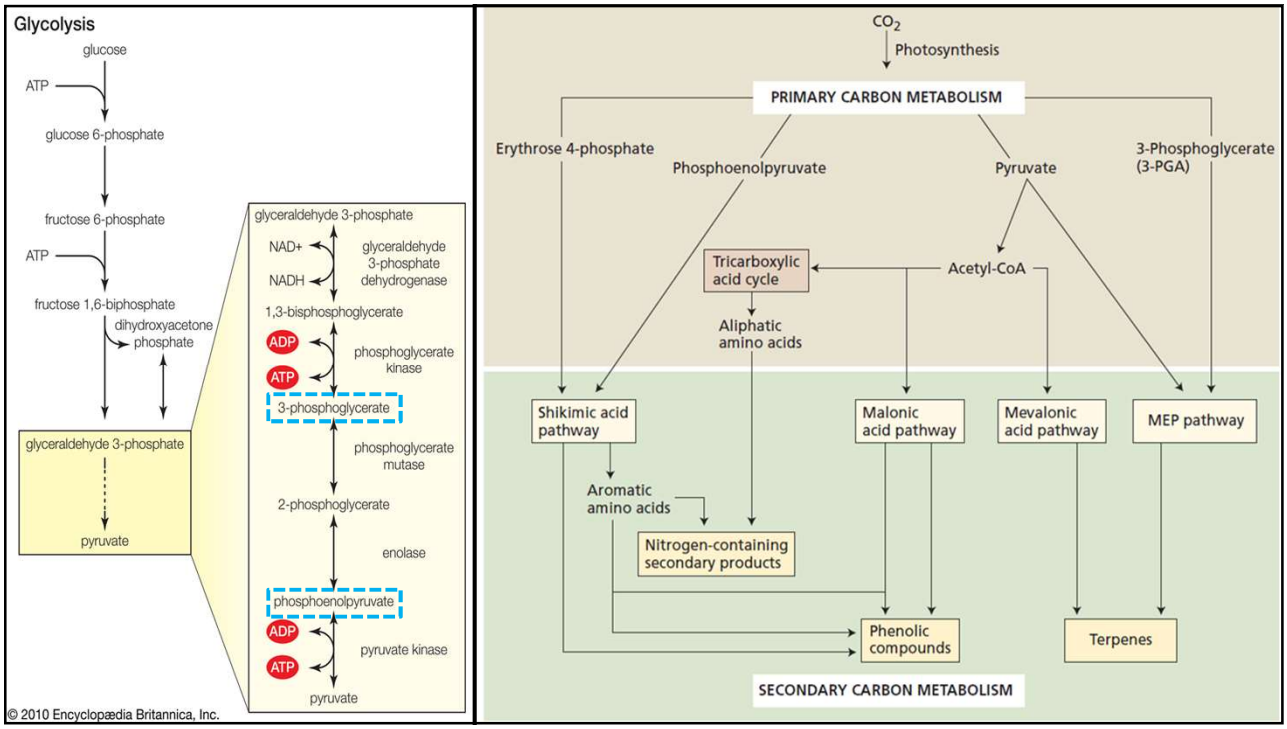
Glycolysis
glucose
ATP
glucose 6-phosphate
fructose 6-phosphate
ATP
fructose 1,6-biphosphate
dihydroxyacetone phosphate
glyceraldehyde 3-phosphate
pyruvate
glyceraldehyde 3-phosphate
NAD+
NADH
glyceraldehyde 3-phosphate dehydrogenase
1,3-bisphosphoglycerate
ADP
ATP
phosphoglycerate kinase
3-phosphoglycerate
phosphoglycerate mutase
2-phosphoglycerate
enolase
phosphoenolpyruvate
ADP
ATP
pyruvate kinase
pyruvate
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CO2
Photosynthesis
PRIMARY CARBON METABOLISM
Erythrose 4-phosphate
Phosphoenolpyruvate
Pyruvate
3-Phosphoglycerate (3-PGA)
Tricarboxylic acid cycle
Acetyl-CoA
Aliphatic amino acids
Shikimic acid pathway
Malonic acid pathway
Mevalonic acid pathway
MEP pathway
Aromatic amino acids
Nitrogen-containing secondary products
Phenolic compounds
Terpenes
SECONDARY CARBON METABOLISM

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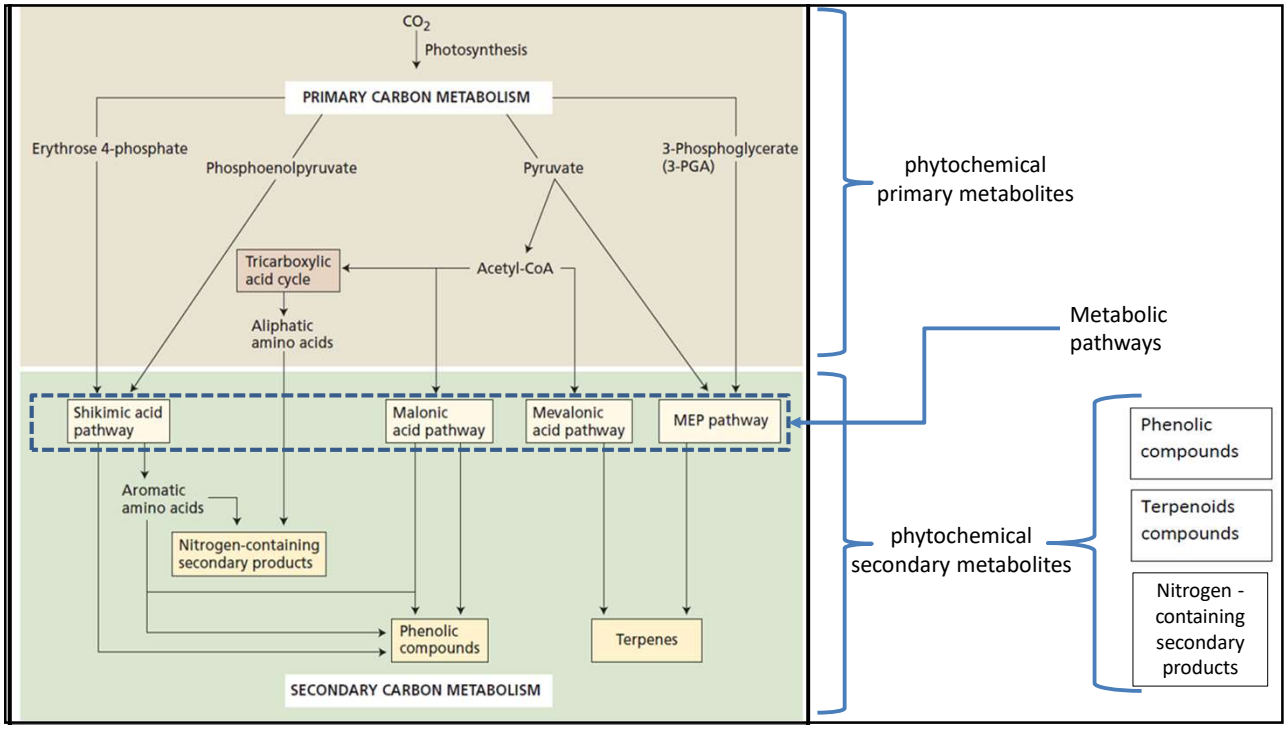
CO2
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Erythrose 4-phosphate
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SECONDARY CARBON METABOLISM
phytochemical primary metabolites
Metabolic pathways
phytochemical secondary metabolites
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Terpenoids compounds
Nitrogen - containing secondary products

10

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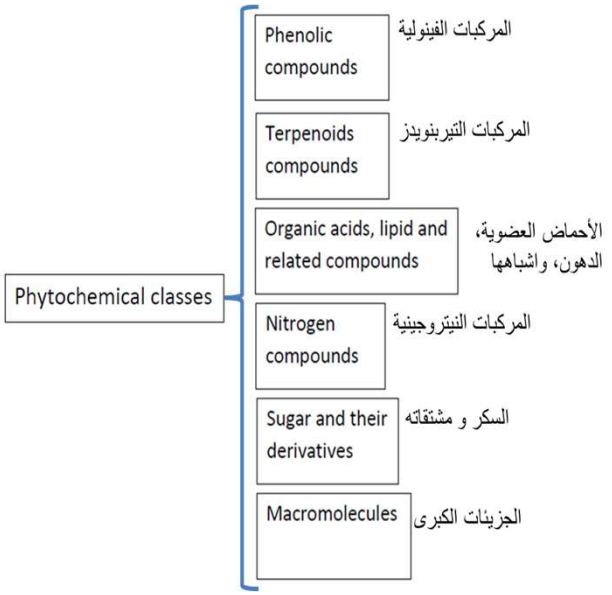
- Based on: **Biosynthetic origin, Solubility properties, Presence of certain key functional groups** → Phytochemicals classes (phytochemists Classification)



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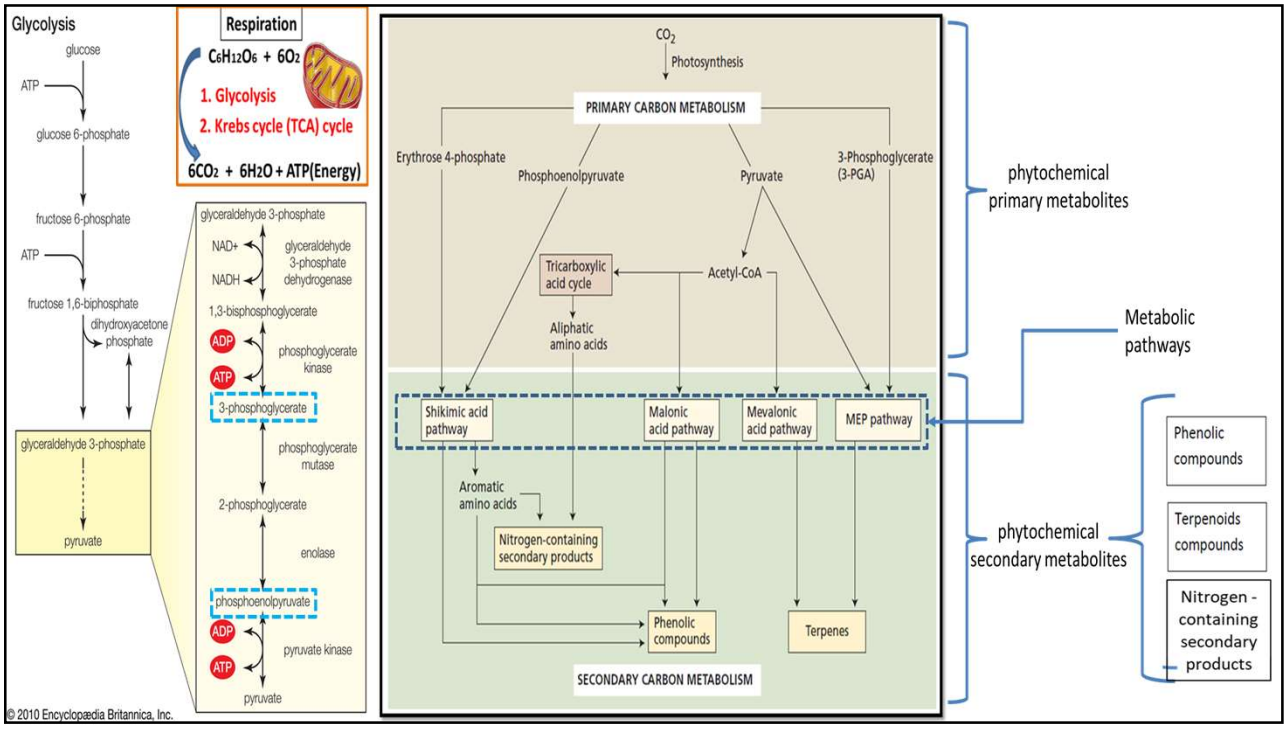

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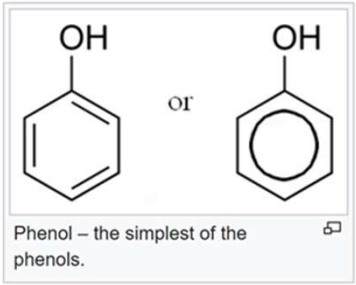
Phenolic compounds or phenols

1) A class of chemical compounds consisting of one or more hydroxyl groups (—OH) bonded directly to an aromatic hydrocarbon group.



Phenol – the simplest of the phenols.

2) They are Aromatic compounds meaning that they contain one or more rings with pi electrons delocalized all the way around them.

3) Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule.

4) The simplest is phenol, C_6H_5OH.

5) Phenolic substances tend to be water-soluble, since they are usually located in the vacuole.

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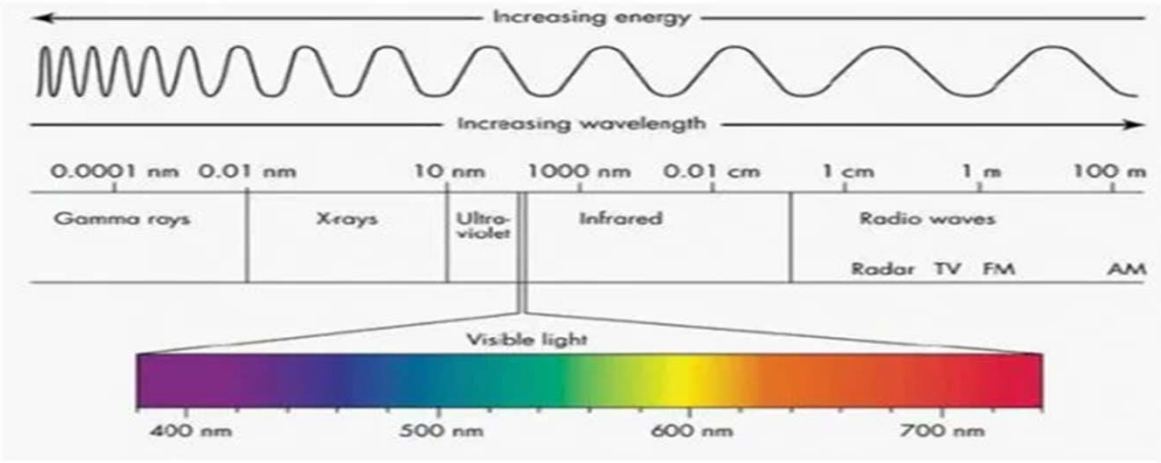
Phenolic compounds or phenols

6) Because phenols are aromatic compounds, they all show intense absorption in the UV region of the spectrum.



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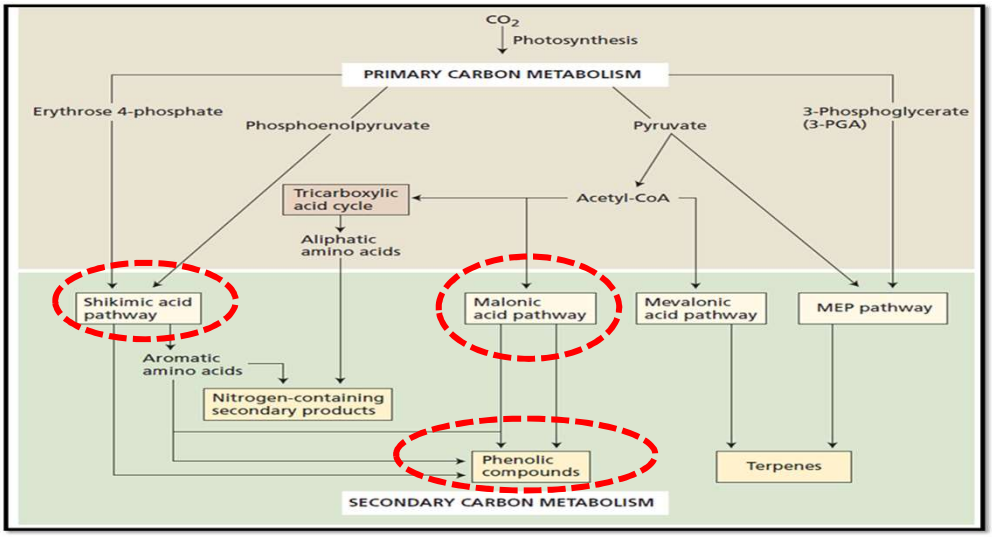
Phenolic compounds or phenols

7) Phenolic compounds formed by the shikimic acid pathway or the malonic acid pathway.



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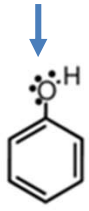
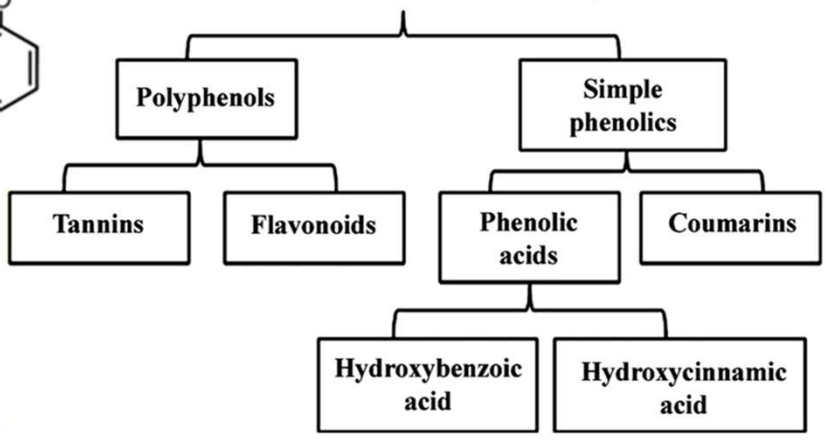


Classification of Phenolic Compounds



- The flavonoids form the largest group.

6

Phenolics (or phenols) functions:

- They produced by plants as defense compounds against herbivores and pathogens.
- Others functions of the phenolic compounds are:
  1. providing mechanical support.
  2. attracting pollinators and fruit dispersers.
  3. absorbing harmful ultraviolet radiation.
  4. reducing the growth of nearby competing plants.

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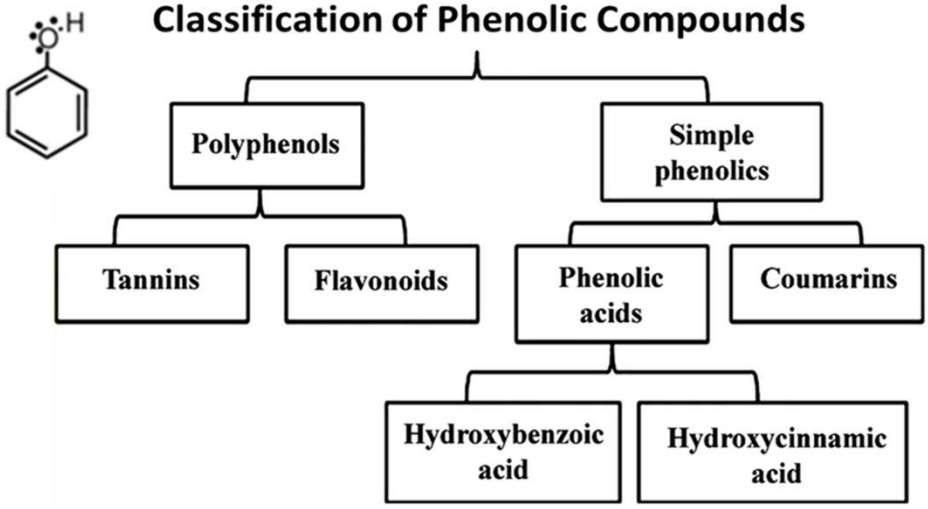

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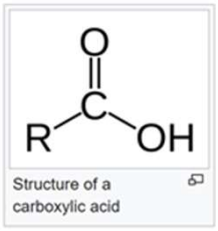
Phenolic acids or phenol carboxylic acids or aromatic acids

- They are types of aromatic acid compound, containing a phenolic ring and an organic carboxylic acid functional group (C(=O)OH), and their basic skeleton based on the number of carbon atoms is (C6-C1 skeleton).



