I REVIEW OF LITERATURE

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A) *Coleus* Lour. :

1. Historical perspectives :

Labiatae is the one of the largest families of dicotyledons. It consists of 200 genera and about 3,000 species, most of which are aromatic annual or perennial herbs. The family is well represented in Britain and in the mediterranean countries, chiefly in warm dry temperate regions. The morphology and taxonomy of the family have attracted the attention of research workers at all time.

With the advent of the discovery of alkaloids, aromatic oils, and it's use in medicine, members of this family have attracted attention of pharmaceutical industrialists. This has opened a new vistas in the field of agriculture and crop improvement. Several members of this family have been reported to contain useful products such as volatile oils and alkaloids (Hutchinson 1969). To cite a few; Mint (*Mentha viridis* L.); Lavender oil (*Lavendula vera* DC.); Patchouli (*Pogostemon heyneanus* Benth.); Pepermint (*Mentha piperita* L.); Marjoram (*Origanum marjorana* L.); Thyme (*Thymus vulgaris* L.); Sage (*Salvia officinale* L.); Sweet basil (*Ocimum basilicum*); Rosemary (*Rosmarinus officinalis* Linn.).

Among 200 genera, the genus *Coleus* has about 200 species, distributed all over the tropical and sub-tropical region of Australia, Asia, Africa and Pacific Island. About

8 species are recorded in India. Many of the species are cultivated for the purpose of edible tubers. They are :

1) C.amboinicus Lour. (Earlier name C.aromaticus Benth)
2) C.forskohlii Briq. (" " C.barbatus)
3) C.parviflorus Benth.(" " C.tuberosus)
4) C.vettiveroides Jacob.
5) C.dazo
6) C.rotundifolius (Earlier name C.dysentricus Baker)
7) C.edulis Vatke.
8) C.spicatus Benth.

It is relevant to give a brief description of these species.

1) C.amboinicus Lour.

This is recognised by different names. SAN - Pashan-bhedi, HINDI - Pathorchur, TAM - Karpuravalli.

A rather large succulent herb with aromatic leaves and small pale purple flowers, commonly cultivated in gardens. It is a native of East Indies. The leaves have a pleasant aromatic odour and pungent taste, and are used for flavouring meat and salad (Burkill 1950). It is good substitute for borage (Borage officinalis Linn.) for flavouring wines and beer. The aromatic properties are attributed to a volatile oil containing carvacrol (Parry 1950). The leaves are reported to be useful for urinary disease. The juice of the leaves mixed with sugar is a powerful carminative. It is employed in dyspepsia, although it is said

to have intoxicating properties. A decoction of the leaves is given for chronic coughs and asthama.

2) C.forskohlii Briq. (Mukerjee 1940)

A perennial herb, 1-2 feet high with a thick root stalk, distributed in sub-tropical Himalayas of Kumaon and Nepal, ascending to 8,000 feet and it is commonly grow on dry, barren hills and is cultivated in Bombay and other region for the roots which are pickled and eaten. This species is considered to be the wild ancestor of all the tuber varieties known as Kaffir potatoes (Greenway 1944).

3) C.parviflorus Benth.

A small herbaceous annual, 1-2 feet high, prostrate or ascending with succulent stem and aromatic leaves. It bears cluster of dark brown tuberous roots. It is grown in India, Ceylon, Java for edible tubers, used as substitute for potato (Radhavachari 1918). This plant is propogated by suckers obtained from tubers.

4) C.vettiveroides K.C.Jacob.

Jacob (1941) reported that it is small succulent herb 45-53 centimeter high, with a procumbent stem and thick, purplish, pubescent leaves. A plant bear long (35-50 cm) fibrous roots which are straw coloured and strongly aromatic when fresh. It is cultivated by vegetative cuttings. The plant has not been seen in flower any-

where. It is cultivated in Madras. The roots are used for the decoration of temple images and for dressing hair (Gopalachetty 1939).

5) C.dazo A.Chev et.

"Rizuka" tubers are eaten by some Europeans, they can be pickled in Sudan and Cango.

6) C.rotundifolius

"Hausa potato", "Fra-fra potato" tubers are consumed like potatoes. A good substitute crop for potatoes in hot climate countries like Malaysia, Indonesia, Mauritis, Madagascar, W. Africa and Sudan.

7) C.edulis Vatke.

Tubers are eaten by E. African natives.

8) C.spicatus Benth.

The Egyptian grew this plant for making chaplets and for food.

2. Distribution :

Out of these eight species which have edible root tubers only Coleus forskohlii have been extensively explored for the antihypertensive steroidal alkaloid (Bhat et al. 1977) Incidentally this species, and only this species is widely cultivated in this region of the country.

Coleus forskohlii Briq. originally called Coleus barbatus Benth. is a native of tropical Africa. It has been under cultivation and found wild in tropical India especially in Deccan plateau and peninsular part of the Indian continent. It is widely cultivated in Gujarat, Bihar, Maharashtra and Karnataka. This plant is also commonly found growing wild on mountainous ranges of Western Ghats of Purandhar.

