Phytochemistry (2)

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Course outlines

- Terpenoids
  - Monoterpenoids (GPP)
  - Irregular monoterpenoids
  - Monoterpenoids-containing drugs
- Sesquiterpenoids (FPP)
- Diterpenes and sesterterpenes (GGPP)
- Triterpenoids
- Steroids
  - Cholesterol
  - Phytosterols
  - Vitamin D
- Cardiac glycosides
- Steroidal hormones
- Tetraterpenes and higher terpenoids

Textbook(s) and Material

- <u>Recommended-but not required:</u>

- “Textbook of Pharmacognosy & Phytochemistry”. Biren Shah & Seth. 2010, 978-81-312-2298-0.

- “Tyler’s Herb of Choice - The Therapeutic Use of Phytomedicinals”. 3rd Ed., Dennis V.C. Awang, Taylor & Francis, Inc., 2009, 978-0-78902-809-9.

- “Medicinal Natural Products: A Biosynthetic Approach”. 3rd Edition. Paul M. Dewick. 1997, 978-0-470-74168-9

Phytochemistry Definition

- **Phytochemistry** is branch of pharmacognosy that deals with chemical and biological characters.

- **Phytochemistry** is the science that studies the classification, structures, physicochemical characters, and isolation of phytogenic compounds, which have therapeutic activity and used in the pharmaceutical industry.

Terpenes/Terpenoids

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Terpenes/Terpenoids

- **Outlines**:
  - Definitions
  - Classification
  - Isoprene rule
  - Metabolic pathways
  - Examples
  - Therapeutic activity
  - Drug-food interaction

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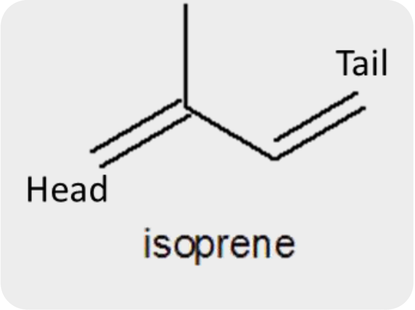
Terpenes Definition

- Large and structurally diverse group of organic compounds which are produced by various plant and animal species, including coniferous plants and some insect species.
- Terpenes contain simple unsaturated hydrocarbon.
- Terpenes originate from turpentine oil.
- Contains **isoprene** $(C_5H_8)_n$ as repeating units.
- Terpenes have a strong odor, and it can protect the plants by deterring herbivores and by attracting predators and parasites of herbivores.



(2-methyl-1,3-butadiene)

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Terpenoids Definition

- **Terpenoids**, also called **isoprenoids** are modified class of terpenes with an additional functional groups usually containing oxygen and oxidized methyl groups.

- Terpenoids are the largest class of plant secondary metabolites, representing about 60% of known natural products.

Terpenoid Functions

- **Why do plants produce so many terpenoid compounds?**
- The presence in the genome of each plant species of a large number of genes already involved in terpene biosynthesis underlies the present ability of plant lineages to make large numbers of terpenoids. It also provides a large platform for the evolution of new terpenes via mutation and selection.
- New terpenoids keep arising in specific plant lineages, potentially as an outcome of coevolution with natural enemies. However, specialized terpenoids tend to occur as cocktails of multiple, related compounds in both radiating species as well as less quickly evolving species, suggesting that terpenoid diversity provides an advantage per se.

Terpenoid Functions

- **Why do plants produce so many terpenoid compounds?**
- These suites of terpenoid defense compounds do not increase in number indefinitely for several reasons, such as excessive cost or the evolution of alternative (non-terpenoid-based) defense pathways.
- As a result of the multifaceted chemical and physical properties of terpenoids, some also have non-defensive functions, including signaling to mutualists, such as pollinators or symbionts.

Classification of Terpenes

- Terpenes have two classification:

  1. **By the number of isoprene units in the molecule**; a prefix in the name indicates the number of isoprene pairs needed to assemble the molecule.
  2. **By the number of rings presents in the structure**.

Classification Based on Isoprene Units

Class	Isoprene Units	Carbon Atoms	Examples
Monoterpene	2	10	Camphor, eucalyptol, geraniol, lavandulol, limonene, menthol, pinene, thymol
Sesquiterpene	3	15	Abscisic acid, bergamotene, cedrol, curcumene, patchoulol, vetivone, farnesol
Diterpene	4	20	Abietic acid, cafestol, caffeol, carnosol, gibberellin, phytol, geranylgeranil
Triterpene	6	30	Betulinic acid, morolic acid, oleanolic acid, ursolic acid, brassinosteroids, saponins, squalane
Tetraterpene	8	40	Carotenoids and xanthophyllus
Polyterpene	>8	>40	Rubber

- Commonly, terpenes contain 2, 3, 4 or 6 isoprene units; the tetraterpenes (8 isoprene units) form a separate class of compounds called **carotenoids**; the others are rare.

