UNIT 1

CHEMICAL & SPECTRAL APPROACHES TO SIMPLE MOLECULES OF NATURAL ORIGIN

A-Chemical approach

Detection of alkaloids:Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates:
Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict 's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides:
Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.
Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.
.4-Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins
Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test:0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

6. **Detection of phytosterols**

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8-Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9**. Detection of flavonoids**

. **Alkaline Reagent Test**: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

. **Lead acetate Test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and aminoacids

. **Xanthoproteic Test**: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

. **Ninhydrin Test**: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

11. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes

B-Chromatographically approach

Chromatography is the separation of a mixture into individual components using a stationary phase and a mobile phase;

Classification of chromatography

1 ADSORPTION CHROMATOGRAPHY

(I)-GSC **(II)** TLC **(III)** HPLC **(IV)** HPTLC **(V)** COLUMN chromatography

2-PARTITION CHROMATOGRAPHY.

GLC (II) PAPER chromatography [3] – **Other** (1)-Ion exchange chromatography (2)- Gel permeationchromatography(3)- Chiralchromatography

C- Spectral Approach

I-Ultra Violet Spectroscopy II-I.R.Spectroscopy (**III)** NMRspectroscopy **IV** mass Spectroscopy **V**-**A**tomic absorption spectroscopy**VI**-flame photometer

TRACER TECHNIQUES & UTILIZATION IN BIOGENETIC STUDIES

Definitions: Living plants considered as biosynthetic laboratory primary as well as secondary metabolite. Different biosynthetic pathway: (**I) Shikmic acid pathway:** Aromatic amino acids (**II)-Mevalonic acid pathway**: Terpenes (**III) Acetate pathway:** Fatty acids

Biosynthesis: Formation of a chemical compound by living organisms Biogenesis: Production or generation of living organisms from other living organisms Primary metabolites: Required for growth & normal physiological activity e.g., carbohydrates, fatty acids, amino acids, nucleic acids, proteins. Secondary metabolites: Biosynthetically derived from primary metabolites. They represent chemical adaptations to environmental stresses, or act as defensive or protective against microorganisms, insect & higher herbivores e.g., alkaloids, glycosides, resins, tannins Various intermediate and steps are involved in biosynthetic pathway in plants can be investigated by means of following techniques: -

1-Use of isolated organ 2- Grafting methods 3-Use of mutant strain 4- Tracer technique 5- Enzymatic studies

ISOLATED ORGAN/TISSUE:

This method is based on using isolated parts of plant e.g., stem, roots. This technique is useful in the determination of site of biosynthesis of particular compounds. Roots and leaves for the study of Nicotiana and Datura, petal disc for the study of rose oil, tropane alkaloids in the root of solanaceae family.

Grafting methods: This method is used for the study of alkaloid formation by grafted plants. Tomato scions grafted on Datura produce alkaloids, while Datura scion grafted on Tomato produce less quantity of alkaloids. This shows that main site of alkaloid biosynthesis is root,.Use of mutant strains: In this mutant strains of microorganisms are produced with the lack of certain enzymes..Gibberella mutant is used to produce isoprenoid compounds, *Lactobacillus acidophillus* is used for mevalonic acid pathway for isoprenoid biosynthesis

TRACER TECHNIQUE:

It can be defined as technique which utilizes a labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate & time. OR In this technique different isotope, mainly the radioactive isotopes which are incorporated into presumed precursor of plant metabolites and are used as marker in biogenic experiments. The labelled compound can be prepared by use of two types of isotopes.

RADIOACTIVE ISOTOPES:

- [e.g. ^{1}H, ^{14}C, ^{24}Na, ^{42}K, ^{35}S, ^{35}P, ^{131}I decay with emission of radiation]
- 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.3H compound is commercially available

Stable isotopes:

- [e.g. ^{2}H, ^{13}C, ^{15}N, ^{18}O]
- Used for labeling compounds as possible intermediates in biosynthetic pathways.
- Usual method of detection are: – Mass spectroscopy [^{15}N, ^{18}O]
- NMR spectroscopy [^{2}H, ^{13}C]

