Shree H.N.Shukla institute of Pharmaceutical Education & Research , Rajkot

B.Pharm

Semester V

Subject Name: Pharmacognosy and Phytochemistry II

Subject code:BP503TP

Chapter 1

Topic covered

Metabolic pathways in higher plants and their determination

a) Brief study of basic metabolic pathways and formation of different secondary metabolites through these pathways- Shikimic acid pathway, Acetate pathways and Amino acid pathway.

b) Study of utilization of radioactive isotopes in the investigation of Biogenetic studies.

Biogenesis of Primary Metabolites

- All organisms need to transform and interconvert a vast number of organic compounds to enable them to live, grow, and reproduce.
- They need to provide themselves with energy in the form of ATP, and supply of building blocks to construct their own tissues.
- An integrated network of enzyme mediated and carefully regulated chemical reactions are used for this purpose, collectively called intermediary metabolism, and the pathways involved are termed metabolic pathways.
- 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 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.
- They represent chemical adaptations to environmental stresses, or serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous predators.
- They are sometimes considered as waste or secretory products of plant metabolism and are of pharmaceutical importance.
- Biosynthetic reactions are replica of common organic reactions like catalytic reactions, phosphorylation, hydride transfer, oxidation, elimination, acylation, alkylation, reduction, condensation, rearrangement etc.
- These are dependent on different conditions such as catalysts, type of energy and nature of medium during the course of reaction.
- The elucidation of biosynthetic pathway in plants for production of various metabolites has been extensively examined by means of isotopically labeled precursors.

Cell Metabolism :

- Cell metabolism is the process (or really the sum of many ongoing individual processes) by which living cells process nutrient molecules and maintain a living state.

- Metabolism has two distinct divisions: anabolism, in which a cell uses energy and reducing power to construct complex molecules and perform other life functions such as creating cellular structure; and catabolism, in which a cell breaks down complex molecules to yield energy and reducing power.

- Cell metabolism involves extremely complex sequences of controlled chemical reactions called metabolic pathways

Metabolic Pathway:

- In biochemistry, a metabolic pathway is a series of chemical reactions occurring within a cell, catalyzed by enzymes, resulting in either the formation of a metabolic product to be used or stored by the cell, or the initiation of another metabolic pathway (then called a flux generating step).

- Many pathways are elaborate, and involve a step by step modification of the initial substance to shape it into the product with the exact chemical structure desired.

- Various metabolic pathways within a cell form the cell's metabolic network. In the metabolic pathway a substrate enters depending on the needs of the cell,

- i.e. the specific combination of concentrations of the anabolical and catabolical end products (the energetics of the flux- generating step).

- Metabolic pathways include the principal chemical, mostly enzyme-dependent, reactions that an organism needs to keep its homoeostasis.

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:

- Metabolites are the intermediates and 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.

  Induction of secondary metabolism: The induction of secondary metabolism is linked to particular environmental conditions or developmental stages.

- For example, when grown in a nutrient-rich medium, most bacteria employ almost solely basic metabolism in order to grow and reproduce. However, when nutrients are depleted, they start

producing an array of secondary metabolites in order to promote survival.

- Plants produce secondary metabolites as a response to adverse environmental conditions or in particular developmental stages. For example, exposure to UV radiation induces the biosynthesis of UV-absorbing compounds

- Secondary metabolites are those chemical compounds in organisms that are not directly involved in the normal growth, development or reproduction of organisms.

- Typically primary metabolites are found across all species within broad phylogenetic groupings, and are produced using the same pathway (or nearly the same pathway) in all these species. Secondary metabolites, by contrast, are often species-specific (or found in only a small set of species in a narrow phylogenetic group), and without these compounds the organism suffers from only a mild impairment, lowered survivability/fecundity, aesthetic differences, or else no change in phenotype at all.

- The function or importance of these compounds to the organism is usually of an ecological nature as they are used as defenses against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes (coloring agents, attractive smells, etc). Since these compounds are usually restricted to a much more limited group of organisms, they have long been of prime importance in taxonomic research.

Importance of photosynthesis in formation of primary metabolites

- Photosynthesis is the process where plants convert sunlight into energy, then store it as carbohydrates, sugars, such as glucose.
- Photosynthesis may be the most important process in ecosystems, both brings in energy needed within the ecosystem, and produce oxygen (O_2) needed for cellular respiration, and the production of more ATP.

Photosynthesis has three basic steps:

1. Energy is captured from the sunlight.
2. Light energy is converted into chemical energy in the form of ATP and NADPH.
3. Chemical energy is used to power the synthesis of organic molecules (e.g. carbohydrates) from carbon dioxide (CO_2).

- Organic natural products are constructed of carbon, hydrogen, and oxygen atoms; frequently nitrogen atoms are also involved, and less frequently sulphur, phosphorus, chlorine, bromine, and iodine atoms. Organometalic compounds, especially metal complexes, also occur. The ultimate sources of these elements are the soil, sea, and the air.

- Biosynthesis begins with photosynthesis. Green plants and other photosynthetic organisms use the energy of absorbed visible light to make organic compounds. These organic compounds are the starting point for all other biosynthetic pathway.

- The products of photosynthesis provide not only the substrate material but also chemical energy for all subsequent biosynthesis.

- The light of appropriate wavelengths is absorbed by chloroplast, carbon dioxide is reduced to the level of sugar, and gaseous oxygen, equal in volume to the CO_2 reduced is liberated. The direction of these changes is exactly the reverse of those accomplished during the oxidation of food-stuffs in the process of respiration, and indeed, plants are important in the balance of nature because they restore to air the O_2 needed for respiration.

➕ Using the formula [CH_2O] to designate the basic unit of carbohydrate molecule (six of these units would yield $C_6H_{12}O_6$), or glucose, the simplest equation for the photosynthesis:

$$CO2 + H2O \xrightarrow{\text{light energy}} [CH2O]n + O2$$
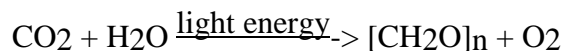
Two fundamental processes which take place in photosynthesis, both of which require light, are the production of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and phosphate and the light-energized decomposition of water (Hill's reaction)

$$H_2O \xrightarrow{\text{hv}} 2[H] + \tfrac{1}{2} O_2$$
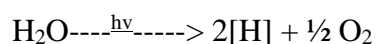
ATP is a coenzyme and the high energy of the terminal phosphate bond is available to the organism for supply of the energy necessary for endergonic reactions. The Hill reaction produces free oxygen and hydrogen ions which bring about the conversion of electron carrier, NADP, to its reduced form NADPH.

In this complicated process, two systems, Photosystem I and Photosystem II (also known as pigment system I and II) are commonly referred to; they involve two chlorophyll complexes chlorophyll a and chlorophyll b which absorb light at different wavelengths, at 680nm and 650 nm.

➕ When light hits a plant, chlorophyll absorbs the photons. The energy that it absorbs is picked up by photosystem II (P680).

➕ Photosystem II takes the energy, along with H_2O, and passes that energy to an electron acceptor, Q. O_2 is released at this point.

➕ The electron acceptor, Q, now has energy. Q takes that energy, and shuttles it off to photosystem I (P700).

➕ As that energy is being shuttled from Q to photosystem I, ADP gets charged, becoming ATP. Photosystem I now takes that energy, and charges NADP+. When NADP+ is charged, it loses hydrogen, and becomes NADPH. This entire process can be summarized with the following chemical equation.

H_2O + light + ADP + P ---> O_2 + ATP + e-
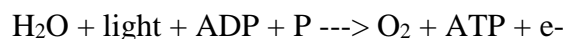
+ After the above steps occur in photosystem II, the electron is finally sent to photosystem I, where the following happens.

e- + $NADP^+$ + H ---> NADPH

+ Now there are two high energy molecules, fully charged and ready to be used. Plants make more energy that it needs immediately, so the NADPH and ATP are used to make glucose as follows:

CO_2 + ATP + NADPH ---> $C_6H_{12}O_6$

+ This happens in Calvin cycle. The photosynthesis and formation of energy molecules are shown in **Fig 1** and **Fig 2** respectively
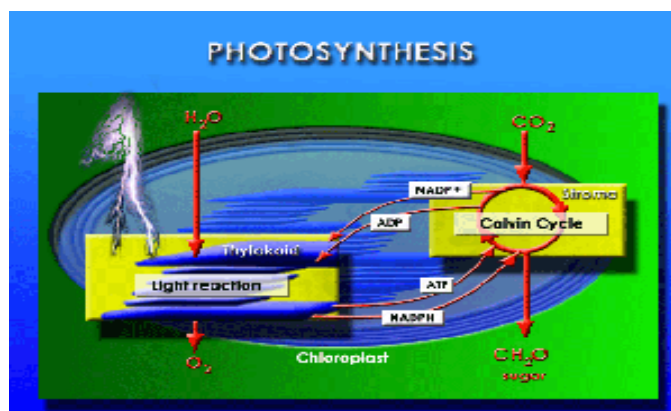
Fig 1: Photosynthesis

