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Monoterpenes in Plants- a mini review

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ABSTRACT

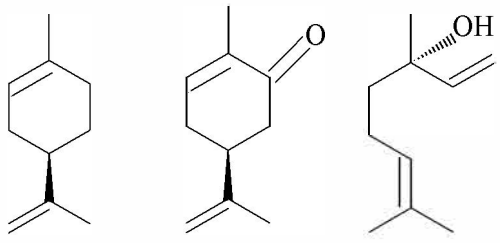
Monoterpenes are abundant chemicals in plants that are formed by a multitude of enzymes and present a challenge both in identification and understanding the synthetic enzymes involves in their formation and fate. They are especially abundant in fragrance-producing plants. The enyme that produce them are numerous but several key enzymes have been identified and are discussed in this short review.

INTRODUCTION

Over 30, 000 terpenoids are known [1] and they constitute the largest family of natural products, exceeding in number the alkaloids and phenylpropanoids combined [2]. Monoterpenes are 10-carbon members of the isoprenoid family of natural products [3]. Most members of this group are synthesized by the monoterpene synthases (cyclases).

Monoterpene synthases catalyze the conversion of the ubiquitous acyclic precursor, geranyl pyrophosphate (GPP) to the cyclic parents of the various monoterpene skeletal types which are often subsequently transformed to a wide range of derivatives by various redox reactions [4].

Monoterpenes can be divided into three major subgroups : linear monoterpenes, monocyclic monoterpenes and bicyclic monoterpenes [5].



(+)- limonene (+)- carvone S- linalool

Figure 1: Chemical structures of monoterpenes ((+)- limonene, (+)- carvone and S-linalool

LINALOOL

Linalool is a naturally-occurring terpene alcohol chemical with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness). It is found in many flowers and spice plants including *Michelia alba*. It was found that 3.183% linalool can be identified through SPME technique and it is one of the main compounds in *Michelia alba* volatiles [6].

STORAGE OF MONOTERPENES IN PLANT

The storage site of monoterpenes in plant are the oil glands, glandular hairs and trichomes cell of the leaf. The biological role of monoterpenes include herbivore defense. Animal ingesting the toxic monoterpenes experience pain and discomfort due to the action of the monoterpenes on cellular enzyme activity- chiefly on the mitochondrial enzymes. To have this effect the monoterpenes must be present at a substantial amount in the organs mention earlier. Consumption of animals lead to rupture of the glands and he monoterpenes will be released into the surrounding environment [7]. Monoterpene emission rate in leaf depends upon its pool size in a particular plant and environmental variation such as temperature plasy an important parameter with a higher temperature generally resulted in a higher rate of emission. Due to this, plants need a large pool size but at the carbon expense for plant growth and reproduction [7].

Several factors that could affect the regulation of monoterpene production include carbon balance, ratio of photosynthetic carbon assimilation and its utilization and plant wounding. In the latter, wounding triggers a large increases in monoterpene synthesis in bark tissues. In addition, it is possible

that other emission-related factors would be affected by the wounding treatment [8].

Broad leaf species that emit monoterpenes to the atmosphere harbour oil glands, glandular hairs and trichomes on the leaf surface. The basal emission rate is dependent upon the pool size of monoterpenes, physical resistance imposed by the tissue and the storage structure [9].

MONOTERPENE BIOSYNTHESIS

Terpene synthase converts the three universal intermediates of the isoprenoid pathway- geranylgeranyl diphosphate (GGPP), geranyl pyrophosphate (GPP) and farnesyl diphosphate (FPP) to diterpenes, monoterpenes and sesquiterpenes, through various mechanism that include a common electrophilic reaction [10].

