4. BIOGENESIS OF SECONDARY METABOLITES

INTRODUCTION

Plant phenolics are biosynthesized by numerous ways and therefore it constitutes a heterogeneous group from a metabolic point of view. The two basic pathways involved are: the shikimic acid pathway and the malonic acid pathway. The shikimic acid pathway involves itself in the biosynthesis of most plant phenolics. The malonic acid pathway is an important source of phenolic secondary products in fungi and bacteria, but to a lesser extends in higher plants.

The **Calvin cycle** or carbon fixation or Calvin-Benson cycle is a series of biochemical reactions that occurs in the chloroplasts of photosynthetic organisms. It was discovered by Melvin Calvin and Andrew Benson. James Bassham has also made important contributions to elucidate this pathway. The calvin pathway is light-independent and occurs inside the stomata. It has three phases. Phase 1 is called the carbonfixation, phase 2 the reduction and phase 3 the regeneration of carbondioxide acceptor, RuBP.

During photosynthesis, light is used to generate chemical free energy, stored in ATP and NADPH. CO_2 comes into the stroma of the chloroplast through the stomata of the leaves. During the "dark" cycle, it uses the energy to convert carbon dioxide and water into organic compounds. The enzyme Rubisco catalyzes the binding of CO_2 to RuBP (a 5-carbon compound) to create an unstable carbon molecule that immediately splits into 3-carbon molecules of 3-PG (3 phospho-glycerate). One molecule of ATP is used to produce an ADP and an inorganic phosphate P_i. 1,3-bisphosphoglycerate is reduced by NADPH and the molecule then accept up a proton (H+) to become glyceraldehyde-3-phosphate and the NADPH is oxidized during the process to $NADP^+$.

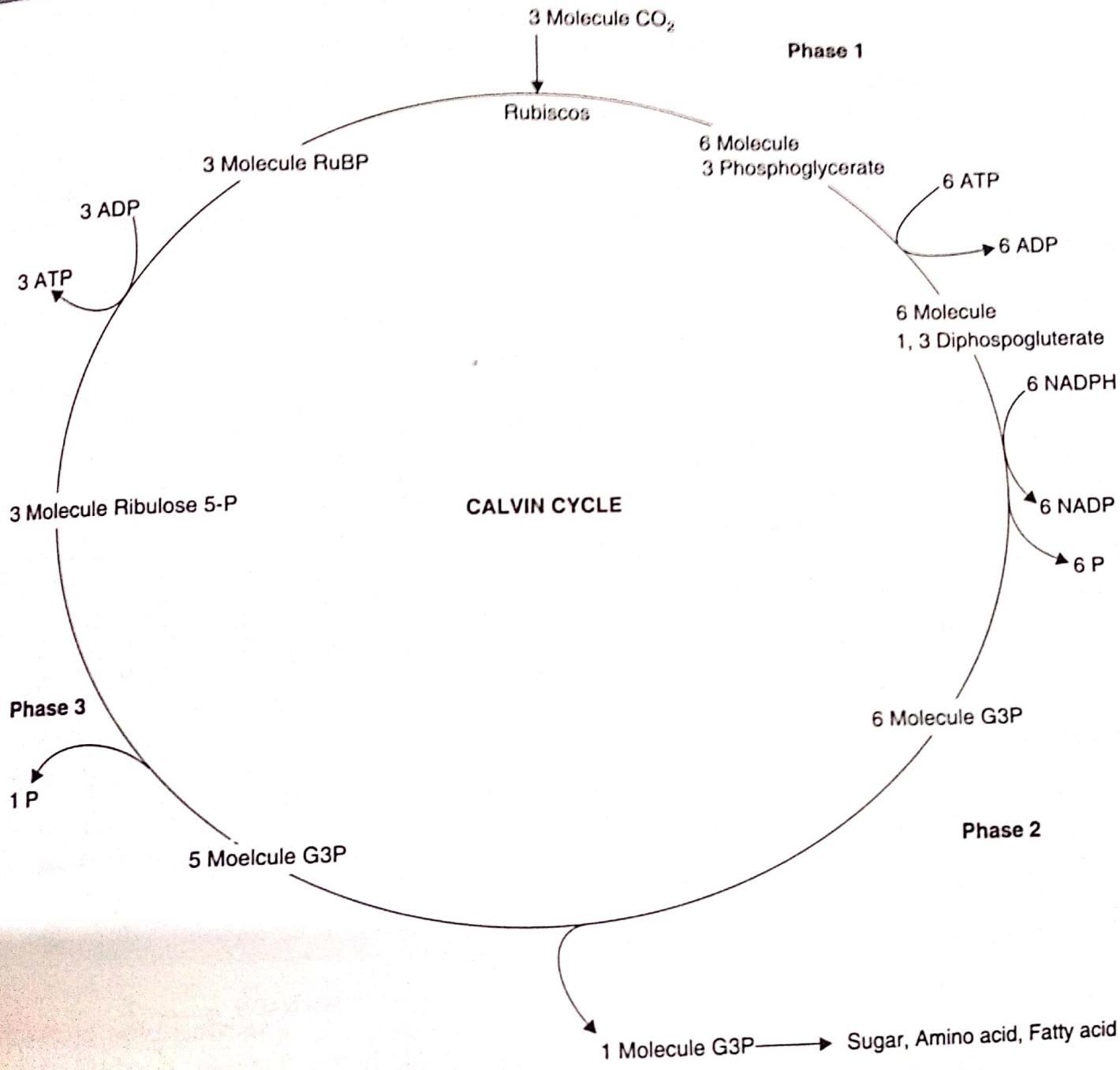
In the following equations, the chemical species (phosphates and carboxylic acids) exist in equilibria among their various ionized states as governed by the pH.

The reactions can be sumarized as:

$$6\ CO_2 + 12\ NADPH + 12\ H_2O + 18\ ATP \rightarrow C_6H_{12}O_6 + 12\ NADP^+ + 18\ ADP + 18\ P_i$$

Output of Calvin Cycle

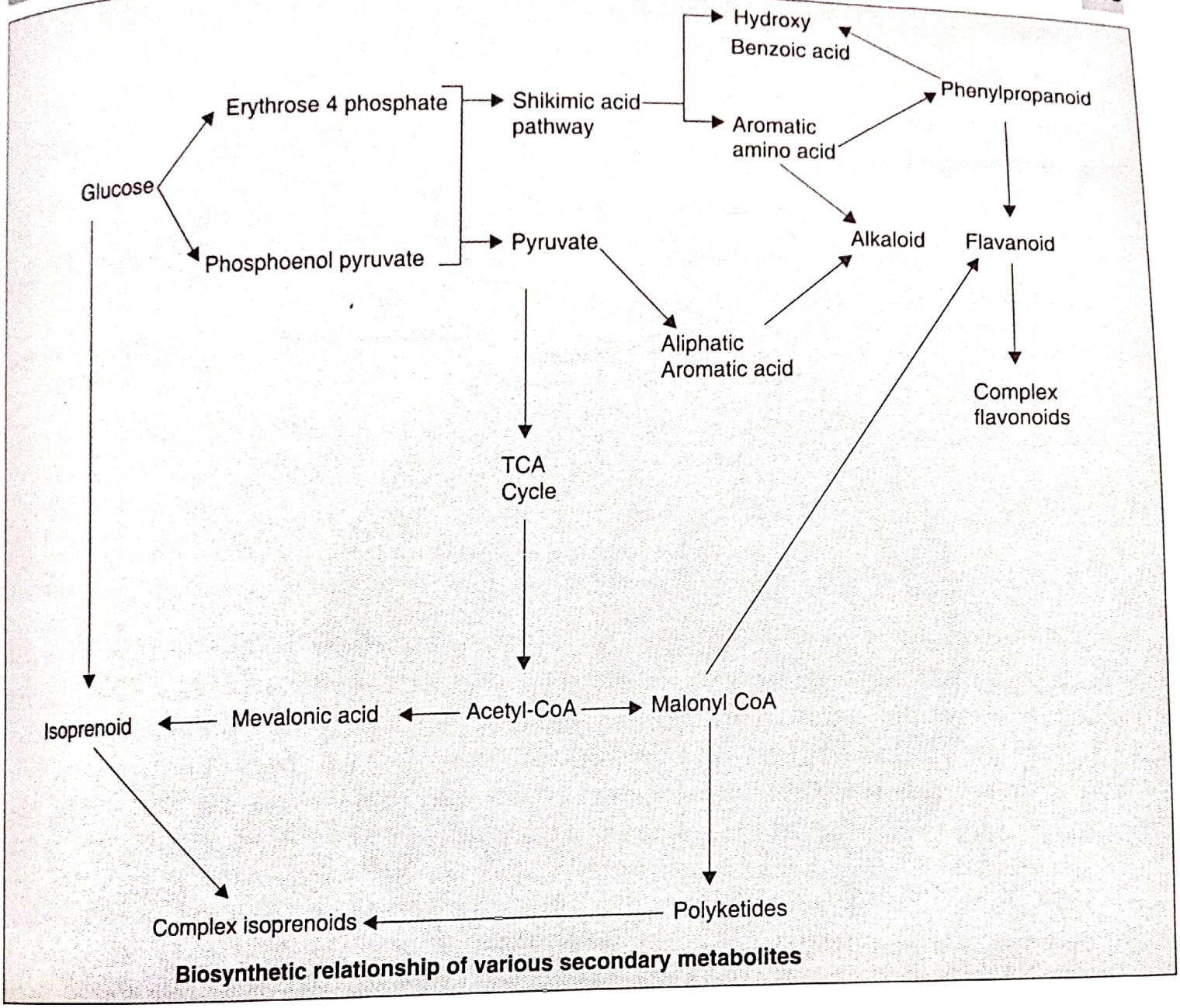
G_3P (glyceraldehydes 3 phosphate) is combined to fructose. Fructose is then rearranged to create glucose. Fructose and glucose then undergo dehydration and form a glycosidic linkage to create the disaccharide sucrose and water. Sucrose is then transported throughout the plant.