Photosynthesis summary
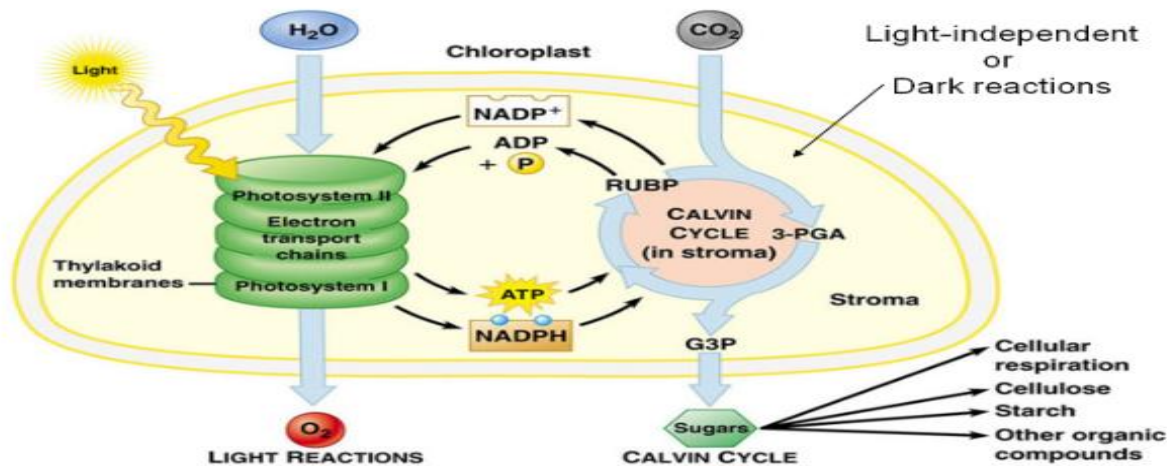
Fig 2: Photosynthesis and light dependent and light independent reactions

Calvin Cycle: The Calvin cycle is the last step in photosynthesis. The purpose of the Calvin Cycle is to take the energy from photosystem I and fix carbon. Carbon fixation means building organic molecules by adding carbon onto a chain. The following formula summarizes the Calvin cycle.

$$C_5 + CO_2 + ATP + NADPH \rightarrow C_6H_{12}O_6$$

Where C_5 is a five carbon molecule, such as pyruvate, when is recycled as glucose is synthesized. The first step in the Calvin cycle is for the $3C_5$ to bind with $3CO_2$, producing a six 3-carbon organic molecules ($6C_3$). Next, 6ATP and 6NADPH energizes the binding of a C_3 to make a 6-carbon molecule (C_6), glucose. The remaining $5C_3$ continues moving through the Calvin cycle, being turned back into the starter C_5 organic molecule. The following chemical equation (**Fig 3**) summarizes the Calvin cycle**.**
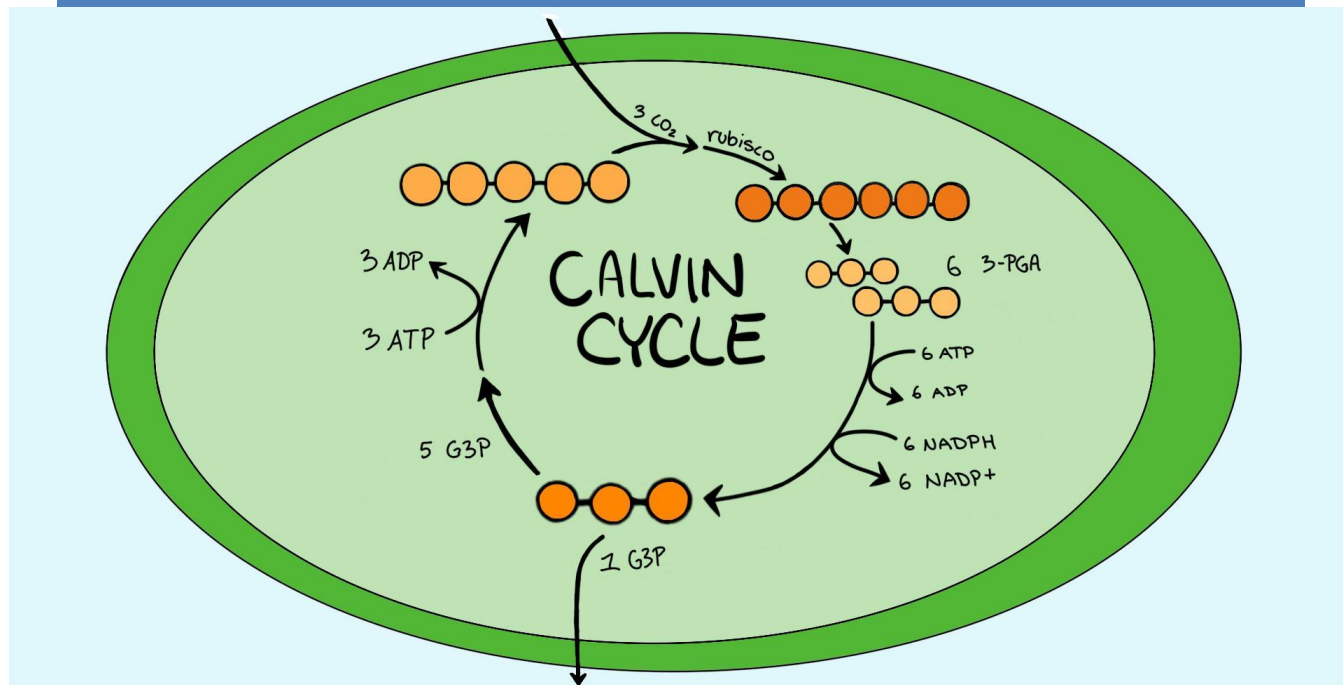
Fig 3: Chemical equation summarizing calvin cycle

Glycolysis (Embden-meyerhoff pathway): Glycolysis represents an anabolic pathway common reactions as outlined in **Fig 4.**
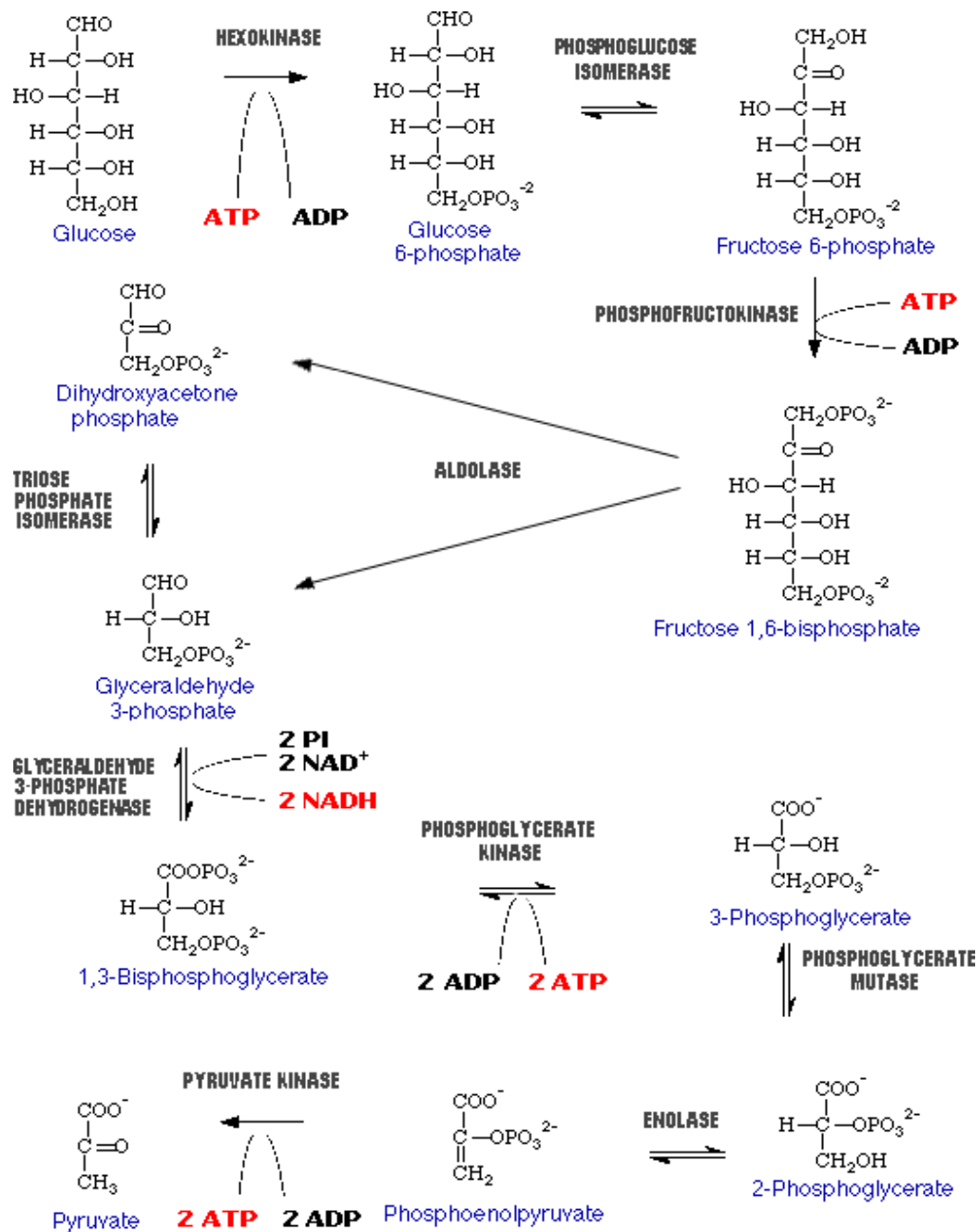

Fig 4: Outline of the Embden- Meyerhoff scheme of glycolysis

- Sugars and polysaccharides are transformed into glucose or one of its phosphorylated derivatives before being processed any further. In the course of degradation, ATP is produced.