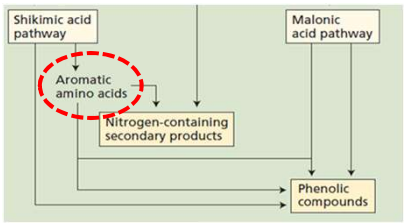
Structure of a carboxylic acid

(C(=O)OH)

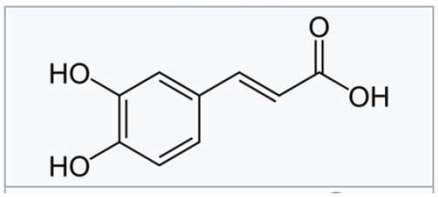
- Two important naturally occurring types of phenolic acids are:

1) Hydroxy-benzoic acids. Example, salicylic acid.
2) Hydroxy-cinnamic acids. They are a class of aromatic acids or phenylpropanoids having a C6–C3 skeleton. Example, Caffeic acid.

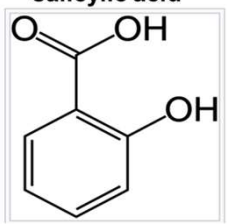
- Phenylpropanoids have an aromatic ring to which a three-carbon side-chain is attached. Derived biosynthetically from the aromatic protein amino acid phenylalanine and they may contain one or more C6-C3 residue.



Caffeic acid



salicylic acid



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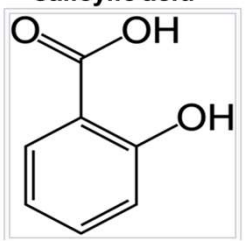
salicylic acid functions as a plant hormone.

salicylic acid



Salicylic acid is involved in

1) It plays a role in the resistance to pathogens (i.e. systemic acquired resistance by inducing the production of pathogenesis-related proteins and other defensive metabolites.

1) Methyl salicylate -- can also diffuse through the air, facilitating plant-plant communication. Methyl salicylate is taken up by the stomata of the nearby plant, where it can induce an immune response after being converted back to salicylic acid.

10

salicylic acid

Salicylic acid الصفصاف الأبيض احد مصادر

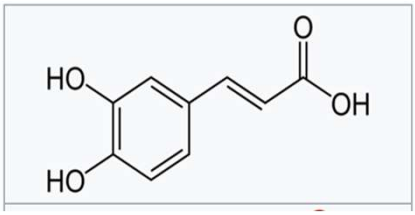
White willow (Salix alba)

White willow (*Salix alba*) is a natural source of salicylic acid.

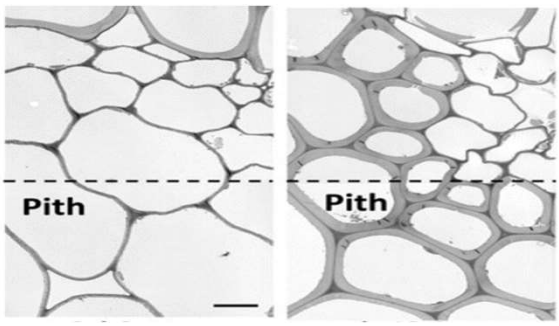
11

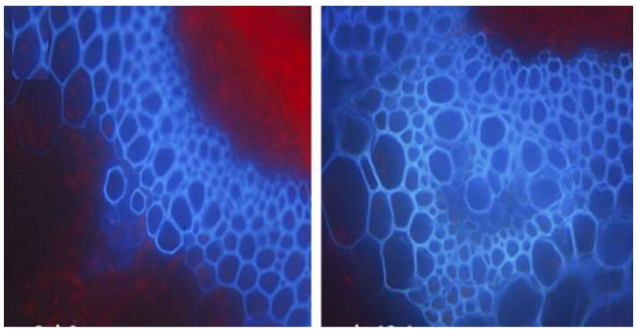
Caffeic acid and mechanisms of stress tolerance

Caffeic acid



- Caffeic acid is actively involved in plant physiology and mechanisms of stress tolerance primarily utilized by plants for the synthesis of lignin which ultimately thickened cell walls and plant become resistant to ion toxicity sodium and heavy metal stress.





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Caffeic acid is found at a high level in some herbs, especially thyme, sage and spearmint.

thyme

sage

spearmint

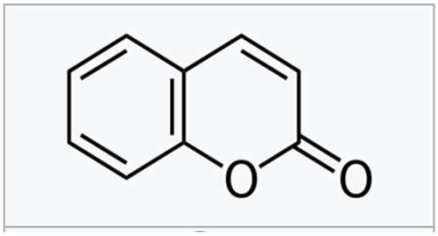
13

Coumarin

1) It is an aromatic organic chemical compound with formula $C_9H_6O_2$.

1) Its molecule can be described as a benzene molecule with two adjacent hydrogen atoms replaced by a lactone-like chain −(CH)=(CH)−(C=O)−O−

$C_9H_6O_2$
Coumarin



14

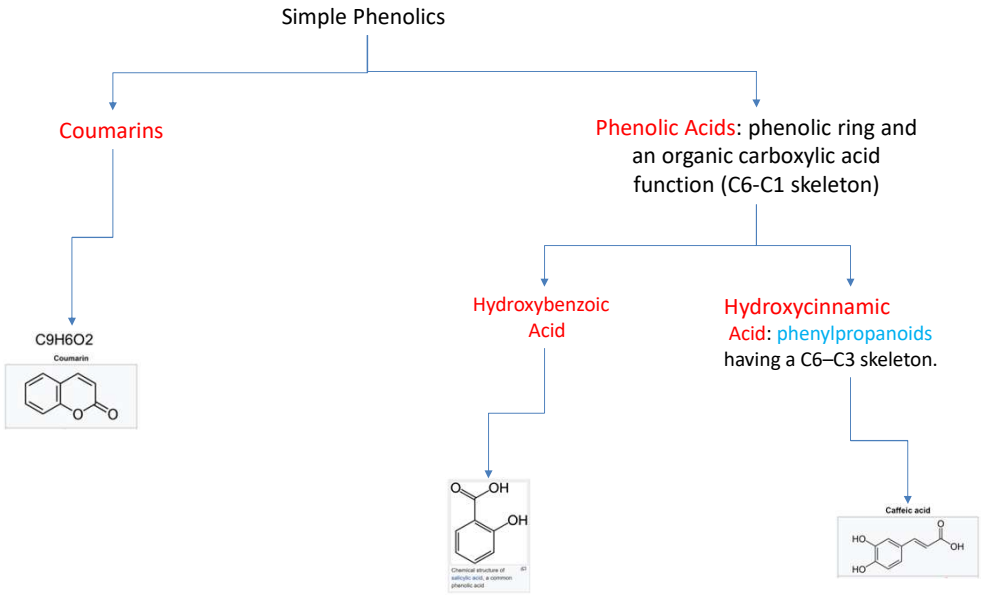
Coumarin is found naturally in many plants, it occurs in vanilla grass (Anthoxanthum odoratum) and sweet grass (Hierochloe odorata).

- It is found in many plants, where it may serve as a chemical defense against predators.
- Coumarin has appetite-suppressing properties, which may discourage animals from eating plants that contain it.

vanilla grass

sweet grass

15



16

In last lecture في المحاضرة السابقة تحدثنا عن

- **Phenolic compounds or phenols**

1) One or more hydroxyl groups (—OH) bonded directly to an aromatic hydrocarbon group.
2) General properties & Biosynthetic origin & Functions in plants & Classification

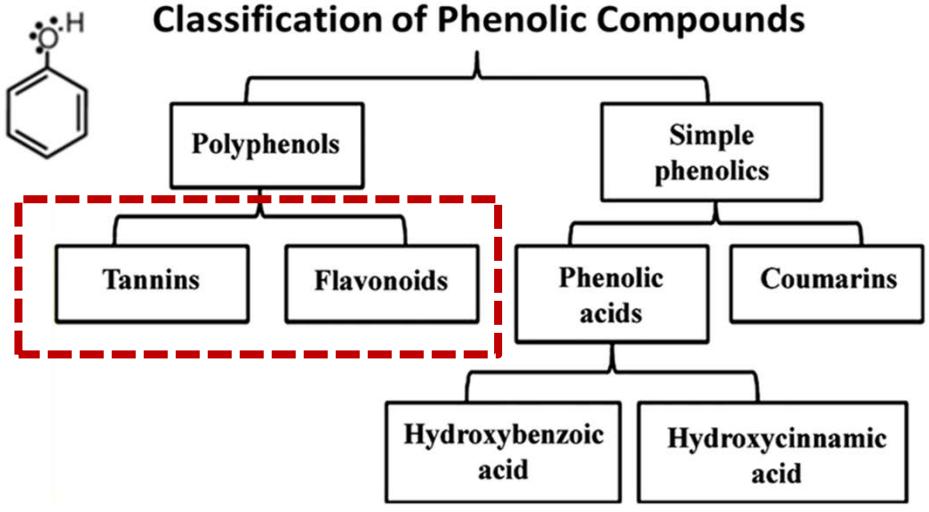
- **Phenolic acids or phenol carboxylic acids or aromatic acids**

1) Basic carbon skeleton (C6-C1 skeleton), & functional group→carboxylic acid (C(=O)OH)
2) Two types of phenolic acids:
   I. Hydroxy-benzoic acids→(e.g. Salicylic acid)
   II. Hydroxy-cinnamic acids(phenylpropanoids) having a (C6–C3 skeleton)→ (e.g.Caffeic acid)
3) Functions in plants & some examples of plant species.

- **Coumarin**

1) An aromatic organic chemical compound with formula $C_9H_6O_2$.
2) Functions in plants & some examples of plant species.

1



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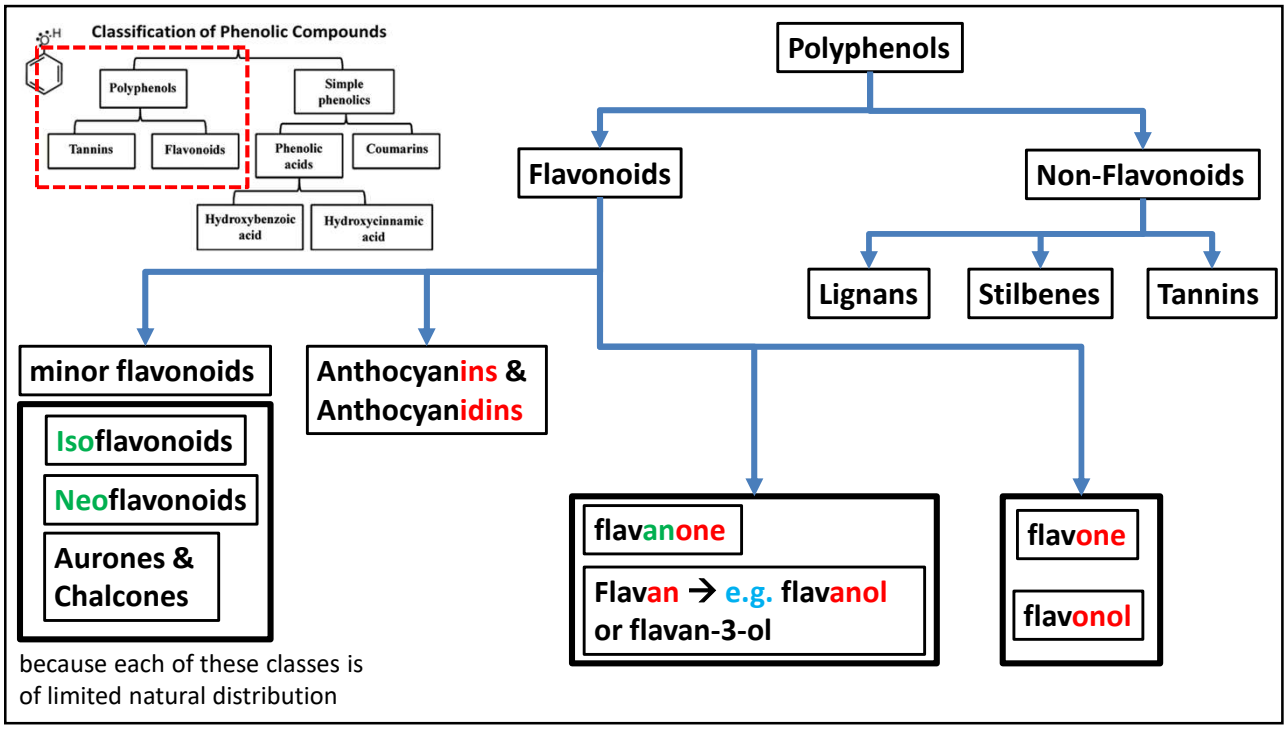
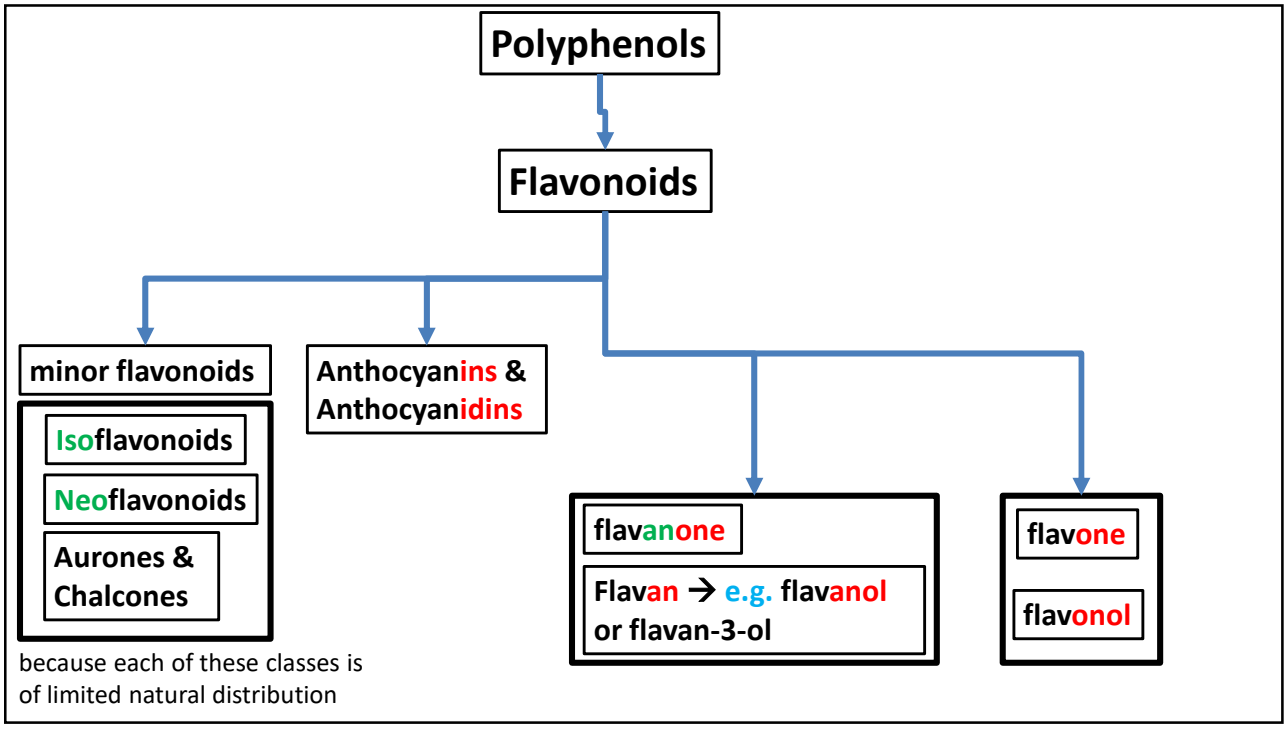

Classification of Phenolic Compounds
Polyphenols
Simple phenolics
Tannins
Flavonoids
Phenolic acids
Coumarins
Hydroxybenzoic acid
Hydroxycinnamic acid
Polyphenols
Flavonoids
Non-Flavonoids
Lignans
Stilbenes
Tannins
minor flavonoids
Anthocyanins & Anthocyanidins
Isoflavonoids
Neoflavonoids
Aurones & Chalcones
flavanone
Flavan → e.g. flavanol or flavan-3-ol
flavone
flavonol
because each of these classes is of limited natural distribution

3


Polyphenols
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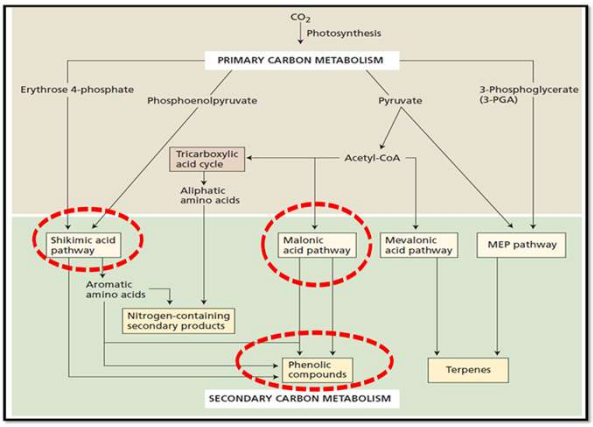
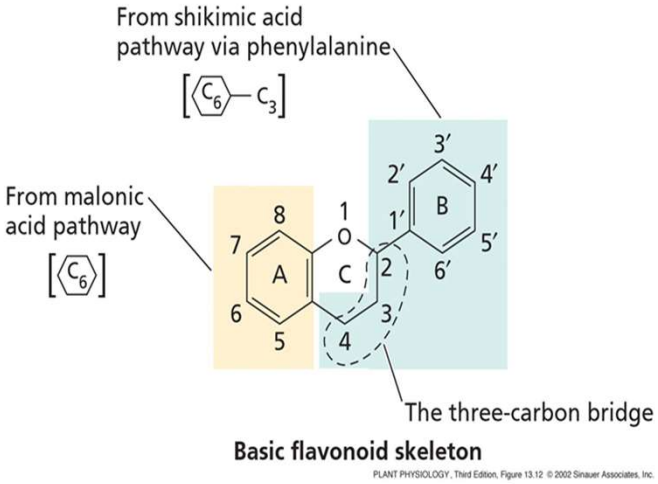
Aurones & Chalcones
flavanone
Flavan → e.g. flavanol or flavan-3-ol
flavone
flavonol
because each of these classes is of limited natural distribution

4

Structure of flavonoids

The flavonoids are possessing 15 carbon atoms; **two benzene rings** joined by a **linear three carbon chain** the skeleton can be represented as the C6 - C3 - C6 system.



