

BIOSYNTHESIS OF SECONDARY METABOLITES IN PLANTS

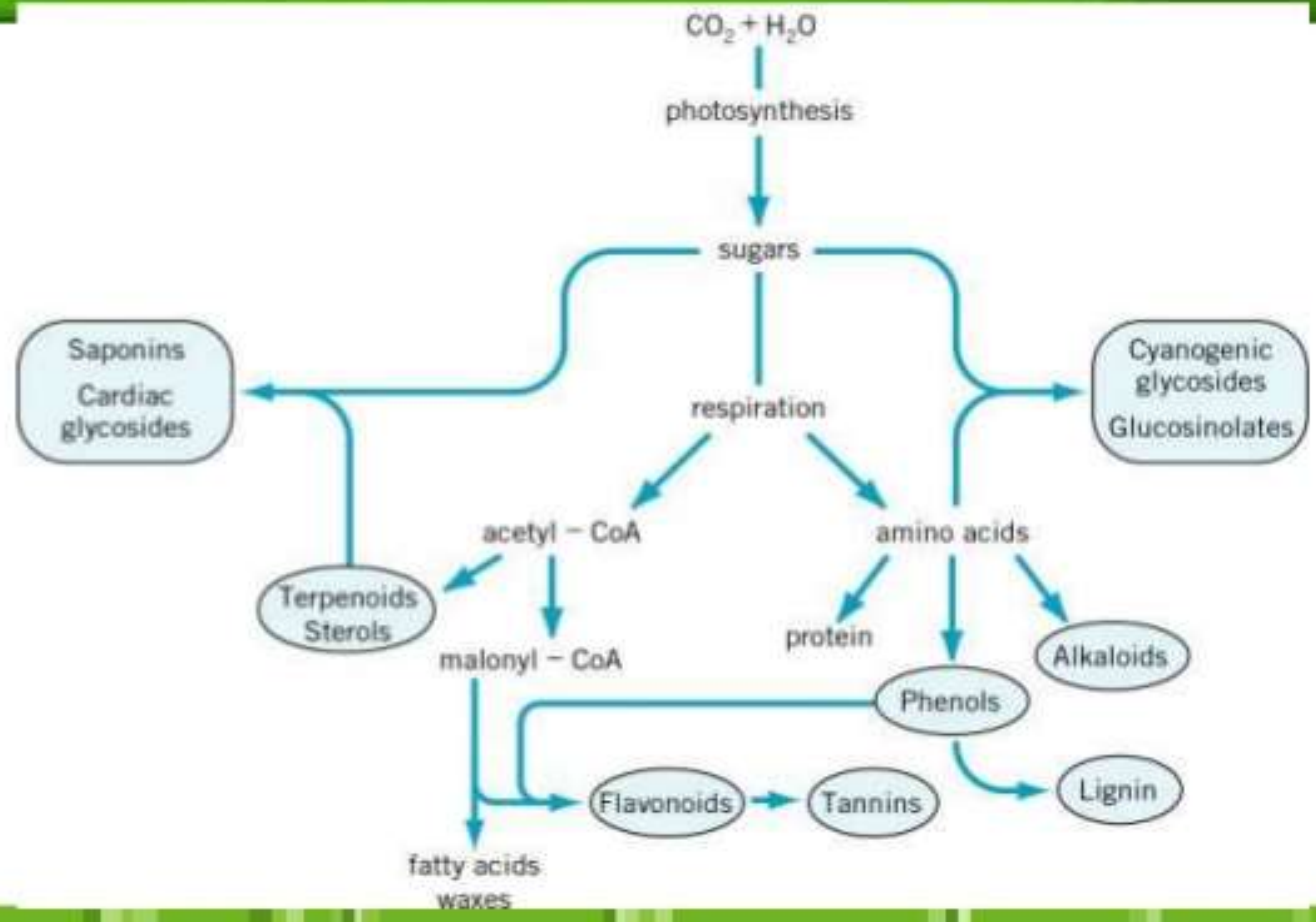
BIOSYNTHESIS

- It is a process of forming larger organic compounds from small subunits within a living organism.
- It is mainly done by enzymes.
- It is also known as anabolism since simple compounds are joined together to form macromolecules by enzymes. Eg. Photosynthesis

BIOSYNTHESIS

- Living plants are solar-powered biochemical and biosynthetic laboratory which manufactures both primary and secondary metabolites from air, water, minerals and sunlight.
- The primary metabolites like sugars, amino acids & fatty acids that are needed for general growth & physiological development of plant which distributed in nature & also utilized as food by man.
- The secondary metabolites such as alkaloids, glycosides, Flavonoids, volatile oils etc are biosynthetically derived from primary metabolites.
- Biosynthetic reactions are replica of common organic reactions like catalytic reactions, phosphorylation, hydride transfer, oxidation, elimination, acylation, alkylation, reduction, condensation, rearrangement etc.

Secondary metabolites are derived from primary metabolites



METABOLIC PATHWAYS

Major Metabolic Pathways:

- Glycolysis
- Anaerobic respiration
- Kreb's cycle / Citric acid cycle
- Oxidative phosphorylation - Creation of energetic compounds from non-living matter
- Photosynthesis (plants, algae cyanobacteria) Chemosynthesis (some bacteria)

Other pathways occurring in (most or) all living cell:

- Fatty acid oxidation (β -oxidation)
- Gluconeogenesis HMG-CoA reductase pathway (isoprene prenylation)
- Pentose phosphate pathway (hexose monophosphate)
- Porphyrin synthesis (or heme synthesis) pathway
- Urea cycle