- Pyruvate may be regarded as the preliminary final product of the degradation.

- Pyruvate is fed into the citric acid cycle via an intermediate product.

- This pathway produces energy in the form of ATP. The starting product glucose is completely oxydized to water and carbon dioxide.

Citric Acid Cycle (Kreb's cycle):

The **citric acid cycle**, also known as the tricarboxylic acid (TCA) cycle or the Kreb's cycle is the common mode of oxidative degradation in eukaryotes and prokaryotes (**Fig 5**).

It accounts for the major portion of carbohydrate, fatty acid and amino acid oxidation and produces at the same time a number of biosynthetic precursors.

- The TCA cycle is showing enzymes, substrates and products.

- The abbreviated enzymes are: IDH = isocitrate dehydrogenase and -KGDH = -ketoglutarate dehydrogenase.

- The GTP generated during the succinate thiokinase (succinyl-CoA synthetase) reaction is equivalent to a mole of ATP by virtue of the presence of nucleoside diphosphokinase.

- The 3 moles of NADH and 1 mole of $FADH_2$ generated during each round of the cycle feed into the oxidative phosphorylation pathway.

- Each mole of NADH leads to 3 moles of ATP and each mole of $FADH_2$ leads to 2 moles of ATP. Therefore, for each mole of pyruvate which enters the TCA cycle, 12 moles of ATP can be generated
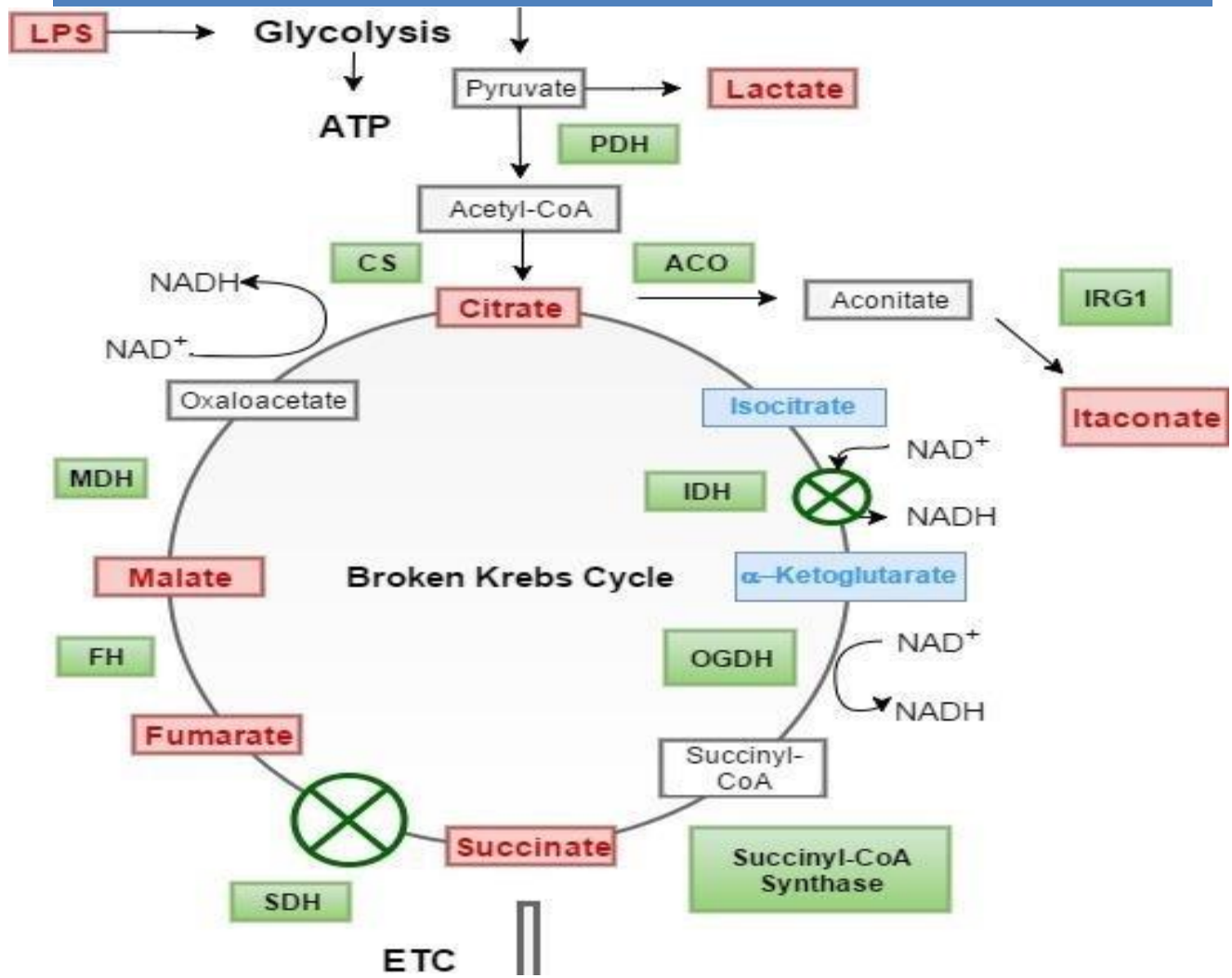
Fig 5: Tricarboxylic acid cycle (TCA) or Krebs cycle

Carbohydrate Utilization

+ Storage carbohydrate such as the starch of plants or the glycogen of animals is made available for energy production by a process which involves conversion to pyruvate and then acetate, actually acetyl coenzyme A, the latter then passing into the tricarboxylic acid cycle.

+ As a result of this, the energy-rich carbohydrate is eventually oxidized to carbon dioxide and water.

+ During the process, the hydrogen atoms liberated are carried by coenzymes into the

cytochrome system, in which energy is released in stages, with the possible formation of ATP and ADP and inorganic phosphate. Eventually the hydrogen combines with oxygen to form water.

➕ A number of pathways for the initial metabolism of glucose are known for various living tissues. One involves compounds which are found in photosynthesis cycle and another is the Embden- Meyerhoff scheme of glycolysis. The overall reaction of glucose in terms of ADP and ATP is