According to Mukerjee (1940) it is common on dry barren hills and is cultivated in the border area fields. Since the time immemorable this species is cultivated in this part of the country for the purpose of roots, which are eaten and pickled because, they are tuberous and fleshy in nature. Because of it's aroma, in Maharashtra, Gujarat and Karnataka, the roots are used as spices and condiments. In recent years this plant is cultivated for the extraction of alkaloid from it's root.

3. General morphology :

Coleus forskohlii have some special distinguishable characters. It is a perennial herb of 1-2 feet hight with a fleshy thick root stocks; stem somewhat cylindrical, stout, green and woody at the base. The branches villous with long hairs. Leaves 1-3 by 0.5 to 1-5 inch, elliptic-oblong, obtuse, pubescent on both sides, petiole 1/4 - 1/2 inch long. Flowers

large showy, in whorls of 6-10 which are at first rather close, afterwards distant, in long spike like recemes 6-12 inch or more long, rachis densely glandular-hairy; bracts large, conspicuous, imbricate before flowering, broadly ovate with a slender mucro about 1/10 inch long, membranous, veined, pubescent, deciduous; pedicel short decurved. Calyx purple, upper lip ovate, entire veined slightly longer than the lower, lower lip trangular with pointed teeth. Corolla, pale purple, nearly 3/4th inch long, the mouth oblique, upper lip short, errect with four shallow rounded lobes; lower lip form a neck at the base, 1/3rd inch long, boat shaped and curved upward. Filaments combined into a sheath at the base. Nutlets globose 1/16 inch in diameter, smooth, nearly black.

Vernacular name of Coleus forskohlii is main-mul and Garmar. It flowers in between September to November. It takes 10 to 15 weeks from the time Coleus sprouts till it flower. Single inflorescence takes 15 days for complete flowering. The flowers are showy, borne in solitary raceme. When near to maturity the flower deepens in colour turning bright violet. Genus Coleus forskohlii takes 6 month for complete maturity. It bears thick, stout, brown tubers with pungent odour, so aromatic.

4. Cytology :

Sixteen species of economically important family Labiatae from Pachmarhi Hills (M.P.) have been studied for

the first time by Bir (1982). He exposed five species for cytological studies. They are namely, Leucas mollissima Wall, var. scaberula Hook, n = 14; Micromeria capetellata Benth, n = 24; Plectranthus mollis spreng., n = 14; Pogostemon purpurescens Dalz., n = 16 and Salvia coccinea Linn. pseudococcinea (Jacq.) Gray, n = 11. They were worked out for the first time. New cytotypes were located in 4 species Coleus barbatus Benth., n = 17 (2x); Lavendula bipinnata O.kze, n = 20 (2x); Ocimum canum Sims., n = 40 + 0 - 1B (10x) and O. Sanctum Linn., n = 18 (4x). In Hyptis sauveolens Poit, n = 14 was recorded in contrast to earlier report of n = 16 from Northern India. For other species previous reports were confirmed. Of these species studied, 31.25% are polyploids. The other species studied were Leucas lanata, Micromeria biflora, P.benghalense, S.plebeia, S. splendens and O.kilomandscharicum.

Cytological work and genetic improvement is scanty in Coleus forskohlii. The only work reported recently is of Bahl and Tyagi (1988). They examine the pachytene chromosomes of the Coleus forskohlii and reported that it has 2n = 30 chromosomes (based on meiotic analysis) and length varied between 50.45 µ to 15.0 µ.

5. Mutation breeding :

Improvement of the cultivated plants largely depends on the extent of genetic variability available within the species.

Ionizing radiations and various chemical mutagens provide a handy tool to enhance the natural mutation rate and thereby, enlarge the genetic variability and increase the scope for obtaining the desired selections. In the past three decades, a large amount of research on induced mutations in cultivated plants has been carried out in India with the view to develop better cultivar.

A systematic study for evaluation of the potential of induced mutations by physical and chemical mutagens for crop improvement was initiated in mid fifties (Swaminathan 1957). A wide range of physical and chemical mutagens have been used by several investigators for inducing mutations in different crop plants. The different physical mutagens used in the various studies were x-rays, gamma-rays, radioactive isotopes like P^{32} and S^{35}, and fast and thermal neutrons. These have also been used in combination with ultraviolet and infra red rays (Bhatia et al. 1963). Alkylating agents are the most commonly used chemical mutagens (Savin 1968). Vegetable oils have also been found to be mutagenic (Natarajan 1958).

Studies of Bhatia and Swaminathan (1963) have shown that the response of varieties to different mutagenic treatments are similar. The mutation rate was highest in the thermal neutron treatment followed by isotopes S^{35} and P^{32} respectively and x-ray treatment. Later studies by Goud (1967)

have, however, shown differences in varietal response to mutagenic treatment.

Shama Rao (1979) had made attempts to induce genetic variations for better selections in sugarcane for increased sugar content, higher yield and resistance to prevailing pests and diseases. Very tender single bud sets were exposed to gamma-rays from a 60 source at a dose rate of 6 k rads/min. and have shown that useful mutants could directly be used as cultivars through mutation breeding in a vegetatively propogated crop such as sugarcane.