Metabolites

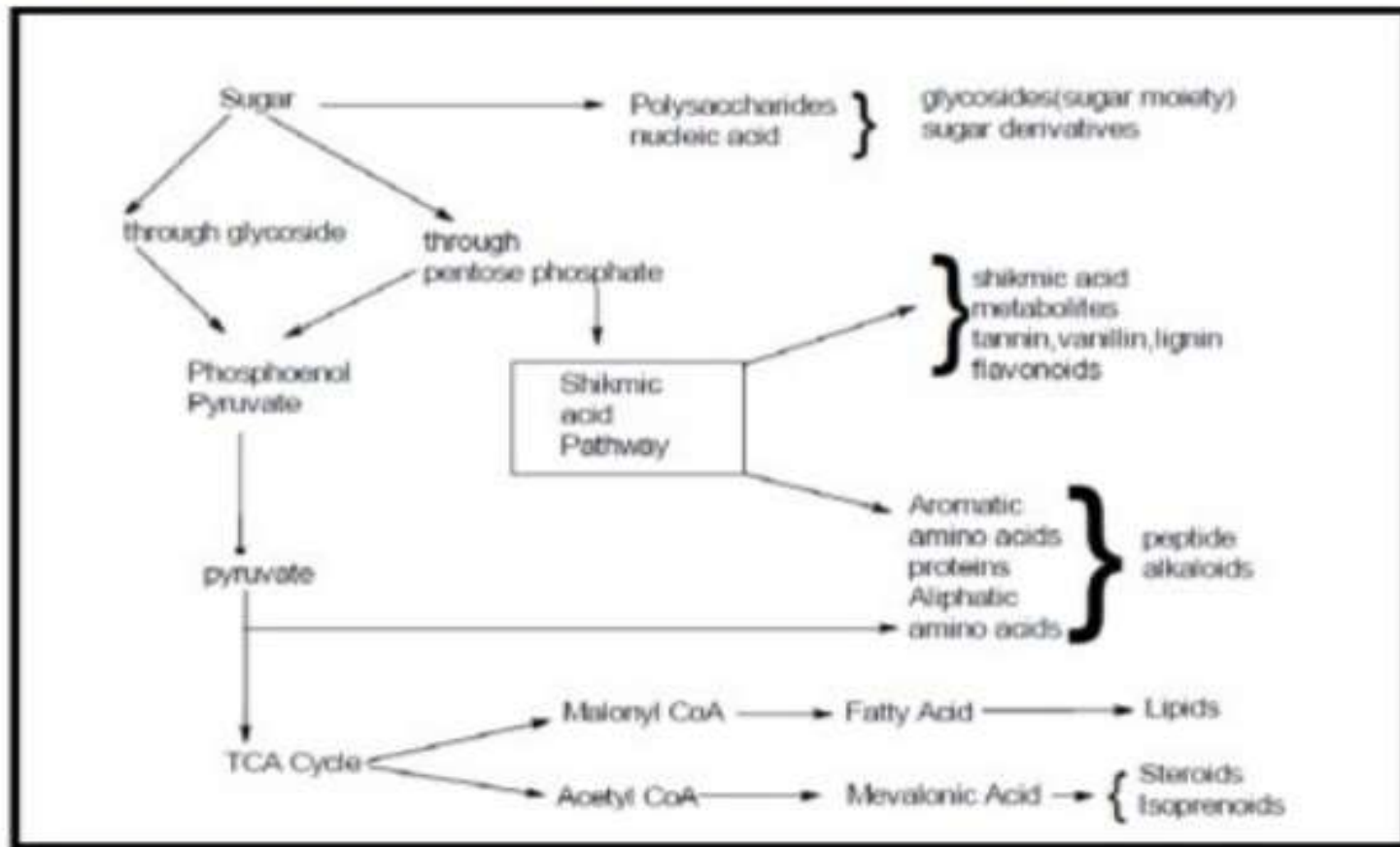
- Metabolites are the intermediates & products of metabolism. The term metabolite is usually restricted to small molecules.
- A primary metabolite is directly involved in the normal growth, development, and reproduction.
- A secondary metabolite is not directly involved in those processes, but usually has important ecological function.

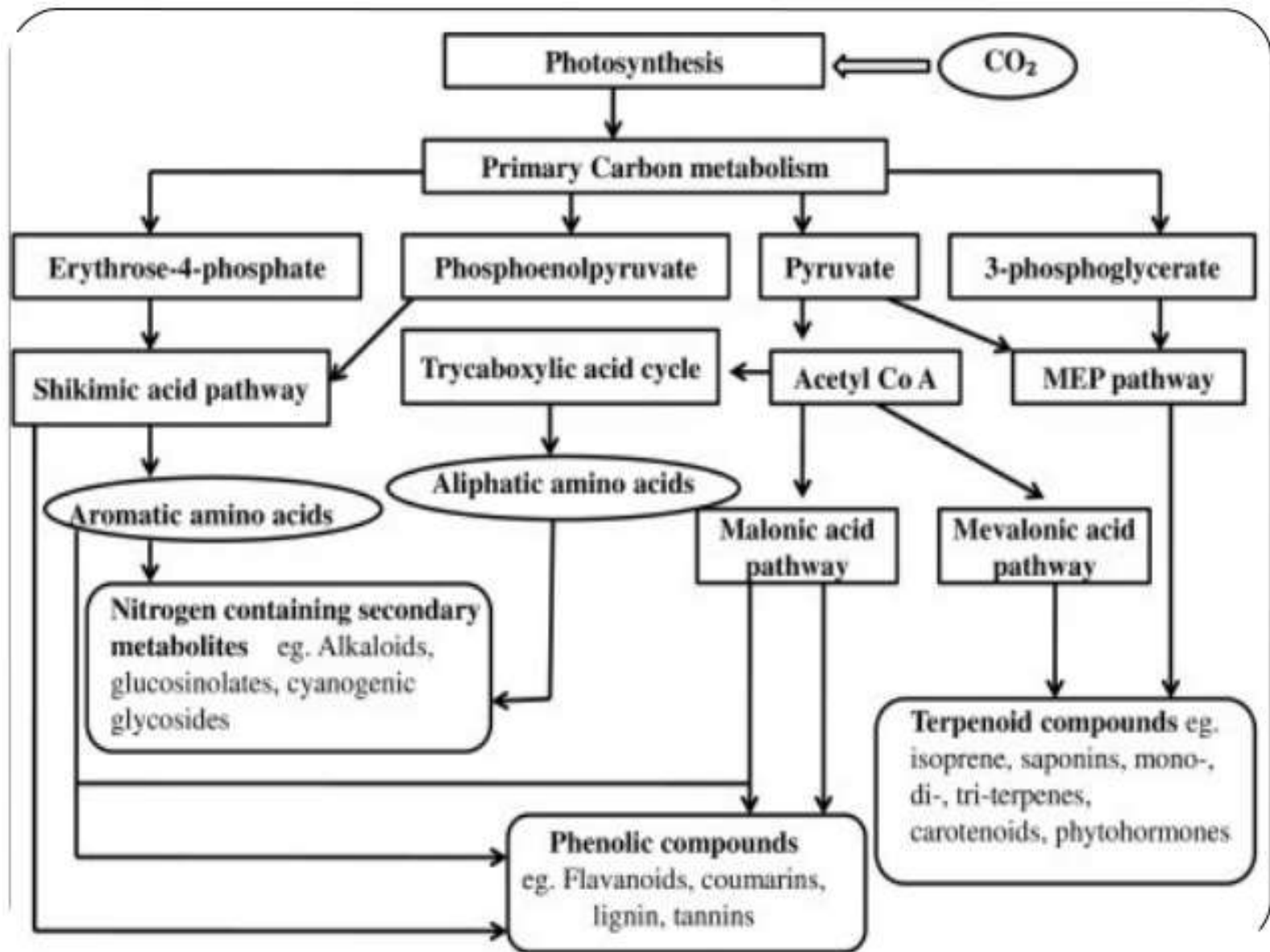
Biosynthetic Pathway of Secondary Metabolites:

It involves 3 basic mechanism:

1. Shikimic acid Pathway
2. Acetate-mevalonate pathway
3. Acetate malonate pathway

The primary and secondary metabolites derived from carbon metabolism





TRACER TECHNIQUES

It can be defined as technique which utilizes radioactive labelled compound (**radiotracers**) to find out or to trace the path that the radioisotopes follow from the reactant (presumed precursor) to different intermediates to products, at a given rate & time.