Tricarboxylic acid cycle or Krebs cycle

In the first step of the TCA cycle, the methyl carbon of Acetyl Co-A is allowed to condense with the keto carbon of oxaloacetate to produce citrate. As the oxaloacetate formation occurs through a thermodynamic unfavourable precursor; the exergonic nature of citrate synthases helps the reaction to proceed forward. The acetyl Co-A present on the mitochondrion is transported to the cytoplasm by the excess of the citrate. This acetyl Co-A which is brought to the cytoplasm activates the enzyme Acetyl Co-A carboxylase and is then utilized for the biosynthesis of fatty acid and cholesterol.

The isomerization of citrate to isocitrate is brought by aconitase, a mitochondrial enzyme which is stereospecific in nature. The enzyme cisaconitate is linked during the reaction and the enzyme aconitase is called the non heme iron protein due to the presence of inorganic iron and sulphur as

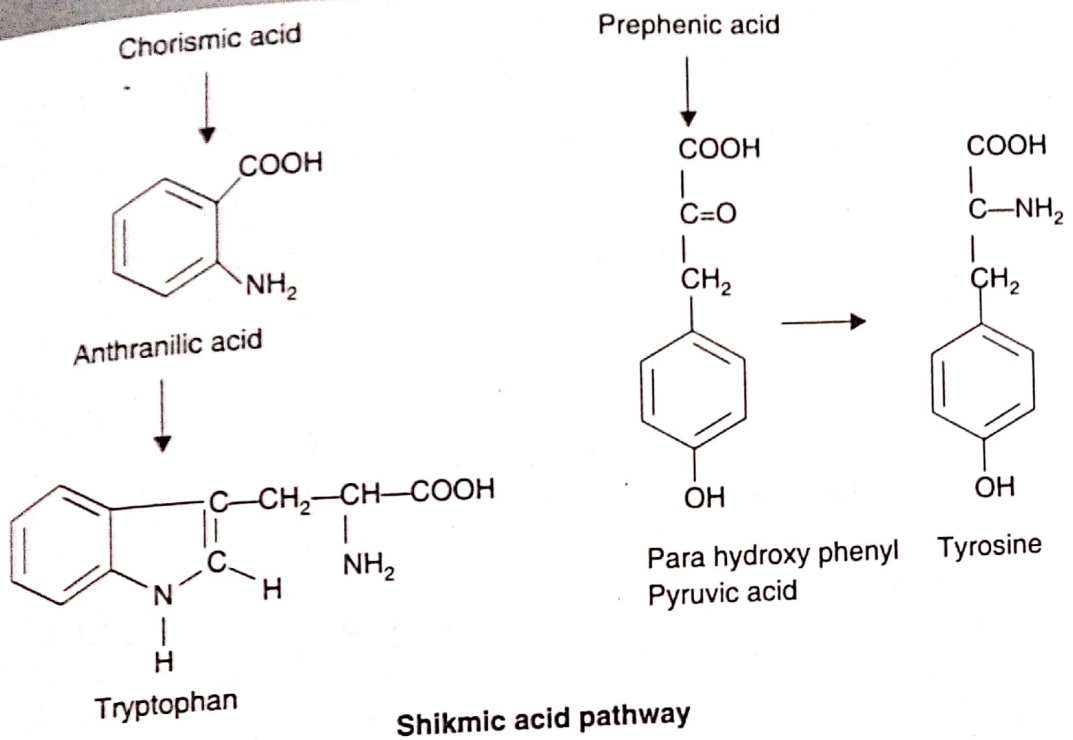


Biosynthetic relationship of various secondary metabolites

Shikimic acid pathway

It converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids like: L-phenylalanine, L-tyrosine and L-tryptophan. Shikimic acid is an intermediate product in this pathway and it is present in plants, fungi, and bacteria but not found in animals. As animals do not synthesize these (phenylalanine, tyrosine, and tryptophan) amino acids, these are classified as essential nutrients in animal diets.

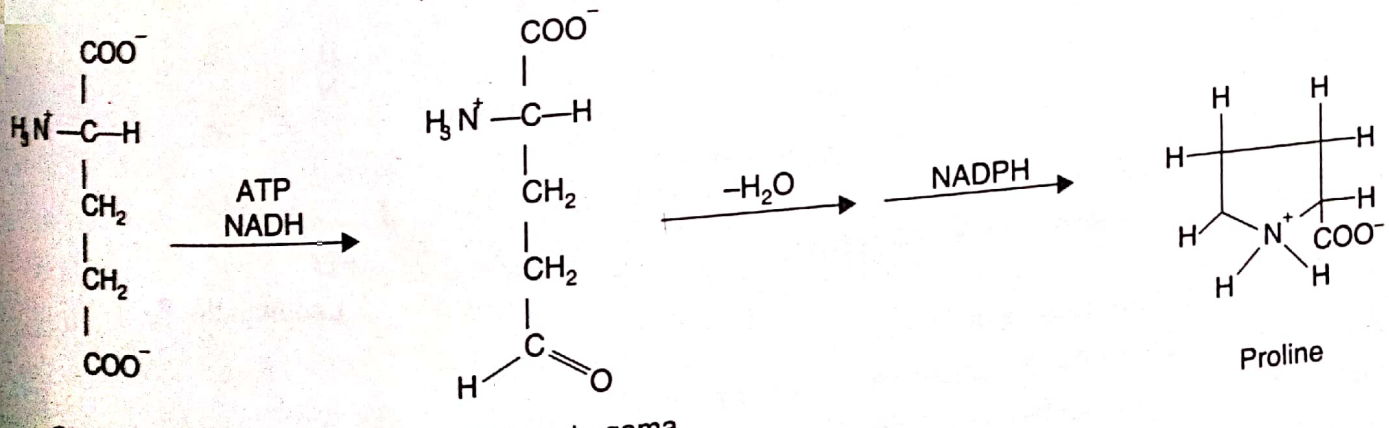
Natural products derived from shikimic acid range in complexity from the very simple products like vanillin; used primarily as a flavoring agent, salicylic acid; the precursor of aspirin, lawsone (a naphthoquinone used in some sunscreens) to a more complex natural products like lignan lactone podophyllotoxin. Shikimic acid pathway also generates cinnamates, anthranilates, common phenylpropanoids in essential oils including methyl chavicol, eugenol, methyl eugenol, methyl cinnamate, vanillin & anethole; lignans & flavonoids.