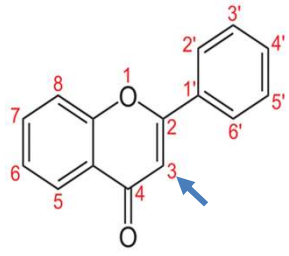


- **have the general structure of a 15-carbon skeleton which consists of two phenyl rings (A and B) and heterocyclic ring (C)**

5

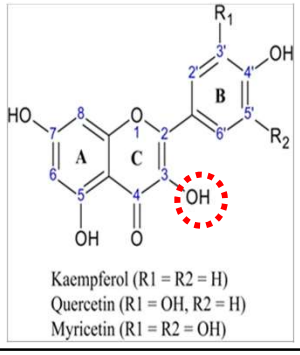
flavone

- The six-membered ring condensed with the benzene ring lacks a 3-hydroxyl substitution.
- e.g.apigenin and luteolin
- Can be found in genus *Citrus*. Such as lemon, orange.



flavonol

- The six-membered ring condensed with the benzene ring have a 3-hydroxyl substitution
- e.g.kaempferol, quercetin, myricetin

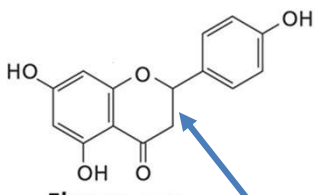


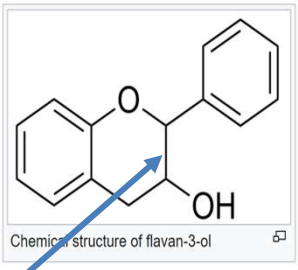
flavanone

flavan

flavanol or flavan-3-ol

- The six-membered ring condensed with the benzene ring is dihydro derivatives
- "dihydro" refers to the two added hydrogen atoms needed to remove one double bond.





remove one double bond

6

flavones and flavonols

UV absorbing
protection against UV (280 - 320 nm)
insect pollinator attraction

mainly colorless co-pigments in flowers; widespread in leaves

How we see the golden eyes…

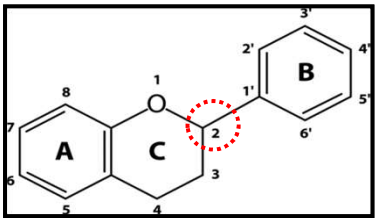
How honeybees see golden eyes…

UV absorbing flavonols are present in the inner part of petals

7

The position of the benzenoid substituent could be:

At the 2-position carries a phenyl ring(B)



flavanone

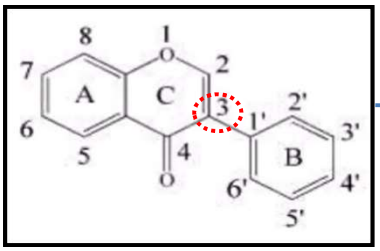
flavone

flavonol

Flavan → e.g. flavanol or flavan-3-ol

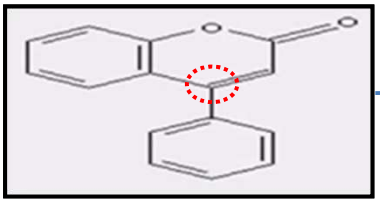
At the 3-position

Isoflavonoids



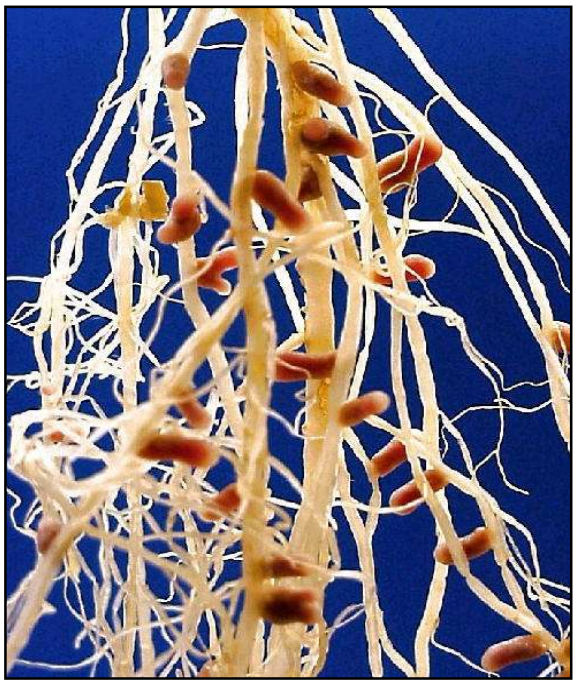
At the 4-position

Neoflavonoids



Isoflavonoids and Neoflavonoids can be regarded as abnormal flavonoids

8



Isoflavonoids

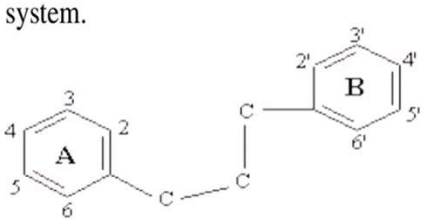
- colorless; often in root; only common in one family, the Leguminosae (common in legumes)
- antimicrobial properties
- also involved in signalling → e.g. attracting rhizobia

➢ Rhizobium is attracted to legumes through signaling by isoflavanoids released from roots.

Rhizobium is a genus of bacteria associated with the formation of root nodules on plants. These bacteria live in symbiosis with legumes. They take in nitrogen from the atmosphere and pass it on to the plant, allowing it to grow in soil low in nitrogen.

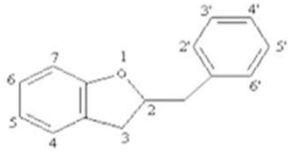
9

The flavonoids are possessing 15 carbon atoms; **two benzene rings** joined by a **linear three carbon chain** the skeleton can be represented as the C6 - C3 - C6 system.

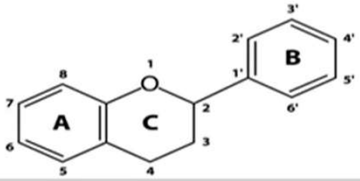


The three-carbon (-C3-) may be included through an oxygen bond between the two phenyl rings into:

1- A five-membered heterocyclic ring (furan) as in **aurones.**



2- A six-membered heterocyclic ring (pyran) to give **flavonoids** which constitute the largest group.

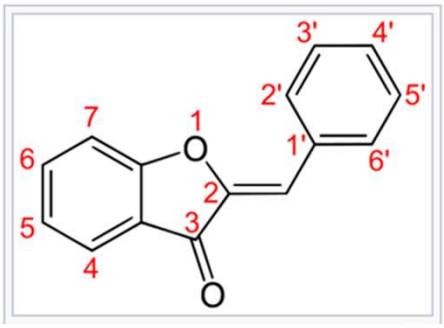


10

Aurones & Chalcones

- Aurones are plant flavonoids that provide yellow color to the flowers (yellow flower pigments).
- Examples → Aurones and Chalcones

Aurone

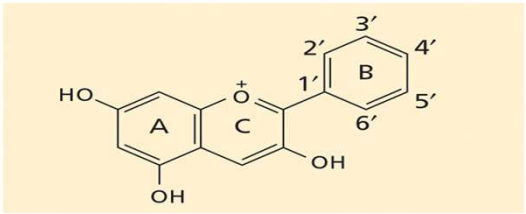


11

Anthocyanins & Anthocyanidins

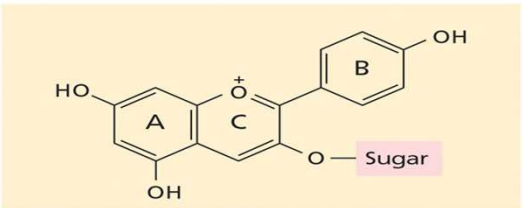
1) Most important and widespread group of coloring matters in plant.
2) Occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits.
3) Water-soluble vacuolar pigments that are found in the cell vacuole, mostly in flowers and fruits, but also in leaves, stems, and roots. In these parts, they are found predominantly in outer cell layers such as the epidermis and peripheral mesophyll cells.
4) Anthocyanins are derived from anthocyanidins by adding sugars.

(A)



Anthocyanidin

(B)



Anthocyanin

PLANT PHYSIOLOGY, Third Edition, Figure 13.13 © 2002 Sinauer Associates, Inc.

Anthocyanidins are common plant pigments, the sugar-free counterparts of **anthocyanins**.

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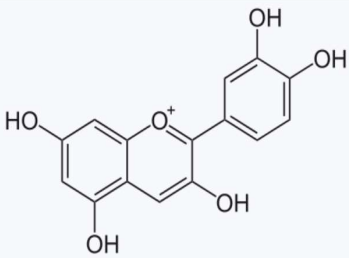
5) The anthocyanins are based chemically on a single aromatic structure, that of cyanidin.
6) Cyanidin is a pigment that has a characteristic reddish-purple color, though this can change with pH; solutions of the compound are red at pH < 3, violet at pH 7-8, and blue at pH > 11
7) The difference in chemical structure that occurs in response to changes in pH, is the reason why anthocyanins often are used as pH indicators, as they change from red in acids to blue in bases through a process called halochromism.
8) A halochromic material is a material which changes colour when pH changes occur. The term 'chromic' is defined as materials that can change colour reversibly with the presence of a factor. In this case, the factor is pH.

Cyanidin

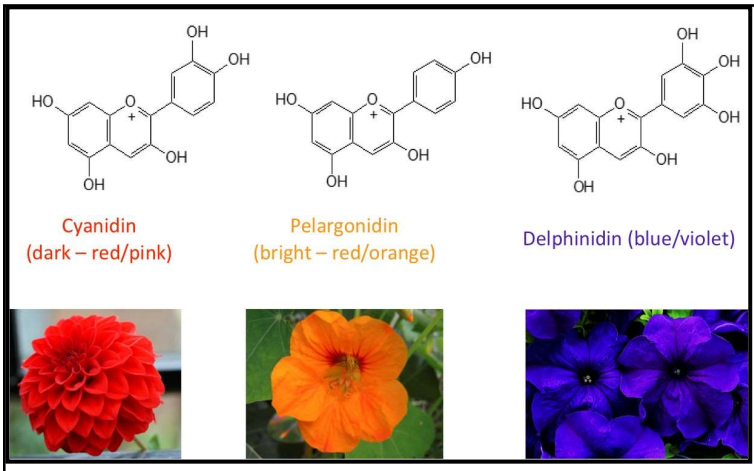


Cyanidin (pH indicator)

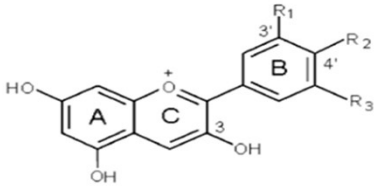
below pH 3 *above pH 11*

3 ⇌ 11

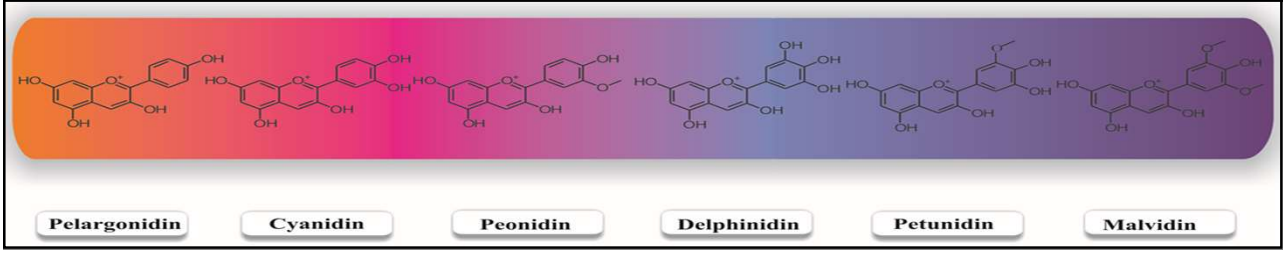
13



Common examples



ANTHOCYANIDIN	R_1	R_2	R_3
Pelargonidin (Pg)	H	OH	H
Cyanidin (Cy)	OH	OH	H
Delphinidin (Dp)	OH	OH	OH
Peonidin (Pn)	OMe	OH	H
Petunidin (Pt)	OMe	OH	OH
Malvidin (Mv)	OMe	OH	OMe

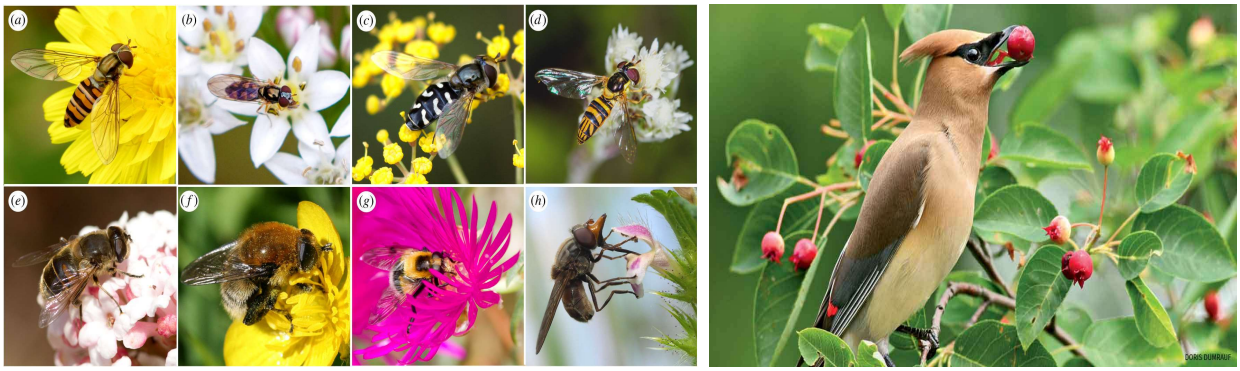


14

Functions of Anthocyanins & Anthocyanidins

Coloration

In flowers, the coloration that is provided by anthocyanin accumulation may attract a wide variety of animal pollinators, while in fruits, the same coloration may aid in seed dispersal by attracting herbivorous animals to the potentially-edible fruits bearing these red, blue, or purple colors.

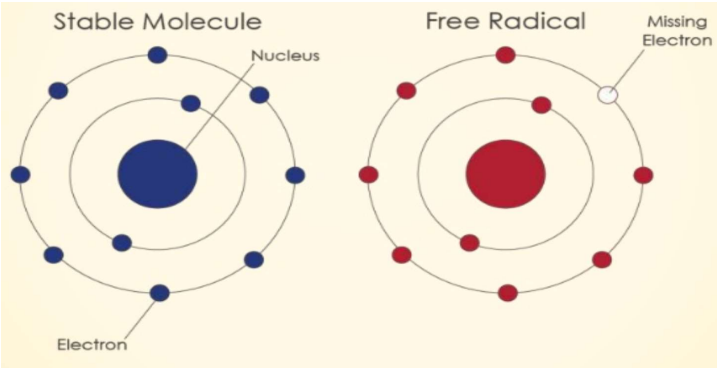


15

Functions of Anthocyanins & Anthocyanidins

Plant physiology

Anthocyanins may have a protective role in plants against extreme temperatures. Tomato plants protect against cold stress with anthocyanins countering reactive oxygen species, leading to a lower rate of cell death in leaves. (**Reactive oxygen species**: Reactive oxygen species are free radicals, a type of unstable molecule that contains oxygen and that easily reacts with other molecules in a cell. A build up of reactive oxygen species in cells may cause damage to DNA, RNA, and proteins, and may cause cell death)



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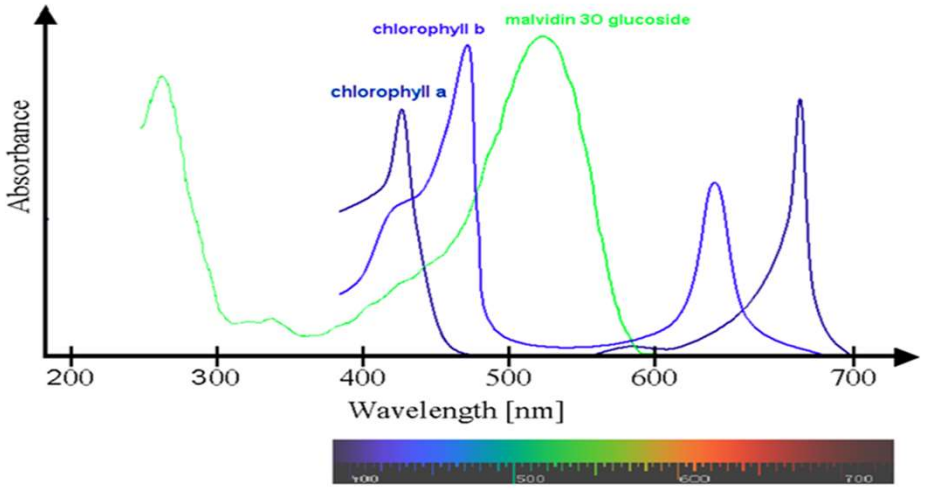
Functions of Anthocyanins & Anthocyanidins

Light absorbance

The absorbance pattern responsible for the red color of anthocyanins may be complementary to that of green chlorophyll in photosynthetically-active tissues. It may protect the leaves from attacks by herbivores that may be attracted by green color



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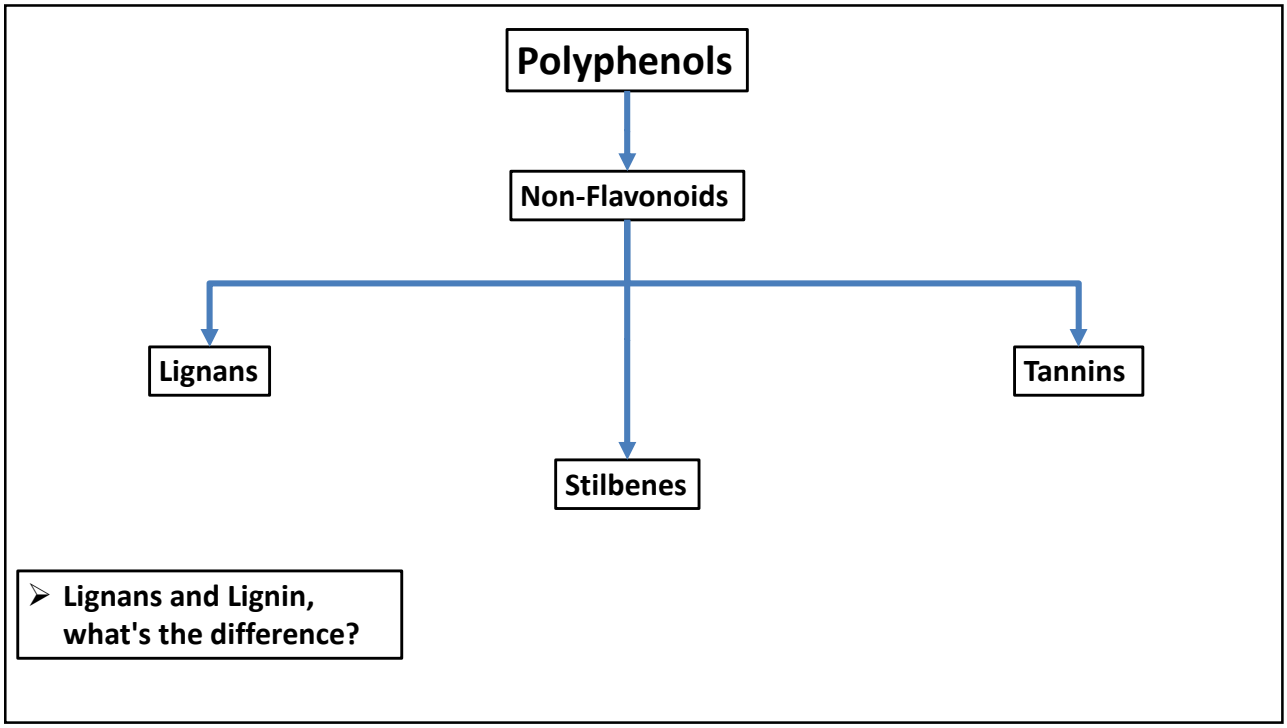

- Lignans and Lignin, what's the difference?

18

Tannins

1) **Tannins have a protein-binding property which makes them:**
   a) Different levels of toxicity to herbivores. And animals can sense high levels of tannins in their food and opt for another food resource because high levels of tannins in diet can kill some animals.
   b) Tannins can bind with salivary proteins and make proteins precipitate and aggregate, producing a rough, "sandpapery", or dry sensation in the mouth, and that makes protein less digestible.(astringent taste)
   c) Tannins can bind digestion enzymes in the gut of herbivores.
   d) Because tannins can bind to proteins making them difficult to digest, thus decreasing the nutritional value of the plant material. (that's why drinking tea with your meal isn't a good idea)

tannins in unripe fruits, lets the fruit mature by deterring eating.