In Nicotiana tabacum L. the predominant alkaloid nicotine, is a major factor in smoking pleasure. The level of nicotine in cigarettes varies from 1.5 to 2.5%. However, in recent years, the trend has been towards cigarettes with low nicotine level, in order to reduce the harmful effects of smoking on human health. Valleau (1949) was the first to report on breeding low nicotine tobacco varieties. Since then, efforts have been made by breeders in several tobacco producing countries to evolve tobacco varieties having acceptable level of nicotine, Krishnamurthy (1978) and Ramavarma (1979) have developed a tobacco mutant with low nicotine content by the treatment of EMS.

Mutation breeding is ideally suited for the improvement of vegetatively propogated crops which are highly heterozygous.

It is impossible to improve some of the vegetatively propogated plants by hybridization on account of problem of sterility, apomixis and cross-barriers etc. The greatest advantage of mutation breeding is that only one or a few characters of an otherwise outstanding cultivar can be improved without affecting its desirable characters.

6. Mineral Nutrition :

The complex structure of secondary products made difficult to study the biogenesis of these compounds, and the enzymes involved in the system. Nevertheless the study of biogenesis of cyclic amino acids and such other complex primary compounds has been known and the important minerals involved as a cofactor are also known. Gasic et al. (1978) extensively serveyed the colchicine content from Colchicum autumnale, in relation to soil mineral composition. They established the +ve correlation between the trace element Cu, Zn and Mn and the total alkaloid content. It is well established in the literature that aromatic amino acids such as phenylalanin, tyrosine and tryptophan play a crucial role in metabolism of large number of complex compounds including alkaloid (Fowden 1965). Zinc is one of the important trace elements which is known to have a role as cofactor in tyrosine synthesis, and Mn has the same role to play in tryptophan synthesis.

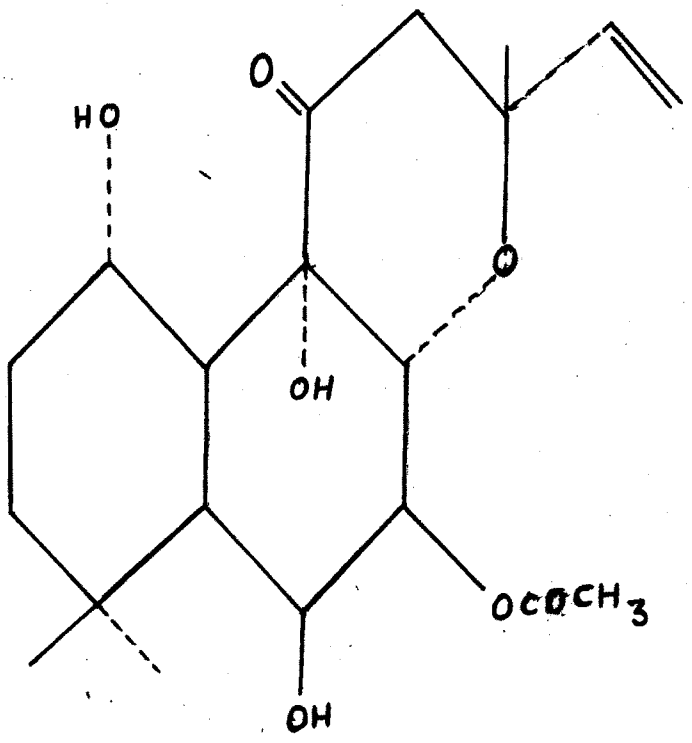
Nicholas (1961), Bennett and Heftmann (1969) and Capstack et al. (1965) have shown that in plants Mavalonic acid is converted to squalene and later to sterols. Capstack et al. (1965) demonstrated that the squalene formed in plants had same lebelling pattern as that of animal. Mavalonic kinase and phosphomavalonic kinase have been shown to be the first two enzymes involved in the conversion of mavalonic acid to mavalonic-5-pyrophosphate through mavalonate-5-phosphate. Both of the reactions are known to require ATP as phosphate donor and Mg^{++} or Mn^{++} promote greater enzyme activity (Tchen 1958). In the activation of the enzymes low concentration of Mn^{++} are more effective than Mg^{++} (Tchen 1958, Williamson and Kekwick 1965). This foregoing brief scanty information provides a clew that some of the mineral elements control the metabolism of secondary compounds.

clue

B) Forskolin as a Drug :

Forskolin, the antihypertensive steroidal alkaloid is extracted from the root tubers of Coleus forskohlii. According to Bhat et al.(1980), it is diterpenoid having the structure given below, the properties are as follows : The molecular formula of this chemical is $C_{22}H_{34}O_7$; molecular weight 410, melting point 230 $\pm$ 2°C. The chemical name is 1α, 6 β, 9α-trihydroxy-7 β-acetoxy-8, 13-epoxy-iabd-14-en-11-one, 1.