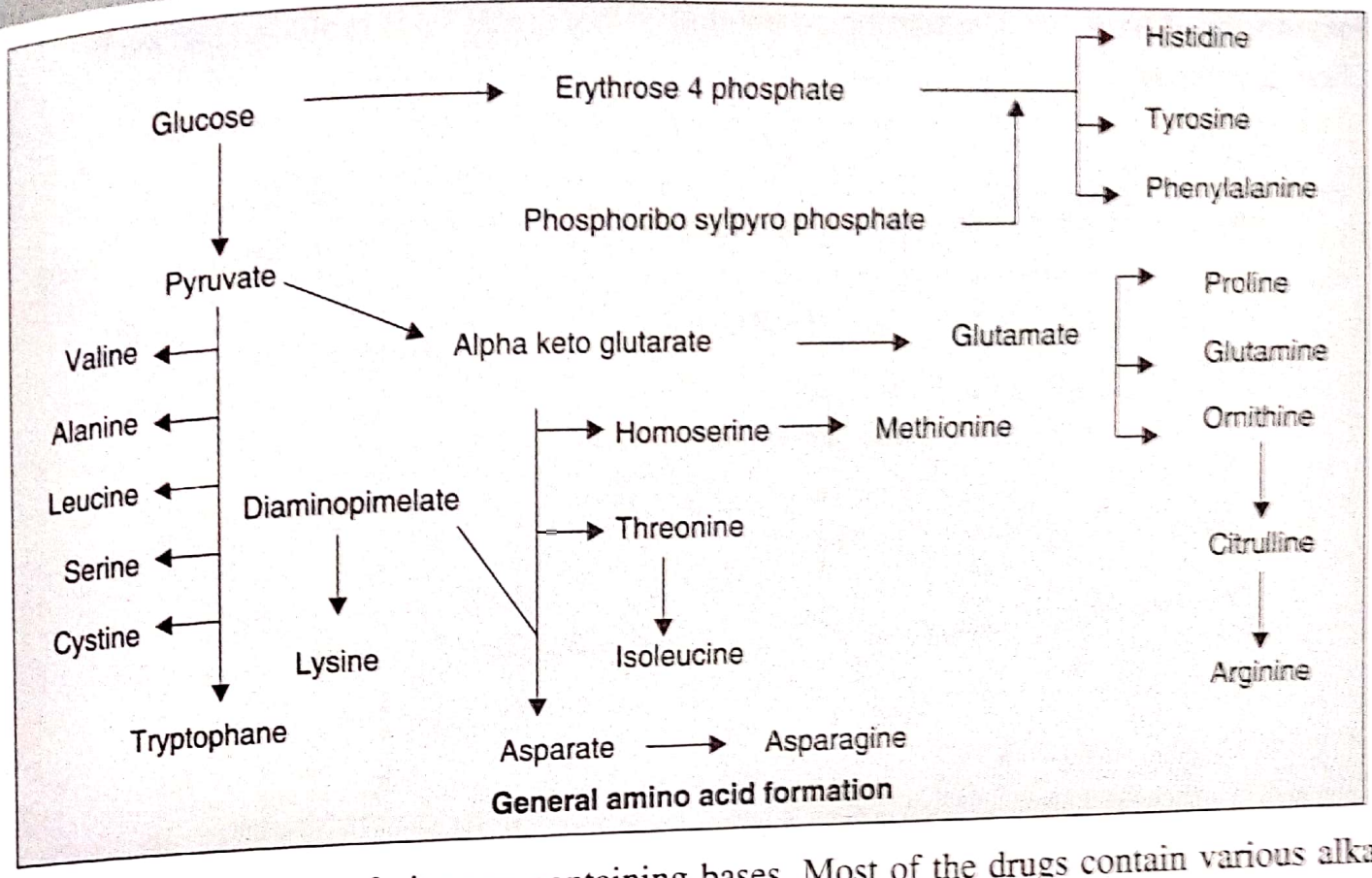
Shikmic acid pathway

Amino acid synthesis in plants

There are about 20 amino acids which are required by the plant for the synthesis of the proteins. The enzyme aminotransferases are very important as they can transfer an amino group to a -Keto acid. or they can also distribute the amino groups. Based on chemical structure and on their precursors, all the amino acids are grouped into five: the glutamate family has *alpha*-keto glutarate as its precursor, the aspartate family with oxaloacetate as its starting product, the alanine-valine-leucine group, the serine-glycine group and the family of aromatic amino acids with pyruvate, 3-phosphoglycerate and phosphoenolpyruvate and erythrose-4-phosphate as the starting products respectively. The amino acids belonging to glutamate family are glutamate, glutamine, proline and arginine. The amino acids like aspartate, asparagine, threonine, isoleucine and methionine are examples of amino acids grouped in aspartate family.