2) **Two types of tannins**
   1. Condensed tannins – occur almost universally in ferns and gymnosperms and widespread among angiosperms especially woody species.
   2. Hydrolysable tannins – limited to dicotyledonous plants

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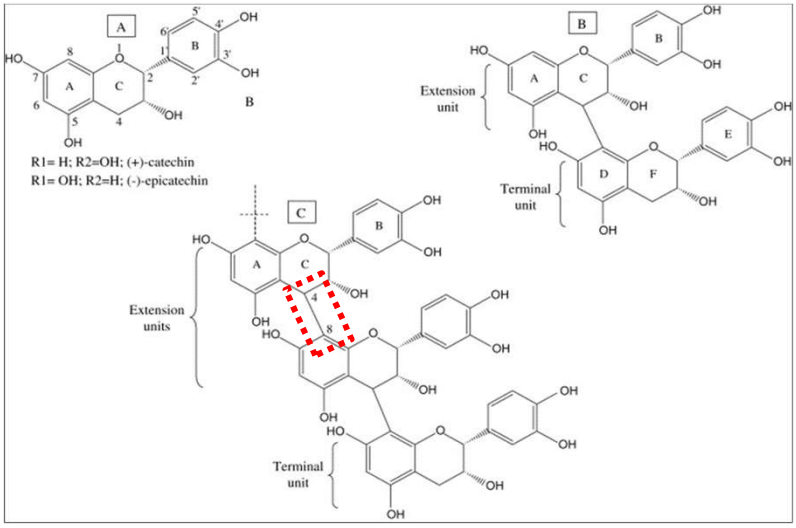
Condensed tannins –

- formed biosynthetically by the condensation of single catechin to form dimers and then higher oligomers, with C-C linking one flavan unit to the next by a 4 – 8 or 6 – 8 link.



formed by polymerization of flavonoid units

A→ monomers

B→) dimer

C→ oligomers with C4-C8 linkage

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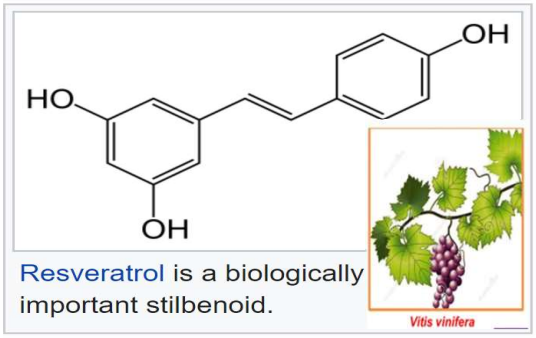
Stilbenoids

1) Stilbenoids are hydroxylated derivatives of stilbene.
2) They have a C6–C2–C6 structure.
3) Can act as **phytoalexins**.
4) Example is resveratrol→ an antifungal

➢ **Phytoalexins**: are antimicrobial substances synthesized by plants, and they accumulate rapidly at areas of pathogen infection.

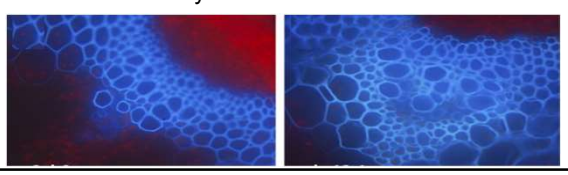


Antifungal assay

21

Lignans and lignin, both are polyphenolic substances.

lignin is
1) a complex high molecular weight polyphenol polymer.
2) Indigestible
3) The main components of plant cell wall occurs in all vascular plants (Structural function)
4) Also protective function because it deters herbivores, pathogens, and toxics due to its toughness.
5) one of the most important secondary metabolite
6) Remember from last lecture, Caffeic acid is actively involved in plant physiology and mechanisms of stress tolerance primarily utilized by plants for the synthesis of lignin which ultimately thickened cell walls and plant become resistant to ion toxicity sodium and heavy metal stress.



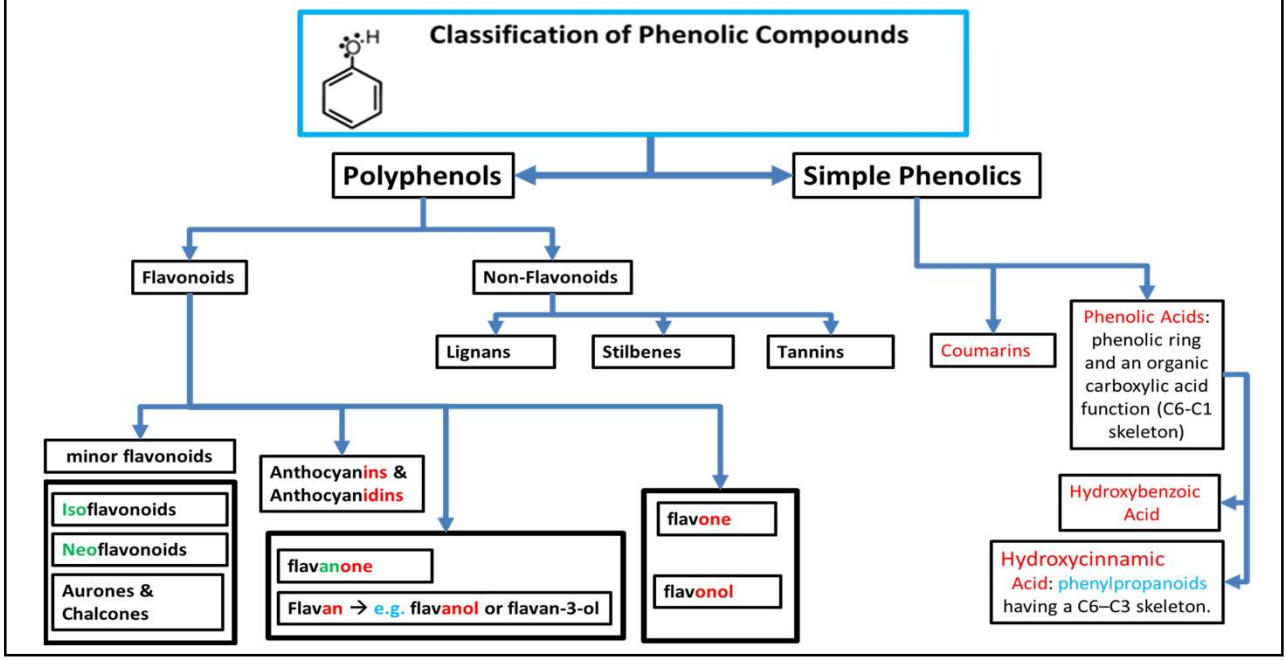
lignan is
1)a large group of low molecular weight polyphenols
2)soluble in water
3)found in plants, particularly seeds, whole grains, and vegetables.
4)They may play a role as antifeedants in the defense of seeds and plants against herbivores. (how?)
5)Lignans are precursors= originators→ to phytoestrogens.

- phytoestrogens→ phyto = plant, estrogen = the hormone which gives fertility to female mammals.
- Phytoestrogens→imitate estrogen
- Phytoestrogens→ have the ability to cause estrogenic and/or antiestrogenic effects
- It has been hypothesized that plants use a phytoestrogen as part of their natural defense against the overpopulation of herbivore animals by controlling female fertility. Also, phytoestrogens may affect male fertility

22

In last lecture

في المحاضرة السابقة تحدثنا عن

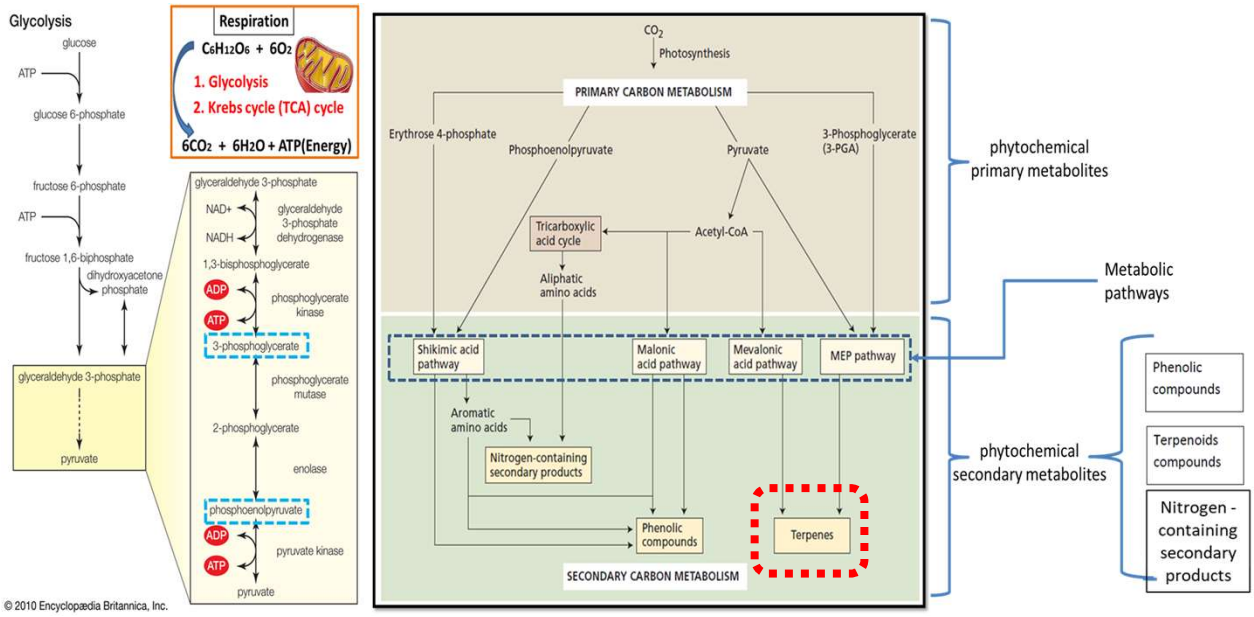


1

Terpenoids compounds

2

1) They produced by the mevalonic acid pathway from the acetyl-CoA.



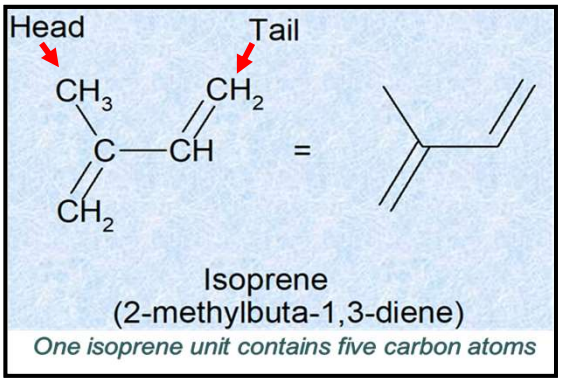
3

Terpenoids compounds

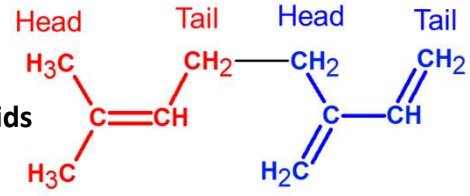
1) The **terpenes**, or **terpenoids**, constitute the largest class of secondary products, while flavonoids constitute the largest class of phenolic compounds.
2) They are generally insoluble in water, but lipid-soluble.
3) terpenoids are unsaturated (double bound between carbon atoms) compounds formed by joining together isoprene units.
4) So, they are derived from the 5-carbon alkene **isoprene**. (thus, the terpenoids also called "isoprenoids").
5) terpenoids are major components of the plant essential oils. and terpenoids are components of a wide variety of fruit and floral flavors and aromas.
6) Often located in cytoplasm of the plant cell
   - Essential oils sometimes occurs in special glandular cells on the leaf surface
   - Carotenoids – associated with chloroplasts in the leaf and with chromoplasts in the petal.

4

Terpenoids are classified based on the number of 5-carbon units (isoprene units)



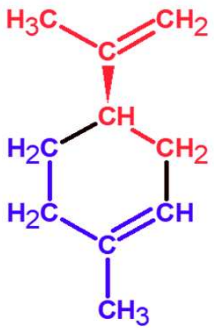
linear Terpenoids



Isoprene units can be linked:

- **head to tail to form linear Terpenoids**
- **in rings to form cyclic Terpenoids**

Cyclic Terpenoids



5

Overall Functions of terpenoids

1) Growth-regulating properties

- abscisins and gibberellins.

2) Agents of communication and defense among insects (distinctive smells and odours)

- They produced in response to herbivores feeding, to attract predatory insects and parasites of the feeding herbivores.
- Some are toxins and feeding deterrents to many plant feeding insects and mammals.

3) Accessory pigments in photosynthesis

- Carotenoids
  - plant color - pale yellow through bright orange to deep red
  - Accessory pigments in photosynthesis

6

Terpenoids are classified based on the number of 5-carbon units (isoprene units)

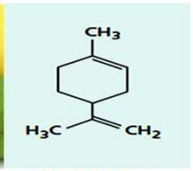
- **Monoterpenoids → have 2 isoprene units (10-Carbons)**
- **Sesquiterpenoids → have 3 isoprene units (15-Carbons)**

Essential oils

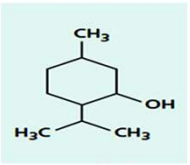
- **Monoterpenoids (C10)–boiling point 140-180 °C**
  - **limonene**
  - **menthol**
  - **menthone**
  - **carvone**
- **Sesquiterpenoids (C15) – boiling point >200 ° C**
  - **abscisic acid** → **hormone controlling dormancy in seed**

Essential oils

- **Volatile with Characteristic scent**
  - A volatile substance is one that evaporates at room temperature or below.
- Commercially important – natural perfumes, and flavoring in food industry
- Mono & Sesqui can be acyclic, monocyclic, or bicyclic.
- Simple mono- are widespread and tend to occurs as majority of essential oils.
- Flower and seed oils tend to have more specialized mono- present



Limonene



Menthol

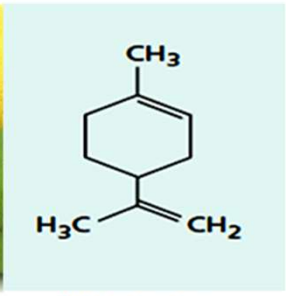
7

1. **Limonene**

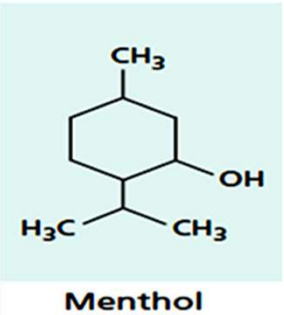
is a non-volatile monoterpene, a major component in oil of citrus fruit peels, and known as distasteful to herbivores.

2. **Menthol**

is a volatile monoterpene, produced by various plants (especially mint). Menthol is a smell that warns herbivores that the plant is toxic to them before herbivore feeding commences.



Limonene



Menthol

8

Terpenoids are classified based on the number of 5-carbon units (isoprene units)

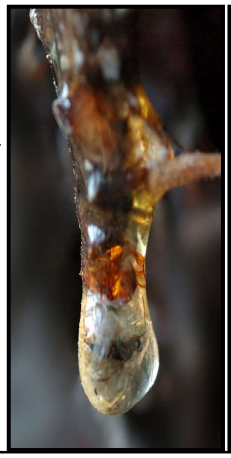
Diterpenoids→have 4 isoprene units (20-Carbons)

Diterpenoids & gibberellins

- **Resin diterpenoids** →The latex of the rubber tree is (natural rubber)
- **Toxic diterpenoids** →Poisonous
- **Gibberellins** →Gibberellic acid-stimulate growth

Terpenoids compounds:
- **Diterpenoids and gibberellins**

- 3 classes of diterpenes – resin diterpenes, toxic diterpenes and gibberellins
- **Resin diterpenes**
  - Have protective function in nature. Plants secrete resins for their protective benefits in response to injury. The resin protects the plant from insects and pathogens.
  - Exuded from wood of trees or latex of herbaceous plants
  - The latex of the rubber tree is the chief source of natural rubber
- **Toxic diterpenes**
  - Poisonous
- **Gibberellins**
  - Group of hormone – stimulate growth
  - Gibberellic acid – the most popular gibberellin



9

Plants secrete resins for their protective benefits in response to injury

10

Terpenoids are classified based on the number of 5-carbon units (isoprene units)

Triterpenoids→have 6 isoprene units (30-Carbons)

Triterpenoids and steroid → **Saponin, Phytosteroids, cardiac glycosides**

Saponin → **feeding deterrents**; **Phytosteroids** → **membrane fluidity**; **cardiac glycosides** → **toxic**

Terpenoids compounds:
- **Triterpenoids and steroid**

❖ **cardiac glycosides**→ toxic nature of these compounds

- Cardiac glycosides affect the sodium-potassium ATPase pump in cardiac muscle cells to alter their function.

❖ **Phytosteroids**, also known as **plant steroids**,→ are naturally occurring steroids that are found in plants

- The richest naturally occurring sources of phytosterols are vegetable oils
- regulates membrane fluidity and permeability in a similar manner to cholesterol in mammalian cell membranes.

❖ **Saponins** →are bitter-tasting usually toxic plant-derived organic chemicals that have a foamy quality when agitated in water.

- Saponins are both water and fat soluble, which gives them their useful soap properties.
- In plants, saponins may serve as (feeding deterrents) and to protect the plant against microbes and fungi. Saponin occur especially in - waxy coatings of leaves and on fruits – protective function in repelling insect and microbial attack

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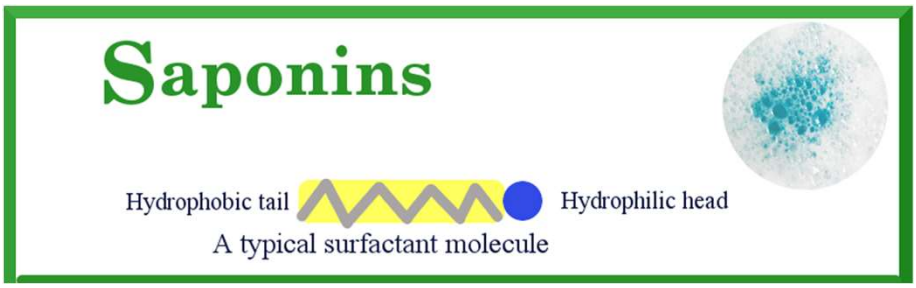
Saponins are both water and fat soluble, which gives them their useful soap properties → protective function & feeding deterrents



Saponins are called saponins because they foam in water and because they are surfactants.