Classification Based on No. of Rings

Class	Rings
Acyclic (Linear)	0
Monocyclic	1
Bicyclic	2
Tricyclic	3
Tetracyclic	4

Isoprene Rule

- In biosynthesis of terpenes, two or more isoprene molecules are linked to one another. Linking between two isoprene molecules could occur in three ways, given that the **head** and the **tail** of the molecule are primarily involved in the linking:

➢ **1-1 linkage**:

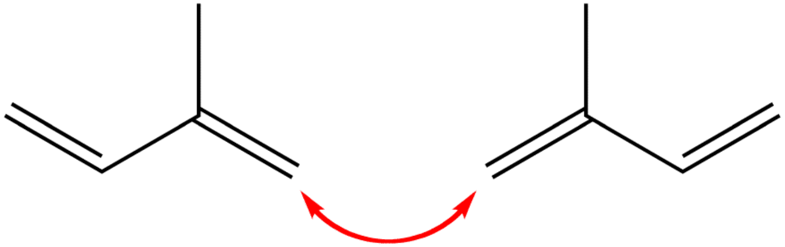
  - The **head** of one isoprene molecule could link with the **head** of another isoprene molecule.

➢ **1-4 linkage**:

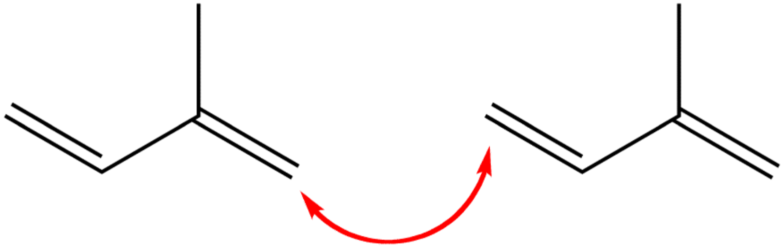
  - The **head** of one isoprene molecule could link with the **tail** of another isoprene molecule.

➢ **4-4 linkage**:

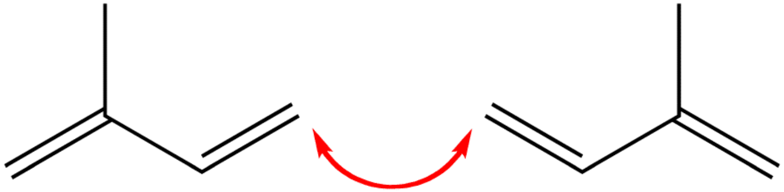
  - The **tail** of one isoprene molecule could link with the **tail** of another isoprene molecule.



head-to-head or 1-1 link



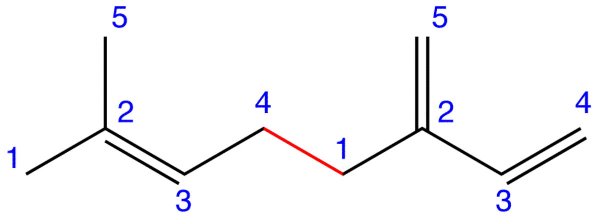
head-to-tail or 1-4 link



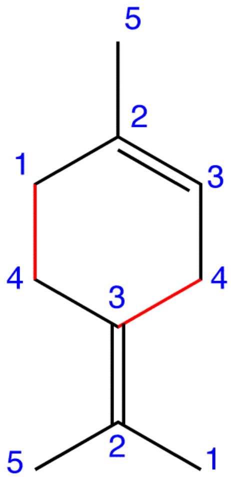
tail-to-tail or 4-4 link

Isoprene Rule (1-4 Linkage)

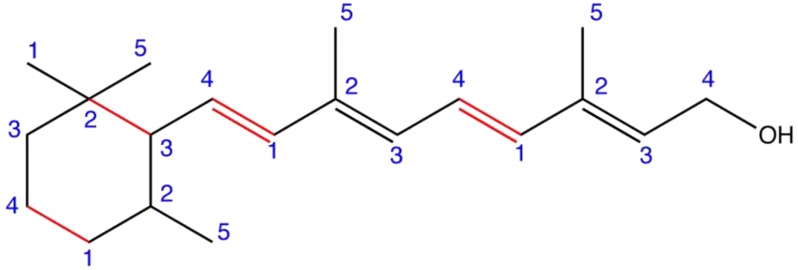
- The isoprene rule states that, in most naturally occurring terpenes, there are **no** 1-1 or 4-4 links.
- In applying isoprene rule we look only for the skeletal unit of carbon.