- $C_6H_{12}O_6 + 6CO_2 + 38\ ADP + 38P$ (inorganic) $\rightarrow 6H_2O + 6CO_2 + 38\ ATP$
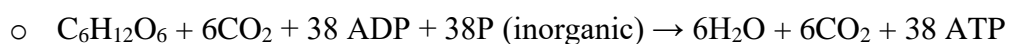
➕ The above scheme, given in barest outline, are the fundamental not only for the building up and breaking down of reserve foodstuffs, but also in that the intermediates are available for biosynthesis of all other groups of compounds found in plants. The primary and secondary metabolites derived from carbon metabolism are shown in **Fig 6**. The interrelationship of biosynthetic pathways leading to formation of secondary metabolites is shown in **Fig 7**.
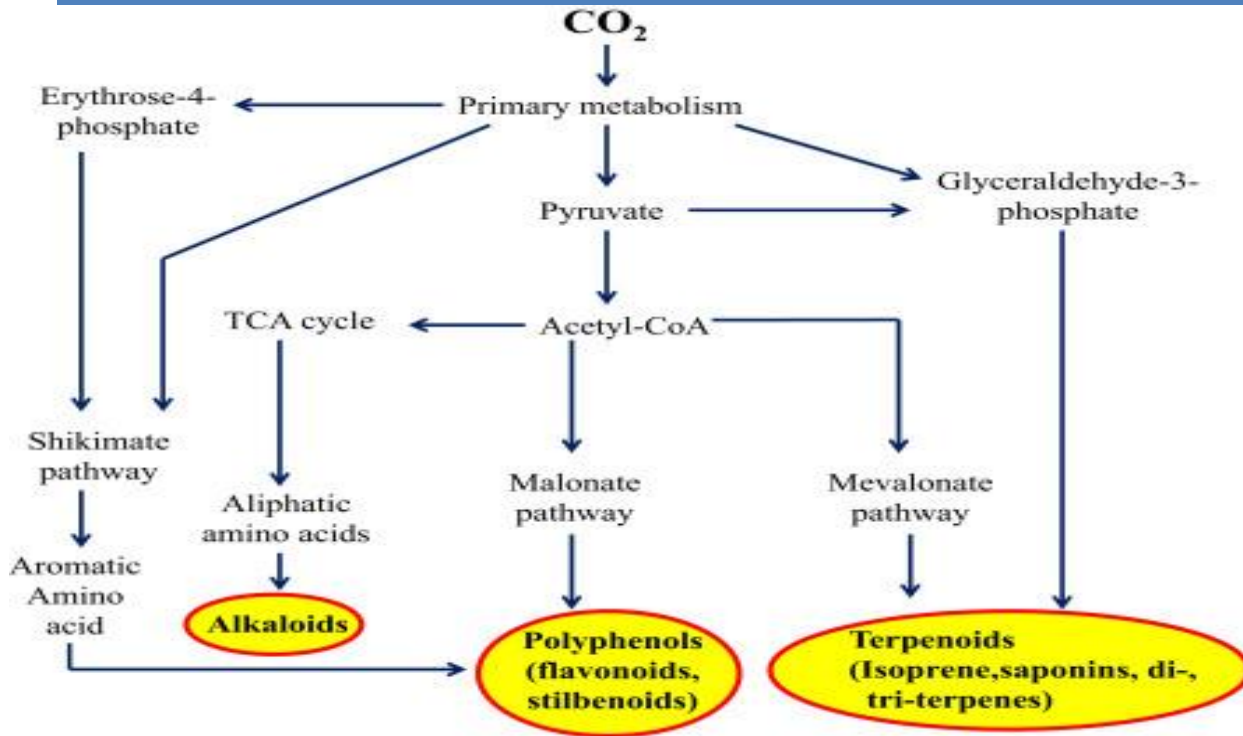
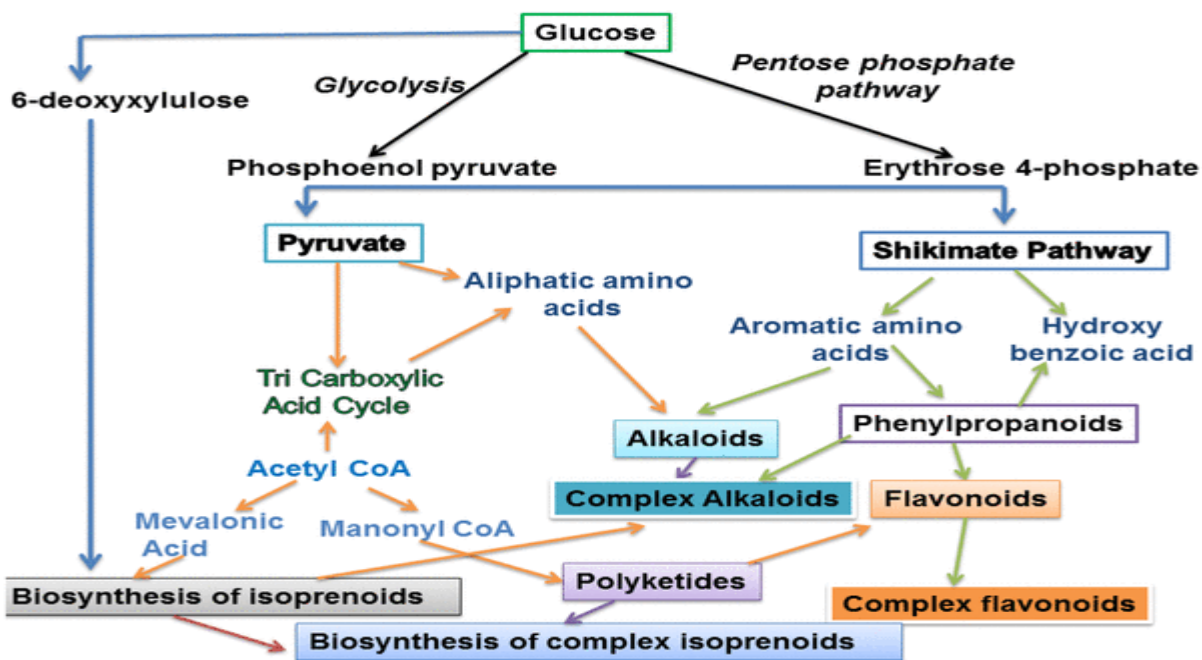**Fig 6: Primary and secondary metabolites derived from carbon metabolism**

Fig 7: Interrelationship of biosynthetic pathways leading to secondary constituents.

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 **Fig 8.**

- 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.

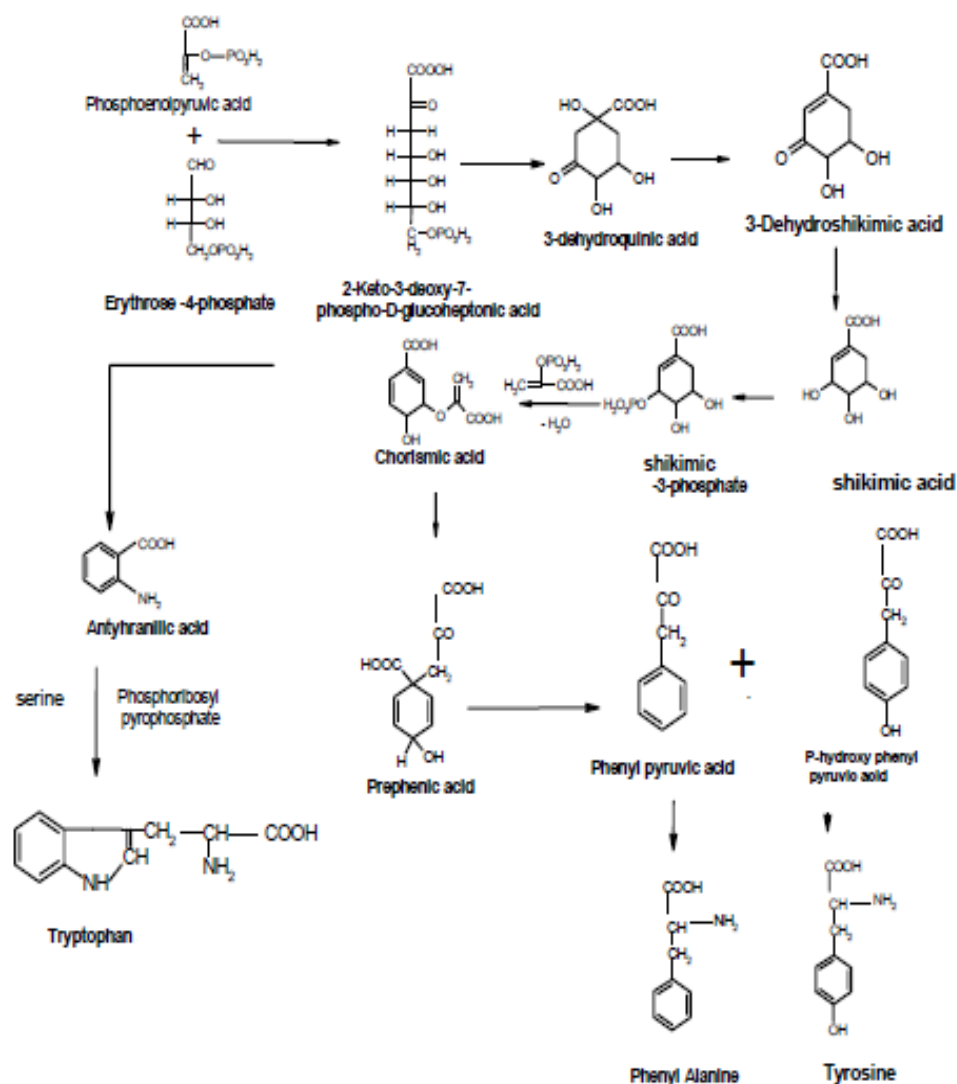Fig 8: Biosynthesis of aromatic compounds via shikimic acid pathway

Amino acids:

- Amino acids occur in plants both in the form of free state and as the basic units of proteins and other metabolites.
- They are compounds containing one or more amino groups and one or more carboxylic acid group.
- Plants are able to generate all 20 amino acids necessary for protein synthesis by themselves. They arise at various levels of glycolytic and TCA systems. All amino acids can be regarded as members of five families:

1. The glutamate family starting with *alpha*-ketoglutarate

2. The aspartate family with the starting compound oxaloacetate

3. The alanine-valine-leucine group (pyruvate)

4. The serine-glycine group (3-phosphoglycerate)

5. The family of aromatic amino acids (phosphoenolpyruvate and erythrose-4-phosphate, an intermediate in the pentose phosphate pathway).

- Plants take up nitrogen as nitrate, and in smaller amounts also as ammonium ions. A few species, mainly of the leguminosae family, live in symbiosis with nitrogen-fixing bacteria that are able to reduce atmospheric nitrogen. All other plants reduce nitrate (NO_3^-).

- Nitrogen appears to enter the metabolism of the organism by reductive amination of alpha keto acids; alpha ketoglutaric acids, pyruvic and oxaloacetic acids.

- In a first step, nitrate is reduced to nitrite (NO_2^-). The reducing agent is $NADH + H^+$ that is gained during glycolysis, the respective enzyme nitrate reductase. This reaction occurs in the cytosol and couples glycolysis and nitrate reduction and has the advantage that the NAD^+ necessary for the progress of glycolysis.

- ♣ In the second step, nitrite is reduced to ammonium by nitrite reductase, an enzyme that is located in plastids.
- ♣ Each type of plastid has its own assimilatory nitrite reductase. In photosynthetic tissues, reduced ferrodoxin functions as the electron donor that is produced in photosynthesis.