Biosynthesis of Proline

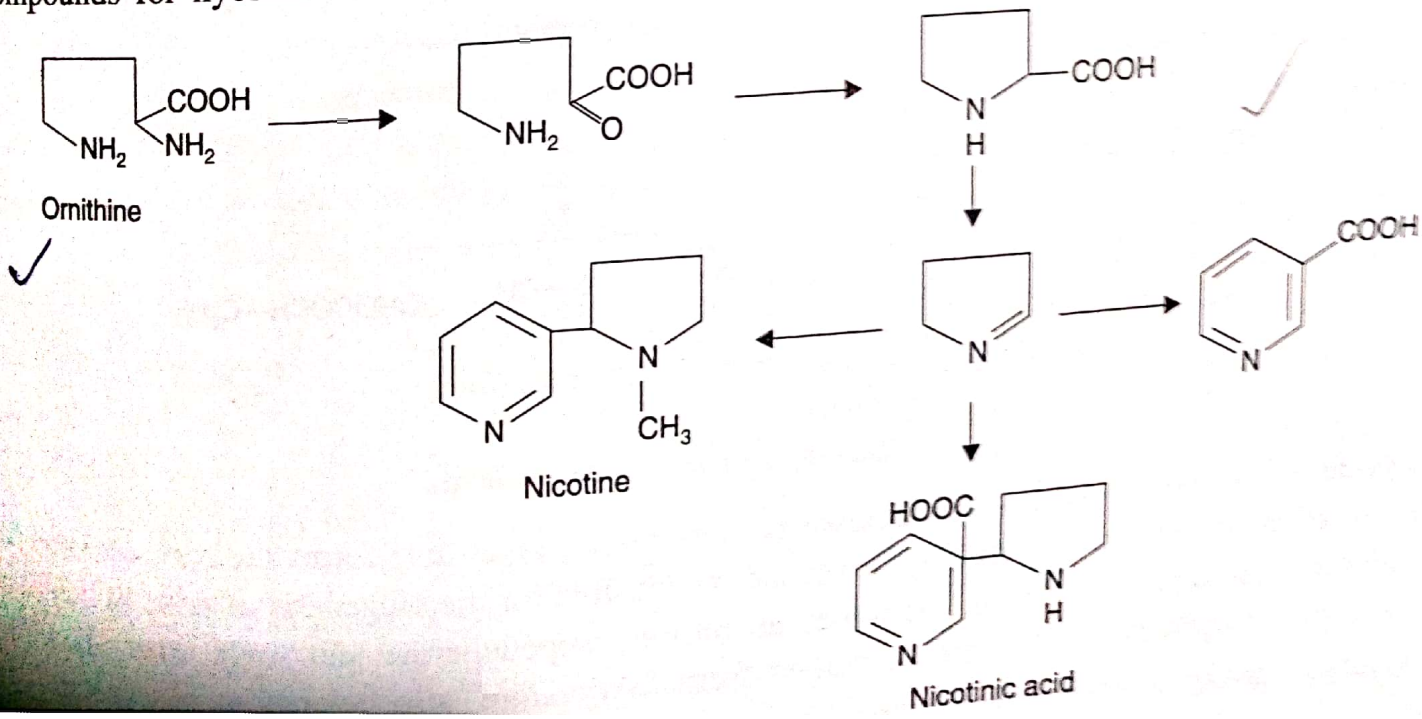


General amino acid formation

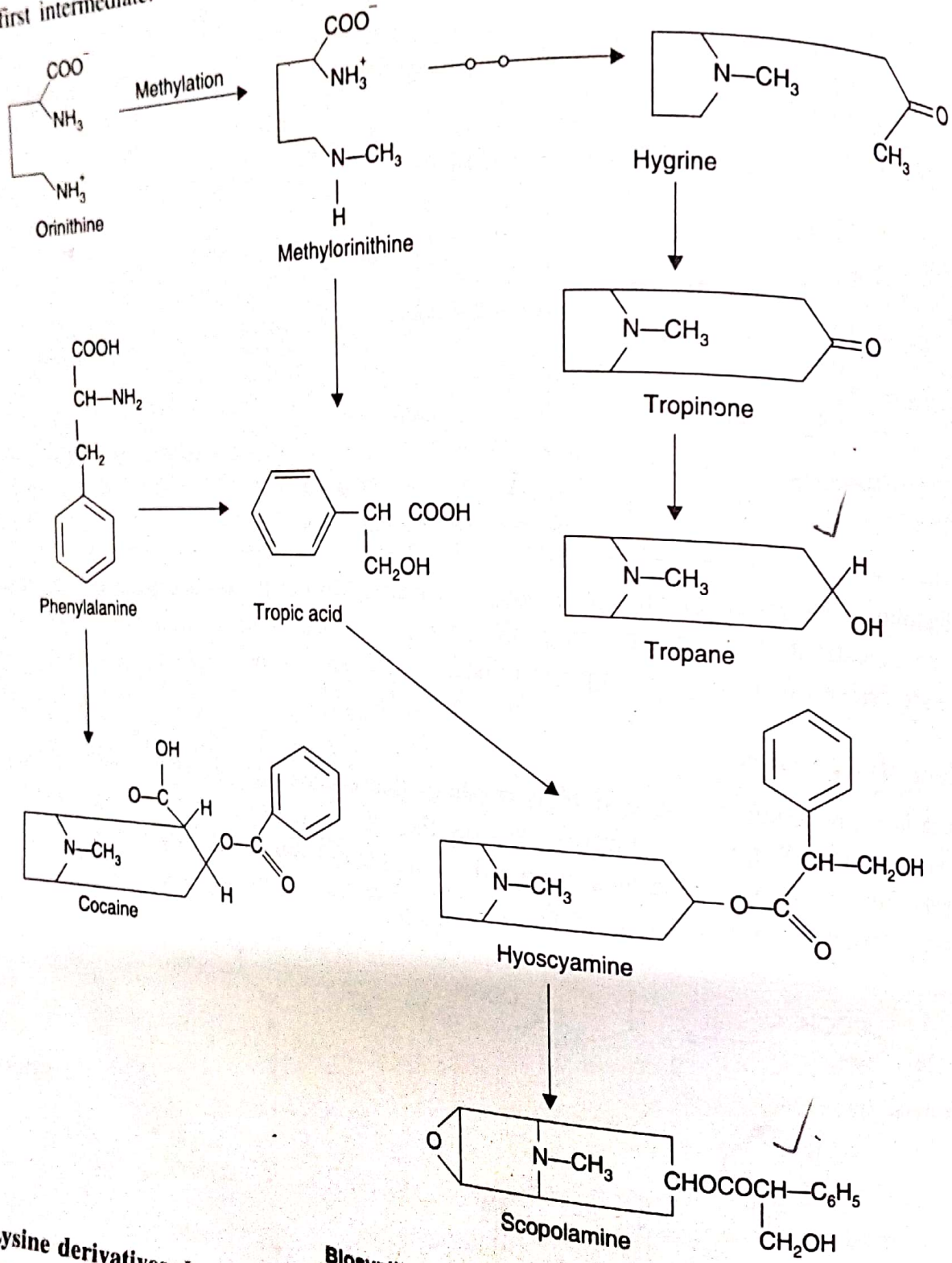
Alkaloids are a group of nitrogen-containing bases. Most of the drugs contain various alkaloids, which are investigated using labelled radioactive precursors. Some of the active constituents like caffeine are derived from purines or pyrimidines, while a large majority is produced from amino acids.

Ornithine derivatives

Ornithine is a precursor of the cyclic pyrrolidines that occur in the alkaloids of tobacco (nicotine, nornicotine) and in Solanaceae family. Most of the tobacco alkaloids have nicotine as the starting compound. Few of the intermediates produced during the biosynthesis of tropane are also the starting compounds for hyoscamine and cocaine.



Biosynthesis of tropane: The starting compound of this synthesis is ornithine and methylornithine is the first intermediate.