Myrcene
Acyclic mono**terpene**



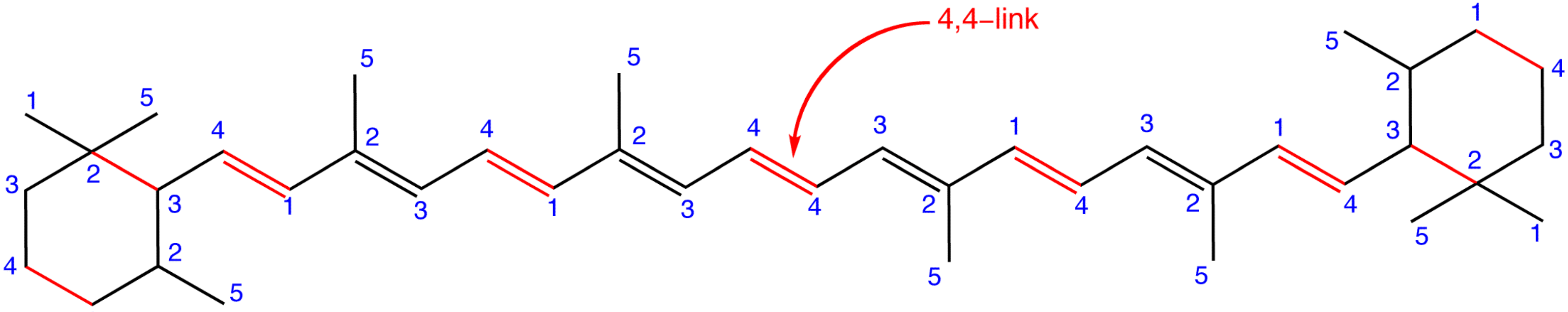
Limonene
Monocyclic mono**terpene**



Retinol
Monocyclic Di**terpenoid**

Irregular Terpene

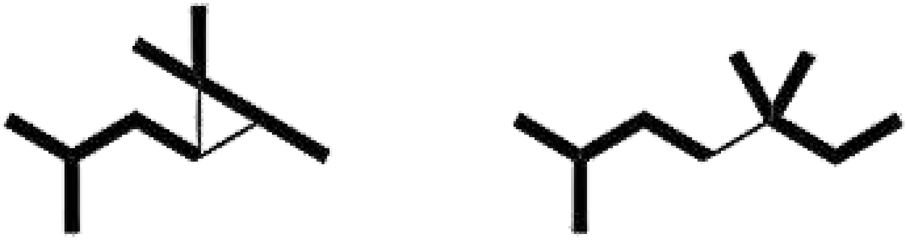
- Irregular terpene: Is a terpene that does not follow the isoprene rule (1-4 linkage).
- Example: Squalene (C_{30}) and phytoene (C_{40}) display a tail-to-tail linkage at the center of the molecules.



β-Carotene

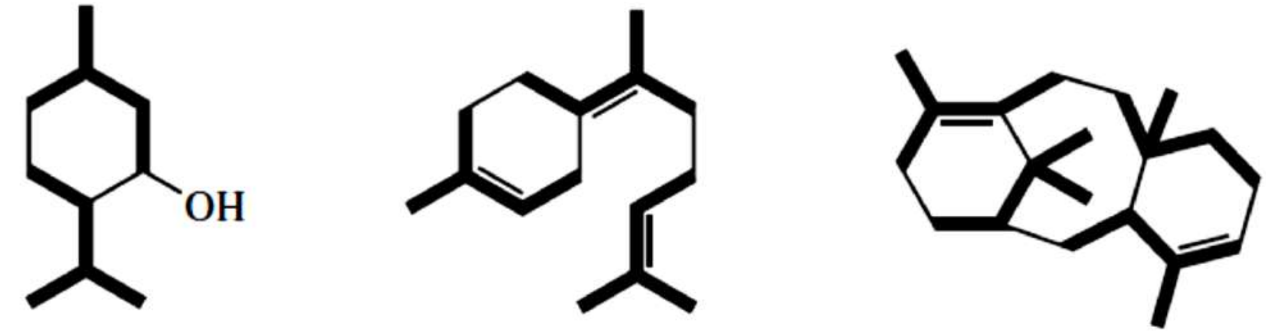
Irregular Terpene

- Carotenoids are joined tail to tail conjugation at their central (4-4 linkage).
- Some terpenoids whose carbon content is not multiple of five.
- Some terpenoid whose carbon content is a multiple of five but can not be divided into isoprene units.