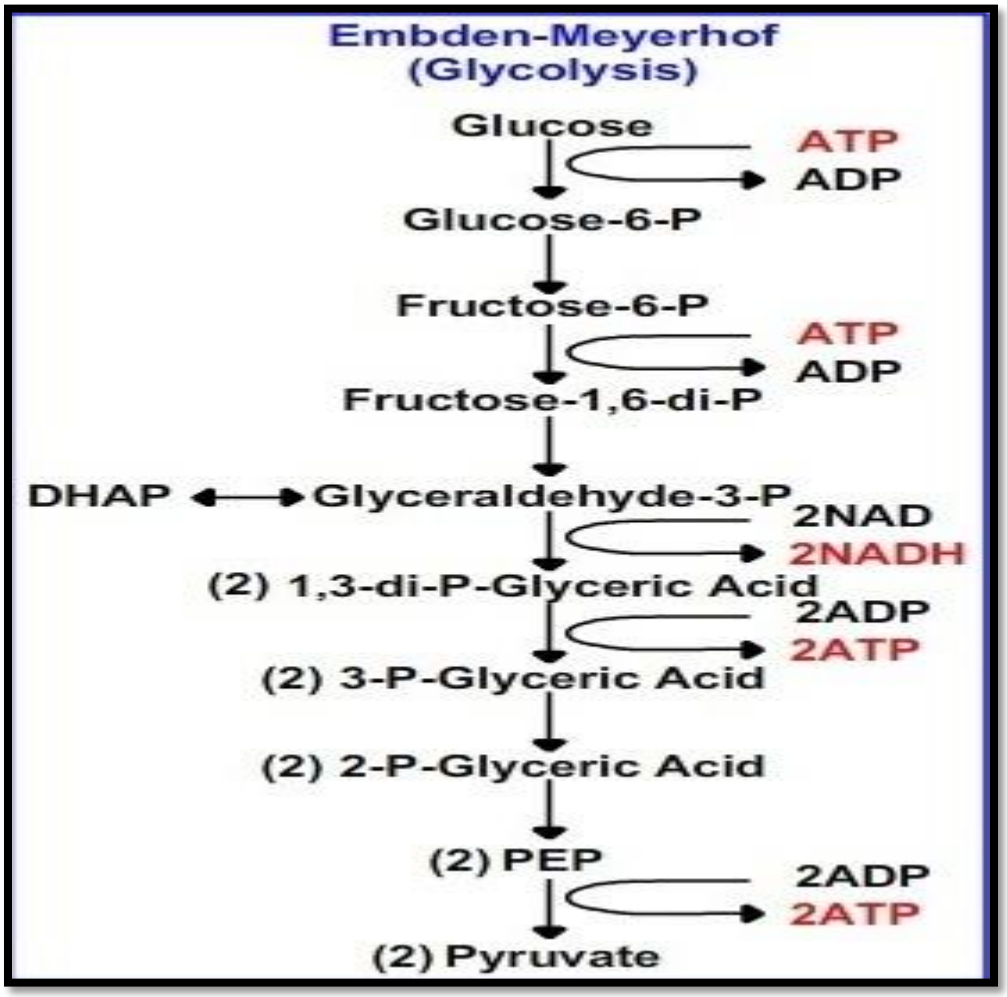
- Tracing of biosynthetic pathway: e.g. By incorporation of radioactive isotope of ^{14}C into phenylalanine, the biosynthetic cyanogenetic glycoside prunasin, can be detected.

Location & quantity of compound containing tracer: ^{14}C labelled glucose is used for determination of glucose in biological system Different tracers for different studies: For studies on alkaloids, proteins nitrogen and amino acid (Labelled nitrogen give specific information than carbon). For terpenoids O atom and glycosides O, N, S & C atom used Convenient and suitable technique