In this technique different isotope, mainly the radioactive isotopes which are incorporated into one or more atoms of presumed precursor of plant metabolites and are used as marker in biogenic experiments. (^3H , ^{14}C , ^{35}S)

ISOTOPES

- Isotopes – Iso means equal, topes means place – occupy same place in atomic table.
- Elements with same atomic number but different atomic weight. (same number of protons but differ in neutrons)
- Example: carbon isotopes ($^{12}_6\text{C}$, $^{13}_6\text{C}$ and $^{14}_6\text{C}$) and Hydrogen isotopes -???
- The labelled compound can be prepared by use of two types of isotopes.
- Radioactive isotopes and stable isotopes
- **Radioactive isotopes** - decay with emission of radiation (α , β and γ form). e.g. ^3H , ^{14}C , ^{24}Na , ^{42}K , ^{35}S , ^{35}P , ^{131}I – unstable isotopes
- For biological investigation – carbon & hydrogen.
- For metabolic studies – S, P, and alkali and alkaline earth metals are used.
- For studies on protein, alkaloids, and amino acid – labelled nitrogen atom give more specific information.
- **Stable isotopes** – do not emit radiation.[e.g. ^2H , ^{13}C , ^{15}N , ^{18}O] – Used for labelling compounds as possible intermediates in biosynthetic pathways.
- Usual method of detection are: – MASS spectroscopy [^{15}N , ^{18}O] – NMR spectroscopy [^2H , ^{13}C]

SIGNIFICANCE OF TRACER TECHNIQUE

- Tracing of Biosynthetic Pathway. Eg. By incorporation of radioactive isotope of ^{14}C into phenylalanine, the biosynthetic cyanogenetic glycoside **prunasin**, can be detected.
- Location & Quantity of compound containing tracer – Eg. ^{14}C labelled glucose is used for determination of **glucose** in biological system
- Different tracers for different studies - For studies on **nitrogen and amino acid**. (Labelled nitrogen give specific information than carbon)
- **Convenient and suitable** technique

CRITERIA FOR TRACER TECHNIQUE

- The starting **concentration** of tracer must be sufficient to withstand resistance with dilution in course of metabolism.
- Proper Labelling - for proper **labelling** physical & chemical nature of compound must be known.
- Labelled compound **should involve in the synthesis** reaction.
- Labelled compound **should not damage the system** to which it is used.

ADVANTAGES

- High sensitivity.
- Applicable to all living organism.
- Wide ranges of isotopes are available.
- More reliable, easily administration & isolation procedure.
- Gives accurate result, if proper metabolic time & technique applied.

LIMITATIONS

- Kinetic effect
- Chemical effect
- Radiation effect
- Radiochemical purity
- High concentration distorting the result.

STEPS INVOLVED IN TRACER TECHNIQUE

- Selection of radioisotopes
- Preparation of labeled compound (precursor or intermediate)
- Introduction of labelled compound into a biological system (plant part) at specific site and at correct time.

PRECAUTIONS

- The precursor should react at necessary site of synthesis in plant
- Plant at the experiment time should synthesize the compound under investigation
- The dose given is for short period.
 - Root feeding – eg. Tobacco and Datura alkaloids
 - Stem feeding – cut end of stem immersed in water, nutrients and labelled compound.
 - Direct injection into hollow stem or capsular fruits
 - Infiltration – plant rooted in soil – compound enters through cotton wick
 - Floating method – leaf discs or chopped pieces are taken in substrate solution.
 - Spray technique – sprayed over leaves

STEPS INVOLVED IN TRACER TECHNIQUE

- Time to time to separation & determination of labelled compound in various biochemical fractions at later time.
 - Geiger – Muller counter
 - Liquid Scintillation counter
 - Gas ionization chamber
 - Bernstein – Bellentine counter
 - Mass spectroscopy
 - NMR eletrodemeter
 - Autoradiography
 - Radio paper chromatography
- Establishment of precursor –intermediate-product relation

METHODS OF TRACER TECHNIQUES

- PRECURSOR PRODUCT SEQUENCE
- DOUBLE & MULTIPLE LABELLING
- COMPETITIVE FEEDING
- ISOTOPE INCORPORATION
- SEQUENTIAL ANALYSIS

APPLICATIONS OF TRACER TECHNIQUES

- Study of **squalene cyclization** by use of ^{14}C , ^3H labelled mevalonic acid.
- **Inter relationship among 4 – methyl sterols & 4, 4 dimethyl sterols**, by use of ^{14}C acetate
- **Terpenoid biosynthesis** by chloroplast isolated in organic solvent, by use of ^{14}C mevalonate.
- Study the **formation of cinnamic acid** in pathway of coumarin from labelled coumarin.
- **Origin of carbon & nitrogen atoms of purine ring system** by use of ^{14}C or ^{15}N labelled precursor.
- **Study of formation of scopoletin** by use of labelled phenylalanine.
- By use of ^{45}Ca as tracer - found that the **uptake of calcium by plants** from the soil. (CaO & CaCO_2).

SHIKIMIC ACID PATHWAY

- The Shikimic acid pathway is a key intermediate from carbohydrate for the biosynthesis of **C6-C3units** (phenyl propane derivative).
- The Shikimic acid pathway converts simple carbohydrate precursors derived from **glycolysis** and the **pentose phosphate pathway** to the aromatic amino acids.
- The shikimate pathway is a **7 step metabolic route** used by **bacteria, fungi, Algae, parasites, and plants** for the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan).
- This pathway is **not found in animals**; therefore, phenylalanine and tryptophan represent essential amino acids that must be obtained from the animal's diet.
- **Animals can synthesize tyrosine from phenylalanine**, and therefore is not an essential amino acid except for individuals unable to hydroxylate phenylalanine to tyrosine).