Terpenoids Arrangement

- Most terpenoids are modified further by cyclization reactions, though the head-to-tail arrangement of the units can usually still be recognized, e.g., **menthol**, **bisabolene**, and **taxadiene**.



menthol (C_{10}) bisabolene (C_{15}) taxadiene (C_{20})

Important Note

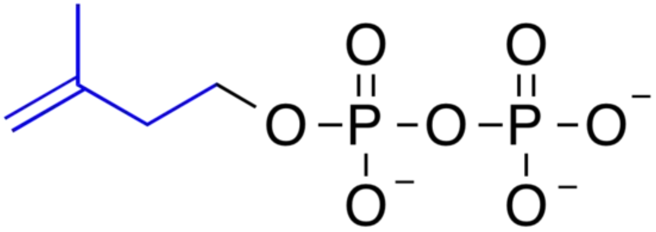
- Isoprene is a natural volatile compound which is released in huge amounts by many species of plants and produced from DMAPP. It is classified as hemiterpene (C_5).
- Isoprene is not involved (as a precursor) in the formation of the big other classes of terpenoids; but its structural unit as a C_5 unit had been suggested as the fundamental building block for these compounds, hence they are also termed isoprenoids.
- The biochemically active isoprene units which is involved in the biosynthesis of the different classes of terpenoids were dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP).

Terpenoids Metabolic Pathways

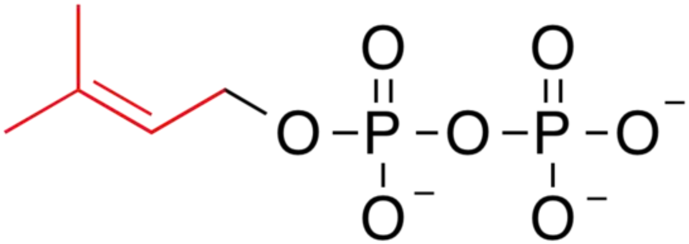
- The biochemical isoprene units may be derived by two pathways:
    1. **Mevalonic acid (MVA) pathway** in cytosol.
    2. **Methylerythritol 4-phosphate (MEP) pathway** in plastid.
- For many years, the early parts of the mevalonate pathway were believed to be common to the whole range of natural terpenoid derivatives in all organisms.
- However, it has since been proven that an alternative pathway to isoprene units' synthesis exists, via MEP, and that this pathway is probably more widely utilized in nature than is the mevalonate pathway.

Terpenoids Metabolic Pathways

- Both pathways shared with producing
  - Isopentenyl-5-pyrophoshate (**IPP**) (C_5).
  - Dimethylallyl-5-pyrophosphate (**DMAPP**) (C_5).



