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 involved in their formation and fate. They are especially abundant in fragrance-producing plants. The enzymes that produce them are numerous but several key enzymes have been identified and are discussed in this short review.

KEYWORD

Monoterpenes; Plants; Linalool

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].

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 plants 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 mentioned earlier. Consumption of animals lead to rupture of the glands and the 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 play 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 monoterpenoid production include carbon balance, ratio of photosynthetic carbon assimilation and its utilization and plant wounding. In the latter, wounding triggers a large increases in monoterpane 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
mechanisms that include a common electrophilic reaction [10].

Some cyclases (pinane, bornane, fenchane, camphene
and thujane families) were studied to formulate the general
stereochemical model of the biosynthesis of monoterpenes [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 course of the
reaction from this universal intermediate may involve additional
electrophilic cyclizations and/or rearrangements before
termination of the cationic reaction sequence [11].

![Diagram](image)

**Figure 2:** Monoterpene (menthol and carvone) biosynthesis
pathway

Metabolic pathway for the conversion of C10 isoprenoid
units through geranyl diphosphate and limonene to the principal
essential oil components (β)-menthol (peppermint) and (α)-carvone
(spearmint). The enzymes responsible are: isopentenyl
diphosphate isomerase (1); geranyl diphosphate synthase (2); 4S-
limonene synthase (3); 4S-limonene-3,5-hydroxylase (4); and 4S-
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 noncleavable
substrate analogs. It is suggested that the highly tertiary allylic
system is produced at the active site where 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 noncleavable substrate
analog 6,7-dihydrogeranyl diphosphate, (DHGPP) and racemic
methanogenderanyl 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 CHEMISTRY 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 tricycylene are some of the
symmetrical monoterpeneoids found. No absolute stereochemical
inferences can be made of the isomerization-cyclization cascade.
Of these symmetrical types, 1,8-cineole (1,3,3-trimethyl-2-
orbicyclo[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 (3S)-linalyl pyrophosphate (and,
potentially, (4R)-and (4S)-α-terpinyl) 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, monoterpenes 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 Ochotodes secundarium, 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 benzyl 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 terpentine 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 pathway. 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 4S(-)-limonene, an intermediate in the biosynthesis of a monoterpoid, 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 benzene pathway and monoterpeneoids. 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 emit 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].

(+)-Sabinene 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- Riccioecarpus natans contains 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 myrcene synthase, a monoterpene synthase, from cultured tissues of the marine red alga Ochotodes secundarium 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 Arabidopsis 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-obcinene, 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 (GYMNOSEMS AND ANGIOSPERMS)

Different enzyme properties are expected for monoterpene synthases from divergent plant sources. But in general, they usually catalyze the stereospecific isomerization of GPP to 3R- or 3S-linalyl pyrophosphate. This is followed by cyclization reactions 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 GPP 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
livewort 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 monoterpene in plant cell tissue cultures.
Constitutively expressed olerocin is synthesized in the epithelial
cells of specialized secretory structures in some plant species such
as the comifer. Induced olerocin appears to originate from
nonsecreted cells. Olerocin is composed of turpentine, largely
monoterpene with some sesquiterpene olefins [16].

In spearmint (Mentha spicata), peppermint (Mentha
xipertisa), and other essential oil plants from the Lamiaceae,
monoterpene 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-storing space in the glandular trichome 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
monoterpene into the oil-storage space and also served as the
actual site for the biosynthesis of monoterpene. 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
monoterpene. This layer contains the epicuticular that covers the
exocarp. The latter is consisted of irregular parenchymatous cells,
and is 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 C15 precursor of the sesquiterpenes and
triterpenes and to geranylgeranyl pyrophosphate synthase
(GGPPS) which condenses three molecules of IPP with DMAPP
to form the C20 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-dependent ionization of the allylic coenzyme and 1’-4
addition to IPP. The product are higher isoprenologue.

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 the 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 olerocin 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
monoterpene found.

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