Surfactants are molecules with both a hydrophilic and a hydrophobic part. This structure allows them to orient at water-oil interphase. (in simple words: being soluble both in water and in oil)

hydrophilic → having a tendency to mix with, dissolve in, or be wetted by water.محبة للماء

Hydrophobic→ tending to repel or fail to mix with water.كارهة للماء

12

Terpenoids are classified based on the number of **5-carbon units (isoprene units)**

Tetraterpenoids→have 8 isoprene units (40-Carbons)

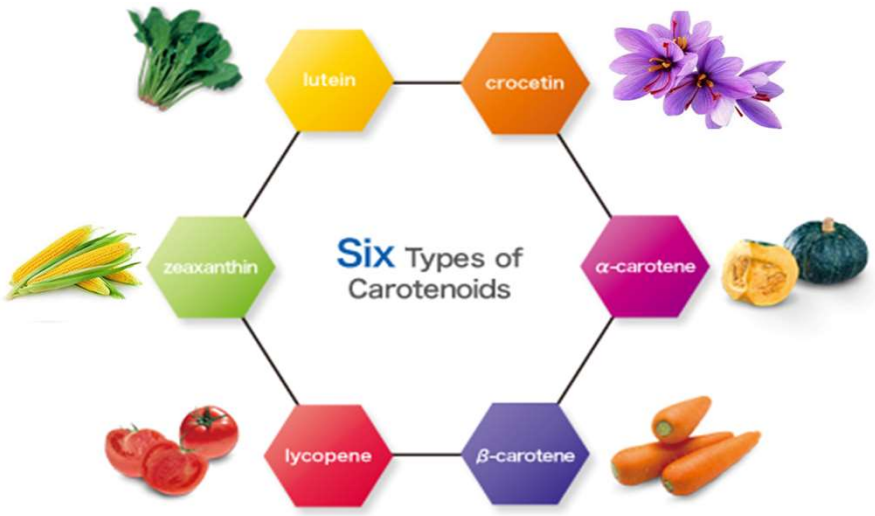
Carotenoids

- **Lycopene**→ Tomato
- **β-carotene**→ carrot
- **α—carotene**→ pumpkin
- **Lutein**→ spinach
- **Crocetin**→ saffron crocus
- **Zeaxanthin**→ Zea mays (corn)

Terpenoids compounds:
- **Carotenoids**

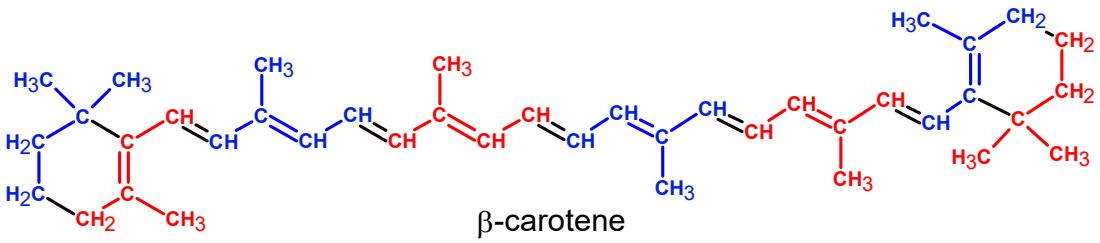
Extremely widely distributed group of lipid-soluble pigment

2 principal function
- As colouring matters in flowers and fruits
- Accessory pigment in photosynthesis



13

β-carotene – a linear terpene

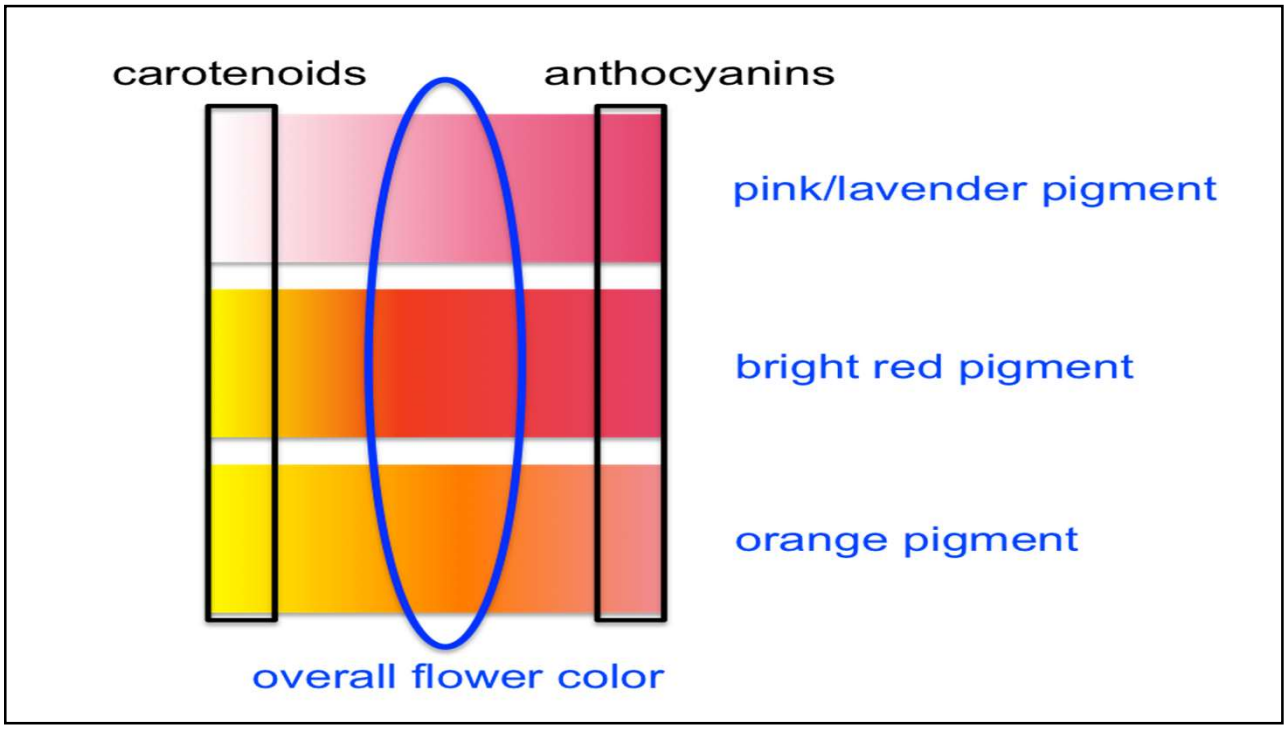


β-carotene

8 isoprene units

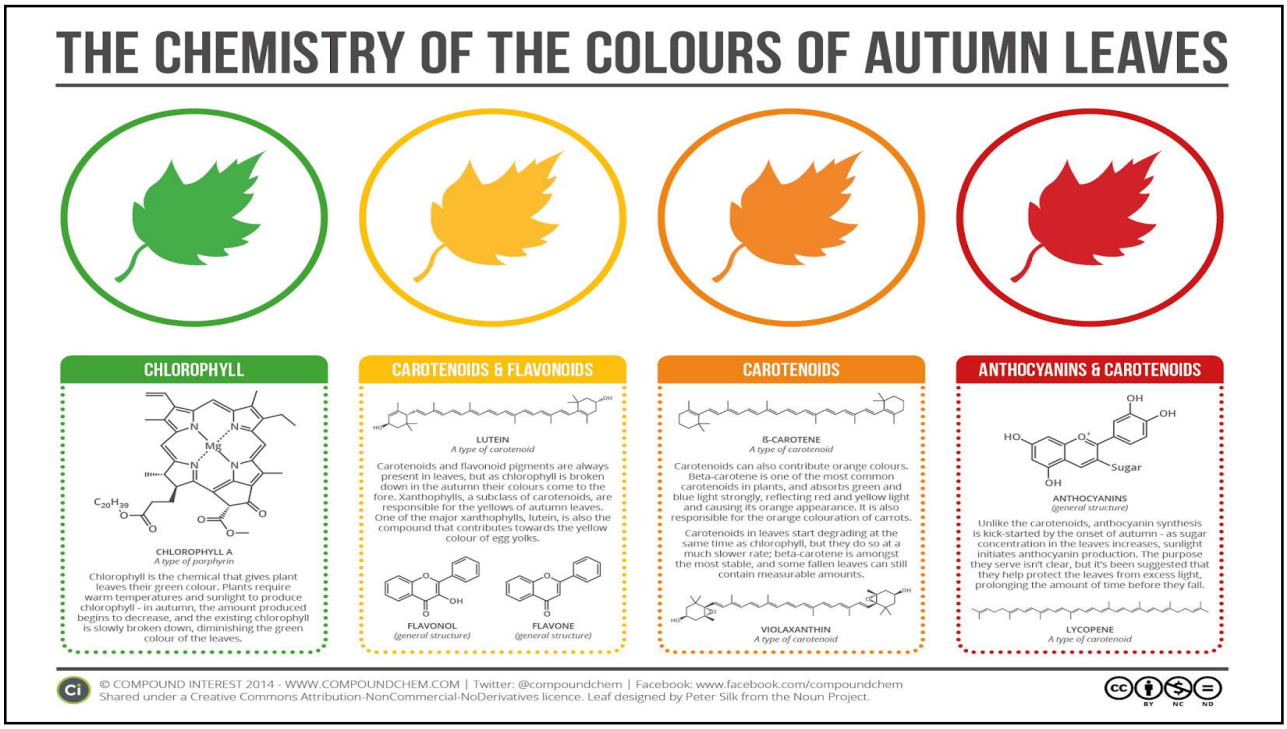
40 carbon atoms

14


carotenoids
anthocyanins
pink/lavender pigment
bright red pigment
orange pigment
overall flower color

15

16



17

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- **Diterpenoids → have 4 isoprene units (20-Carbons)**
- **Triterpenoids → have 6 isoprene units (30-Carbons)**
- **Tetraterpenoids → have 8 isoprene units (40-Carbons)**
- **Polyterpenoids → have more than 8 isoprene units (more than 40-Carbons)**

Essential oils
- Volatile
- Characteristic scent

Monoterpenoids (C10)–boiling point 140-180 °C
- limonene

- menthol
- menthone
- carvone

Sesquiterpenoids (C15) – boiling point >200 ° C
- abscisic acid → hormone controlling dormancy in seed

Diterpenoids & gibberellins
- Resin diterpenoids → The latex of the rubber tree is (natural rubber)

- Toxic diterpenoids → Poisonous
- Gibberellins → Gibberellic acid-stimulate growth

Triterpenoids and steroid → Saponin, Phytosteroids, cardiac glycosides
- feeding deterrents
- membrane fluidity
- toxic

Carotenoids
- Lycopene → Tomato
- β-carotene → carrot
- α—carotene → pumpkin
- Lutein → spinach
- Crocetin → saffron crocus
- Zeaxanthin → Zea mays (corn)

- As colouring matters in flowers and fruits
- Accessory pigment in photosynthesis

18

In last lecture — في المحاضرة السابقة تحدثنا عن

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- Accessory pigment in photosynthesis

1

N-containing compounds

- Nitrogen compounds – nitrogen element

N-containing Primary Compounds
- Amino acids → Peptides → Protein
- Chlorophylls

- directly involved in normal growth, development, and reproduction of plant species

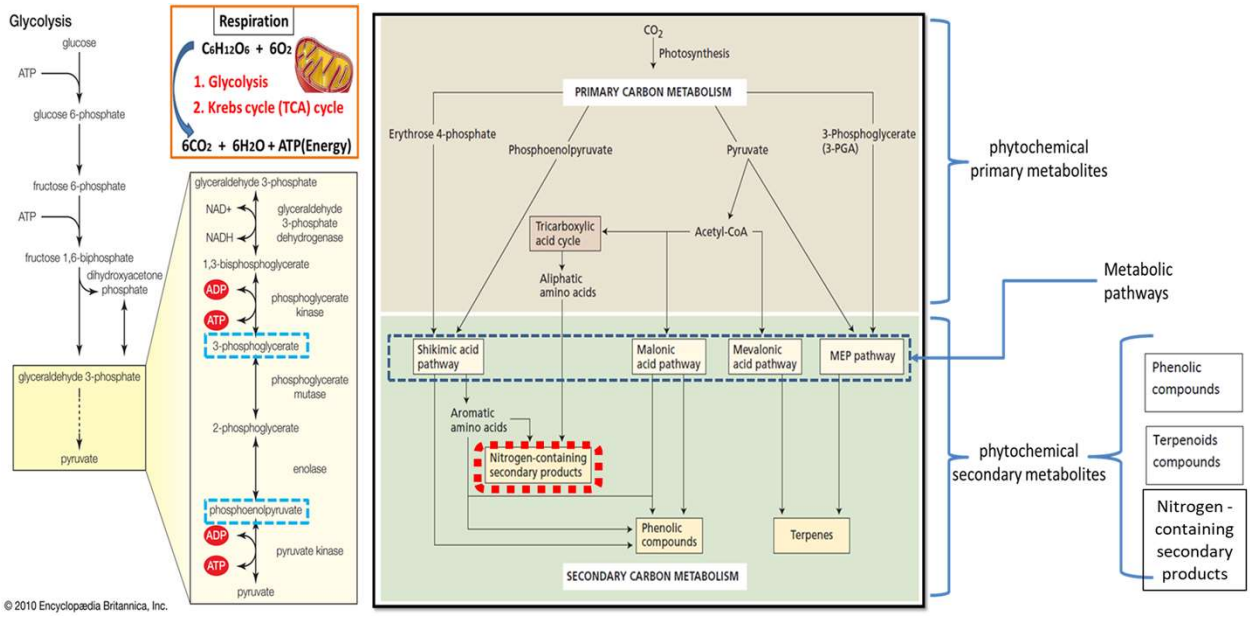
N-containing Secondary Compounds
- Alkaloids
- Cyanogenic glycosides
- Glucosinolates
- Amines

- not directly involved in those processes, but usually has important ecological functions

- They are less common in plants than Terpenes and Phenolics.
- They are synthesized from aliphatic and aromatic amino acids.

2

N-containing Secondary Compounds are synthesized from aliphatic and aromatic amino acids.

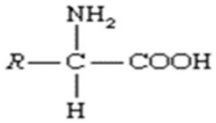


3

Amino acids

1. Amino acids → a group of organic molecules that consist of a basic amino group (—NH2), an acidic carboxyl group (—COOH), and an organic R group (or side chain) that is unique to each amino acid.
2. The formula of a general amino acid is: **RCH(NH2)CO2H**
3. The amino acids differ from each other in the particular chemical structure of the R group.
4. Amino acid are colorless ionic compounds & water soluble
5. Amino acid have difference charge properties, amino acid mixture can be divided into neutral, basic and acidic.
6. Plant amino acid conveniently divided into two groups:

RCH(NH2)CO2H



a. Protein amino acid

1. Generally recognized to be twenty in number
2. Found in plant and animal

b. Non-protein amino acid (not incorporated into proteins)

1. They are not part of the 20 such molecules that are translated into proteins by the standard genetic code.
2. Their role in plant is not clear, although present in high concentration in seeds
3. May be important as nitrogen storage material, or act as protective substances
4. found in plants of the family Leguminosae, e.g., Canavanine (non-protein amino acid) resemble in structure with arginine (protein amino acid).

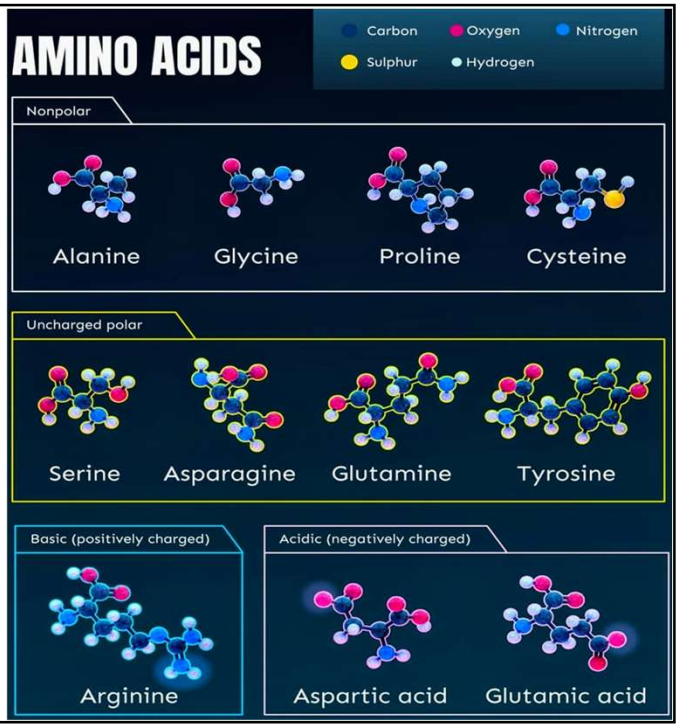
4

Protein amino acid

Red color → present in larger amount and represent a storage form of nitrogen

Blue color → low amount in plant tissue and cannot be readily detected.

Protein amino acid	Charge properties
Glycine	neutral
Alanine	neutral
Serine	neutral
Cysteine	neutral
Threonine	neutral
Valine	neutral
Leucine	neutral
Isoleucine	neutral
Methionine	neutral
Aspartic acid	acidic
Asparagines	neutral
Glutamic acid	acidic
Glutamine	neutral
Arginine	basic
Lysine	basic
Proline	neutral
Phenylalanine	neutral
Tyrosine	neutral
Tryptophan	neutral
Histidine	basic



5

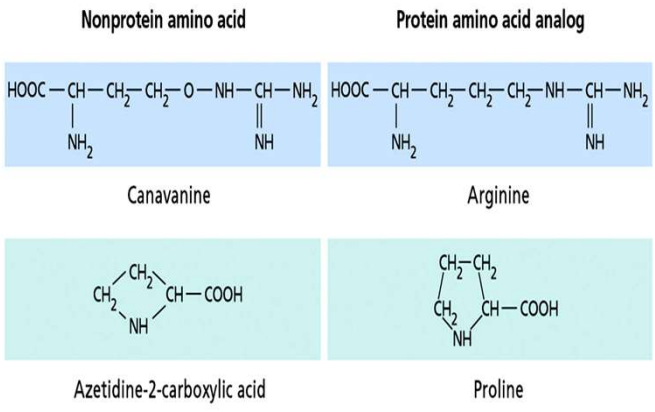
Protein amino acid

Red color → present in larger amount and represent a storage form of nitrogen

Blue color → low amount in plant tissue and cannot be readily detected.