Structural Formula



After determining the structure of this diterpenoid, method to isolate and purify from the roots of Coleus forskohlii was also developed by Bhat et al. (1980) and in the process they characterised another compound from the same root called Naphthopyron. According to Inamdar et al. (1980) forskolin is a novel, positive inotropic and blood pressure lowering agent. They developed different precise methods for quantitative estimation of forskolin in which application of gas liquid chromatography is one of the important approaches. Inamdar et al. (1984) have also developed the technique of TLC and HPLC (High Performance Liquid Chromatography) and

compaired the efficiency of all the three systems. Shah et al. (1980) screened different plants belonging to Coleus and Plectranthus genera of the Labiatae for the presence of forskolin by semi-quantitative assay method. According to them Coleus forskohlii is the only species which contained high forskolin. According to Dubey and Shrimal (1981) Coleus species were used in Ayurvedic medicine for heart diseases, spasmodic pain, painful micturition and convulsions. Pharmacological properties of this diterpene extracted from Coleus forskohlii were investigated, and they showed that it's prominent effect is to lower the blood pressure. They experimented on anesthetized cat and rat and noted the spontaneous effect of relaxation even in hypersensitive rate due to relaxation of vascular smooth muscle. In small doses it has positive inotropic effect on isolated rabbit and cat heart in vivo. Coleonol exhibited nonspecific spasmolytic activity on smooth muscles of the gastrointestinal tract in various species. Trivedi et al.(1982) determined coleonol in roots of Coleus forskohlii by the technique of spectrophotometry and showed that the roots contained about 0.16% of the compound. Singh et al.(1982) made a comparison of physicochemical properties of coleonol and forskolin, both extracted from Coleus forskohlii and they confirmed that these two compounds are structurally non-identical but are sterioisomers. Singh et al.(1984) also reported that besides coleonol some other diterpenes are also present in Coleus forskohlii. They are Coleonol, Coleol,

Coleosol, Coleonol D, Coleonol E and Coleonol F. However, these chemicals differed in their property.

Bhat et al. (1979,1980) developed the technique of purification and isolation of forskolin from the root.

Seaman et al.(1981), Litoach et al.(1982), Hersay and Miller (1983), Moger and Anakwe (1983), Whetton et al. (1983) Meisler and Terry (1984), Boige et al.(1984) have studied the mode of action of this hypertensive diterpane forskolin. According to them forskolin is known to activate adanyl cyclase. Seamon et al.(1981) showed that 5 to 10 µm of forskolin is a maximaly effective concentration which activates adenyl cyclase (ATP pyrophosphatelyase) in rat cerebral cortical membranes in a rapid and reversible manner. Activation is not dependent on exogenous guanyl nucleotides. Forskolin and guanyl nucleotide, do not have additive effect in activation of adenylate cyclase but activation by forskolin and Florine are partially additive. The responses of adenylate cyclase to forskolin of F^{2+} are not inhibited by Mn^{2+}.

Litoach et al.(1982), showed that forskolin increased cyclic AMP (cAMP) accumulation in isolated adipocytes and markedly potentiated the elevation of cAMP due to isoproterenol. They also determined the concentration required to stimulate adenylcyclase activity as $\geqslant$ to 0.1 µm. Whetton et al.(1983) demonstrated that both forskolin and ethanol elicit the activation of basal and ligand-stimulated adenylate

activities in rat liver plasma membranes. Forskolin exerts its greatest effect on basal activity. It increases bilayer fluidity at high concentrations. Based on their study they speculated that as forskolin is a potent perturber of the organization of the lipid bilayer. It is possible that this could modulate its effect on adenylate cyclase and might affect the activity of other membrane enzymes.

The interaction of forskolin with adenyl cyclase was studied by Award et al.(1983). In their study they evaluated its effect on metal and metal ATP kinetics and measured its protective effect when the enzyme was subjected to denaturation. The solubilised calmodulin and forskolin sensitive adenylate cyclase from brain and particular enzymes from platelets were inactivated upon preincubation with N-ethylmaleimide. They showed that forskolin protected against this activation in a concentration 6.3 to 7.6 μm for the brain and platelet adenylcyclase respectively. Forskolin was shown to protect against thermal inactivation of the adenyl-cyclase of the rat brain. The effect of forskolin to stimulate adenyl-cyclase and on electrolyte transport across the isolated polonic mucosa of rat colon descendents were investigated by Bridges et al.(1983). According to them forskolin over a concentration of 10^{-7} to 10^{-5} M dose dependently increased short circuit current (Ise) and transmural potential difference (Vms). Nearly 2-fold increase in Isc and Vms caused by forskolin was accompanied by a small increase in transmural conductance.

The effect of forskolin were rapid and completely reversible without any loss in tissue sensitivity. Forskolin at a concentration of 5×10^{-6} Mole inhibited the absorption of Na^+ and reversed Cl^- absorption to secretion. According to them these effects were due to an inhibition of the mucosal-to-serosal fluxes of Na^+ and Cl^-. Ion substitution experiments revealed that the effects of forskolin were both Na^+ and Cl^- dependent and these ions were required in the serosal solution.

Meister and Terry (1984) also studied the effect of forskolin on adenylate cyclase and showed that it stimulates the activity in mouse.

Moger and Anakwe (1983) studied the effect of forskolin on androgen production by mouse interstitial cells <u>in vitro</u>. They showed that micromolar concentrations of the diterpene, forskolin activates adenylate cyclase in cells and cell membrane. It stimulated androgen production by collagenase-dispersed mouse testicular interstitial cells.with maximum stimulatory concentrations, forskolin and lutenising hormone (LH) increased androgen production with similar time courses and to similar extent.