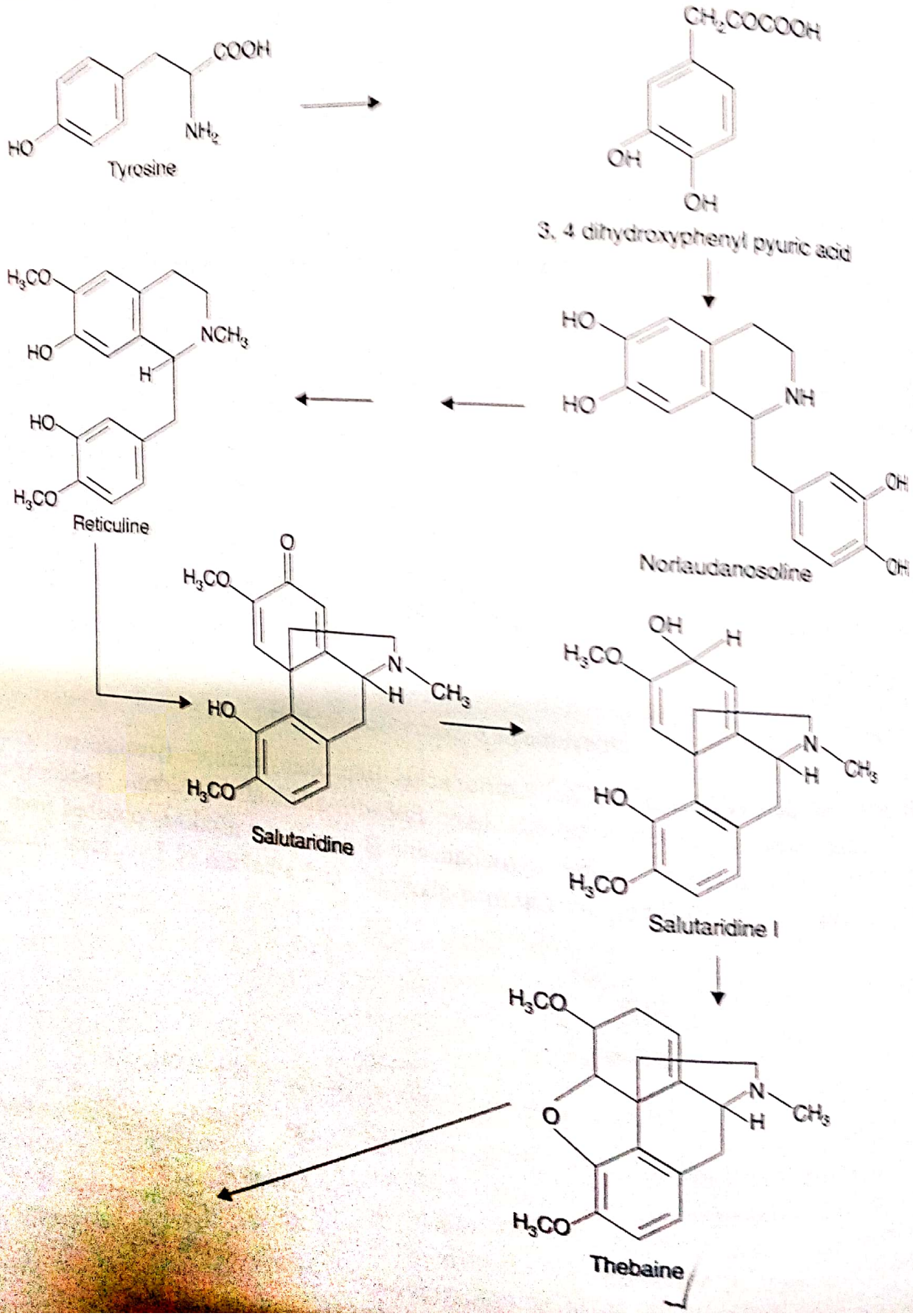
Biosynthesis of tropane alkaloids

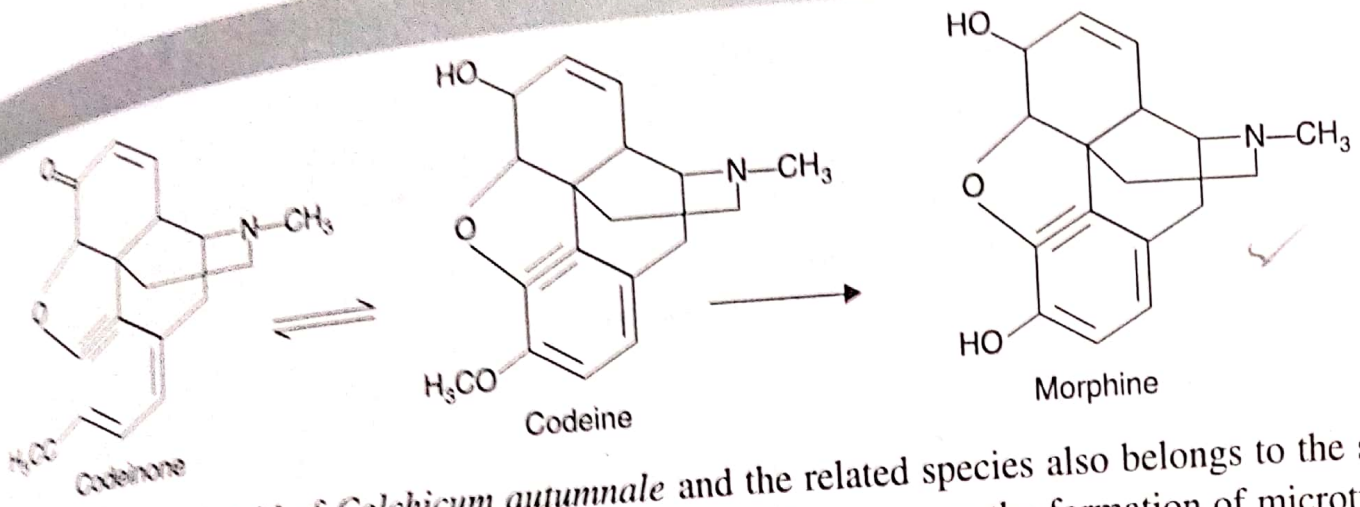
Lysine derivatives: Lysine is a precursor for piperidine. Piperidine forms the basic skeleton for numerous alkaloids. Lysine and its derivatives are responsible for the biogenesis of some of the bitter principles of the lupine, lupinine, lupanine; anabasine; isopelletierine and some other alkaloidal components. Lycopodine, a substance obtained from *Lycopodium* also belongs to this group.

A further group is constituted by the **pyrrolizidine alkaloids:**

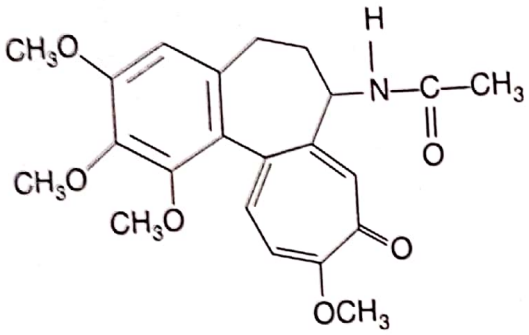
Tyrosine derivatives: The first essential intermediate is dopamine; dopamine is the precursor in the biosynthesis of papaverine, berberine and morphine. Tyrosine is considered to be a precursor for huge family containing alkaloids.

Biosynthesis of morphine: A range of similar compounds like the opium alkaloids, codeine, are derived during the formation of morphine.





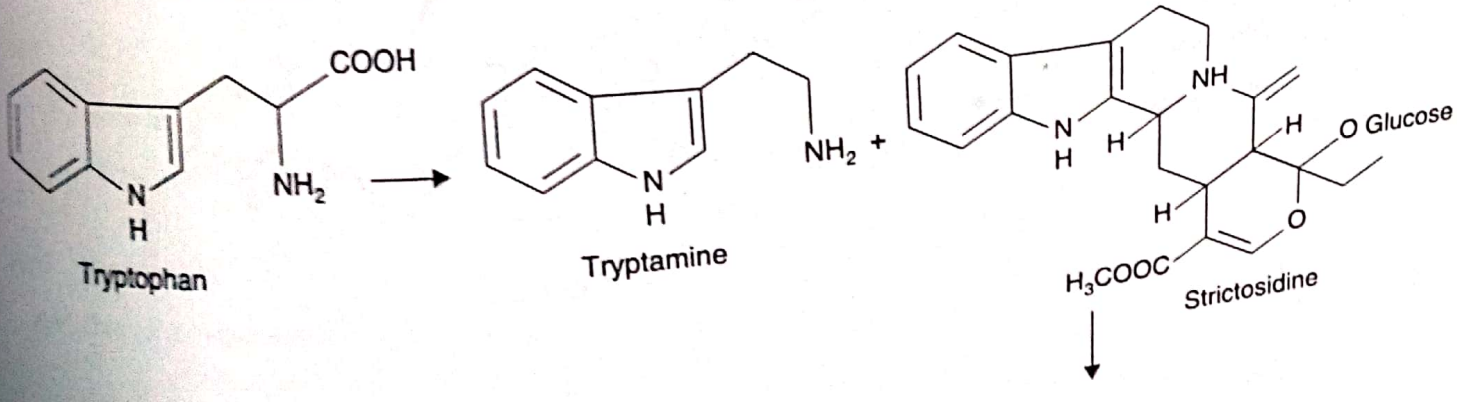
Colchicine, an alkaloid of *Colchicum autumnale* and the related species also belongs to the same class. It is a best-known poison, as it can inhibit mitosis. It also prevents the formation of microtubuli by the aggregation of tubuline dimers.

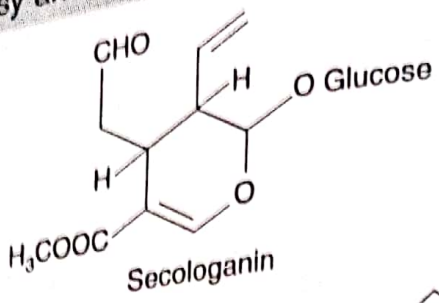


Colchicine

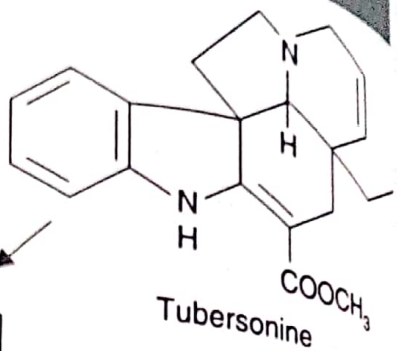
Indole (derivatives of tryptophane, etc.): About 1200 dissimilar compounds, the entire of which are tryptophane derivatives have been isolated till today. The tryptophane derivatives correspond to 25 percent of all known alkaloids and many of them are medicinally valuable. Tryptophane and its decarboxylated product (tryptamine) are precursors for the biosynthesis of broad range of indole alkaloids of which the *Vinca*, *Rauvolfia* and *Catharanthus* alkaloids are examples; and also in the alkaloids belonging to families like: Apocynaceae, Loganiaceae and Rubiaceae. D-tubocurarine, the active components of curare, is also a tryptophane-derivative. Tryptamine on condensation with secologanin produces vincoside a nitrogenous glucoside. Some of the indole alkaloids in vinca are formed from vincoside. Lot of well-known fungi poisons also belong to this group.