IPP

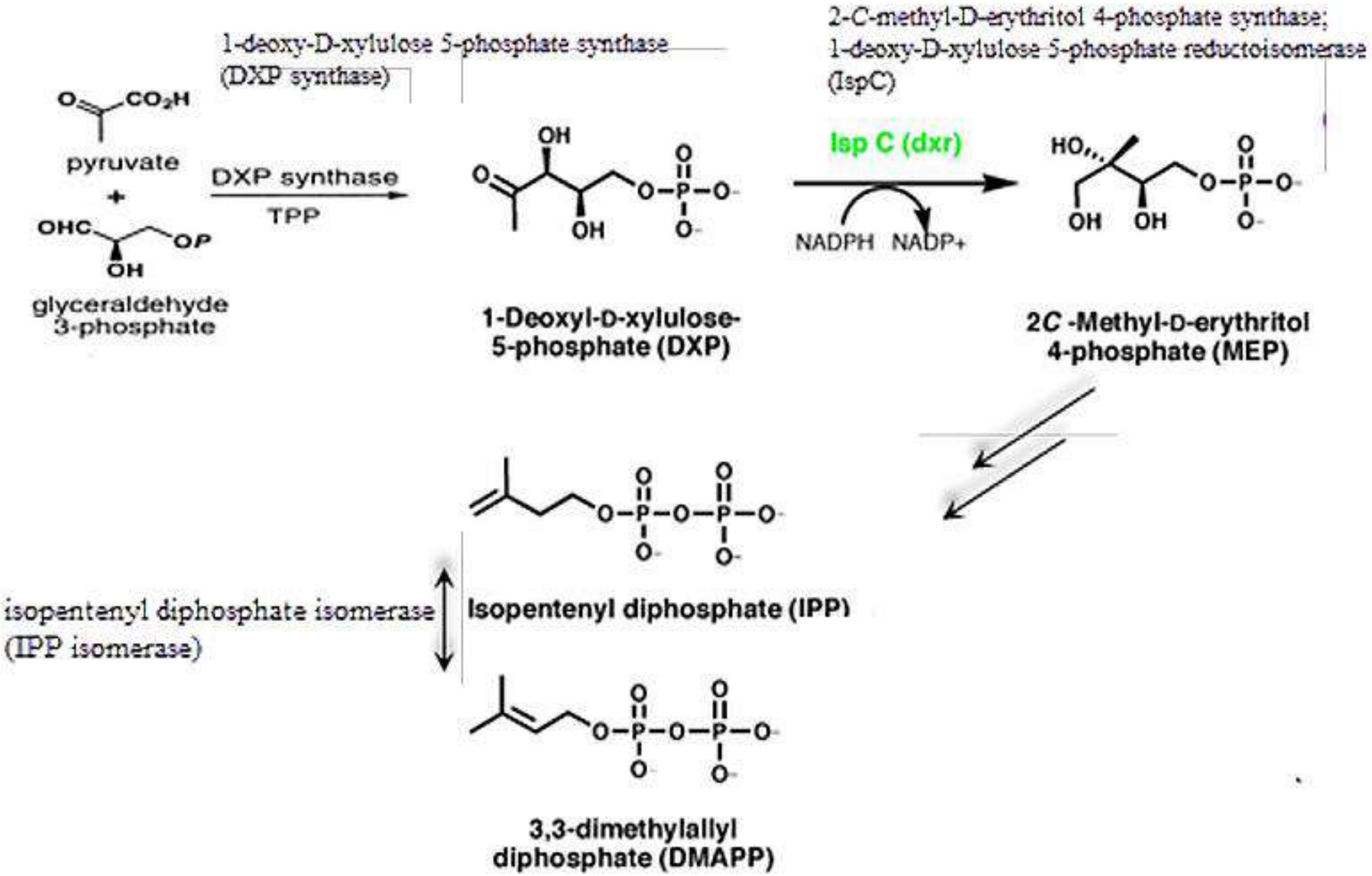


DMAPP

Methylerythritol Phosphate (MEP) Pathway

- Is also referred to as the **mevalonate-independent pathway** or the **deoxyxylulose phosphate pathway.**
- **T**he terminology MEP pathway is preferred because deoxyxylulose phosphate is also used for the biosynthesis of pyridoxal phosphate (vitamin B6) and thiamine (vitamin B1).
- Animals and fungi appear to **lack** MEP pathway, so utilize the mevalonate pathway (MVA) exclusively.
- The MEP pathway is present in **plants**, **algae**, and most bacteria that employ both pathways, often concurrently.