Protein amino acid	Charge properties
Glycine	neutral
Alanine	neutral
Serine	neutral
Cysteine	neutral
Threonine	neutral
Valine	neutral
Leucine	neutral
Isoleucine	neutral
Methionine	neutral
Aspartic acid	acidic
Asparagines	neutral
Glutamic acid	acidic
Glutamine	neutral
Arginine	basic
Lysine	basic
Proline	neutral
Phenylalanine	neutral
Tyrosine	neutral
Tryptophan	neutral
Histidine	basic

Non-protein amino acid



Non-protein amino acids and their protein amino acids analogs

6

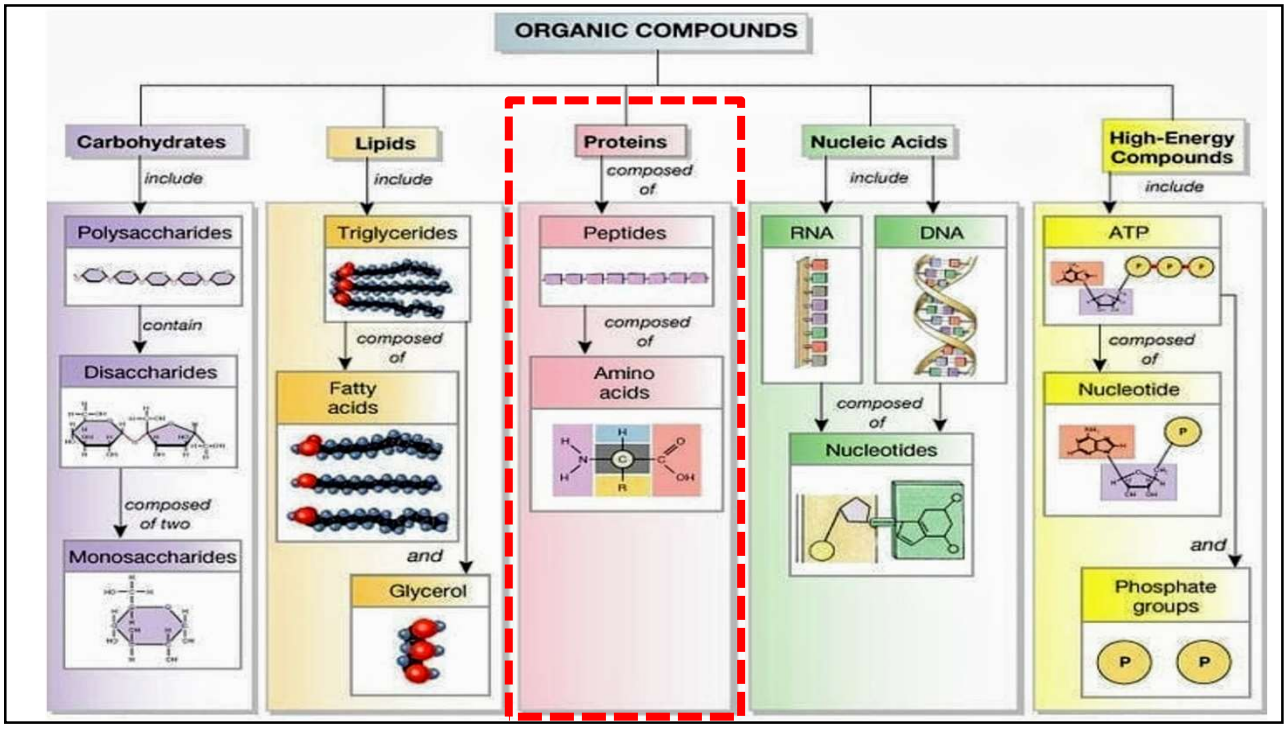
Amines vs. Amino acids

- Considered simply as the products of decarboxylation of amino acid, formed by the reaction:

  $RCH(NH_2)CO_2H \rightarrow RCH_2NH_2 + CO_2$

  - Have an unpleasant fish-like smell (offensive odors)
  - Function in flowers as insect attractants

7



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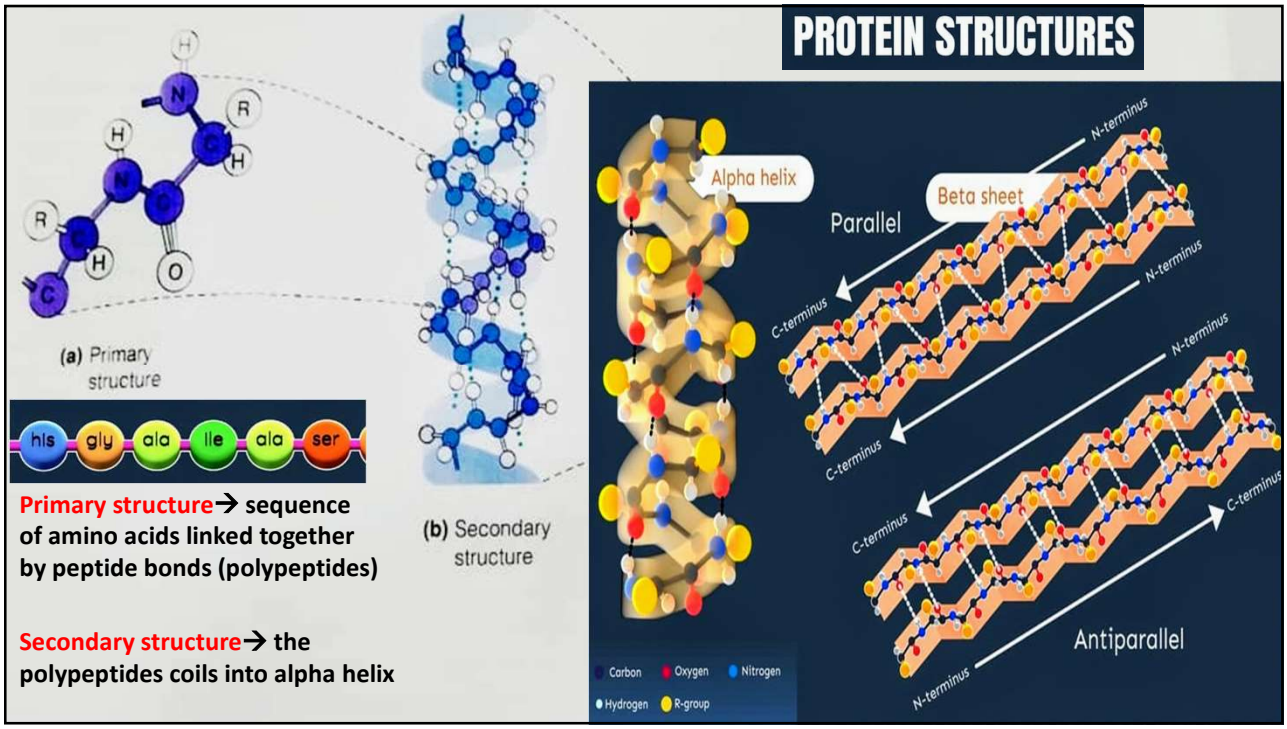
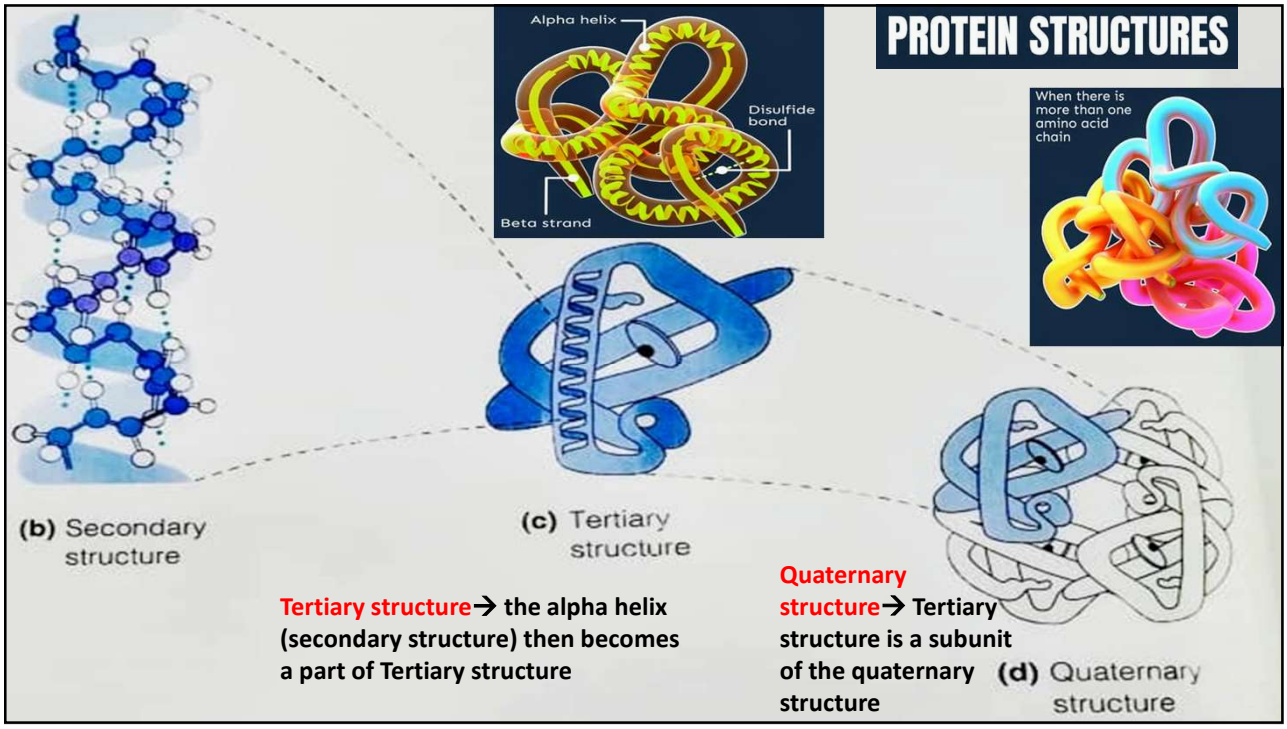


Primary structure→ sequence of amino acids linked together by peptide bonds (polypeptides)

Secondary structure→ the polypeptides coils into alpha helix

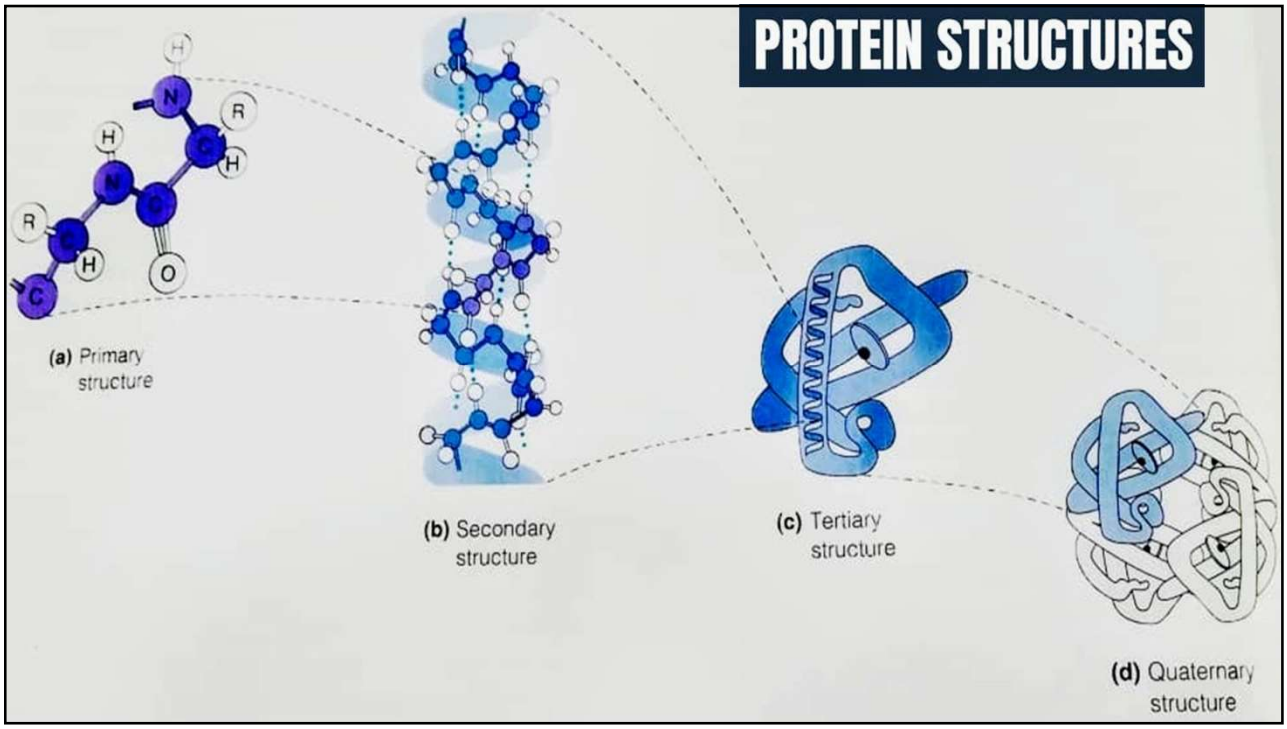
9



Tertiary structure→ the alpha helix (secondary structure) then becomes a part of Tertiary structure

Quaternary structure→ Tertiary structure is a subunit of the quaternary structure

10



11

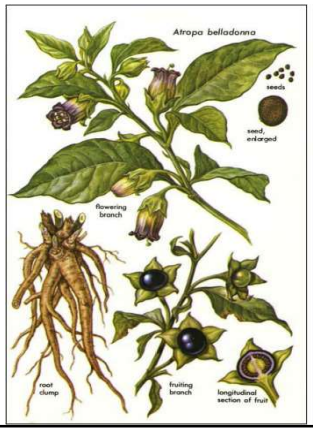
Alkaloids

1) The alkaloids are a large family of more than 15,000 nitrogen-containing secondary metabolites, & the nitrogen atom in these compounds is usually part of a heterocyclic ring, a ring that contains both nitrogen and carbon atoms. And Alkaloids are classified depending on the type of heterocyclic ring system present in the molecule
2) Most alkaloids are alkaline and are weak bases which formed salts with an acid. . At the pH values commonly found in the cytosol (pH 7.2) or the vacuole (pH 5–6), the nitrogen atom is protonated; hence alkaloids are positively charged and are generally water soluble, & almost all the alkaloids have a bitter taste.
3) Alkaloids are usually synthesized from one of a few common amino acids—in particular, lysine, tyrosine, or tryptophan.

12

Alkaloids

4) They are found in approximately 20% of vascular plant species. Mostly in angiosperm, generally absent or infrequent in gymnosperms, ferns, mosses and lower plant. Specific to one family or to a few related plants – name of alkaloid types are often derived from plant source – eg nicotine (*Nicotina tabacum*), atropine (*Atropa belladonna*).



13

1) The role of alkaloids in plants has been a subject of speculation for at least a century.

a) Alkaloids were once thought to be nitrogenous wastes (analogous to urea and uric acid in animals),

b) nitrogen storage compounds, or

c) growth regulators, but there is little evidence to support any of these functions.

d) Most alkaloids are now believed to function as defenses against herbivores, especially mammals, because of their general toxicity and deterrence capability. Livestock deaths due to over-consumption of alkaloid containing plants such as lupines and groundsels.

e) Insect repellents or attractants

2) Often alkaloids are used as medicines for **humans**. Some examples: morphine.

3) Cocaine, nicotine, and caffeine used as stimulants and sedatives.

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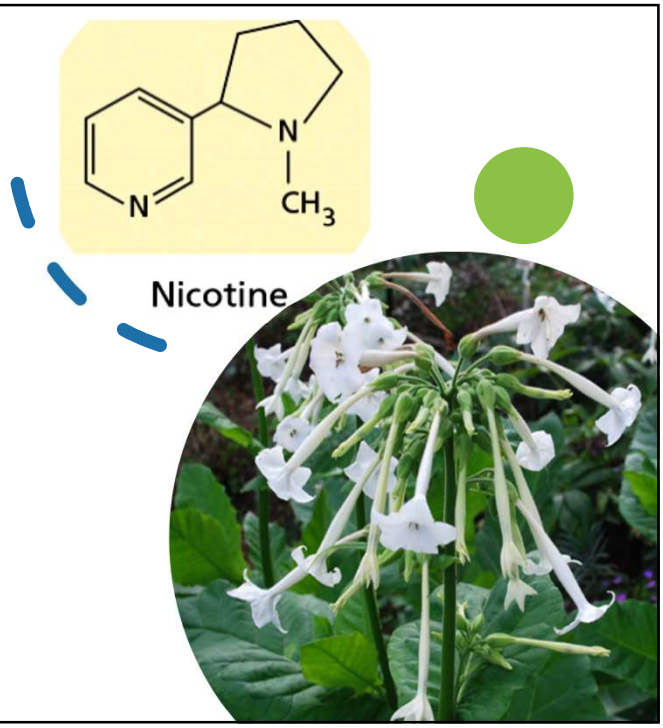
Alkaloids → Nicotine

- The tobacco plants (*Nicotiana spp*.) normally produces nicotine in response to herbivore feeding.
- But if nicotine-tolerant caterpillars are feeding, the tobacco produces terpenes instead.
- These terpenes can attract the predators of the herbivore.



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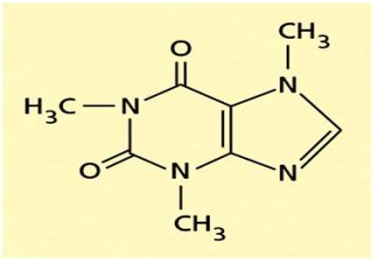
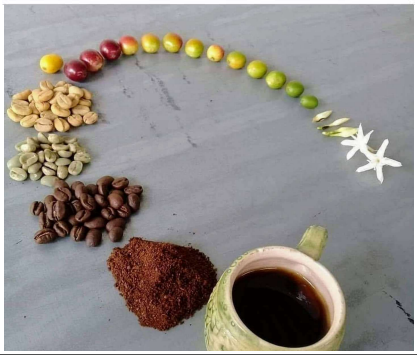
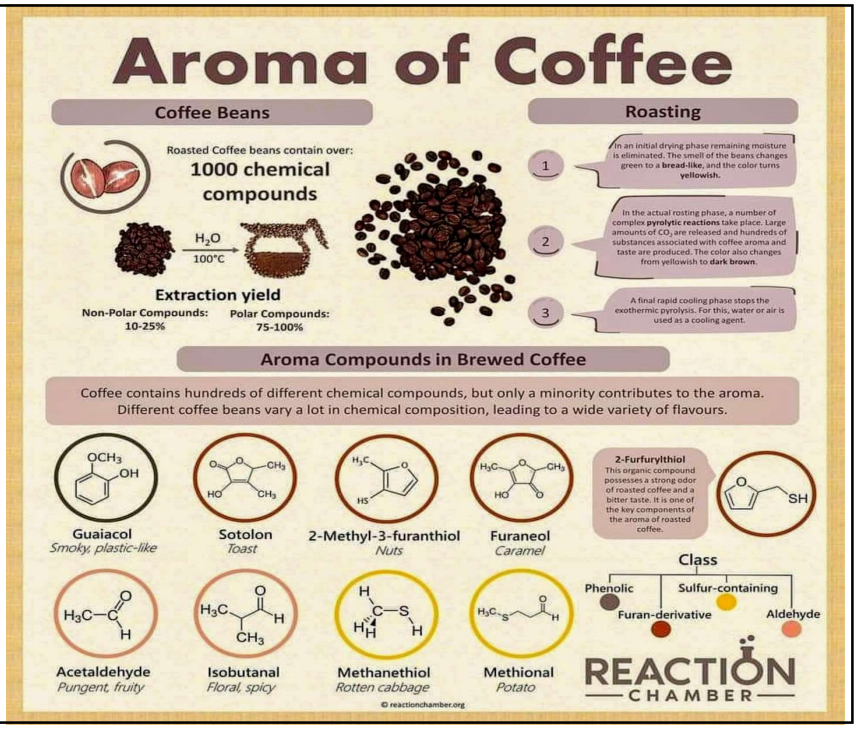
Alkaloids → caffeine



Caffeine





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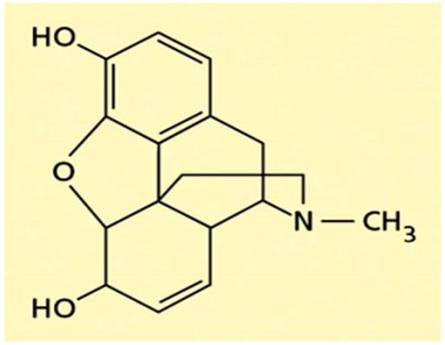
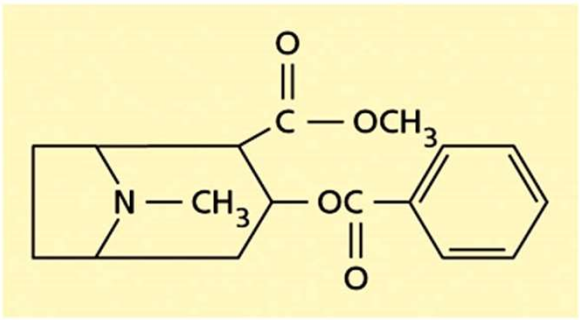
Alkaloids → Morphine & Cocaine



Morphine



Cocaine

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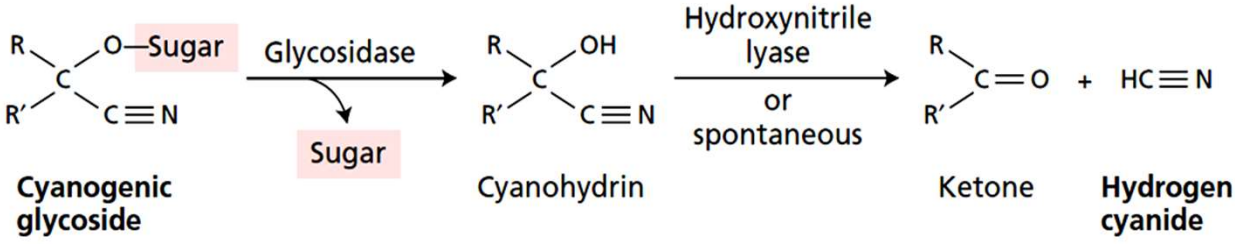
Cyanogenic glycosides

1. The plants convert these compounds into the **toxic gas** hydrogen cyanide (HCN) in response to herbivore feeding.
2. Plants that produce cyanogenic glycosides also produce the enzymes that convert these compounds into hydrogen cyanide, including **glycosidases** and hydroxy nitrile lyases.
3. These enzymes and cyanogenic glycosides are stored in separate compartments or tissues within the plant; when herbivores feed on these tissues, they produce the lethal hydrogen cyanide.