Some cyclases (pinane, bornane, fenchanene, camphene and thujane families) were studied to formulate the general stereochemical model of the biosynthesis of monoterpene [4]. This multistep transformation of geranyl pyrophosphate, (GPP) is considered to involve the initial ionization of the primary allylic ester to generate an ion pair that, by syn-migration of the pyrophosphate, provides the enzyme bound tertiary allylic isomer, either (3R)- or (3S)-linalyl pyrophosphate (LPP) depending on the stereochemistry of the enzyme. Rotation about the newly formed C2-C3 single bond renders the bound intermediate topologically competent to cyclize, ionization and cyclization via the cisoid, anti endo-conformer generates the corresponding monocyclic (4R)- or (4S)-α-terpinyl cation, respectively. The further cource of the reaction from this universal intermediate may involve additional electrophilic cyclizations and/or rearrangements before termination of the cationic reaction sequence [11].

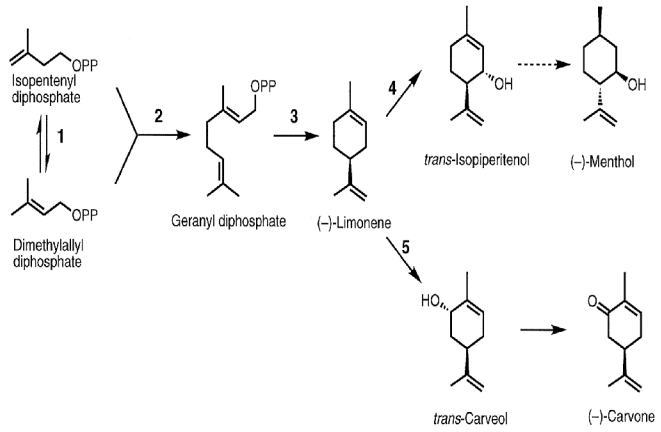


Figure 2: Monoterpene (menthol and carvone) biosynthesis pathway

Metabolic pathway for the conversion of C_5 isoprenoid units through geranyl diphosphate and limonene to the principal essential oil components (−)-menthol (peppermint) and (−)-carvone (spearmint). The enzymes responsible are: isopentenyl diphosphate isomerase (1); geranyl diphosphate synthase (2); 4*S*-limonene synthase (3); 4*S*-limonene-3-hydroxylase (4); and 4*S*-limonene-6-hydroxylase (5). The broken arrow indicates five enzymatic steps. OPP, Diphosphate moiety.

Based on the studies of different monoterpene synthases and their biosynthesis pathways, this basic biosynthesis mechanism is proved to be responsible for the formation of about 30-40 monoterpenes from substrate geranyl pyrophosphate (GPP). Thus far, relevant chemical model studies and all investigation with cell-free cyclase preparations have fully supported this mechanistic scheme [7].

Linalyl diphosphate is an important intermediate in monoterpene synthase catalysis. All studies to observe directly this product in the mandatory isomerization step have failed. One example of the research was the utilization of some noncyclizable substrate analogs. It is suggested that the highly tertiary allylic system is produced at the active site wehere ionization and cyclization occur. Preliminary attempts have been carried out using directed mutagenesis to dissect the cryptic isomerization step of the normally coupled reaction sequence by the monoterpene cyclases have produced significant results [11].

Evaluation of monoterpene synthases that carry out only this partial reaction are used to examine the isomerization step of the reaction. No linalyl diphosphate synthase has yet been described. However, two linalool synthases, capable of transforming a substrate geranyl pyrophosphate, (GPP) to the tertiary allylic alcohol as the sole product have been cloned. The 3S-linalool synthase of *Clarkia breweri* flower is involved in floral scent production in this species [8]. A detailed analysis of this linalool synthase gene suggests that it is a composite structure of recent origin, which more closely resemble a diterpene synthase than a monoterpene synthase [8]. The noncyclizable substrate analogs 6,7-dihydrogeranyl diphosphate, (DHGPP) and racemic methanogeranyl diphosphate, (MGPP) have been used to dissect the cryptic isomerization step of the normally coupled reaction sequence but the inability to resolve chiral monoterpene products and the limited product available from native monoterpene synthases have plagued early researchers [12].

ALTERNATIVE TERMINATION CHEMISTRIES OF MONOTERPENE SYNTHASE

The first step of monoterpene cyclization reactions include ionization and isomerization of the substrate geranyl diphosphate, (GPP). This is followed by the cyclization of bound linalyl diphosphate, (LDP) through a series of carbon intermediates. Ultimately, termination of the multistep cascade is carried out through processes such as deprotonation or nucleophile capture [13].