Biosynthesis of indole alkaloids

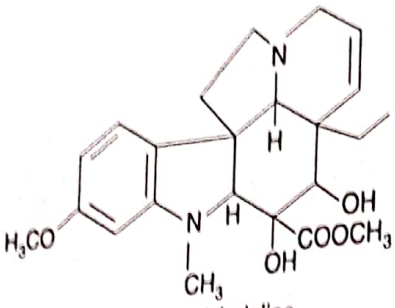




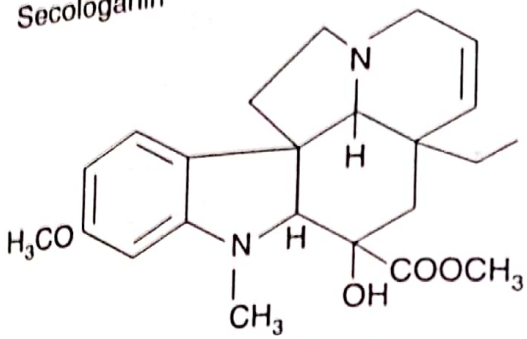
Secologanin



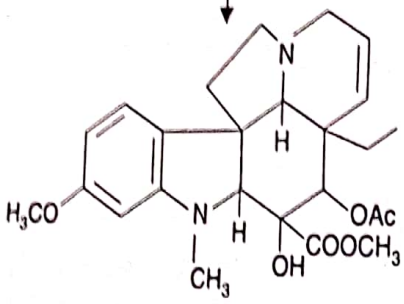
Tubersonine



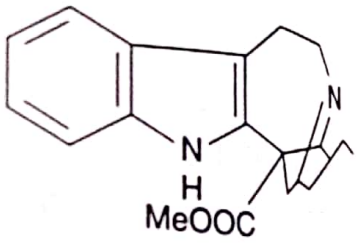
Deacetylvindoline



Deacetooxyvindoline

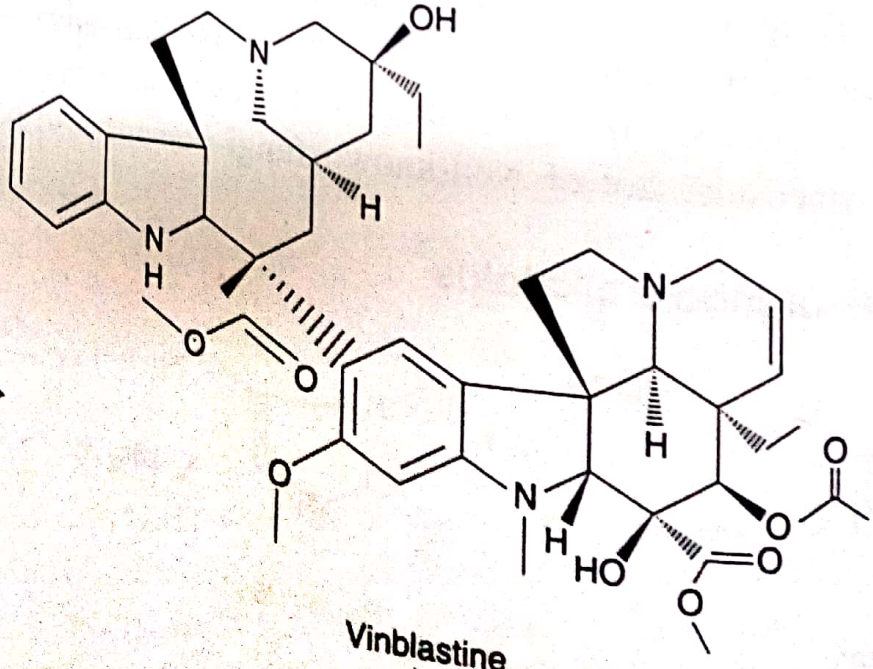


Vindoline

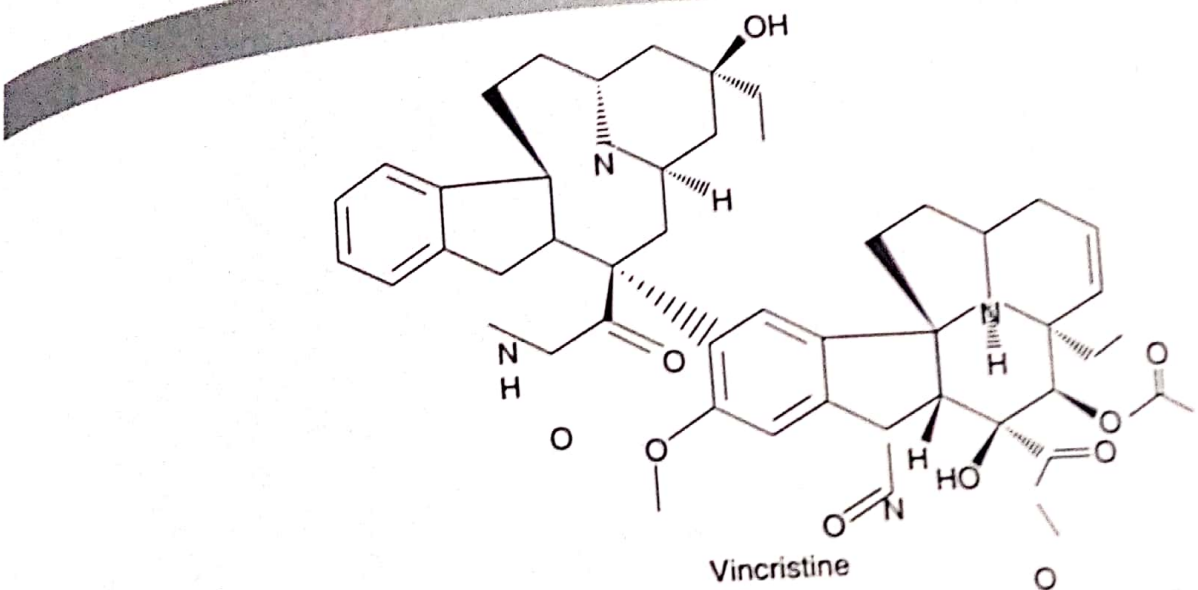


Catharanthine

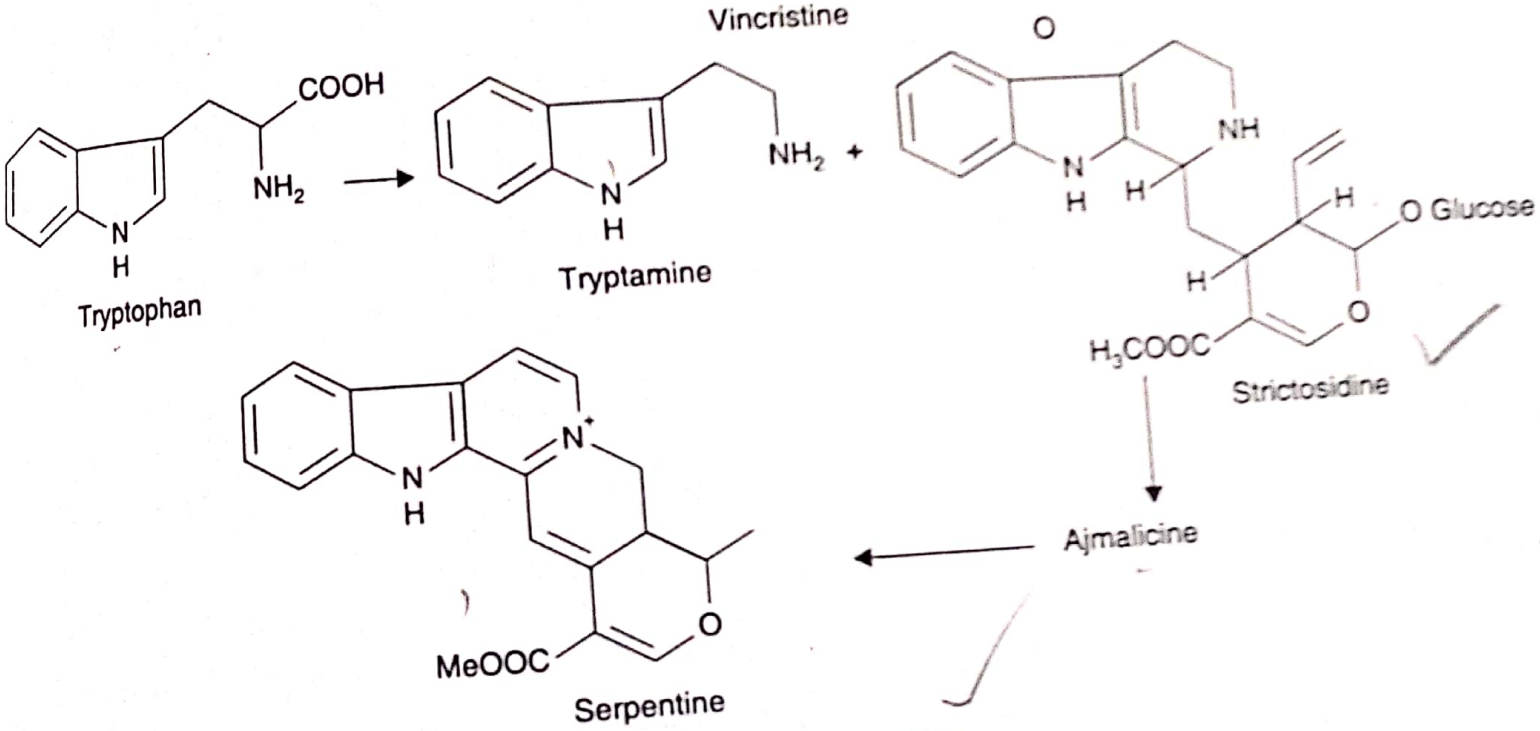
Vindoline
+
Catharanthine



Vinblastine



Vincristine



Tryptophan

Tryptamine

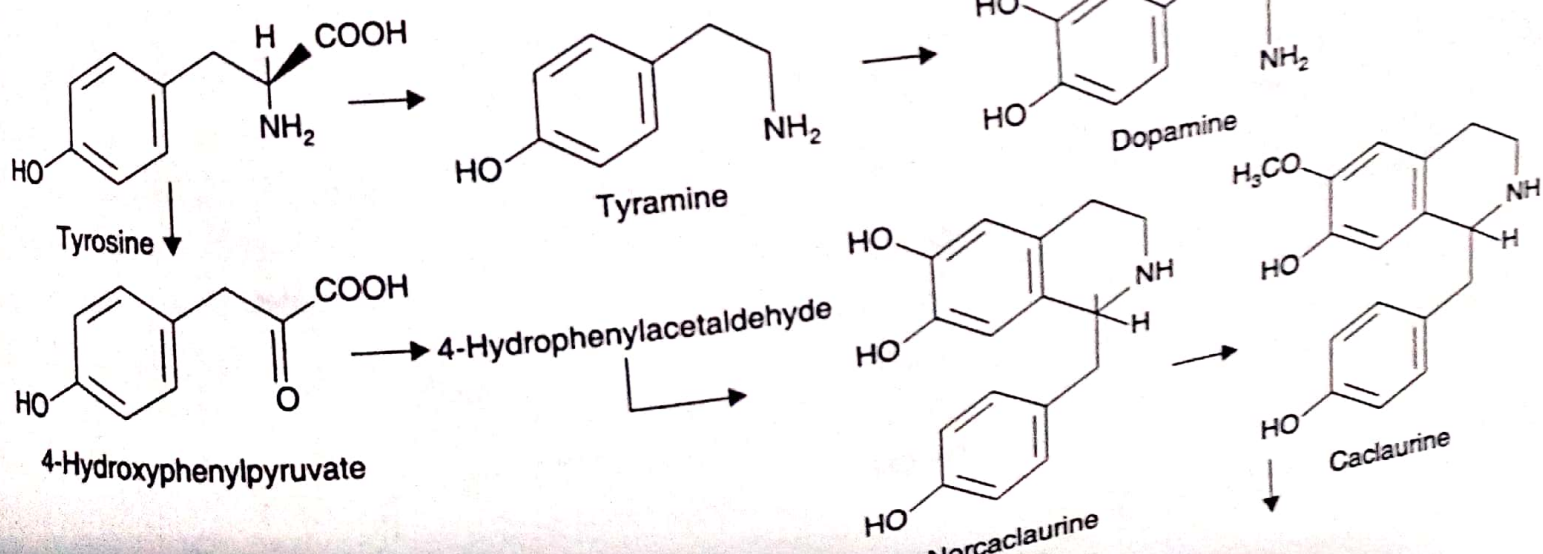
Strictosidine

Ajmalicine

Serpentine

Biosynthesis of serpentine

Biosynthesis of isoquinoline alkaloid



Tyrosine

Tyramine

Dopamine

4-Hydroxyphenylpyruvate

4-Hydrophenylacetaldehyde

Norcaclaurine

Caclaurine

Mevalonic Acid Pathway to Terpenoids and Steroids

Several thousands of different molecules from various plants have been isolated and characterized. In spite of its varied structures, it is found that a few pathways synthesize all of them. Isoprenes could be regarded as a fundamental building block for the isoprenoid compounds as well as many sterols as triterpenes. By 1950 it was established that the acetic acid was involved in the synthesis of cholesterol, squalene, sterol, and rubber. The use of methyl and carboxyl labelled acetic acid with animal tissues indicated that the methyl and the carboxyl carbons alternated the skeleton of cholesterol and squalene and that the lateral carbon atoms all arose from the methyl group of acetic acid. The discovery of acetyl co enzyme A, the so called active acetate gave further support to the role of acetate in