Mersay and Miller (1983) demonstrated forskolin stimulated acid formation and pepsinogen secretion by gastric glands in rabbit. The stimulation was rapid, reversible and dose dependent with an ED50 of approximately 1 μm. The efficiency of forskolin according to them was similar to that of more commonly used secretagogues such as histamine, carbachol, AMP

derivatives. They also recorded stimulation of adenylate cyclase by forskolin in a more effective way then histamine, isoproterenol or NaF. They also recorded 100 fold increase in tissue cAMP levels indicating thereby that the forskolin activates adenylatecyclase in intact cells.

Boige et al.(1984) demonstrated that forskolin stimulates adenylate cyclase in human colonic crypts. According to them forskolin in the 10^{-8} - 10^{-4} M concentration range (i.e. ED_{50} 2 μM) strongly stimulated the cAMP production of epithelial crypts isolated from the human colan. The foregoing discussion makes it clear that forskolin is extracted from the roots of Coleus forskohlii and it is a potent antihypertensive adenylate cyclase stimulating diterpenoid.

1. Biogenesis of diterpene :

Diterpenes are widely distributed through out the plant and animal kingdom in exceptionally diversified form. Though, for the years the word "terpene" has become associated with fragrant, steam-volatile substances from higher plants, with the advent of modern biochemistry and recognition of the significance of isoprenoid structures to animal physiology, this association has diminished (Nicholas 1967).

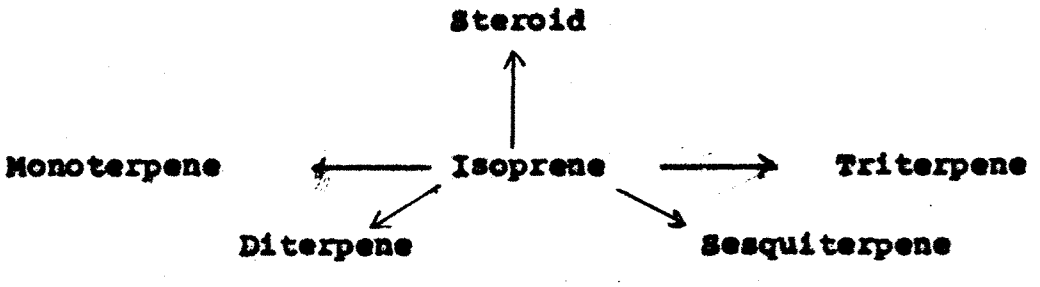
Studies on terpene biogenesis in plants has been accelerated by the use of the ^{14}C isotope technique and the discovery of mevalonic acid (Trease and Evans 1972).

The pathways for the formation of alkaloids have been proposed for many years. Although, many hundreds of individual alkaloids have now been characterized the investigations to date indicate that the majority of nitrogen units of the alkaloids are derived from a relatively small group of amino acids with acetate, mevalonate and methyl donors. These small carbon skeleton have considerable role to play. Many pharmacologically active alkaloids arise from various amino acids (Trease and Evans 1972).

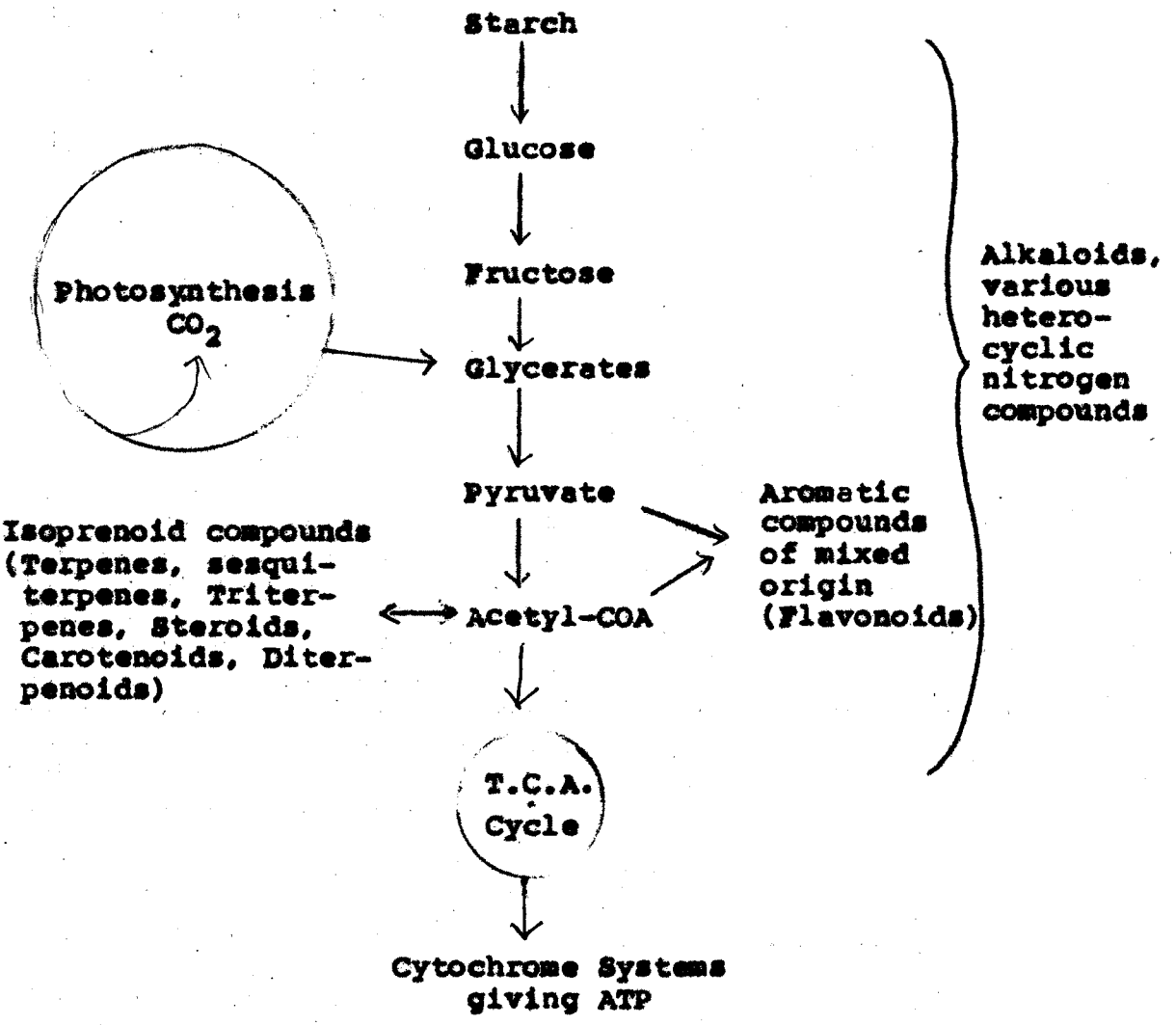
i) Orinthine derived alkaloids
ii) Lysine derived alkaloids
iii) Phenylalanine, Tyrosine and Dihydroxy-phenylalanine-derived alkaloids
iv) Tryptophan - derived alkaloid