- ♣ The reactions are part of an electron transport chain that involves both NADP and FAD. The exact coupling of the steps is still partly unknown.
- ♣ Free ammonium ions are toxic to plant cells and are rapidly incorporated into organic compounds. The most important quantitative way in green plants is the reductive amination of *alpha*-ketoglutarate to produce glutamate catalyzed by the $NADP^+$-dependent glutamate dehydrogenase. Glutamate is accordingly the amino acid generated first **(Fig 9)**
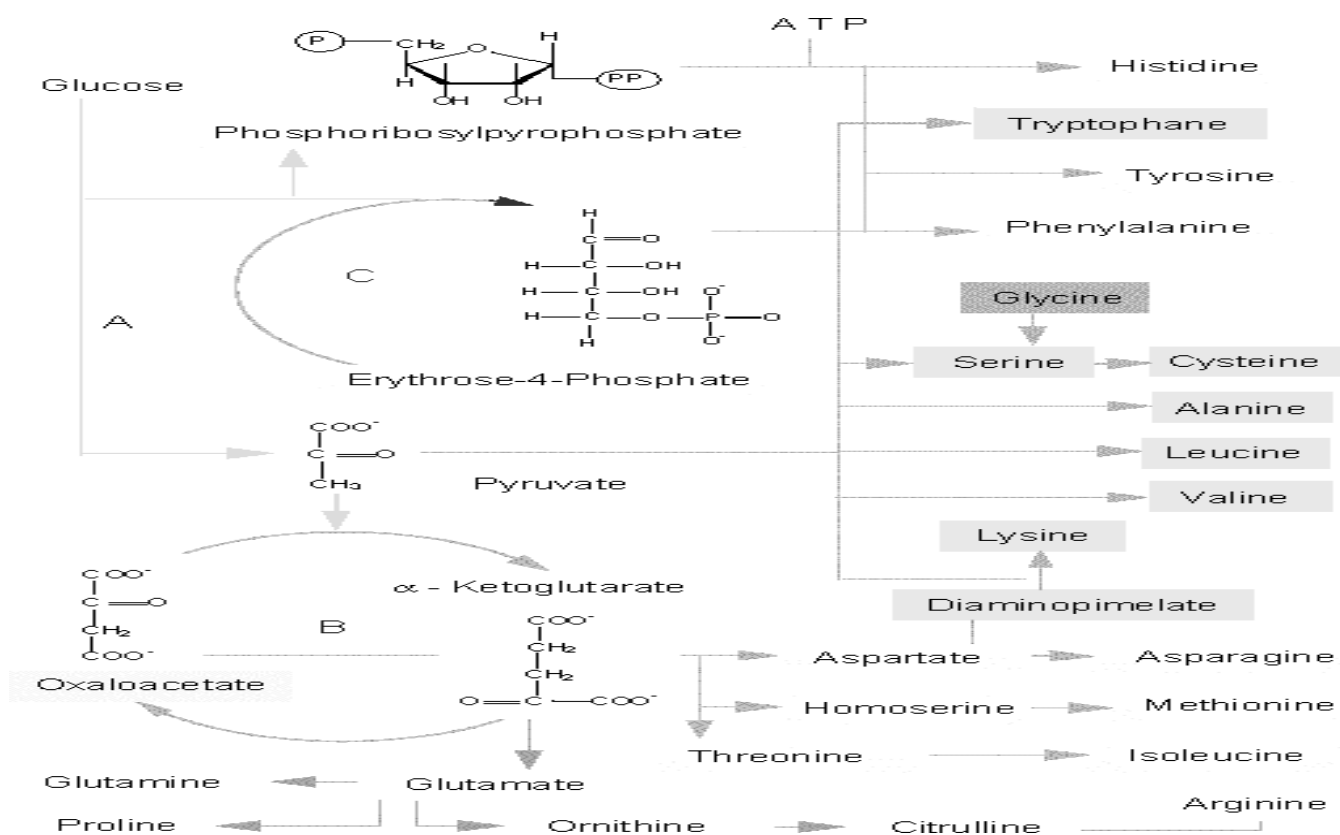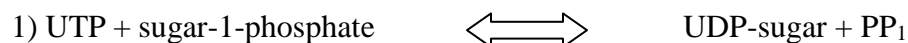

Fig 9: Formation of amino acids

Biosynthesis of Glycosides

- ♣ The term 'glycoside' is a very general one which embraces all the many and varied combinations of sugars and aglycones.

- ♣ The metabolic process of glycoside formation essentially consists of two parts.

- ♣ The first part of biosynthesis is the reactions by means of which various type of aglycones are formed, where as the other part of biosynthesis process takes into account metabolic pathway involving coupling of aglycone with sugar moiety.

- ♣ The synthesis of glycosides in plant cells involves interaction of nucleotide glycoside such as UDP-glucose with alcoholic or phenolic group of a second compound aglycone.

- ♣ Such glycosides, called as O- glycosides, are commonly found in plant. The other glycosides also occur in nature in which the linkage is through carbon (C- Glycosides), nitrogen (N-glycosides) or sulphur (S- Glycosides).

- ♣ The principal pathway of glycoside formation involves the transfer of uridylyl group from uridine triphosphate (UTP) to sugar-1-phosphate and the enzymes catalyzing this reaction are known as uridylyl transferases. The subsequent reaction controlled by enzymatic system glycosyl transferases involves transfer of sugar from uridine diphosphate to aglycone moiety resulting in formation of glycoside.

Uridylyl transferases

1) UTP + sugar-1-phosphate $\Longleftrightarrow$ UDP-sugar + PP_1

Glycosyl transferases

2) UDP + sugar + aglycone $\Longleftrightarrow$ Glycoside UDP
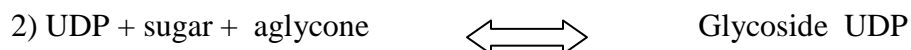
- The sugars present in glycosides may be monosaccharides such as rhamnose, glucose and fucose or deoxysugars such as digitoxose or cymarose as in cardiac glycosides.

- More than one molecule of such sugars may be attached to the aglycone either by separate linkages, which is rare, or, more commonly, as di-, tri- or tetrasaccharide.

- Such complex glycosides are formed by stepwise addition of sugars to aglycone. Since sugars exist in isomeric α- and β- forms, both types are theoretically possible. Practically all natural glycosides, however, are of the β- type.

Cardiac glycosides:

- The aglycones of cardioactive glycosides are steroidal in nature. They are the derivatives of cyclopentenophenanthrene ring containing an unsaturated lactone ring attached to C_{17}, a 14- β hydroxyl group and a cis juncture of rings C and D.

- The knowledge of steroidal biosynthesis is derived from studies of cholesterol production through Acetate → Mevalonate → Isopentenyl pyrophosphate → squalene pathway.

- The biosynthesis of cholesterol involves cyclization of aliphatic triterpene-squalene. The scheme for biogenesis of some of the aglycones is given in **Fig 10.**

Saponins:

- In plants, sapogenins occur in the form of their glycosides, i.e. saponins.

- The neutral saponins are derivatives of steroids with spiroketal side chains, whereas acid saponins possess triterpenoid structures.

- The main pathway for biogenesis of both type of sapogenins is similar. However, a branch occurs, probably after formation of triterpenoid hydrocarbon-squalene which leads to cyclic triterpenoids in one direction and spiroketal steroids in other direction.

- The bioproduction of squalene, cholesterol and various steroidal compounds including the aglycones is outlined in **Fig 10.**
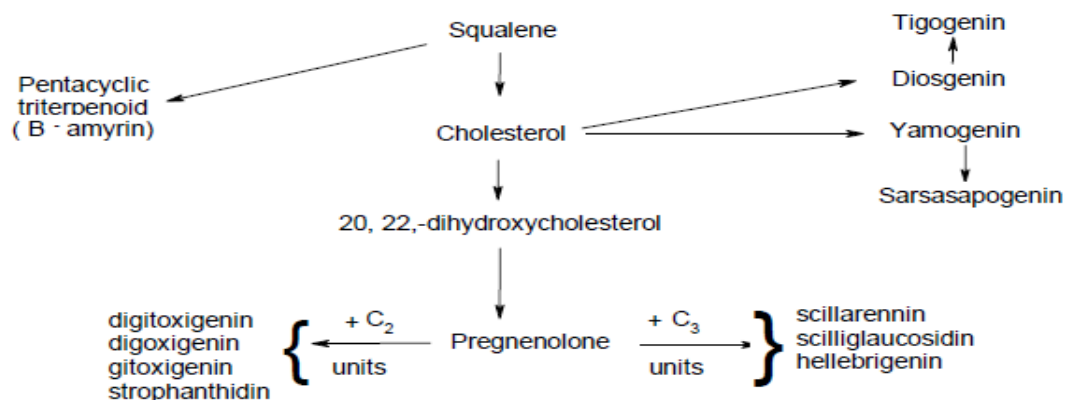
Some Secondary Products of Cholesterol metabolism

Fig 10: Biogenesis of aglycones of cardiac glycosides and saponins

Anthracene aglycones:

An intermediate poly β ketomethylene acid is probably produced from 8 acetate units which on intramolecular condensation forms anthraquinones. A second metabolic pathway for production of anthraquinone is through shikimic acid as operative in some plants of Rubiaceae.