SYMMETRICAL MONOTERPENOID

1,8-cineole, 1,4-cineole and tricyclene are some of the symmetrical monoterpenoids found. No absolute stereochemical inferences can be made of the isomerization-cylization cascade. Of these symmetrical types, 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane) is by far the most common, occurring in numerous essential oils. Given the relative stereochemical elements of the suprafacial isomerization to the linalyl intermediate and the anti endo-cyclization of the latter, two possible stereochemical routes to 1,8-cineole can be formulated. These two alternatives, mirror-image pathways can be distinguished by examining the fate of C1 of the geranyl substrate, through which asymmetry is introduced, and by evaluating the relative utilization of (3R)- and (3R)-linalyl pyrophosphate (and, potentially, (4R)-and (4S)-α-terpineol) as alternate substrates [14].

IMPORTANCE AND FUNCTIONS OF MONOTERPENE

Monoterpenes are 10-carbon members belonging to the isoprenoid family of natural products. They are often responsible for the characteristic odors of plants and are widespread in the plant kingdom. Monoterpenes are used as flavorings, fragrances, and pharmaceuticals and this has stimulated works to increase their yield in plants [15,16]. As previously mentioned, monoterpene act as semiochemicals in plant defense. In the Grand fir (*Abies grandis*), at the site of injury, defensive oleoresin formation in conifers occurs through processes involving constitutive accumulation of resin (pitch) in specialized secretory structures followed by the biosynthesis of sesquiterpenes (turpentine), monoterpenes and diterpene resin acids in nonspecialized cells. Following the evaporation of the monoterpene solvent, the mixture is hardened to form a mechanical barrier that seal the wound or the infected site [10].

In marine red alga *Ochtodes secundiramea*, the acyclic monoterpene myrcene is the progenitor of the unusual cytotoxic halogenated monoterpenes that function as feeding deterrents to herbivore. These compounds often possess unusual biological activities consistent with their roles in the chemical defenses of the soft bodied, sessile inhabitants of this highly competitive environment, and several have already found important biomedical applications [17]. The response of plants towards various stress factors is also mediated by secondary metabolites that include monoterpenes. The role of terpenes in forest decline phenomena has also been studied [18]. Monoterpenes of commercial value include their use as industrial raw materials, essential oil for perfumery and flavoring. For example, lemon essential oil is widely used as flavoring agent in bakery, pharmaceutical applications and as fragrance in perfumery [19]. The famous turpentine (consisting of monoterpene) is source of industrial chemical intermediate and a high-grade commercial solvent [20,21]. Other examples include is *Perilla frutescens* Britton (*Labiatae*), which is used as a food, Chinese crude drug, natural pigment, and spice in Asian countries [22]. The essential oil from *Eucalyptus*, chiefly 1,8-cineole, is used for medicinal and pharmaceutical purposes [23]. Previous research also revealed that the benzylic ether derivatives of 1,8-cineole could be used as agrochemicals [24]. The caraway fruits contain the monoterpene (+)-carvone can be used as an effective sprouting inhibitor of potatoes [15]. Traditionally, monoterpene rich essential oils have been used for treatment of disease and maladies. However, the development of new and more effective drugs quickly replaced the monoterpene containing mixture [24].

The natural emission of turpentine monoterpenes, such as α-pinene, from the forest trees is a major source of tropospheric organic compounds, which upon oxidation have significant consequences for the ozone balance. For this reason, the biogenesis of turpentine monoterpenes and their genetic origins have received considerable recent attention [20].