Isoprenoid compounds :

As a result of the extensive pioneering investigations into plant terpene structures, Ruzicka (1953) published his 'Biogenetic Isoprene Rule'. This indicated that application of isoprenoid units could be used to explain the formation of monoterpenes, diterpenes, sterols and triterpenes, with complex constitutions.

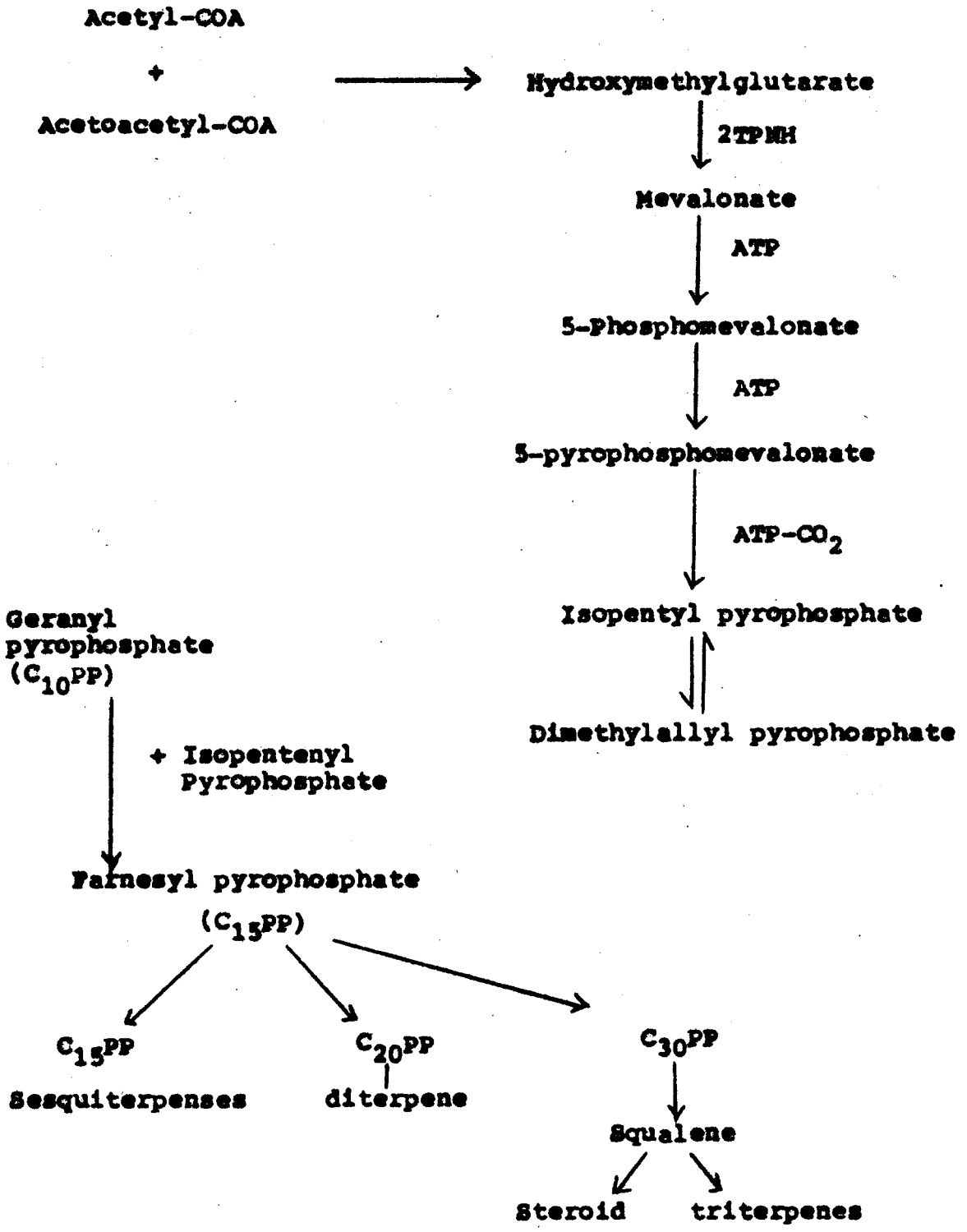


Some basic metabolic pathways appear to be similar in both plants and animals (Trease and Evans 1972), whereas, others are more restricted in their occurrence. It is to the secondary plant products the majority of vegetable drugs owe their therapeutic activity. The production of these secondary metabolites is dependent on the fundamental metabolic cycles of the living tissue, including photosynthesis from which carbon skeleton is derived. The broad outline of the metabolic links is given below.



Adapted from Trease and Evans 1972.

The discovery of acetyl-COA, in 1950 the so called "active acetate" gave support to acetate in biosynthetic processes. The next major advance in this field was the discovery in 1956, of mevalonic acid (3,5-dihydroxy-3-methyl valeric acid) and the demonstration of its incorporation. Mevalonic acid is the 6 carbon acid which forms the basic building block of the isoprenoid compounds. During next four years research involving the use of tracer technique, inhibitor studies, cell free extracts, partition chromatography, ionophoresis and synthetic organic chemistry, it was established that the 5-carbon compound for which biochemists had been seeking so long, was isopentenyl pyrophosphate. It is derived from mevalonic acid pyrophosphate by dehydration and decarboxylation. Isoprenoid synthesis then proceeds pyrophosphate to yield geranylpyrophosphate. These prilliminary stages in the bio-synthesis of isoprenoid compounds, as envisaged by Lynen and Cornforth are shown as below.



Adapted from Trease and Evans, 1972.

2. Cultivation of medicinal plants :

Use of plants and plant parts for the purpose of medicine has been in practice in India from the Vedic time. The very Ayurvedic principle is based on this, even in the Middle East and Europe. From the Egyptian time the plants were variously used for medicinal purpose. Similar to that of Ayurveda, Unani literature also deals with the same. However, the medicinal plants in those days were being tapped from natural resources. The systematic survey of natural products of India mainly the medicinal plants, were carried out during the British time by George Watt. The cultivation of medicinal plants aromatics and etheral oil yielding plants, were started in mediterranean