the biosynthetic process in 1950. In 1956 mevalonic acid and the demonstration of its incorporation by living tissues into those compounds possibly formed from isoprenes units, was reported. Mevalonic acid which is 3, 5, dihydroxy –3-methyl valeric acid, is a 6 – carbon acid and as such is not the active isoprene unit which forms the basic building block of the isoprenoid compound. During the next few years of research, it was established that the 5 –carbon compound for which biochemist had been seeking so long was isopentenyl pyrophosphate by the decarboxylation and dehydration. Steroids, carotenoids, gibberelic acid are some of the members of polymeric isoprene derivatives. Mevalonic acid upon phosphorylation is transformed into a phosphorylated isoprene, which undergoes subsequential polymerization. All green plants are able to generate linear isoprenoids in this way. The terpenes with more than five isoprene units are fairly wide-spreaded; but a lot of the simpler terpenes are limited to some plant groups. Sesqueterpenes are common in mosses but also occur in higher plants. In a similar pattern, monoterpenes (iridians, iridoid compounds) also occurs in higher plants. Gibberellins are diterpenes, which are grouped as phytohormones. Steroids are triterpenes or triterpenoids. Triterpenes are a group of molecules that contain 30 C-atoms. The triterpenoids are generated by the polymerization of six isoprene units. Steroids are found in both gymnosperms and in angiosperms. The terpenoids are hydrocarbons with one or more C=C double bonds, while the terpenoids are oxygen-containing analogues of the terpenes.