STUDIES ON MONOTERPENE SYNTHASES

Several studies on the monoterpene biosynthesis pathways from various plant sources were conducted by researchers around the world [7,8,25]. The biosynthesis of the monoterpenes, limonene and carvone in the fruit of *caraway* (*Carum carvi* L.) proceeds from geranyl pyrophosphate, (GPP) via a three-step pathways. The pathway of (+)-limonene and (+)-carvone biosynthesis in caraway has been assumed to be analogous to the biosynthesis of (-)-limonene and (-)-carvone in spearmint. In this process, GPP is the ubiquitous precursor of the monoterpene [15]. Another molecular biological study on limonene synthase, that catalyzes the cyclization of geranyl pyrophosphate to yield the olefin 4(S)-limonene, an intermediate in the biosynthesis of a monoterpenoid, perillaldehyde, in *Perilla frutescens* has also been carried out [22].

A study has been carried out to observe the scent in the flowers of *Clarkia breweri*. The strong, sweet fragrance consists of at least 12 volatiles that fall into two groups, derivatives of the benzoate pathway and monoterpenoids. Linalool forms a major component of the scent of *C. breweri* flowers. It is an acyclic monoterpene alcohol common to the floral scents of many plant species. In addition to linalool, *C. breweri* flowers emits two linalool oxides that are precursors of linalool. The enzyme-S-linalool synthase (LIS) activity in flower parts was previously observed catalyzes the cation-dependent and stereoselective conversion of GPP to S-linalool [8,26].

(-)-Sabine is the major monoterpene produced by the liverwort *Conocephalum conicum*. A cell-free crude extract of cultured plants *in vitro* was found to catalyze the cyclization of GPP to sabinene [27]. Another liverwort- *Ricciocarpos natans* contain 4S-(-)-limonene as a major monoterpene and a cell-free extract from this nonvascular plant cultured in vitro catalyzes the cyclization of GPP to limone with limonene synthase [28].

In the study of the biosynthesis of marine natural products, isolation and characterization of mycene synthase, a monoterpene synthase, from cultured tissues of the marine red alga *Ochtodes secundiramea* was conducted. The conversion of the monoterpene precursor geranyl pyrophosphate to myrcene represents the first *in vitro* demonstration of a monoterpene synthase from any marine source and provides a basis with which to further explore the biosynthesis of halogenated monoterpenes [17].

In the study of characterization and functional expression of monoterpene synthases from *Arabidopisis thaliana*, functional expression of several *Arabidopsis* terpene synthase like sequences in yielded an active monoterpene synthase enzyme in *Escherichia coli*, that catalyze the conversion of GPP into the acyclic monoterpenes (E)-β-ocimene, β-myrcene and small amounts of cyclic monoterpenes [29]. Similar studies have been carried out in the lodgepole pine (*Pinus contorta*) to further define specific structural and mechanistic differences among monoterpene synthases from divergent plant sources [30].

MONOTERPENE SYNTHASE FROM DIVERGENT PLANT SOURCES (GYMNOSPERMS AND ANGIOSPERMS)

Different enzyme properties are expected for monoterpene synthases from diverge plant sources. But in general, they usually catalyze the stereospecific isomerization of GPP to 3R- or 3S-linalyl pyrophosphate. This is followed by cyclization reaction products related to the chirality of the linalyl intermediate. However, amino acid-specific chemical-modifying reagents have been demonstrated to act differently to this enzyme from gymnosperms and angiosperms [31].

An arginyl residue at the active site of monoterpene synthase from gymnosperms has been found. A GGPP synthase from *Taxus canadensis* was cloned, expressed and characterized and was found to harbor similar requirement for the amino acid at the active site. Since gymnosperms produce terpenes, they must

have substantial prenyltransferase activities involved in the secondary metabolism of terpenoid [31].

Monoterpene synthase (prenyltransferase) from numerous angiosperm species such as 4S-limonene synthase from peppermint and the pinene synthases from sage have been purified and characterized [31]. In general, monoterpene synthase from angiosperms requires histidyl and cysteinyl residues at the active site. In addition they have at least some catalytically important arginyl residues at a site not protected by substrate [30].