countries for the purpose of systematic study and development of industry. In India it has never being practiced either by government organization or by any private organization. The multinational pharmaceutical companies have only exploited the natural resource rather than undertaking cultivation of them. A few companies such as Roussel Pharmaceuticles have undertaken wide range cultivation of some medicinally important plants such as Gloriosa. However, many of the other multinational pharmaceutical companies have not taken up possibly, due to the lack of necessary systematic study. In the Europe and Russia the line of thinking have changed and advanced. They are thinking on the line of control of collecting, culturing, stocking and distribution of the plants (Tetenyi 1988).

Certain drugs are now obtained almost exclusively from cultivated plants. These includes cardamoms, Indian hemp; Ginger, Cinchona and Opium. Flax, opium poppy and coca have been cultivated from time immemorable. To meet the demand wild plants are cultivated. Climatic factor such as temperature, rainfall, day length, altitude and soil condition affects the plant growth.

Experiment carried out in the phytotron at Fif-sur-Yvette indicated that with Datura tatula long exposure to intense light brought about a sharp increase in hyoscine content at the time of flowering. In long day conditions pepermint leaves contain menthone, menthol and traces of menthofuran. Plant grown under short day conditions contain menthofuran as a major component of volatile oil. In the case of Cinchona succirubra the plants grow well at low altitude level but produce practically no alkaloid. Rainfall also have a great influence on vegetation.

Soil differ from each other both in physical and chemical properties. Fine soil rich in humus and having a permeable substratum is generally favourable for plants.

Preparation of soil :

Land is cleared and tilled. Moisture content in the soil is regulated by adding humus or by irrigation. Tillage operation is done to assist drainage, and to loose soil,

suitable for germination of seeds and free growth of root. The fertility of soil is restored by adding suitable doses of fertilizers and manures containing nitrogen, potassium and phosphate or lime. The farmyard manure is a general manure in that it contains all the elements required by the plants. If the soil is deficient in micronutrients, the analysis of soil is done and deficient micronutrients are added. Sometime impoverished soil is kept fallow to restore its fertility. Sometimes intercropping is done with leguminous crops for increasing nitrogen in the soil.