Cyanogenetic glycosides: The aglycones of pharmaceutically significant cyanogenetic glycosides are phenyl propanoid compounds derived from amino acid phenylalanine and tyrosine

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Shikimic acid pathway
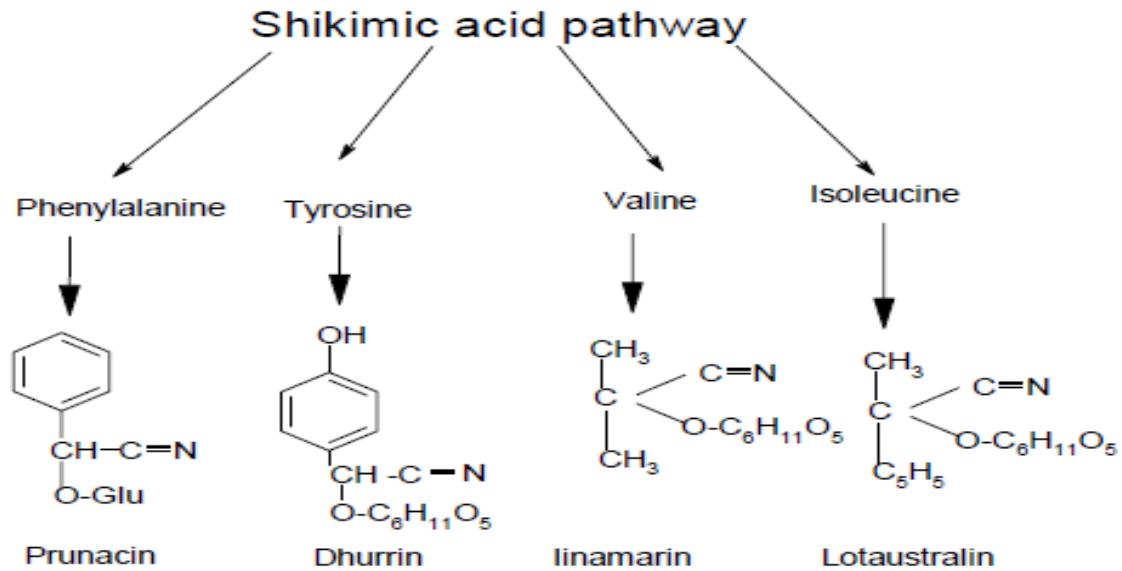
Biosynthesis of cyanogenetic glycosides

Fig 11: Biosynthesis of cyanogenetic glycosides

which are the product of shikmic acid pathway. The aglycone of linamarin is derived from valine and that of lotaustralin from isoleucine **(Fig 11)**

Isothiocynate glycosides: The aglycone or isothiocyanate glycosides may consist of either aliphatic derivatives biosynthesized via acetate pathway or aromatic derivatives produced biosynthetically via shikmic acid **(Fig 12)**.
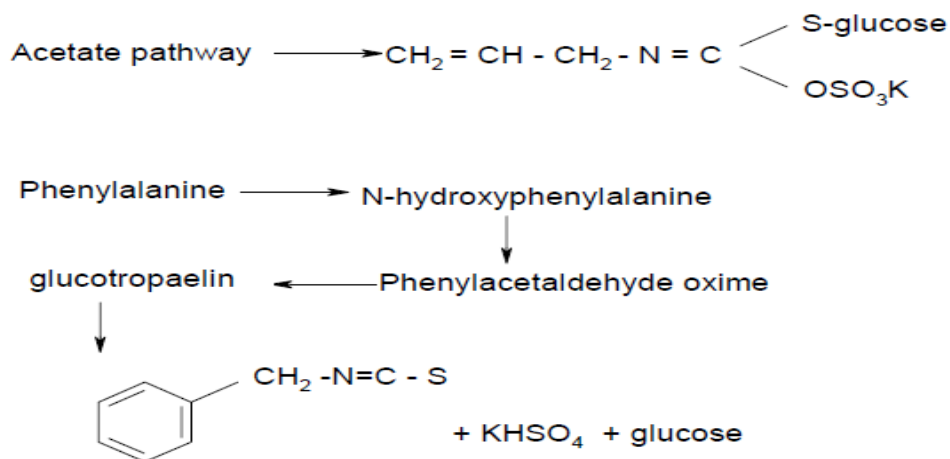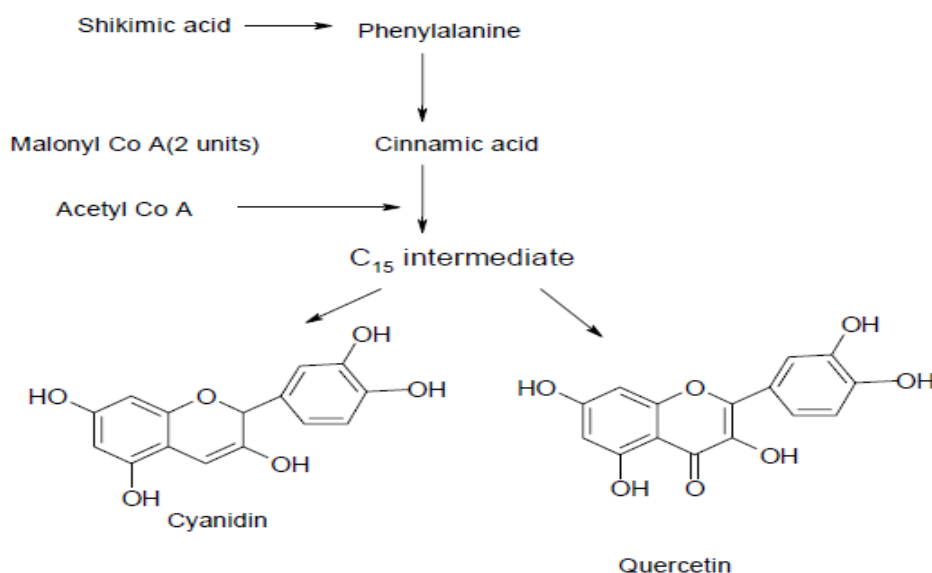
Fig 12: Biosynthesis of isothiocyanate aglycones

Flavones: The aglycones of flavonol glycoside are derived from both acetate metabolism and shikimic acid pathway **(Fig 13)**. One ring arises by head –to-tail condensation of two malonyl CoA units and acetyl Co-A. The other ring and C_3 unit come from a C_6-C_3 precursors, which may be cinnamic acid.

Biosynthetic pathways to Flavonoid Aglycones

Fig 13: Biosynthesis of flavonoid aglycones

The aromatic nuclei of alcohol, aldehyde, lactone and phenol glycosides are derived from C_6-C_3 precursors formed via shikimic acid pathway.

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 are grouped together and are shown in **Fig 14**.
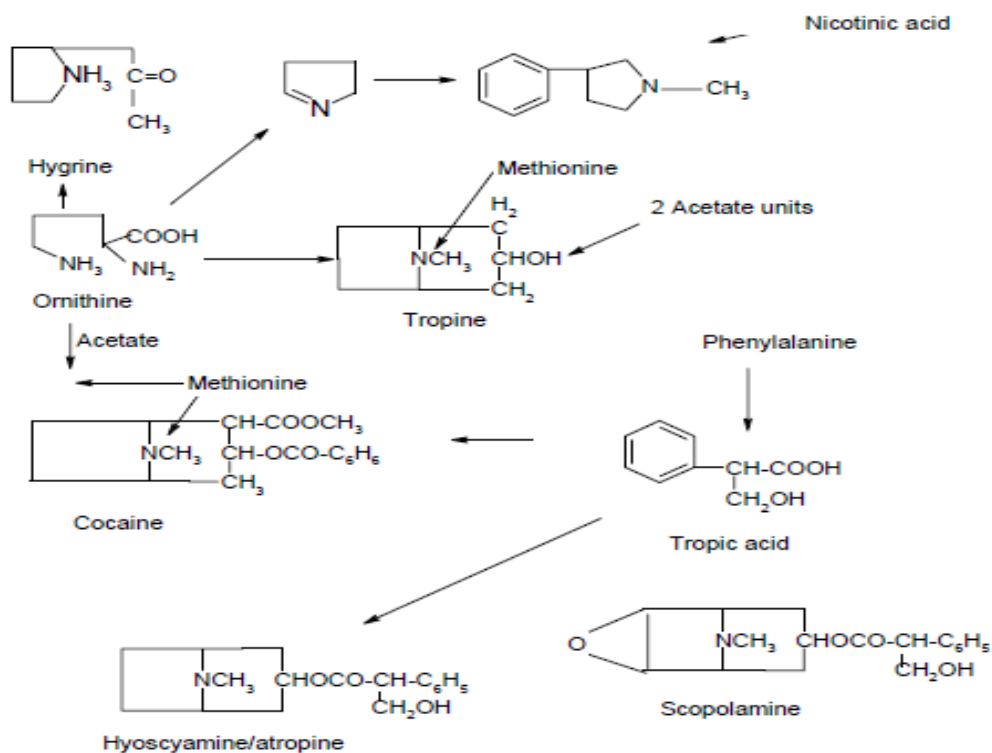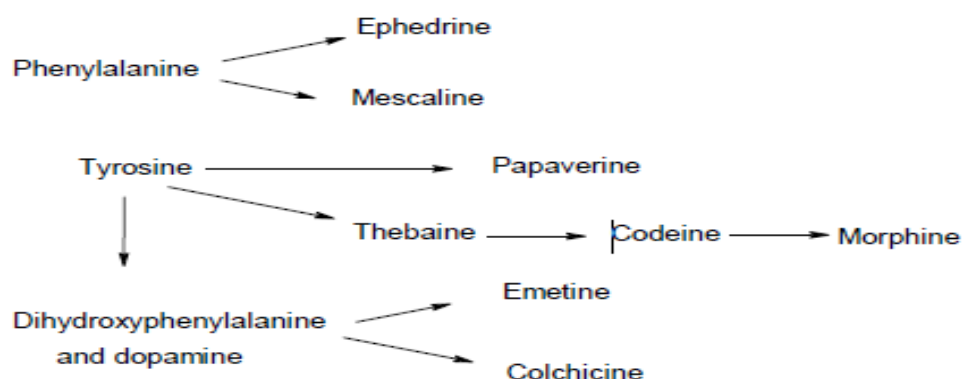
Some alkaloids derived from ornithine

Fig. 14: Biosynthesis of 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 **(Fig 15).** 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 **Fig 16.**

Some alkaloids derived from phenylalanine, tyrosine and their derivatives

Fig 15: Alkaloids derived from phenyl alanine, tyrosine and their derivatives.