The degradation process of Cyanogenic glycosides



Cyanogenic glycoside | Cyanohydrin | Ketone | **Hydrogen cyanide**

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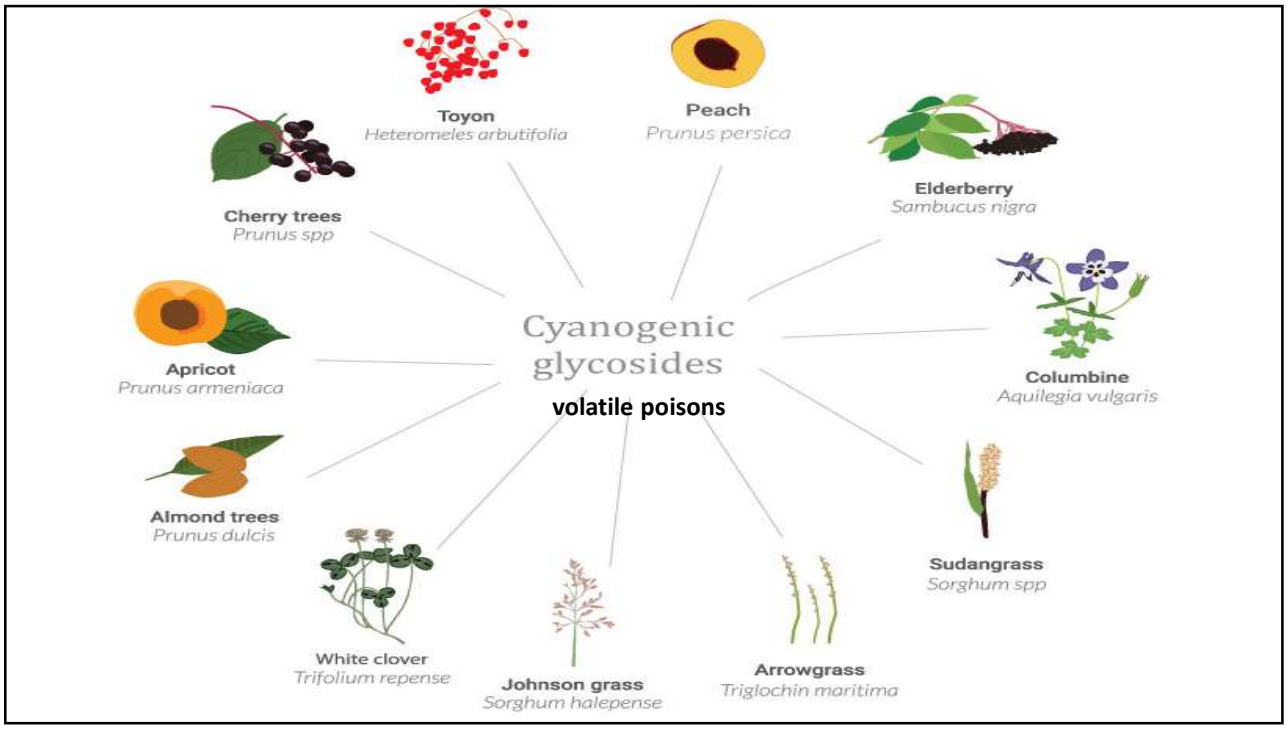

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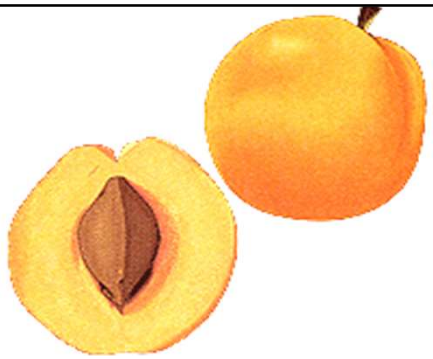
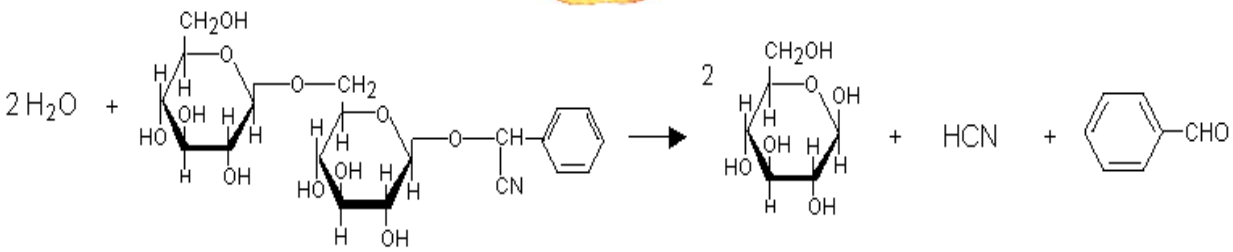
Cyanogenic Glycosides





Water + amygdalin → 2 glucose + cyanide + benzaldehyde

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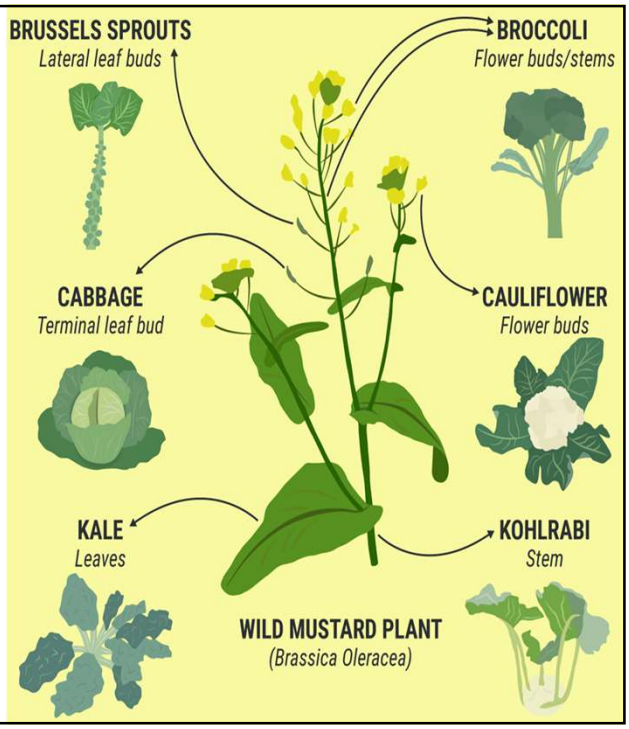
Glucosinolates

- These compounds release **volatile defensive** substances, "**mustard oils**", (often herbivore repellents).
- Plants like cabbage, broccoli, and radishes (*Brassicaceae family*) have these compounds.
- Glucosinolates are **sulfur-** and **nitrogen**-containing secondary metabolites involved in plant defense against herbivores.
- Glucosinolates are present in plant tissues but are also induced to higher levels by herbivores attack.



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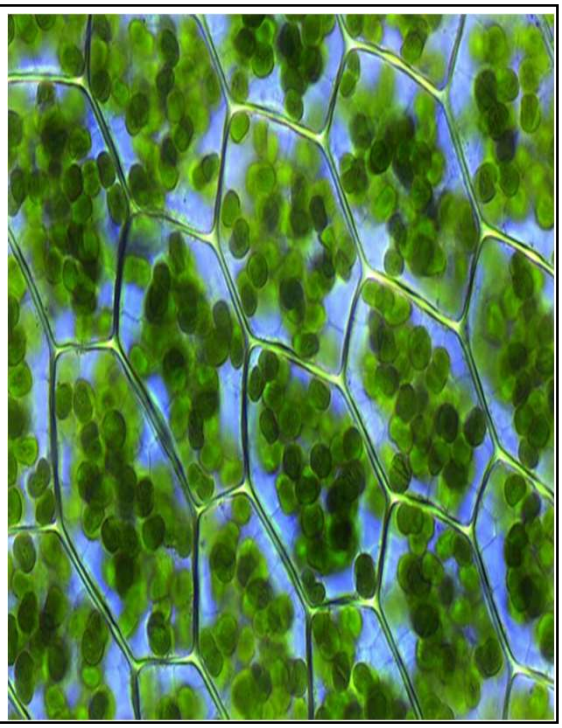
Chlorophylls

- Essential catalysts of photosynthesis
- Occur as green pigment in all photosynthetic plant tissue
- Occur abundantly in the chloroplast
- Two types of chlorophyll exist in the photosystems of green plants: chlorophyll a and b



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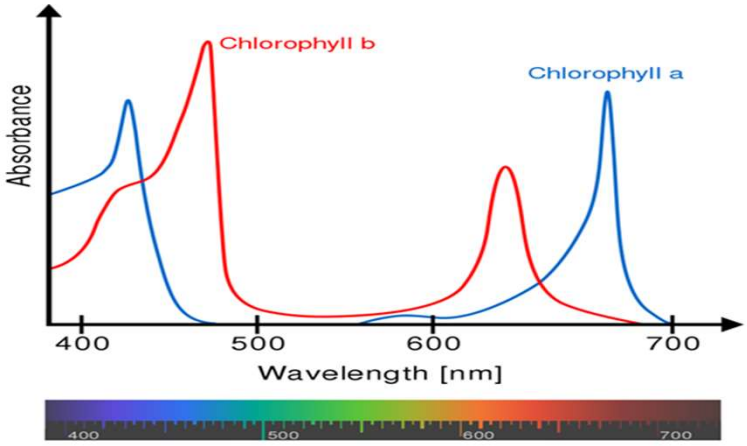
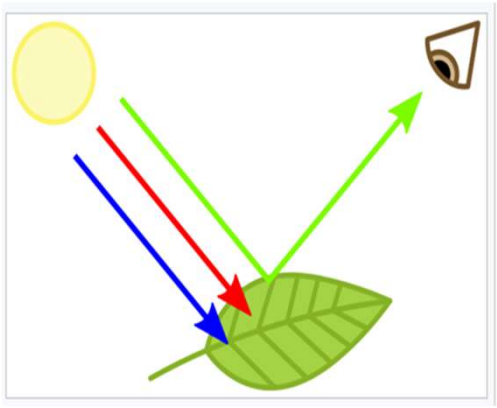
- Chlorophylls absorb light most strongly in the blue portion of the electromagnetic spectrum as well as the red portion.
- Conversely, it is a poor absorber of green and near-green portions of the spectrum. Hence chlorophyll-containing tissues appear green because green light is less absorbed.





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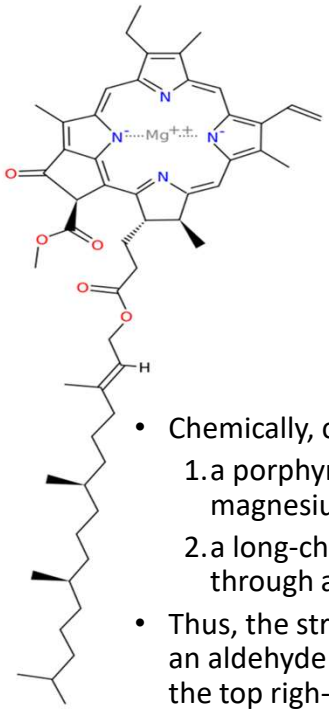
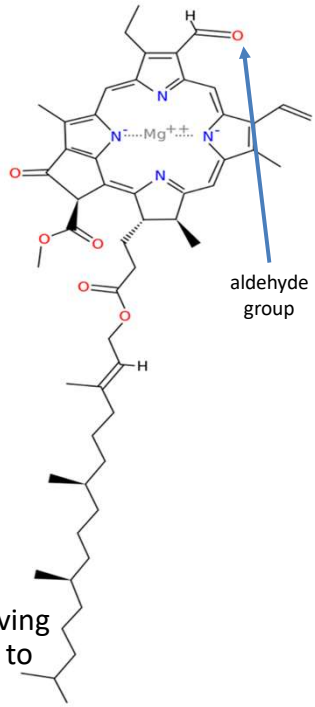


	← Chlorophyll a	Chlorophyll b →
Molecular formula	$C_{55}H_{72}O_5N_4Mg$	$C_{55}H_{70}O_6N_4Mg$
C2 group	$-CH_3$	$-CH_3$
C3 group	$-CH=CH_2$	$-CH=CH_2$
C7 group	$-CH_3$ methyl	$-CHO$ aldehyde
C8 group	$-CH_2CH_3$	$-CH_2CH_3$
C17 group	$-CH_2CH_2COO-$Phytyl	$-CH_2CH_2COO-$Phytyl
C17–C18 bond	Single (chlorin)	Single (chlorin)



- Chemically, chlorophyll contain
  1. a porphyrin (tetra-pyrrole) nucleus with a chelated magnesium atom in the centre and
  2. a long-chain hydrocarbon (phytyl) side chain attached through a carboxylic acid group.
- Thus, the structure of chlorophyll b only differs from a in having an aldehyde group instead of a methyl substituent attached to the top righ-hand pyrrole ring

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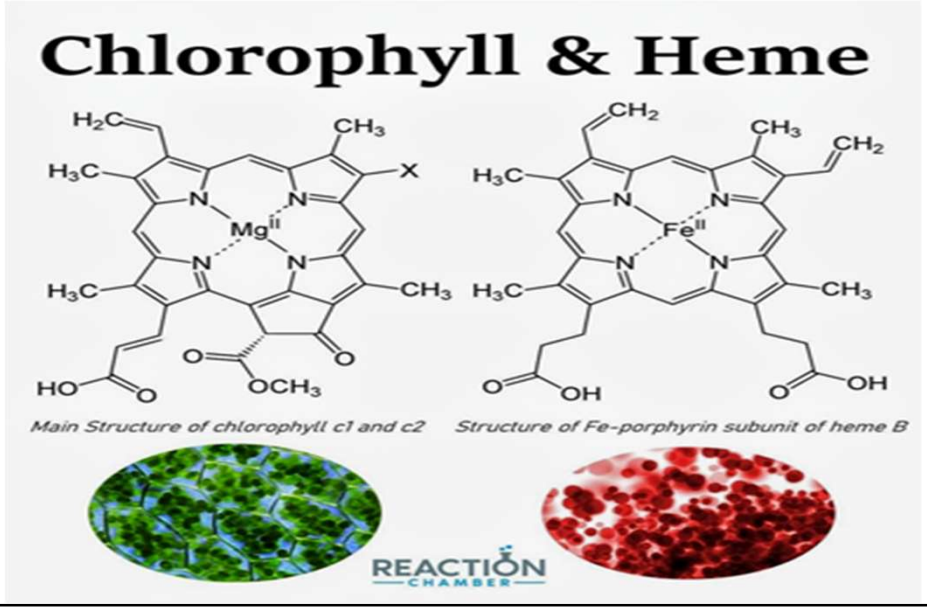
Unlike hemes, which feature iron at the center of the tetrapyrrole ring, chlorophylls bind magnesium.



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In last lecture

في المحاضرة السابقة تحدثنا عن

N-containing compounds

- Nitrogen compounds – nitrogen element

N-containing Primary Compounds

- Amino acids → Peptides → Protein
- Chlorophylls

- directly involved in normal growth, development, and reproduction of plant species

N-containing Secondary Compounds

- Alkaloids
- Cyanogenic glycosides
- Glucosinolates
- Amines

- not directly involved in those processes, but usually has important ecological functions

- They are less common in plants than Terpenes and Phenolics.
- They are synthesized from aliphatic and aromatic amino acids.

1

- **Today, we will cover the following:**

Fatty acids and lipids

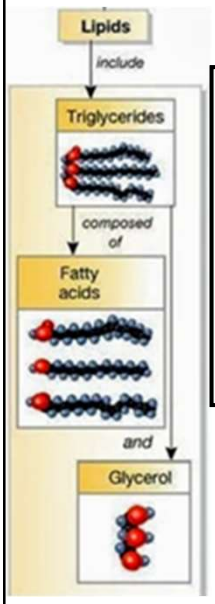
Carbohydrates

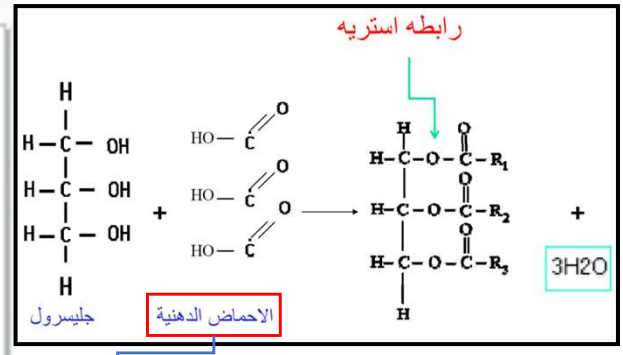
Methods of extraction, separation, and identification - The plant material

2

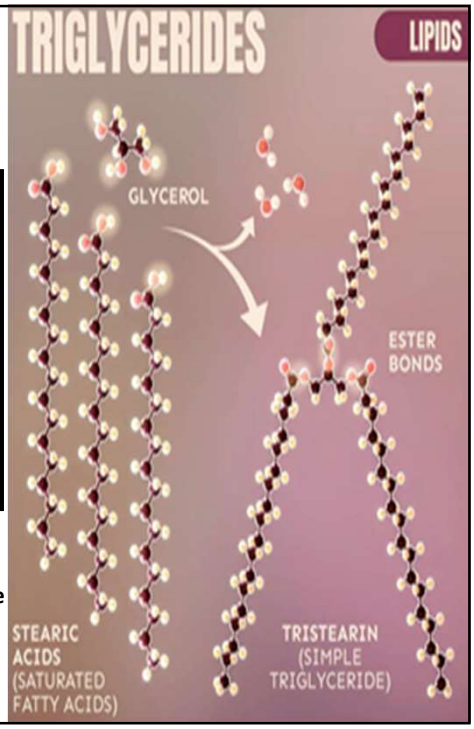
Fatty acids and lipids

- Mainly occurs in bound form, esterified to glycerol as lipid





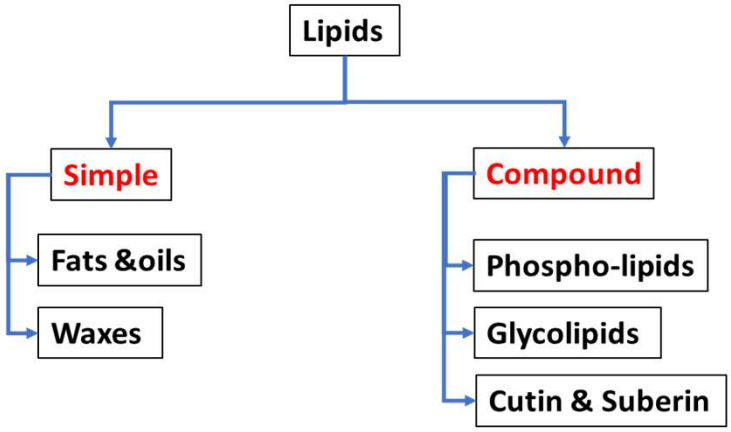
- Fatty acids
  - Saturated مشبعة
    - Lack double bonds between the individual carbon atoms
    - Tend to be solid at room temperature
  - Unsaturated غيرمشبعة
    - At least one double bond in the fatty acid chain
    - Usually liquid



3

Fatty acids and lipids

- Comprise up to 7% of the dry weight in leaves in higher plants and are important as membrane constituents in chloroplast and mitochondria.
- Abundantly in seeds or fruit to provide plants with a storage form of energy to use during germination.