LOCATIONS OF MONOTERPENE SYNTHASES IN PLANT

The plastids in plants are the site of geranyl pyrophosphate (GPP) synthase and monoterpene synthase. S-linalool, is abundant in the stigma of freshly opened flowers of *Clarkia breweri* [8]. Similarly, lipophilic metabolites such as monoterpene from the liverwort *Conocephalum conicum* are deposited in specialized intracellular oil bodies [28]. Compartmentation of these enzymes in specialized organs offer an explanation for the lack of production of monoterpenes in plant cell tissue cultures. Constitutively expressed oleoresin is synthesized in the epithelial cells of specialized secretory structures in some plant species such as the conifer. Induced oleosin appears to originate from nonspecialized cells. Oleoresin is composed of turpentine, largely monoterpenes with some sesquiterpene olefins [16].

In spearmint (*Mentha spicata*), peppermint (*Mentha xpiperita*), and other essential oil plants from the Lamiaceae, monoterpenes are produced and accumulated in specialized glandular trichomes. There are two types of nonphotosynthetic glandular trichomes in mint. The first is a small capitate type that have a limited capacity to store secreted material, and the second is the peltate type that contains a stalk cell, a basal cell, and eight secretory cells that are arranged in a disc. The peltate type develops a large oil-storage space in the glandular trichome and is responsible for the production of the bulk of the monoterpene essential oil [21]. The secretory cells of isolated peppermint peltate glandular trichomes are responsible for the secretion of monoterpenes into the oil-storage space and also served as the actual site for the biosynthesis of monoterpenes. Oil gland leucoplasts are composed of nonpigmented plastid with few internal membranes and have been implicated in monoterpene biosynthesis in nearly 50 plant species [13].

Leucoplast and other plastids, when isolated, are capable of monoterpene biosynthesis when supplied with exogenous precursors. The glands of the fruit flavedo from Lemon (*Citrus limon*) possesses a high content and large variety of monoterpenes. This layer contains the epidermis that covers the exocarp. The latter is consisted of irregular parenchymatous cells, and are completely enclosed glands or oil sacs. In maturing fruits, just beneath the green layer is a thick spongy white mass of tissue-the albedo layer (mesocarp) that are rich in pectins [19].

GERANYL PYROPHOSPHATE SYNTHASE

Geranyl pyrophosphate synthase (GPPS) catalyzes the condensation of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) to generate geranyl pyrophosphate (GPP), the essential precursor of monoterpene biosynthesis. This enzyme is similar to farnesyl diphosphate synthase (FPPS) which condenses two molecules of IPP with DMAPP to form the C_{15} precursor of the sesquiterpenes and triterpenes and to geranylgeranyl pyrophosphate synthase (GGPPS) which condenses three molecules of IPP with DMAPP to form the C_{20} precursor of diterpenes and tetraterpenes. These enzymes, referred to collectively as short-chain prenyltransferase, function at the branch points of isoprenoid metabolism. These enzymes are considered to play a regulatory role controlling the flux distribution of IPP into the various terpenoid families. In addition, all of the short-chain prenyltransferases share sufficient primary sequence identity to suggest that they are evolutionarily related [21]. Their catalytic mechanism involves the divalent metal ion-dependant ionization of the allylic cosubstrate and 1'-4 addition to IPP. The product are higher isoprenologue.

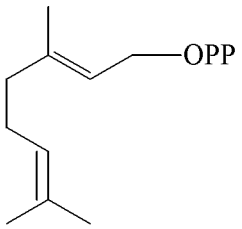


Figure 3: Chemical structure of geranyl pyrophosphate (GPP).

A study of geranyl pyrophosphate synthase from *Abies grandis*, including the cDNA isolation, functional expression, and characterization was conducted. The pairwise comparison between the grand fir GPP synthase and GGPP synthase suggests that relatively few residues determine the chain length specificity of these prenyltransferases and encourages a direct mutagenesis approach to explore the structure-function differences. Directed mutagenesis of an FPP synthase to produced GPP and GGPP, and mutagenesis alteration of a GGPP synthase to produced FPP, have been achieved. However, the mutagenesis of a GGPP synthase to produce GPP has not yet been demonstrated [21]. GPP synthase from the principal precursors of the oleoresin monoterpene, were isolated, partially purified and characterized from the stems of 2-year-old grand fir saplings [31]. This shows the variety of enzymatic synthesis route in plants that lead to the numerous monoterpenes found.

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