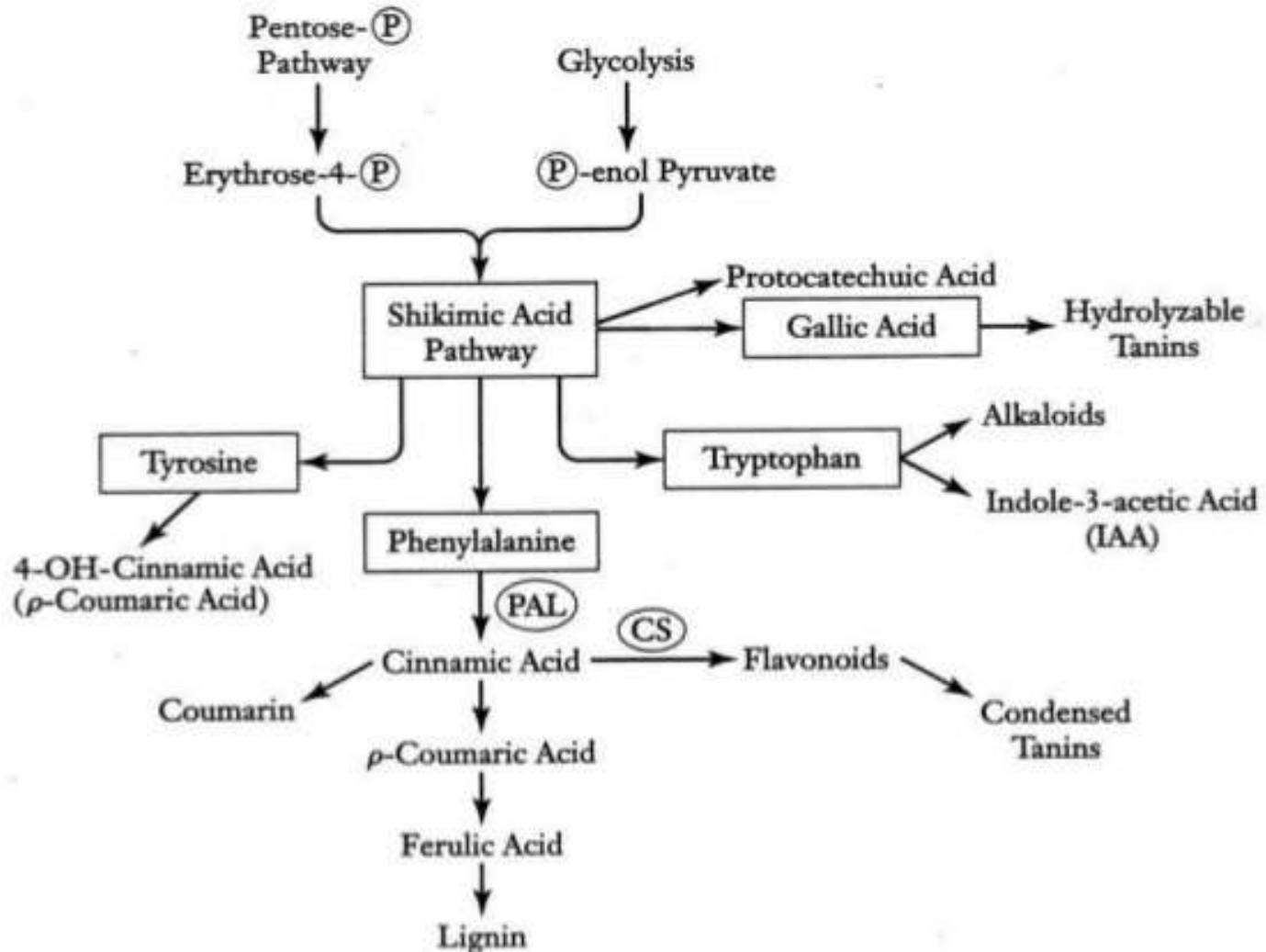
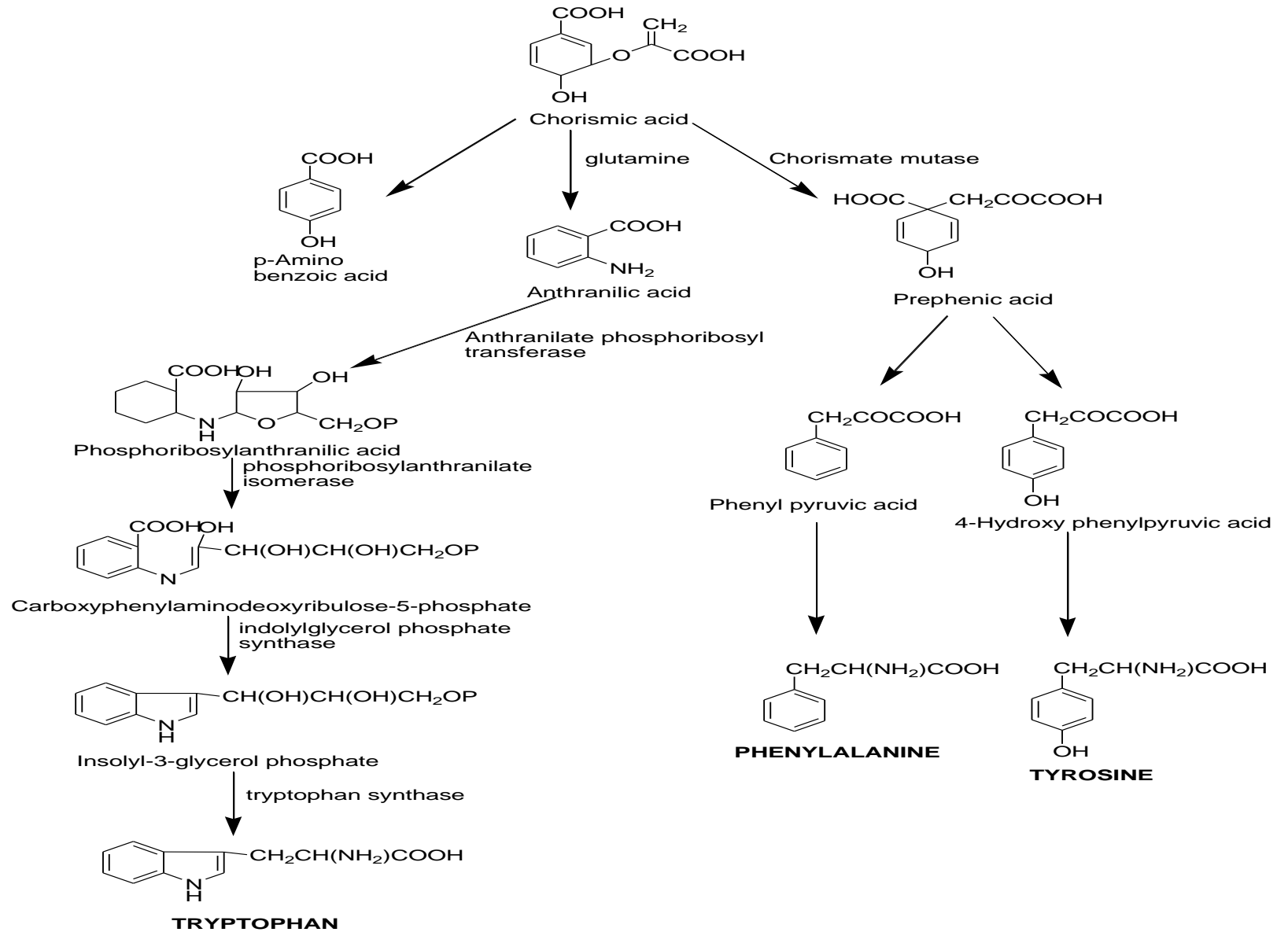


FIGURE 14.14 The central role of the shikimic acid pathway in the synthesis of various primary and secondary metabolites. PAL = phenylalanine ammonia lyase. CS = chalcone synthase.

ROLE OF SHIKIMIC ACID PATHWAY

- Starting Point in The Biosynthesis of Some Phenolics Phenyl alanine and tyrosine are the precursors used in the biosynthesis of phenylpropanoids.
- The phenylpropanoids are then used to produce the flavonoids, coumarins, tannins and lignin.
- **Gallic acid biosynthesis:** Gallic acid is formed from 3-dehydroshikimate by the action of the enzyme shikimate dehydrogenase to produce 3,5-didehydroshikimate. The latter compound spontaneously rearranges to gallic acid.
- Other compounds - Shikimic acid is a precursor for: Indole, indole derivatives and aromatic amino acid tryptophan and tryptophan derivatives such as the psychedelic compound dimethyltryptamine.