Methylerythritol Phosphate (MEP) Pathway



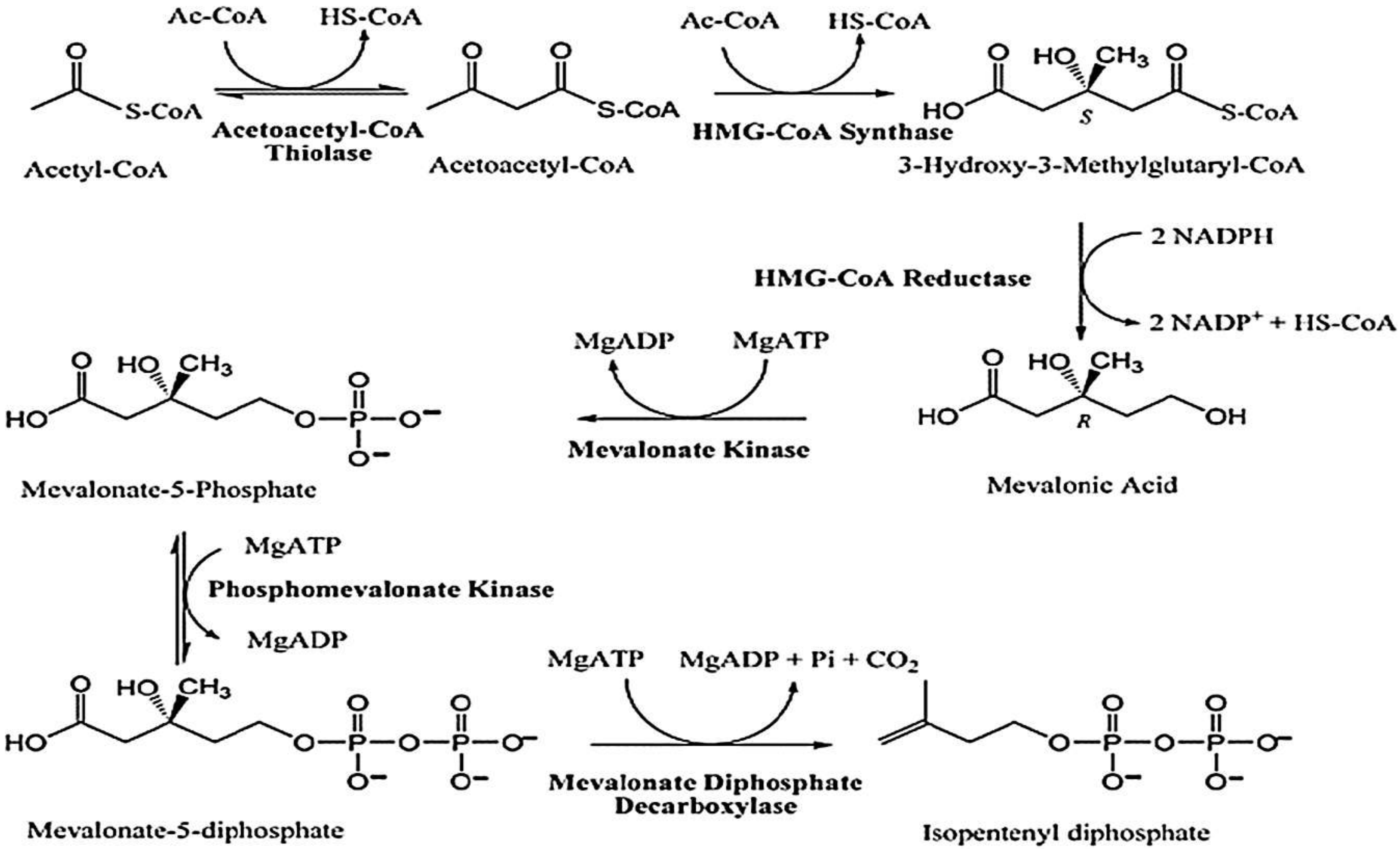
Mevalonic Acid (MVA) pathway

- Three molecules of acetyl-coenzyme A are used to form mevalonic acid (MVA). Two molecules combine initially in a Claisen condensation to give acetoacetyl-CoA, and a third is incorporated via a stereospecific aldol addition giving the branched-chain ester 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA).
- The conversion of HMG-CoA into (3R)-MVA involves a two-step reduction of the thioester group to a primary alcohol and provides an essentially irreversible and rate-limiting transformation.