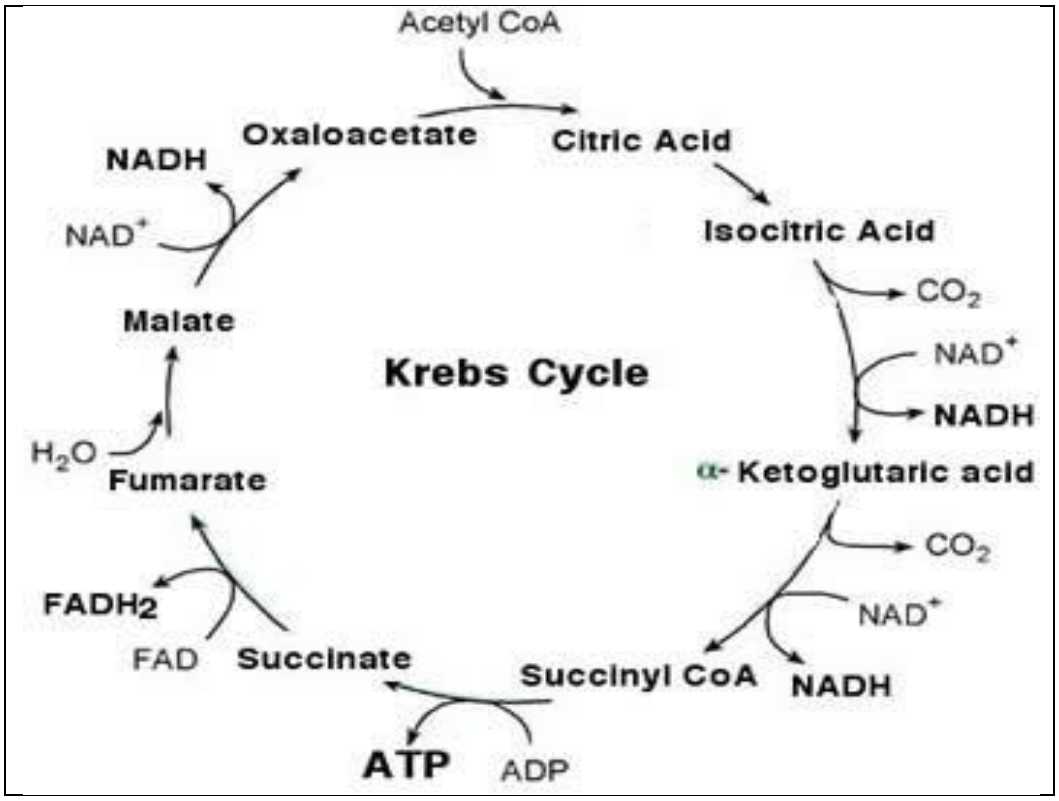
BASIC METABOLIC PATHWAYS

The primary metabolites like sugars, amino acids and fatty acids that are needed for general growth and physiological development of plant are widely distributed in nature and are also utilized as food by man. The secondary metabolites such as alkaloids, glycosides, flavonoids, volatile oils etc are biosynthetically derived from primary metabolites.

Major Metabolic Pathways Cellular respiration: **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 organisms include: **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:



OUTLINE OF THE EMBDEN- MEYERHOFF SCHEME OF GLYCOLYSIS



TRICARBOXYLIC ACID CYCLE (TCA) OR KREBS CYCLE

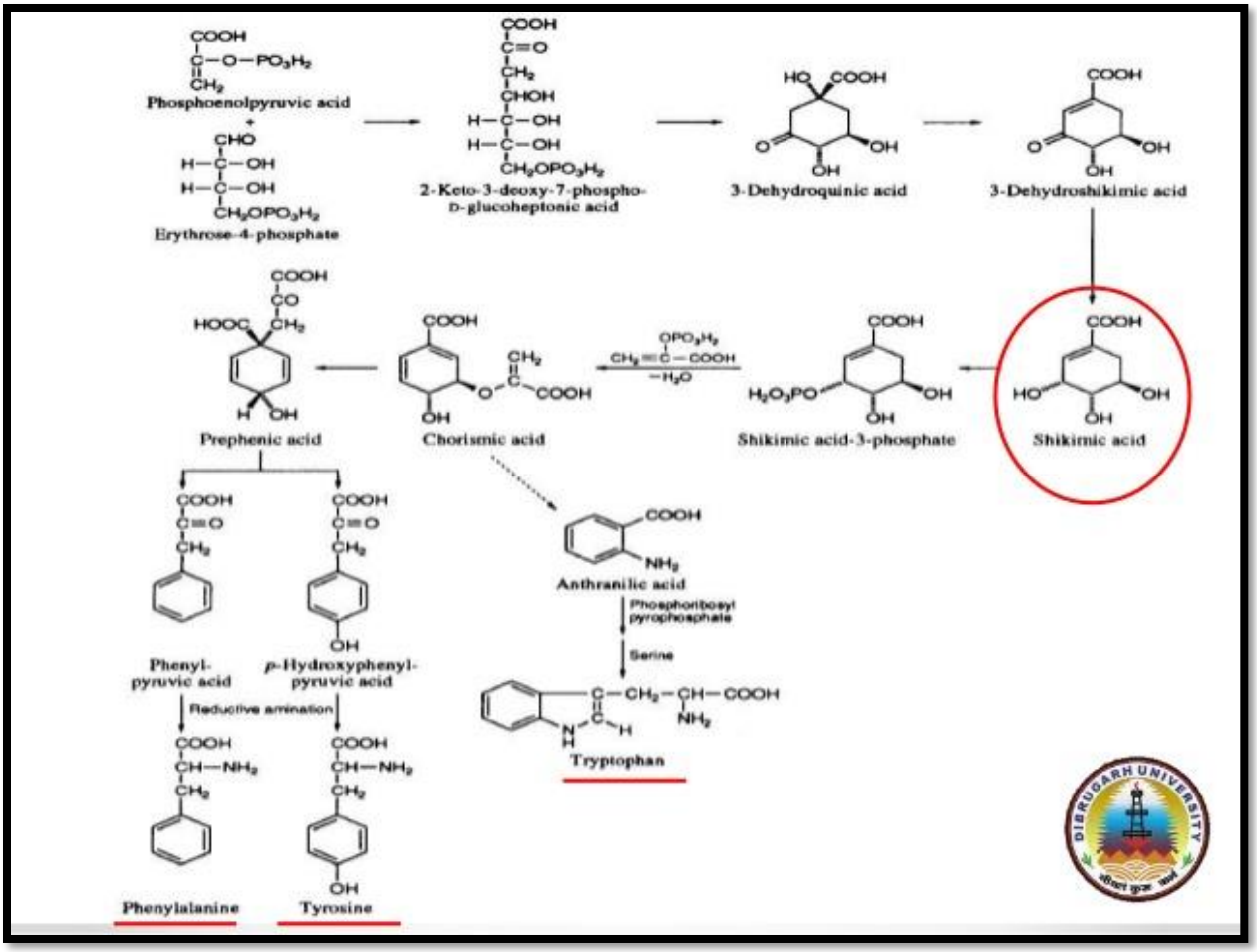
BASIC METABOLIC PATHWAY OF DRUG

I ACETATE PATHWAY II- SHIKIMIC ACID PATHWAY III CITRIC ACID

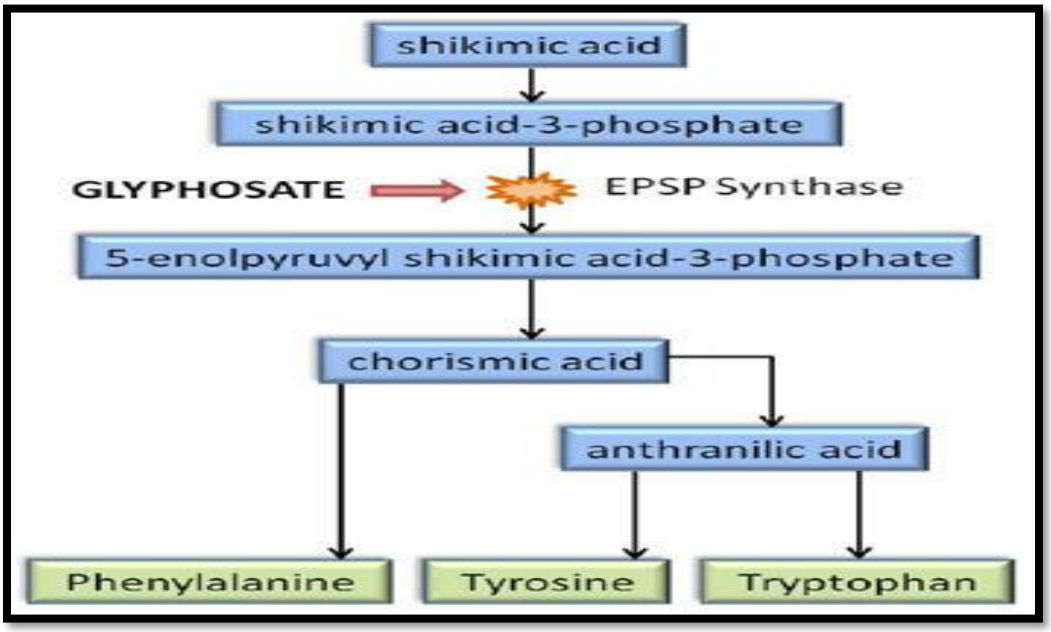
Biosynthesis of Aromatic Compounds

Shikimic Acid Pathway: The shikmic acid pathway is a key intermediate from carbohydrate for the biosynthesis of C_6-C_3 units (phenyl propane derivative). Besides serving as precursor for the biosynthesis of amino acids Shikmic acid is also an intermediate in production of tannins, flavones, coumarins and vanillin. The shikimic acid pathway converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids as shown in The shikimic acid pathway is present in plants, fungi, and bacteria but is not found in animals. Animals have no way to synthesize the three aromatic amino acids—phenylalanine, tyrosine, and tryptophan—which are therefore essential nutrients in animal diets.

Acetate hypothesis: Acetate occupies a central position in relation to the general metabolism of plants. Acetate condensation occurs in many possible routes which give rise to variety of aromatic compounds. Acetic acid is the starting unit in the biosynthesis of a wide variety of straight chain and aromatic natural compounds. Acetate hypothesis: building block of acetate biosynthesis is linear poly acetic chain $CH_3CO\text{-}(CH_{2}\text{-}CO)_n\text{-}CH_{2}\text{-}COOH$ formed by repeated head to tail condensation of acetate units.



Interrelationships of biosynthetic pathways leading to secondary constituents in plants



PRIMARY AND SECONDARY METABOLITES DERIVED FROM CARBON METABOLISM

BIOSYNTHESIS OF ALKALOIDS

Alkaloids are a group of nitrogen-containing bases. Most of them are drugs. Only a few (like caffeine) are derived from purines or pyrimidines, while the large majority is produced from amino acids. The biosynthesis of different groups of alkaloids of pharmacognostically important alkaloids is given below:

Alkaloid derived from ornithine: Ornithine is incorporated into both pyrrolidine specifically and asymmetrically into pyrrolidine ring of tropane nucleus, the α-carbon of ornithine becoming the C_1 of tropine nucleus. The remaining three carbon atoms are derived from acetate, thus completing piperidine moiety. Methionine serves as the methyl group donor, whereas phenyl alanine is the precursors of the tropic acid. The different alkaloids derived from ornithine.