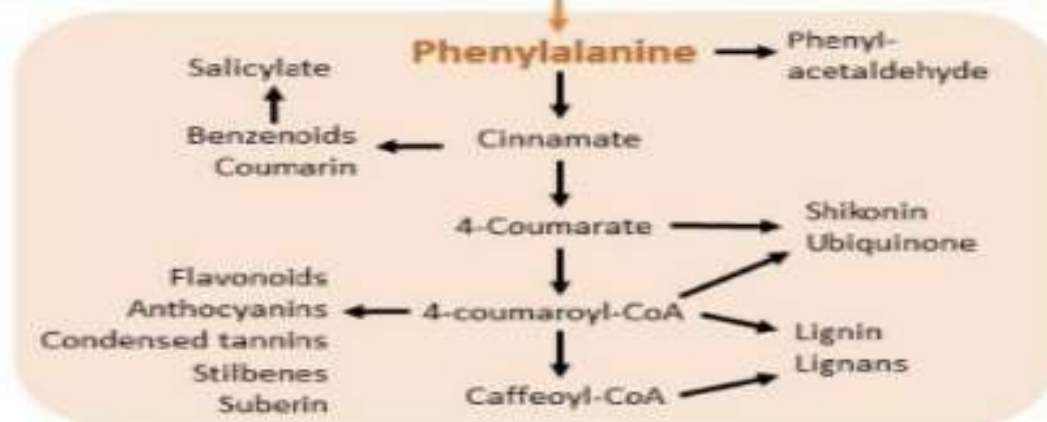
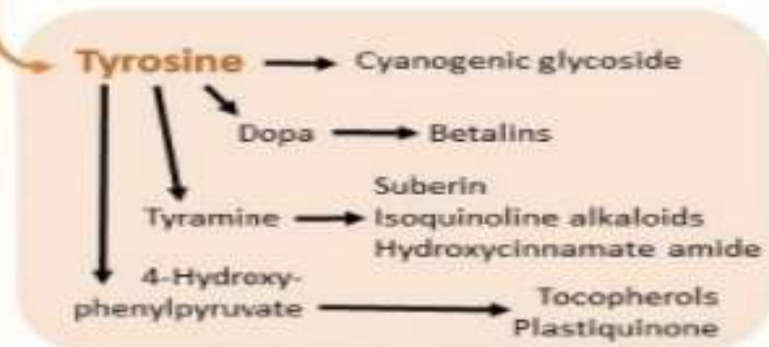
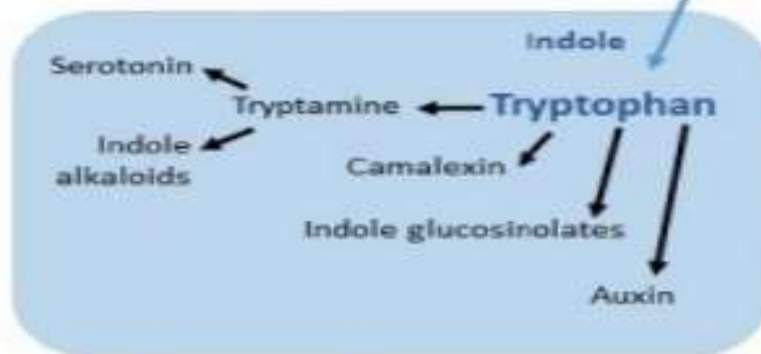
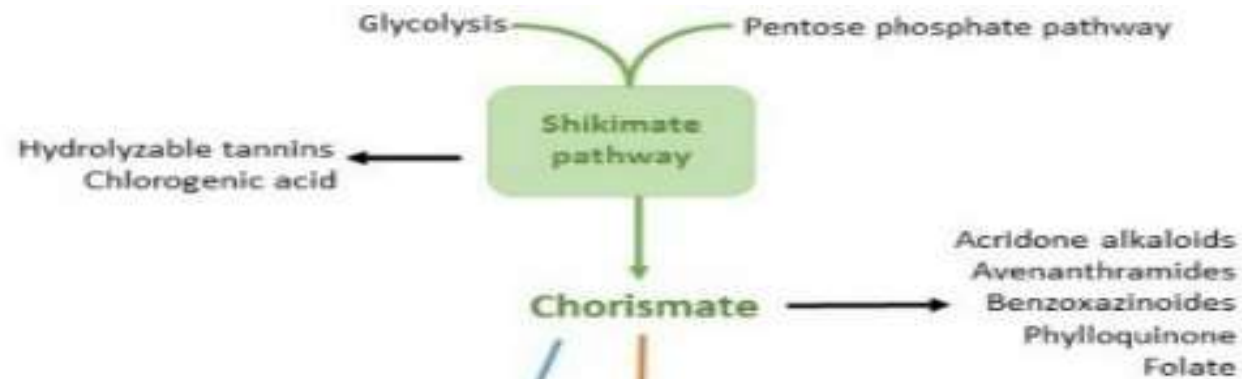


Applications:

- In the pharmaceutical industry, shikimic acid from the Chinese star anise (*Illicium verum*) is used as a base material for **production of oseltamivir (Tamiflu)**.

Target for drugs

- Shikimate can be used to synthesize **(6S)-6-Fluoroshikimic acid**, an antibiotic which inhibits the aromatic biosynthetic pathway.
- **Glyphosphate**, the active ingredient in the herbicide Roundup, kills plants by interfering with the shikimate pathway in plants. More specifically, glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). "Roundup Ready" genetically modified crops overcome that inhibition.



ACETATE – MEVALONATE PATHWAY

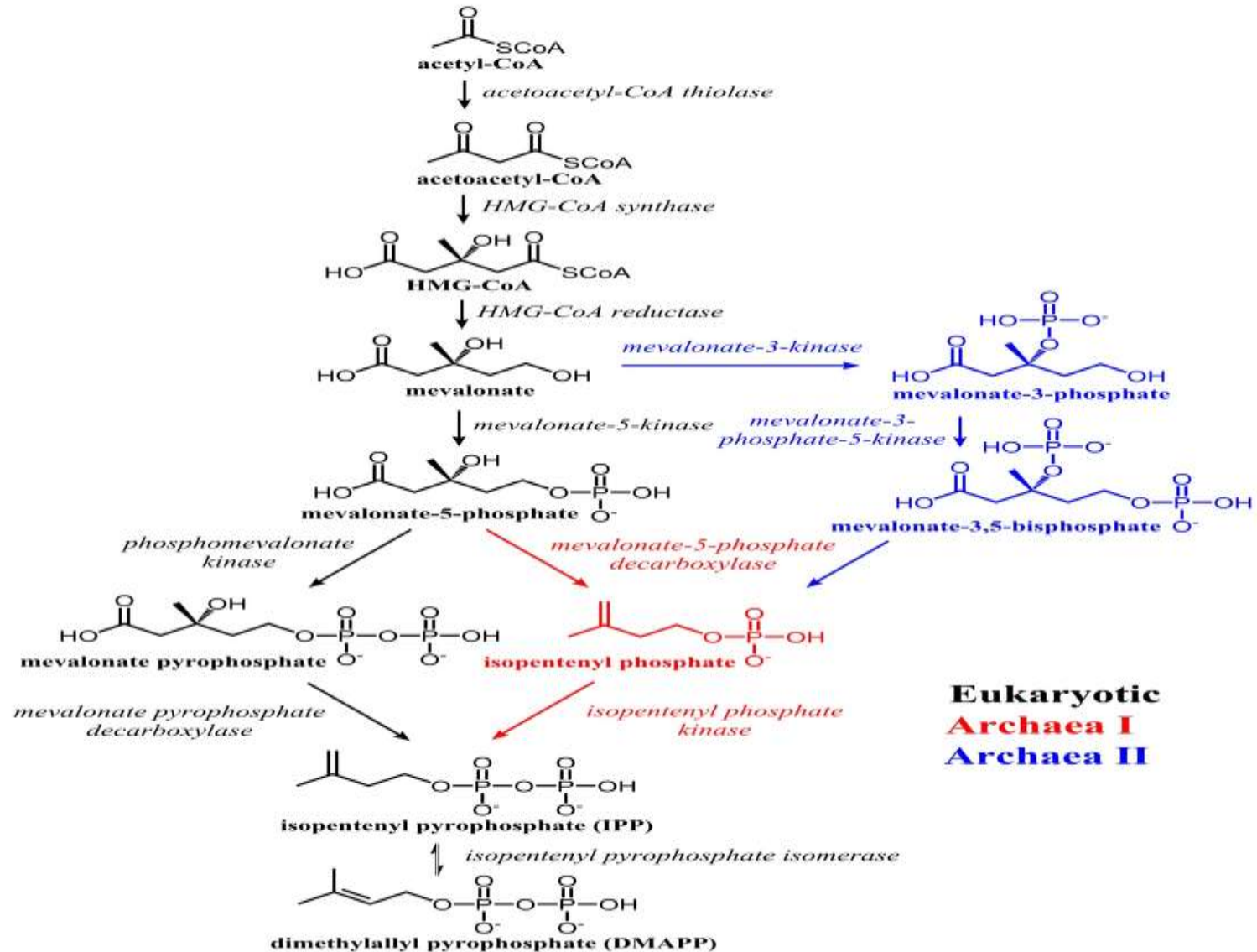
The mevalonate pathway, also known as the **isoprenoid pathway** or **HMG-CoA reductase pathway** is an essential metabolic pathway present in eukaryotes, archaea and some bacteria.