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. The biogenesis of reserpine is shown in **Fig 17**.

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 is shown in **Fig 18** and biogenesis of various isoprenoid compounds are shown in **Fig19.**
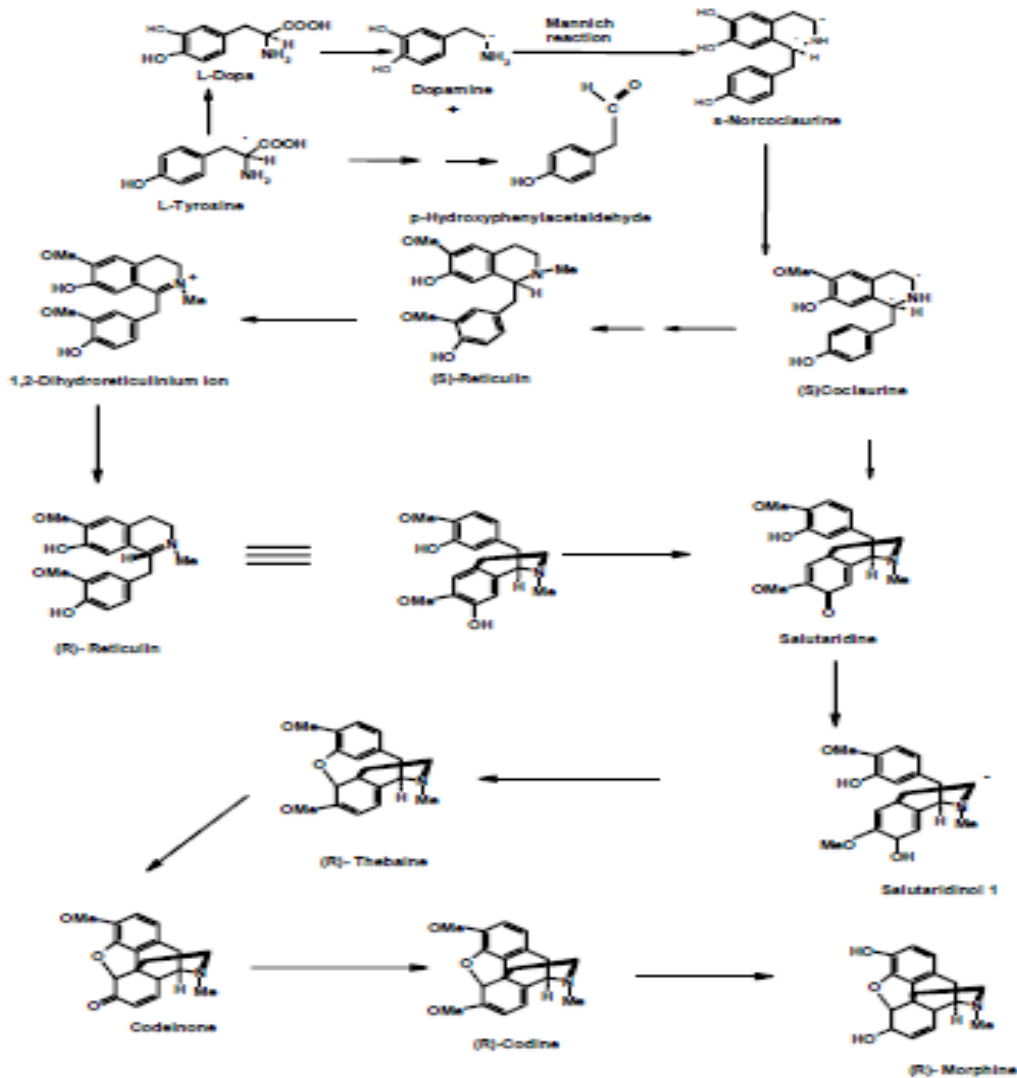

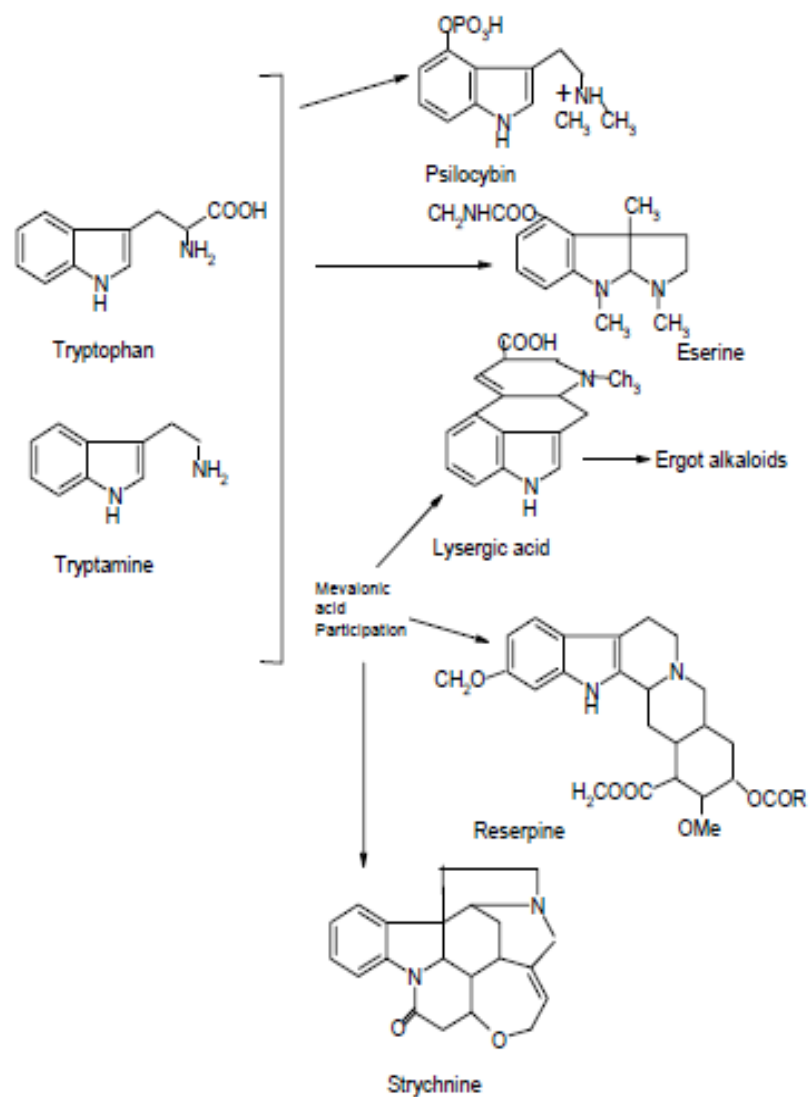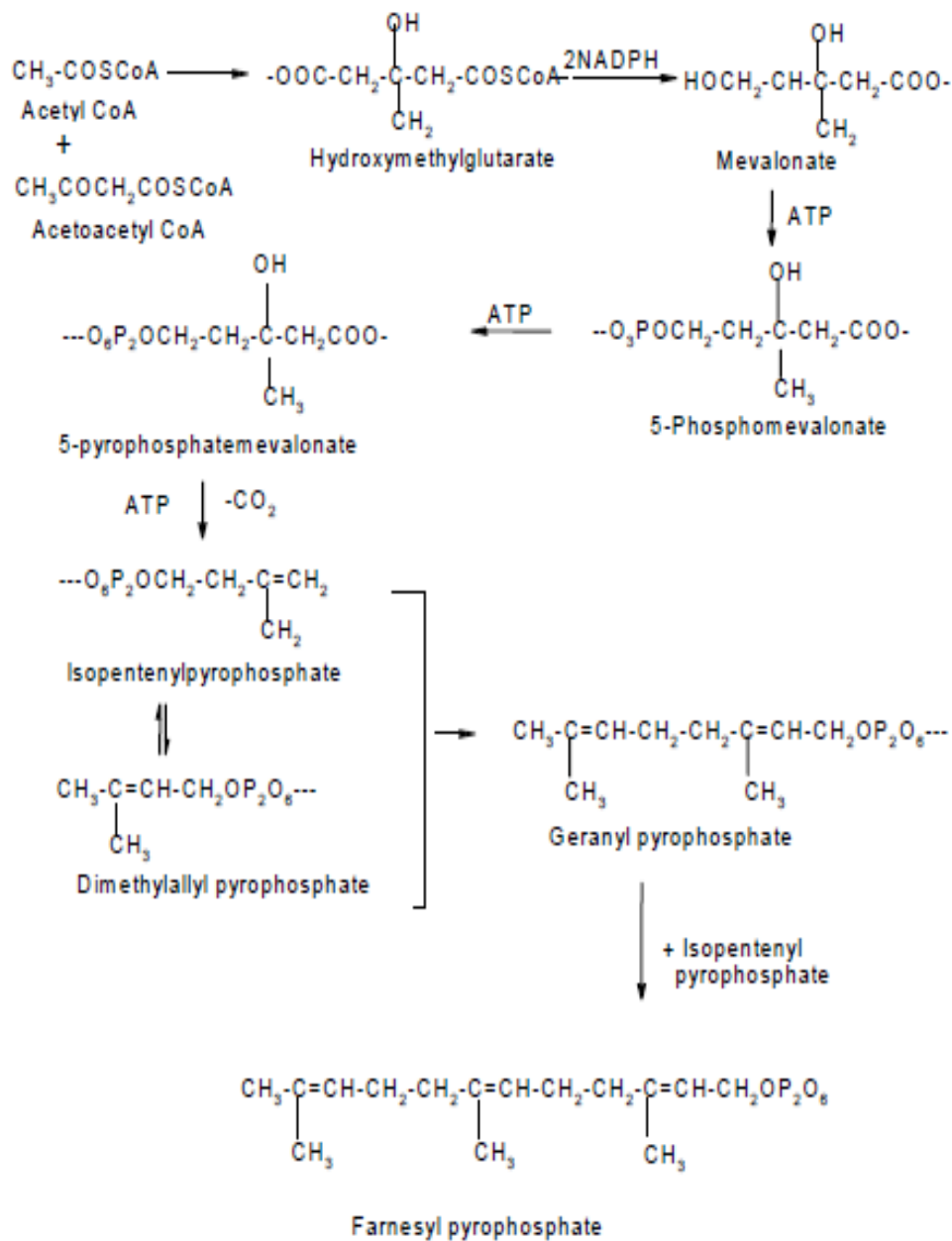**Fig 18: Preliminary stages of isoprenoid biosynthesis**