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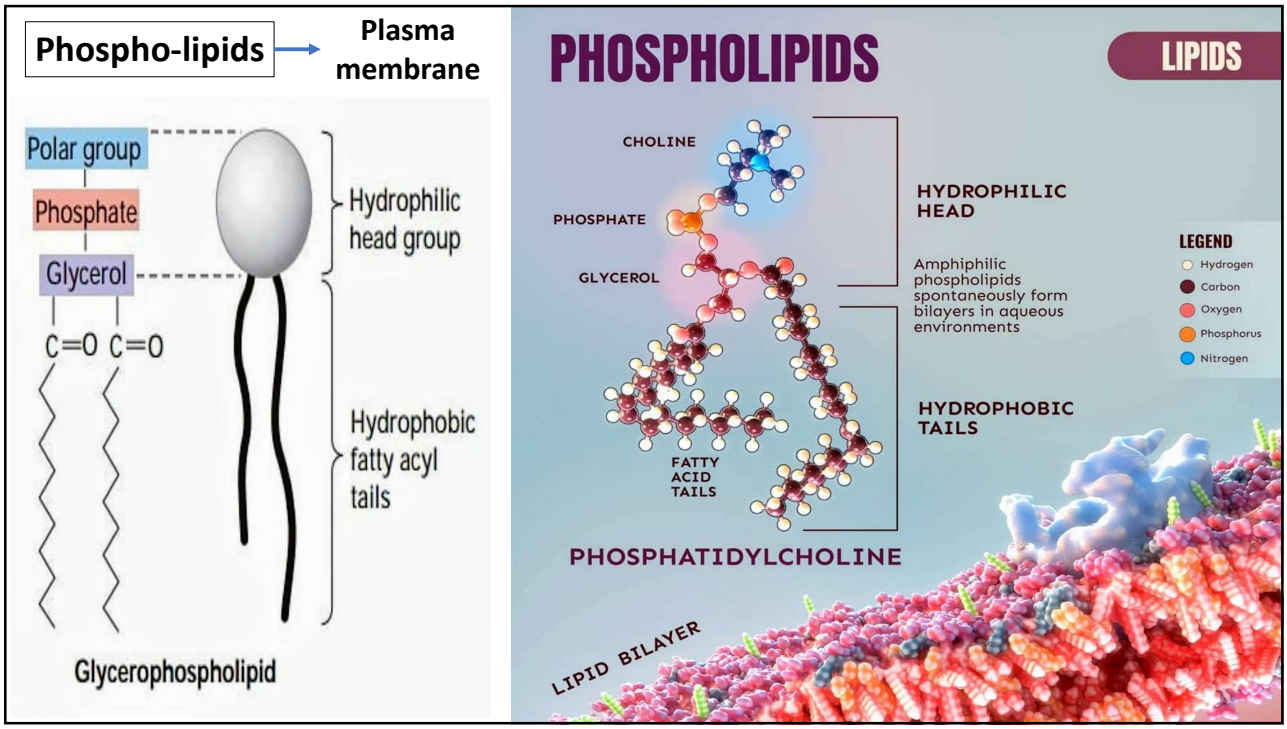

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Surface protection

- All plant parts exposed to the atmosphere are coated with layers of lipid materials that reduce water loss and help block the entry of pathogenic fungi and bacteria.
- The principal types of coatings are ;
  1. **Cutin :** found on most aboveground parts.
  2. **Suberin :** suberin is present on underground parts, woody stems, and healed wounds.
  3. **Waxes :** associated with both cutin and suberin.

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Cutin

- The cutin is a macromolecule,

  a polymer consisting of many long-chain fatty acids that are attached to each other by ester linkages, creating a rigid three-dimensional network.

- The cutin is a major component of plant cuticle.

- The cuticle is a multilayered secreted structure that coats the outer cell wall of epidermis on the areal parts

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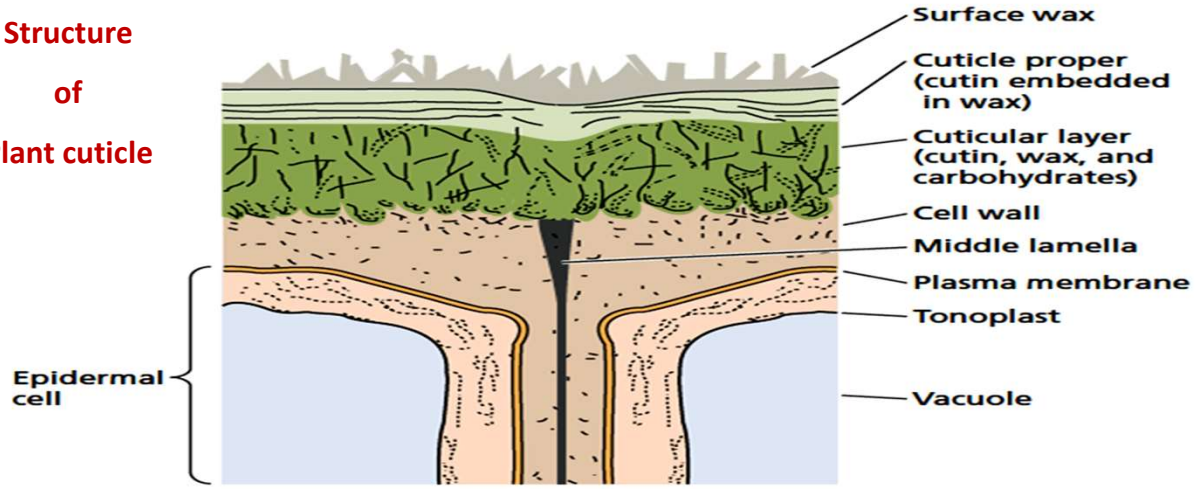
Cutin

Structure of Plant cuticle



The cuticle is composed of: 1- a top coating of wax, 2- a thick middle layer containing cutin embedded in wax (**the cuticle proper**), and a lower layer formed of cutin and wax blended with the cell wall substances pectin, cellulose, and other carbohydrates (**the cuticular layer**).

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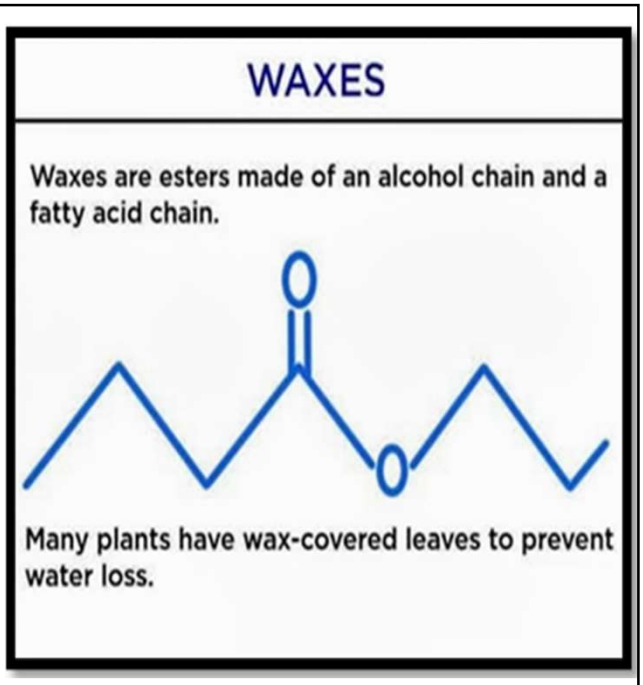
Waxes

- The waxes are complex mixtures of long-chain lipids that are extremely hydrophobic.
- They are synthesized by epidermal cells.
- They leave the epidermal cells as droplets that pass-through pores in the cell wall.



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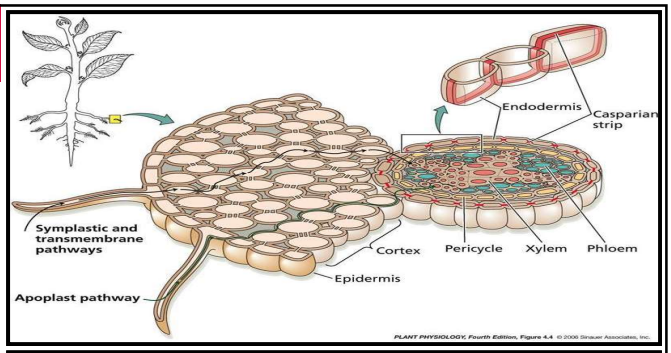
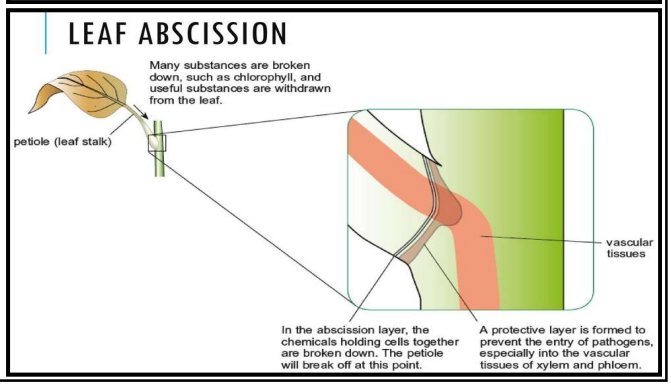
Suberin

- Suberin is also formed from fatty acids but has a different structure from cutin.
- Suberin is a principal component of the outer cell walls of all underground organs.
- Older parts of roots more suberized.
- Suberin is present in the Casparian strip of root endodermis.
- Suberin is also found at sites of leaf abscission and in areas damaged by disease or wounding.





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Cutin, Suberin and Waxes

- Cutin, suberin, waxes are made of hydrophobic compounds.
- They form barriers between the plant and its environment that function to keep water in and pathogens out.
- The cuticle and suberized tissue are important in excluding fungi and bacteria.
- However, many fungi can penetrate directly through the plant surface by mechanical means, and others fungi produce the cutinase enzyme that hydrolyzes cutin and thus facilitates entry of the pathogen into the plant.

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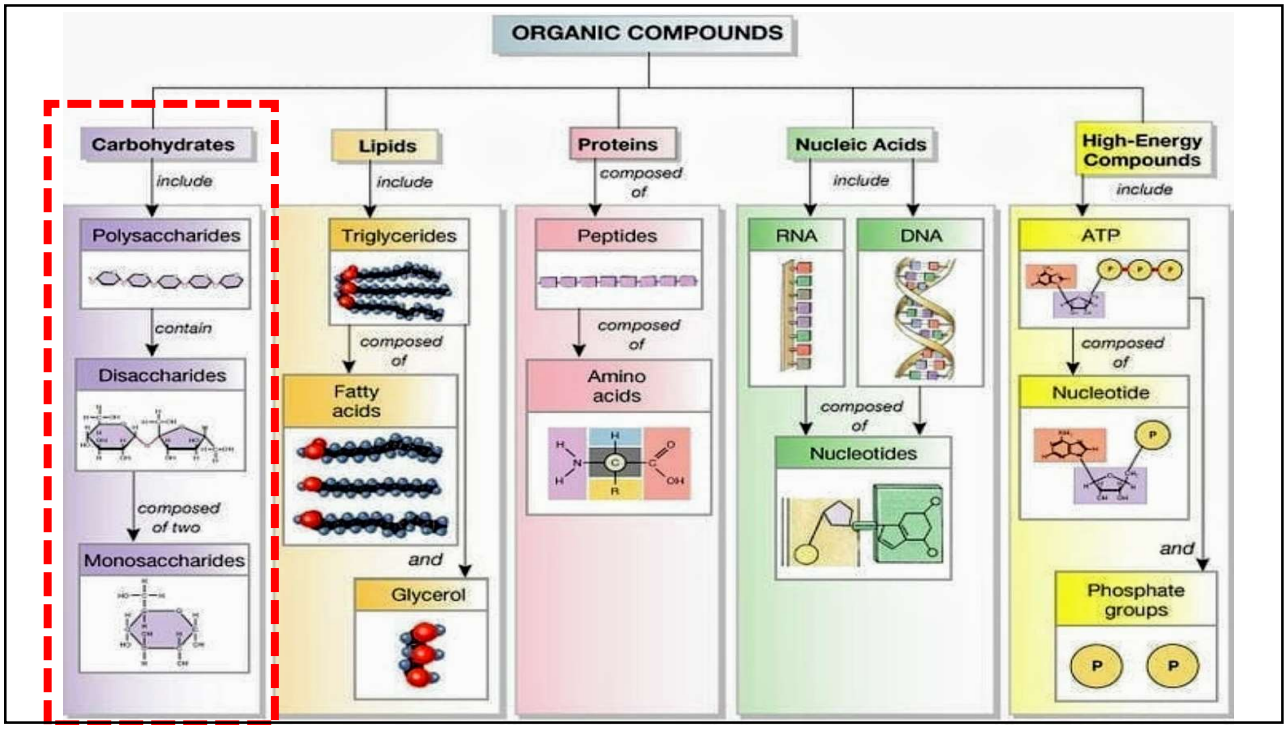

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الكربوهيدرات

Carbohydrates

- وتعني ماءات الكربون

$(CH_2O)_n \quad n \geq 3$

(Carbo)
كربون

(Hydrate)
ماء

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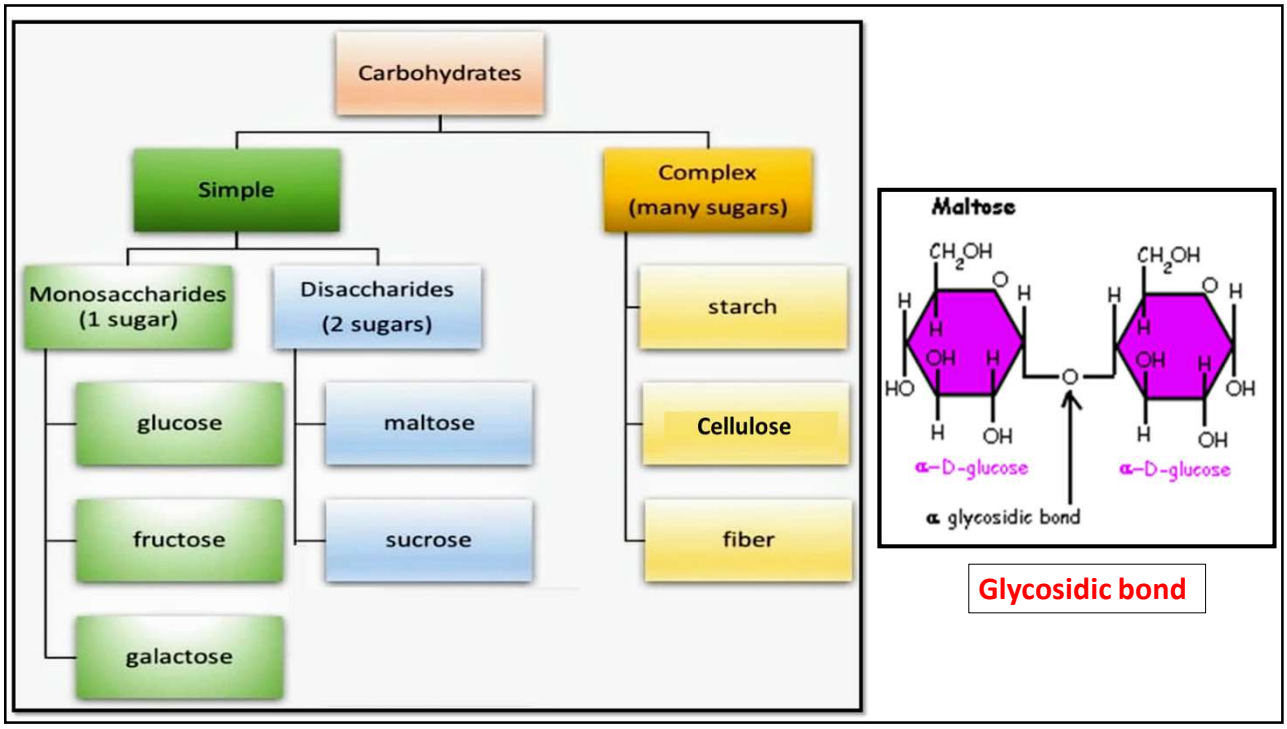

Glycosidic bond

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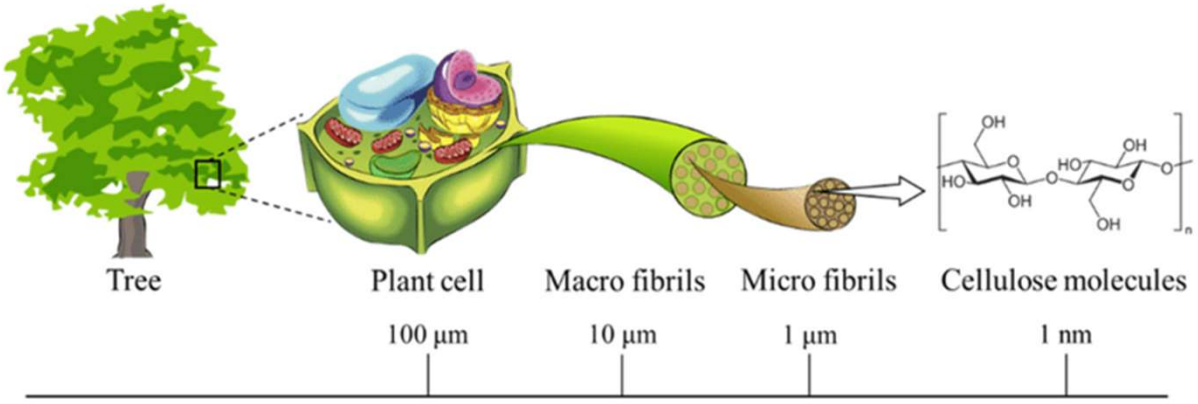
➢ **Plant cell walls are primarily made of cellulose, which is the most abundant macromolecule on Earth. Cellulose fibers are long, linear polymers of hundreds of glucose molecules.**



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Methods of extraction, separation, and identification -The plant material

Extraction

Phytochemicals	Petroleum ether	Chloroform	Ethanol
Phenol/polyphenols	+	+	+
Terpenoids/steroids	-	+	+
Flavonoids	+	-	+
Saponins	+	+	+
Alkaloids	+	-	-
Tannins	-	+	+

- Take note for the nature of the compounds:
  - Essential oil – sensitive to temp change and decreased over time– avoid drying
  - Flavonoids and alkaloids – remarkably stable with time
  - Tannin – better to extract from vacuum-dried fresh leaves rather than air-dried

Separation

- Chromatography
  - Paper chromatography (PC)
  - Thin layer chromatography (TLC)
  - Gas chromatography (GC)
  - High performance liquid chromatography (HPLC)
- The choice of technique depends on the solubility properties and volatilities of the compounds
  - PC – applicable to water-soluble compounds
  - TLC – separating lipid-soluble compounds
  - GC – volatile compounds
  - HPLC – less volatile compounds and polar compounds

Identification

- Methods
  - UV and visible spectroscopy
  - Infrared spectroscopy (IR)
  - Mass spectroscopy (MS)
  - Nuclear magnetic resonance spectroscopy (NMR)

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