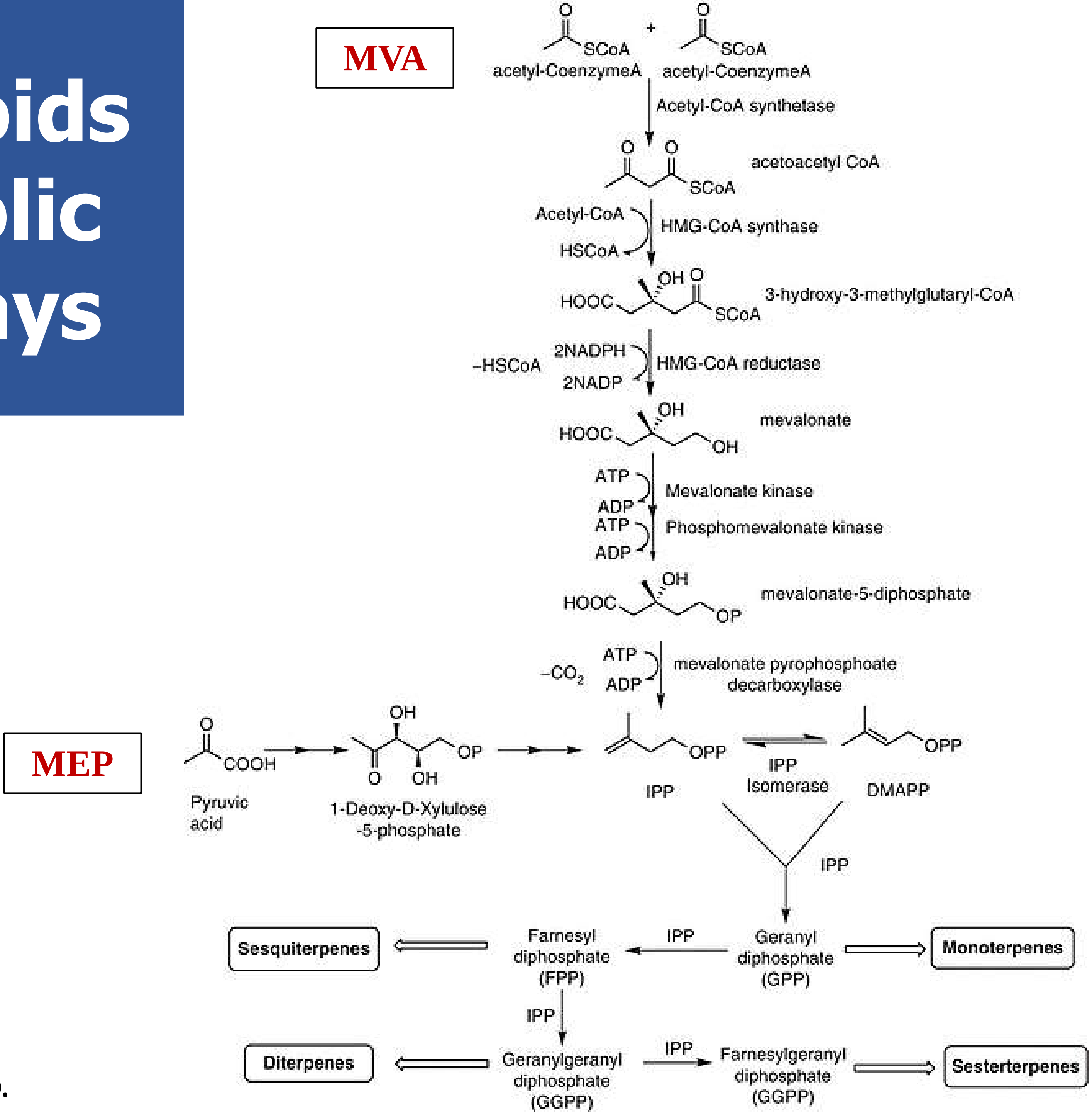
Mevalonic Acid (MVA) pathway

- The six-carbon compound MVA is transformed into the five-carbon phosphorylated isoprene units in a series of reactions, beginning with phosphorylation of the primary alcohol group. Two different ATP-dependent enzymes are involved, resulting in mevalonic acid diphosphate, and decarboxylation–elimination then follows to give IPP.

Mevalonic Acid (MVA) pathway



Terpenoids Metabolic Pathways



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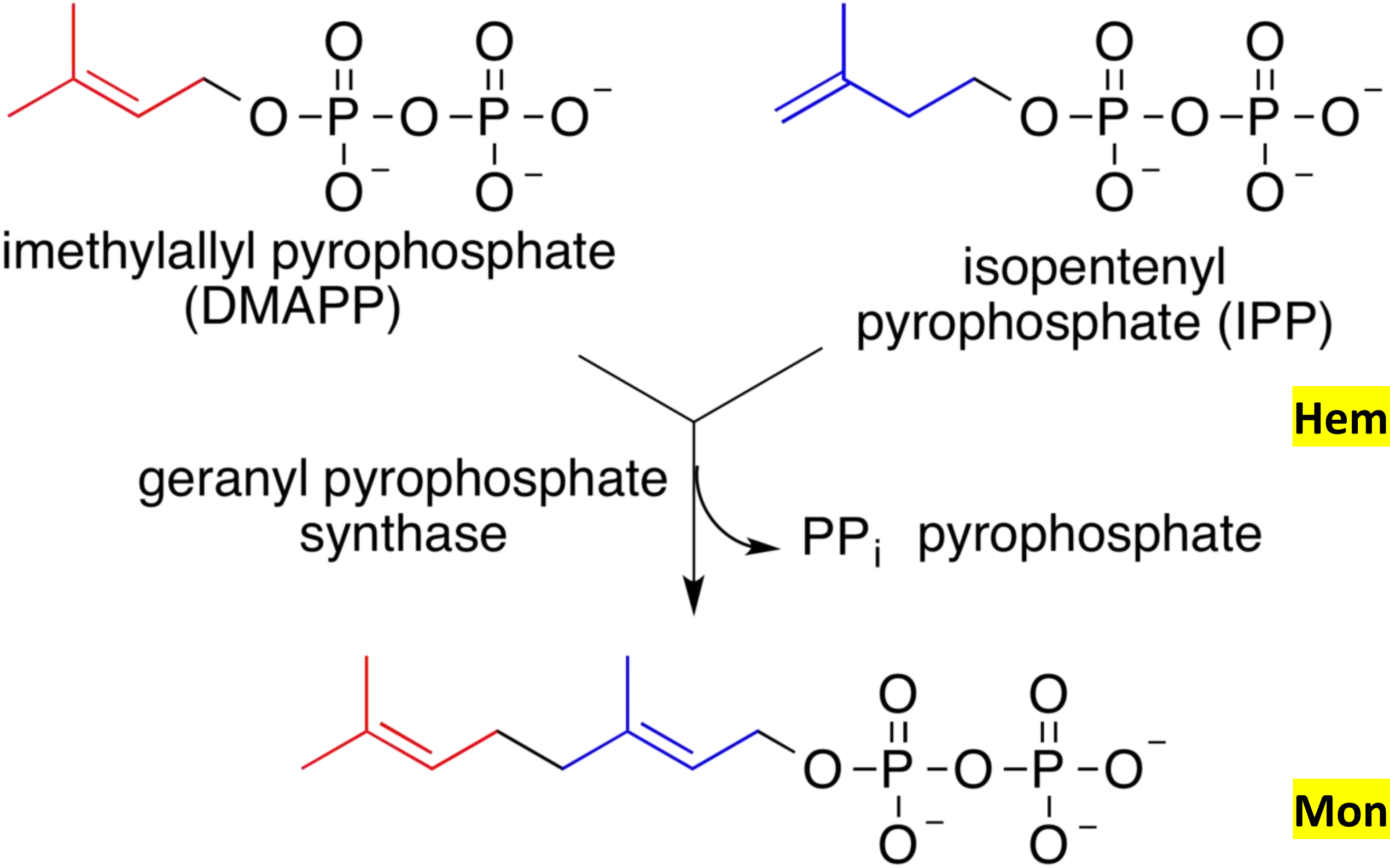
Terpenoids Metabolic Pathways