This pathway produces **two five carbon building blocks** called isopentenyl pyrophosphate (**IPP**) and dimethylallyl pyrophosphate(**DMAPP**), which are used to make isoprenoids, a diverse class of over 30,000 biomolecules such as **cholesterol, Vitamin K, Coenzyme Q10, and all steroidal hormones.**

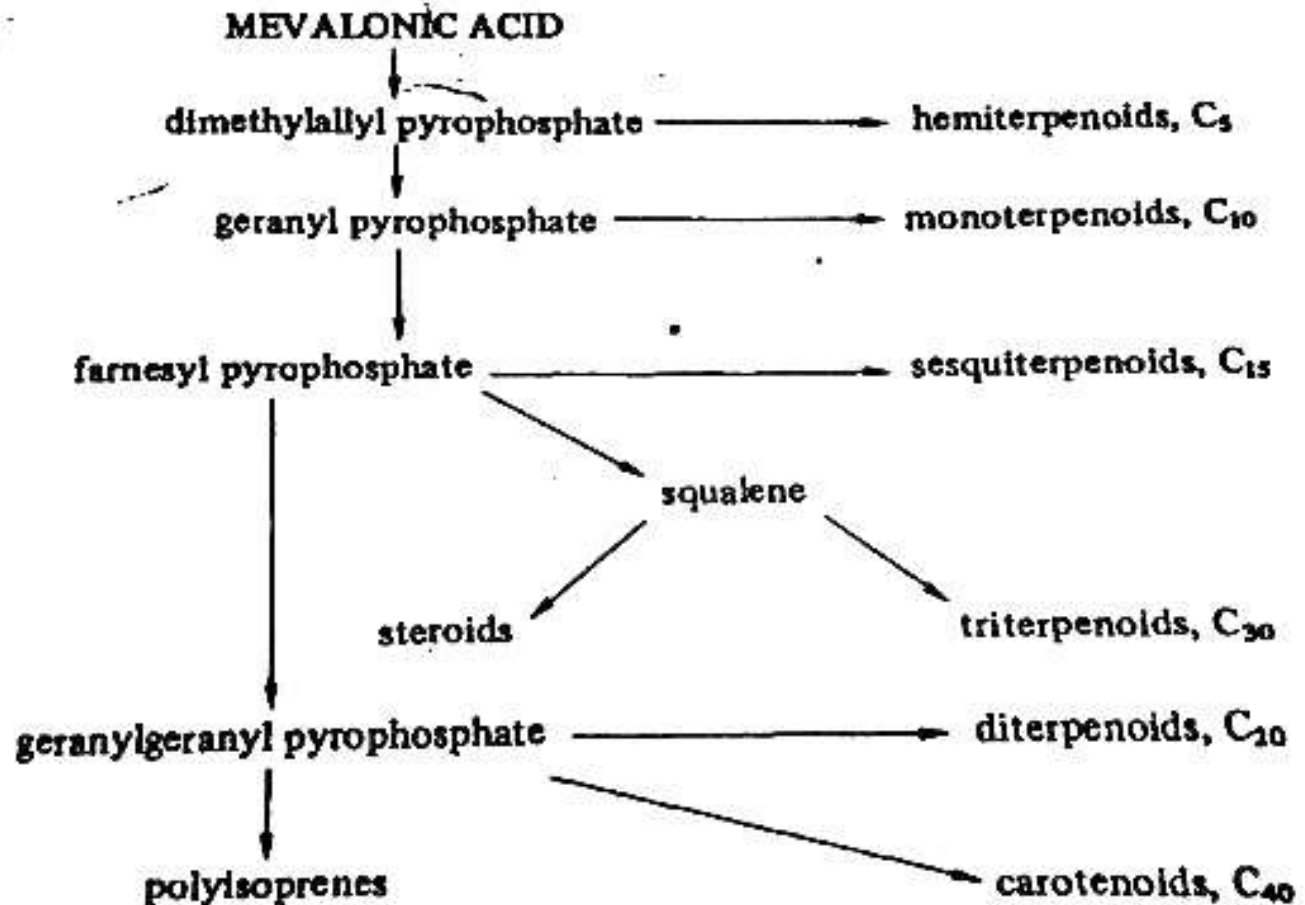
The pathway **starts with acetyl CoA** or active acetate and ends with formation of IPP and DMAPP.

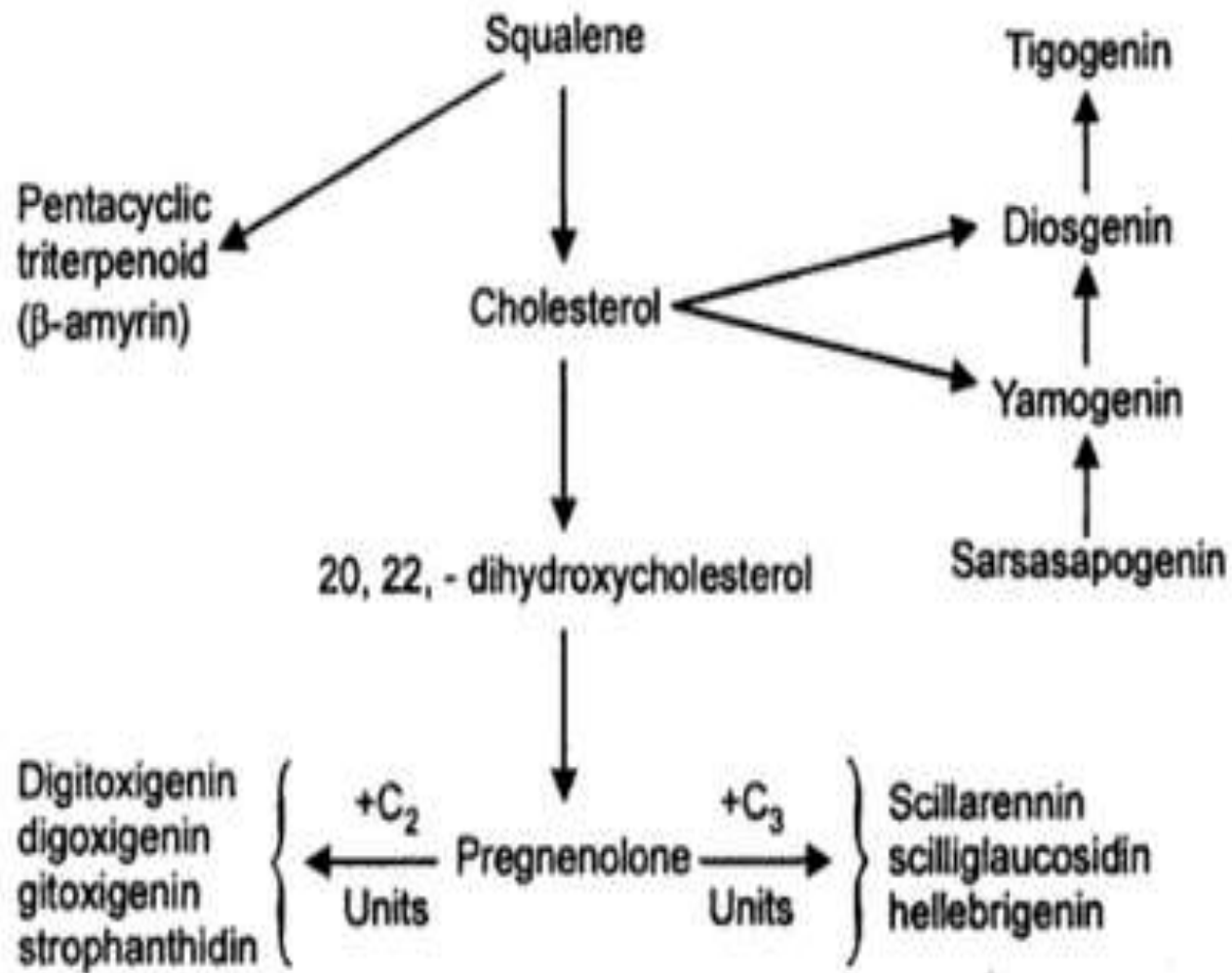
The acetate mevalonate pathway thus works through IPP and DMAPP via squalene to produce two different skeleton containing compounds, that is, **steroids and triterpenoids.** It also produces vast array of **monoterpenoids, sesquiterpenoids, diterpenoids, carotenoids, polyprenols,** and also the compounds like **glycosides and alkaloids in association with other pathways**