Propogation :

Plants are reproduced from seed or by vegetative means.

a) From seeds :

To ensure success in germination seed must be perfectly ripe, air dried, and fresh. Before sowing germination percentage is tested. If seeds have hard coat they are usually treated with hot water or sulphuric acid. The small seeds are mixed in the soil at the time of sowing. Generally speaking, seeds may be burried to the depth of their smallest diameter, but as a protection against birds etc. it is sometimes advisable to sow them deeper.

b) By vegetative method :

Vegetative propogation is the rule in some plants. The plants with higher genetic variability and heterozygosity

can not produce viable seeds. Therefore, they usually reproduce by means of vegetative propogation. For vegetative propogation various types of plant parts are used such as bulb, corm, tuber, rhizome, root stocks, stem cuttings, runners, suckers etc. For propogation plant part is used carefully, it is always better to select healthy, disease free, soft and fresh material.

For easy rooting in case of cuttings plantation is done in equal part of silver sand and peat and rooting medium is added. Sometimes vegetative propogation is done in glass house for maintaining temperature and humidity. Afterwards they are transplanted in the field. Grafting, layering and budding method is also applied in most of the times.

In case of microorganisms such as bacteria and moulds for manufacture of antibiotics, fermentation method is used. Also for valuable organisms agar nutrient is used for multiplication.

To facilitate the proper growth, the growth regulators such as Auxins, Gibberellins, Cytokinins, Abscisic acid, Ethylene is used in proper concentrations.

Weed killers or herbicides (selective or non-selective) such as Mercuric chloride, Sodium chlorate, Auxins and 2:4-dichlorophenoxyacetic acid are applied for good healthy growth.

Plate 1 : Commercial plot of *Coleus forskohlii*

Plate 2 : Plate exhibiting distance between rows

1

2

Plate 3 : Flowering of *Coleus forskohlii*

Plate 4 : Harvesting of *Coleus forskohlii*

3

4

In India, Central Drug Research Institute, Lucknow, National Botanical Research Institute, Lucknow, have been working on different medicinal plants and their active principles as well as cultivation. But maintenance of medicinal plants on large scale is scanty. Hoechst Pharmaceutical Company which has for the first time detected the hypertensive principle in Coleus is also not maintaining the large scale cultivation of Coleus forskohlii.

The plant, by and large, is propogated by cuttings, by the farmers, it grows both in black acidic clayey soil to red loam. Prior to the mansoon the terminal stem cuttings are planted in the field in rows. Normally there is no practice of fertilizing the Coleus, however, before planting them farmyard manure is applied to the field.

3. The collection, drying and storage of drugs :

Pharmacognosy is concerned with both the living plant and the dried drug prepared from it. Many plant constituents are relatively stable and therefore, occur both in fresh plant and in the dried drug. Others may undergo changes brought about by enzymes, heat and moisture during drying and extraction processes. Sometimes, these changes are very complex and conditions must be strictly controlled to give the product desired. Familiar examples of this are the preparation of tea, cocoa and tobacco.

In plant breeding, and the scientific control of the cultivation, collection, drying and storage will do much to improve the quality of the medicaments derived from it. The season at which each drug is collected is usually a matter of considerable importance, since the amount and sometimes the nature, of the active constituents is not constant throughout the year. The age of plant also affects the quality and proportion of the drug in active mixture.

There is increasing evidence that the composition of number of secondary plant metabolites varies appreciably throughout 24 hour and is studied.

a) Collection :

In plant breeding, and the scientific control of the cultivation, collection, drying, and storage of drugs will do much to improve the quality of the medicaments derived from them. Studies on the collection of Podophyllum ephedra, wild cherry and aconite have shown that the season at which drug is collected is an important factor. The plant rhubarb contains anthranols in winter and are converted by oxidation into anthraquinones. Betts and Fairbairn (1964) have shown that the contents of C-glycosides, O-glycosides and free anthraquinone in the developing shoots and leaves of Rhamnus purshiana fluctuates markedly throughout the year. The age of plants also affect quality and proportion of the active constituents in the active

mixture. From the studies in Digitalis, Pinus, Poppy and Salvia it has been reported that there is interconversion of the various alkaloids or glycosides present in it.

b) Drying :

Before drying plant part should be free from disease. Drying should be done slowly at a moderate temperature since drugs containing volatile oil are liable to loose their aroma. Fresh drug contains 60 to 90% of water. In drugs such as Clove, Cardamom and Cinnamon open air drying is used. Drying by artificial heat is more rapid and suitable heat may be applied by open fire or hot water pipes. For rapid drying at low temperature a vacuum dryer may be used. Fairly rapid drying helps flowers and leaves to retain their colour and aromatic drugs, their aroma. The temperature used in each case must be governed by the constituents and the physical nature of the drug. As a general rule, leaves, herbs and flowers may be dried between 20° to 40°C and barks and roots between 30 to 65°C.

Exactly how far drying is to be carried out is a matter for practical experience. For drying purpose generally trays of variable size are used, also suitable permanant construction is also done.

c) Storage :

Drugs are frequently examined during storage. Drugs are stored in the usual containers such as sacks, bales, wooden

cases, cardboard boxes and paper bags can reabsorb 10-12% or more moisture. Drugs such as digitatis and Indian hemp are never allowed to air dry so that they may be kept in a sealed containers with dehydrating agents. For large quantities the bottom of case may be filled with quicklime and seperated from the drug by a perforated grid or sacking. If the time becomes moist it should be renewed. Volatile oils should be stored in sealed, well filled containers in a cool place, sometimes air in the container is replaced by an inert gas.