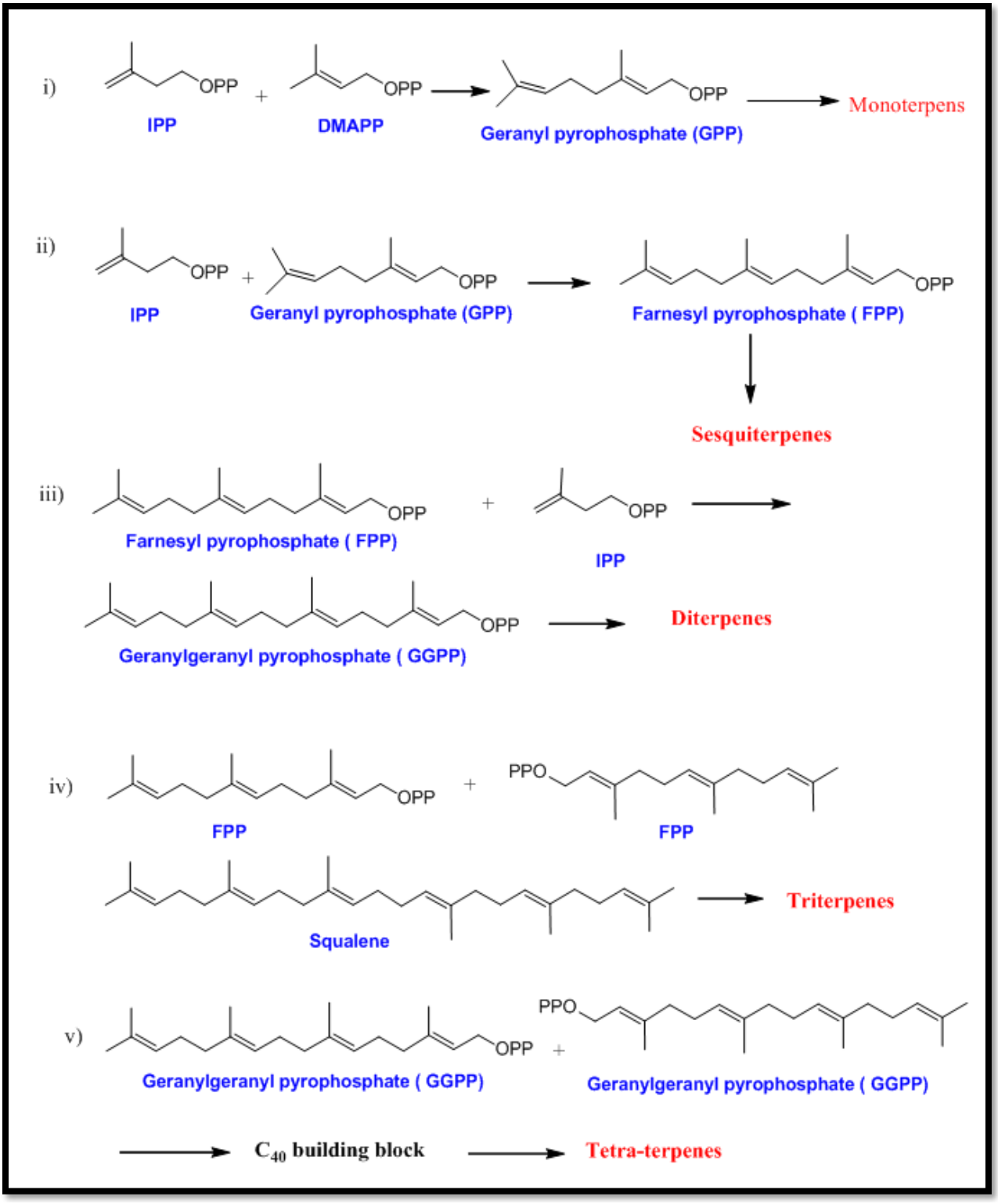
Alkaloids derived from Lysine: Lysine and its associated compounds are responsible for the biogenesis of the anabasine, lupinine, iso- pelletierine and other related alkaloids.

Alkaloids derived from phenylalanine, tyrosine and related amino acids: These amino acids and their corresponding decarboxylation products serve as the precursor for a large number of alkaloids including ephedrine, colchicines and opium alkaloids. Earlier it was shown that tyrosine and Dopamine could serve as a precursor of morphine. It was also proved that the first of the opium alkaloids synthesized is thebaine followed by codeine and morphine. Biosynthesis of morphine, codeine and thebaine is shown in

Alkaloids derived from Tryptophan: Tryptophan and its decarboxylation product tryptamine, serve as the precursor for biosynthesis of a large class of indole alkaloids. The non-tryptophan portions of the alkaloids are, however, derived from monoterpenoid precursors which are designated as the Coryanthe, Iboga and Aspidosperma types. Condensation of tryptamine (or tryptophan) with secologanine, a monoterpene glucoside, gives rise to a nitrogenous glucoside, vincoside, from which a great variety of indole alkaloids, including monomeric alkaloids in Catharanthus roseus, are formed. The rauwolfia alkaloids such as reserpine, rescinnamine, serpentine, ajmaline etc are derived from Coryanthe type monoterpenoid precursor.

BIOSYNTHESIS OF ISOPRENOID COMPOUNDS

The "biogenetic isoprene rule" is the basis for formation of various isoprenoid compounds. The discovery of mevalonic acid (3,5-dihydroxy-3-methylavaluric acid) demonstration of its incorporation by living tissues into these compounds to which the isoprene rule applied, were milestones in understanding biogenesis of terpene derivatives. It is established by research involving tracer techniques, inhibitor studies and ionophoresis that C_5 compound-isopentenyl pyrophosphate- is derived from mevalonic acid pyrophosphate by decarboxylation and dehydration. The C_{10} compound geranyl pyro phosphate is formed by condensation of isopentnyl pyrophosphate with dimethyl allyl pyrophosphate. Further, C_6 units are added by participation of more isopentnyl pyrophosphate units. From geranyl and farnesyl pyrophosphates various isoprenoid structures are synthesized. The preliminary stages in biosynthesis of isoprenoid compouns.



ISOPRENOID BIOSYNTHESIS

STEROID BIOSYNTHESIS