Mevalonate Pathway



Compounds biosynthesised by the Acetate- mevalonate pathway





Some secondary products of cholesterol metabolism

ACETATE MALONATE PATHWAY

It operates functionally with the involvement of acyl carrier protein (ACP) to yield fatty acyl thioesters of ACP.

The biosynthetic reaction pathway to a compound is usually not a simple opposite of its breakdown.

In fatty acid synthesis, acetyl-CoA is the direct precursor only of the methyl end of the growing fatty acid chain.

All the other carbons come from the acetyl group of acetyl-CoA but only after it is modified to provide the actual substrate for fatty acid synthase, malonyl-CoA.

Malonyl-CoA contains a 3-carbon dicarboxylic acid, malonate, bound to Coenzyme A.

Malonate is formed from acetyl-CoA by the addition of CO_2 using the biotin cofactor of the enzyme acetyl-CoA carboxylase.

ACETATE MALONATE PATHWAY

Formation of malonyl-CoA is the **commitment step** for fatty acid synthesis, because malonyl-CoA has no metabolic role other than serving as a precursor to fatty acids. Fatty acid synthase (FAS) carries out the chain elongation steps of fatty acid biosynthesis. FAS is a large **multienzyme complex**. In mammals, FAS contains two subunits, each containing multiple enzyme activities. In bacteria and plants, individual proteins, which associate into a large complex, catalyze the individual steps of the synthesis scheme.

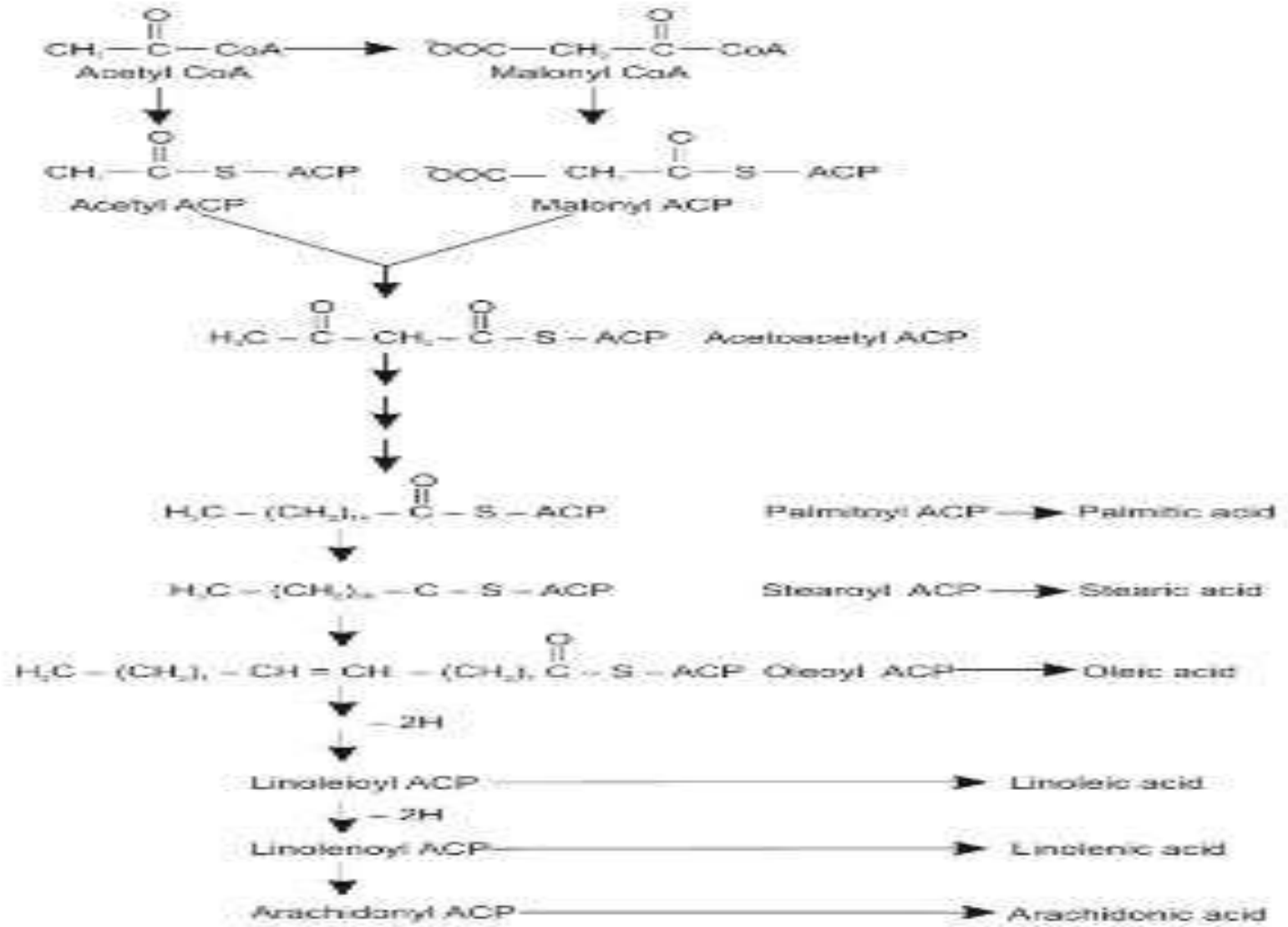
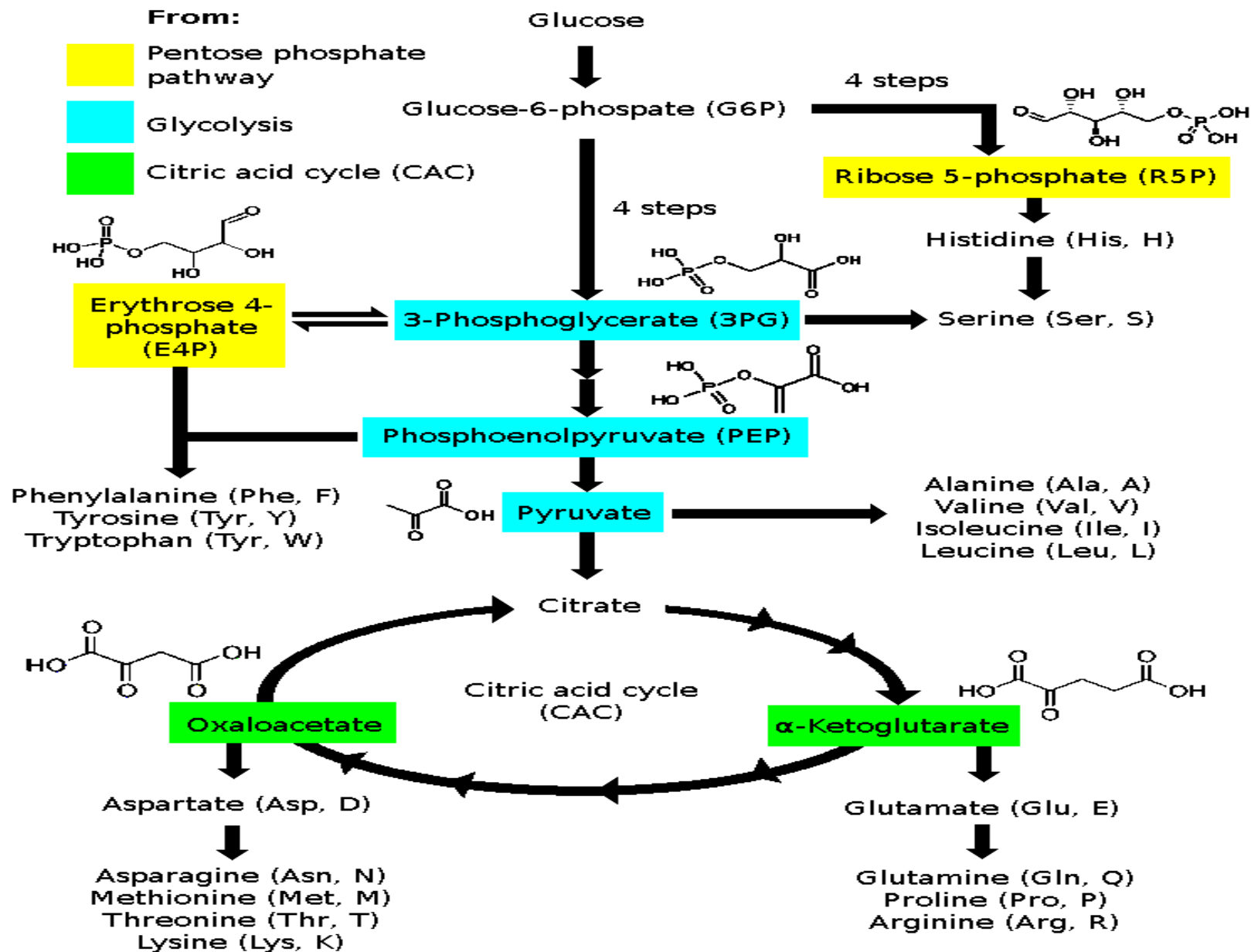