Fig 17: Biosynthesis of indole alkaloids

Prliminary stages in biosynthesis of isoprenoid biosynthesis

Fig 18: Preliminary stages of isoprenoid biosynthesis

Biosynthesis of Triglycerides (Fats and Fatty acids)

It occurs in two stages. First stage is biosynthesis of fatty acid molecule while second stage is associated with formation of a triglyceride structure. For the formation of a fatty acid molecule, acetate is a precursor and reaction proceeds with presence of $NADPH_2$, Mn^{++}, ATP and two enzyme complexes (CoASH and Acetyl CoA) and carbon dioxide. The total reaction is represented in the following steps and is depicted in **Fig 20.**

Principle steps

1. Formation of 3-carbon malonyl-CoA by Acetyl-CoA carboxylase (ACCase)

2. Attachment of malonyl to acyl carrier protein (ACP)

3. Repetitive condensation of 2-carbon units to ACP-linked intermediates to yield palmitoyl-S-ACP (C16:0) and stearoyl-SACP (C18:0)

4. Desaturation

- The reaction occurs only in the formation of saturated fatty acids.

- In case of unsaturated, branched chain or other type of fatty acids, the biosynthetic pathways are not clearly known. Second stage is the formation of triglyceride structure where required glycerol enters the reaction in the form of L-α-glcro phosphate, while fatty acid comes as fatty acyl –CoA. Depending on type of fatty acyl- CoA (value of R), simple or mixed glycerides are formed.
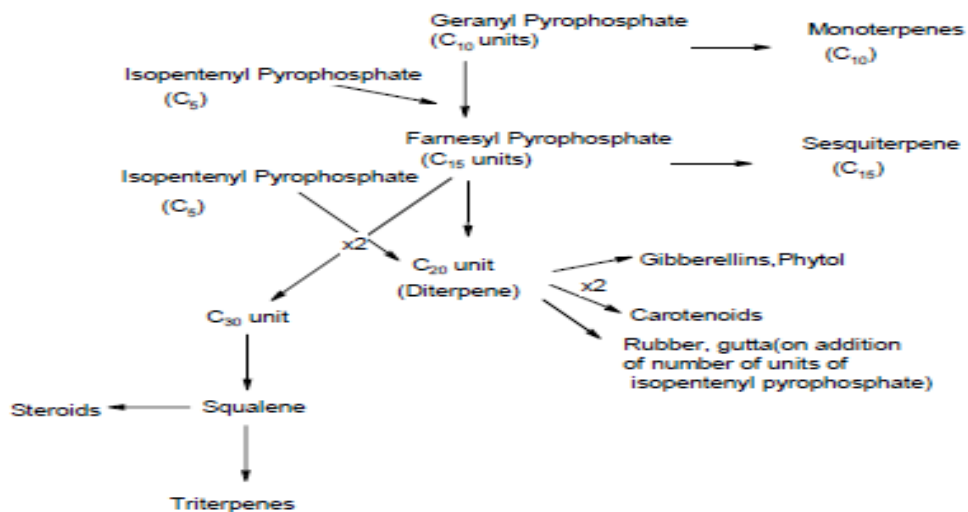
Biogenesis of Various Isoprenoid Compounds

Fig 19: Biogenesis of various isoprenoid compounds

Stress compounds

- These are compounds which accumulate in the plant to a higher than normal level as a result of some form of injury, or disturbance to the metabolism; they may be products of either primary or secondary metabolism.

- Common reactions involved in their formation are the polymerization, oxidation or hydrolysis of naturally occurring substances. Chemically, stress compounds are, in general, of extreme variability and include phenols, resins, gums, oleoresins, coumarins triterpenes and steroidal compounds.
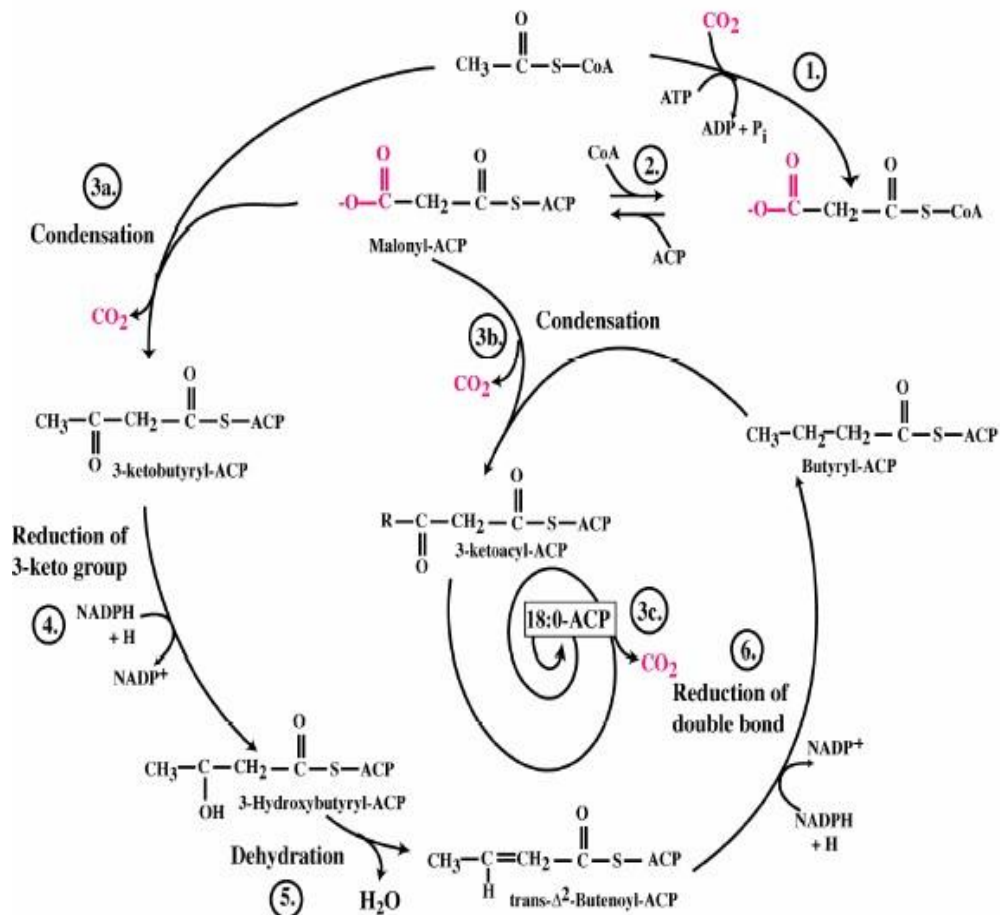
Fig 20: Biogenesis of fatty acids