Generally the class of terpenes has 5-carbon units, often called isoprene units put together in a regular pattern of head-to-tail in terpenes up to a maximum of 25 carbons

Mevalonic acid

Isopentenyl pyrophosphate or 3-methylbutenyl pyrophosphate is regarded as the biogenic equivalent to isoprene. e.g. for monoterpenoids:

Glucose → Acetyl CoA

Acetyl CoA → Acetylacetyl CoA + Acetyl CoA

Acetylacetyl CoA + Acetyl CoA → 3-hydroxy-3-methylglutaryl CoA

3-hydroxy-3-methylglutaryl CoA → mevalonic acid

mevalonic acid → 5-phoshomevalonate

5-phoshomevalonate → 5 pyrophospho mevalonate

5 pyrophospho mevalonate → IPP (isopentenyl pyrophosphate) + dimethylallyl pyrophosphate (DMAPP)

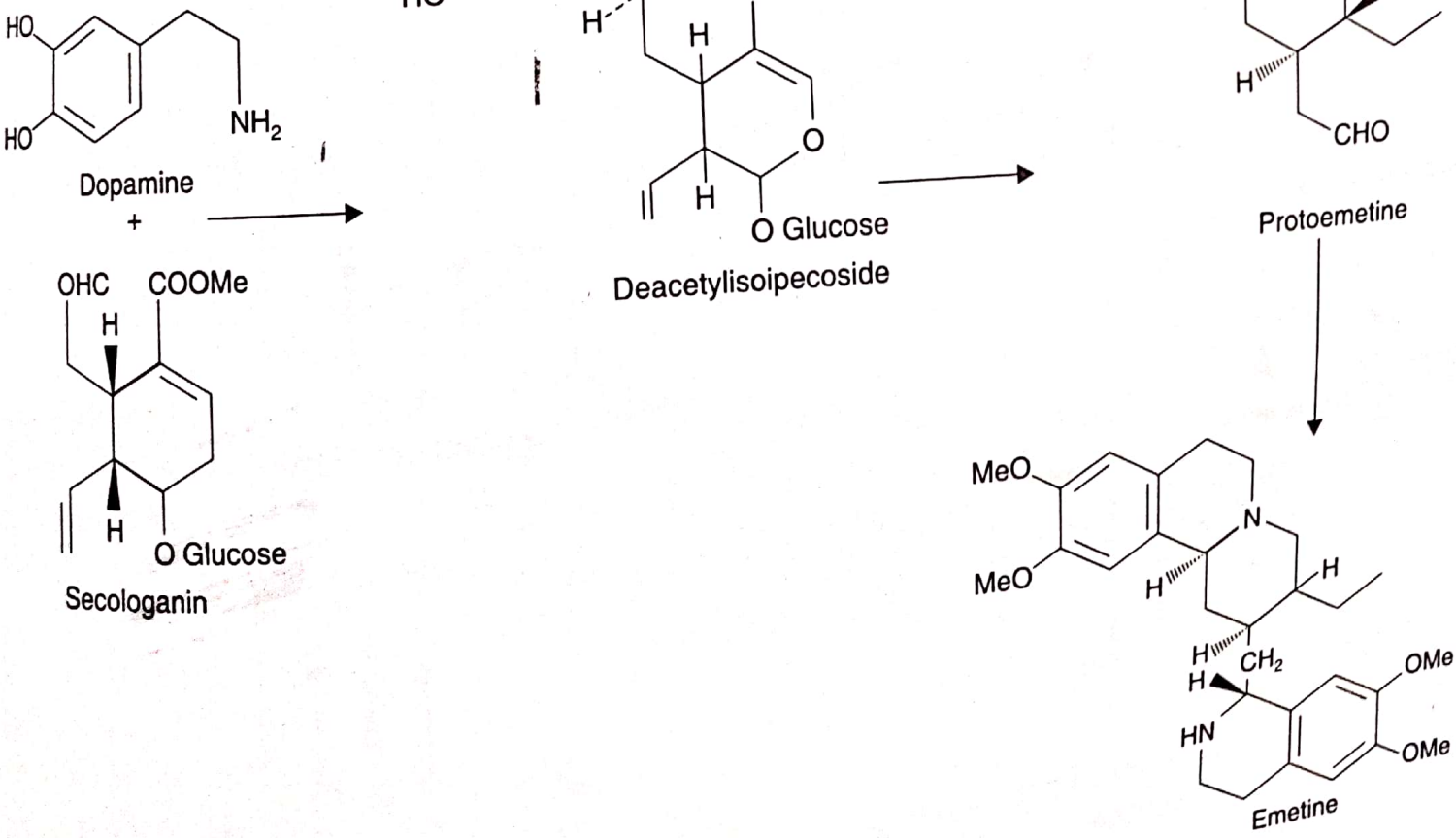
Biosynthesis of Rutin

Naringenin → Dihydrokaempferol → Kaempferol → Quercetin → Quercetin 3-O-glucoside → Rutin

Biosynthesis of Taxol

Terpenoid Biosynthesis → Geranyl geranyl pyrpphosphate → Taxol-4(5) 11(12) diene → Taxol-4(20), 11(12) diene 5 alpha diol → 10-Deacetyle-2-debenzoyl beccatin III → 10 Deacetyle beccatin III → Baccatin III → Taxol

Biosynthesis of Emetine



Aconitine

phosphate → Coptyl PP → → Aconitine.

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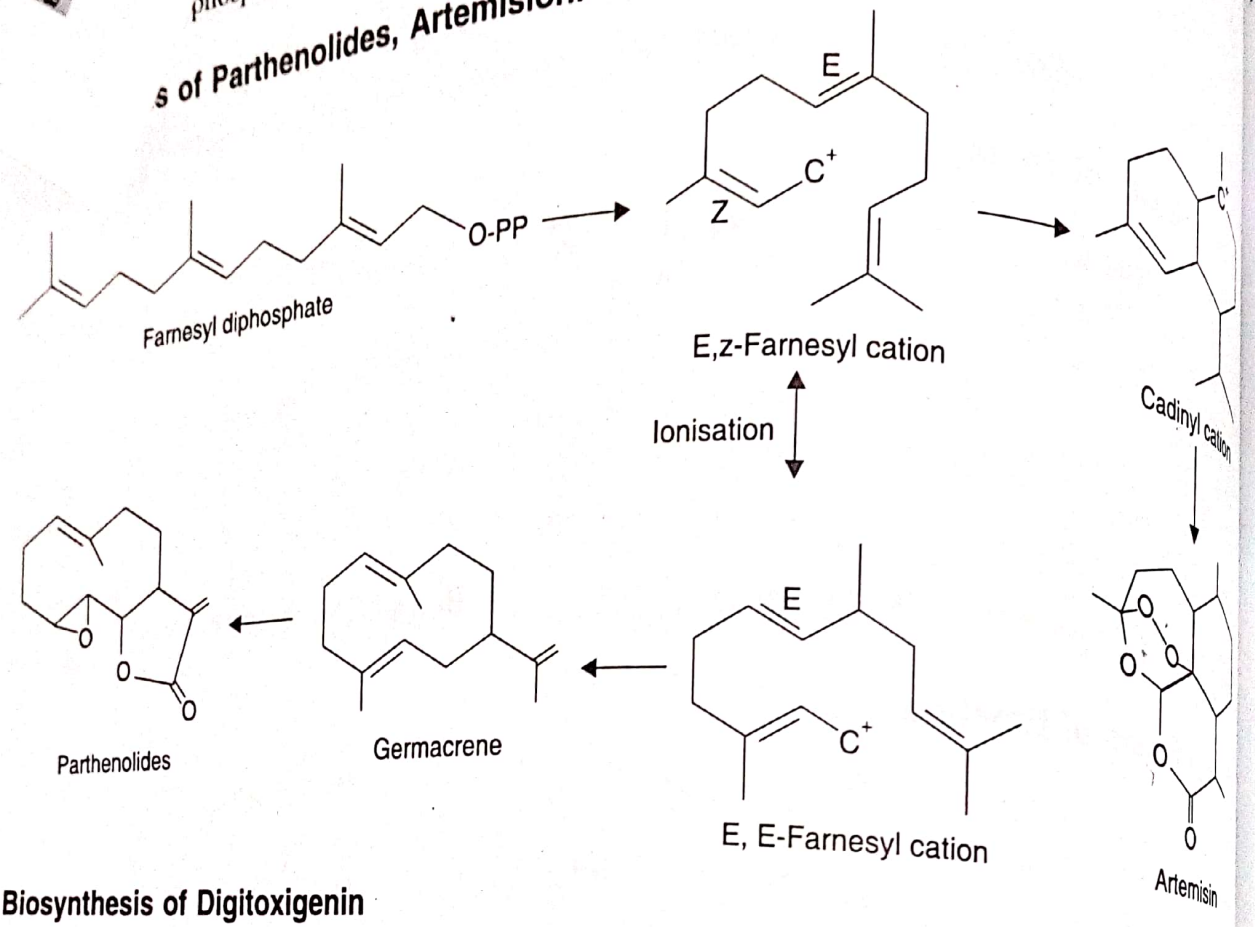

Biosynthesis of Digitoxigenin