FIG. 13.7 Acetate-malonate pathway

AMINO ACID PATHWAY

- Amino acids are organic compounds containing amine (-NH₂) and carboxyl (-COOH) functional groups, along with a side chain (R group) specific to each amino acid.
- Nonpolar/hydrophobic amino acids - glycine, alanine, valine, leucine, methionine, phenylalanine, proline, isoleucine, tryptophan
- polar/hydrophilic amino acids - serine, cysteine, tyrosine, glutamic acid, aspartic acid, lysine, arginine, threonine, histidine
- Sulphur containing amino acids - Cystine, methionine
- All the 20 protein amino acids are derived from intermediates in glycolysis, citric acid cycle or the pentose phosphate pathway
- Nitrogen enters these pathways by way of glutamate and glutamine.
- Organisms vary greatly in their ability to synthesize the 20 common amino acids. Whereas most bacteria and plants can synthesize all 20, mammals can synthesize about half of them generally those with simple pathways.



Unit 1 - Assignment questions

1. What are tracer techniques? Write the applications.
2. Write the role and importance of shikimic acid pathway.
3. Explain acetate-mevalonate pathway
4. Summarize the steps involved in acetate pathway?
5. Illustrate shkimic acid pathway

1. Eugenol is obtained by-----pathway. []
a) flavonoidal b) phenolic c) stilbene d) monolignol
- 2 Putrescine is involved in formation of_____. []
a) terpenoids b) alkaloids c) flavonoids d) lipids
- 3 Tryptamine + Secologanin forms----- []
a) Sanguinarine b) Strictosidine c) Serpentine d) Strychnine
- 4 Triterpens obtained from -----pathway. []
a) malonyl b) HMP c) mevolonate d) shikimic acid
- 5 Tropane alkaloid formation can be investigated by----- []
a) mutant strains b) sequential analysis c) grafting method d) competitive feeding
- 6 Exposure time of $^{14}\text{CO}_2$ in menthol formation is for -----min []
a) 5 b) 15 c) 10 d) 20
- 7 An amino acid found in convolvulaceae family is----- []
a) tryptophan b) ornithinine c) phenylalanine d) tyrosine
- 8 Which among the following is a stable isotope used in tracer techniques? []
a) ^{15}N b) ^{14}C c) ^{24}Na d) ^{35}S
- 9 Which method determines the site of 1° & 2° metabolism for plant metabolites?
[]
a) Isolated organ b) Tracer techniques c) Grafts d) Mutants
- 10 Secologanin + dopamine is involved in formation of -----. []
a) vinblastine b) emetine c) tropane d) quinine

- 11** An amino acid derived from an intermediate prephenate. []
a) tryptophan **b)** proline **c)** phenylalanine **d)** leucine
- 12** Squalene cyclization studied by -----labelled mevalonic acid. []
a) 1 H **b)** 14C **c)** 2 H **d)** 13C
- 13** Macrolide antibiotics are synthesised by -----pathway. []
a) Acetate **b)** Malonyl **c)** Shikimic **d)** Amino acid
- 14** Formation of scopoletin was studied by labeled ----- []
a) phenylalanine **b)** proline **c)** tyrosine **d)** tryptophan
- 15** Ornithine is the precursor for ---- []
a) Atropine **b)** Ipecac **c)** Reserpine **d)** Squalene
- 16** Terpenoidal Indole alkaloid is ----- []
a) Ergot **b)** Morphine **c)** Vincristine **d)** Quinine
- 17** HMG CoA is formed from which pathway? []
a) Phenylpropanoid **b)** Malonyl **c)** Shikimic acid **d)** Mevalonate
- 18** Folates are biosynthesized from----- acid. []
a) chorismic **b)** cinnamic **c)** acetic **d)** pyruvic
- 19** Hexose mono pathway is involved in the formation of -----compounds. []
a) alkaloids **b)** flavonoids **c)** glycosides **d)** triterpenes
- 20** Ipecac and Opium contains-----alkaloids. []
a)quinoline **b)**isoquinoline **c)**phenanthrene **d)**tetrahydroquinoline