dimethylallyl pyrophosphate (DMAPP)

isopentenyl pyrophosphate (IPP)

geranyl pyrophosphate synthase

PP_i pyrophosphate

geranyl pyrophosphate (GPP)

Hemiterpenes C_5

Monoterpenes C_{10}

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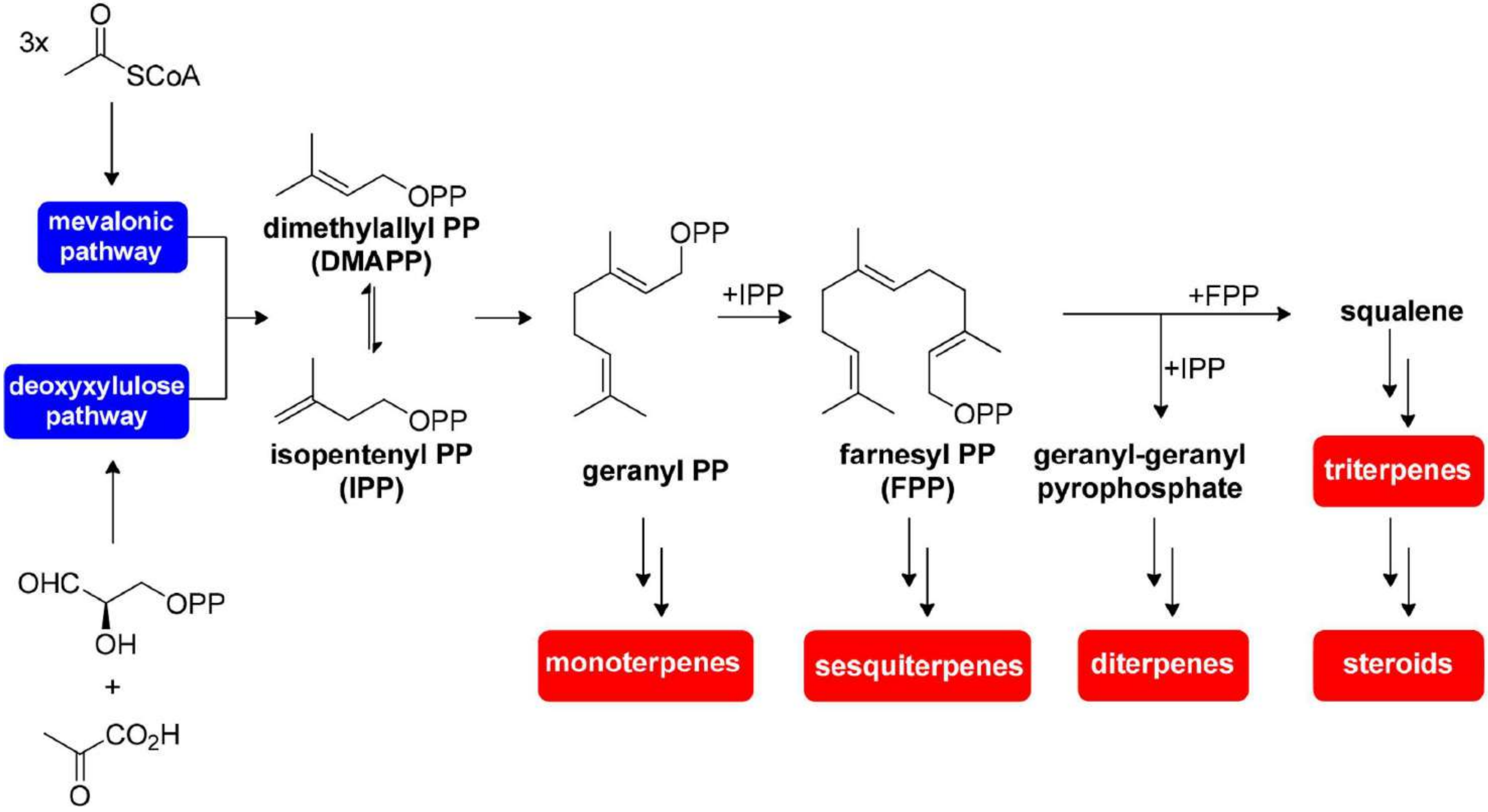
Terpenoids Metabolic Pathways



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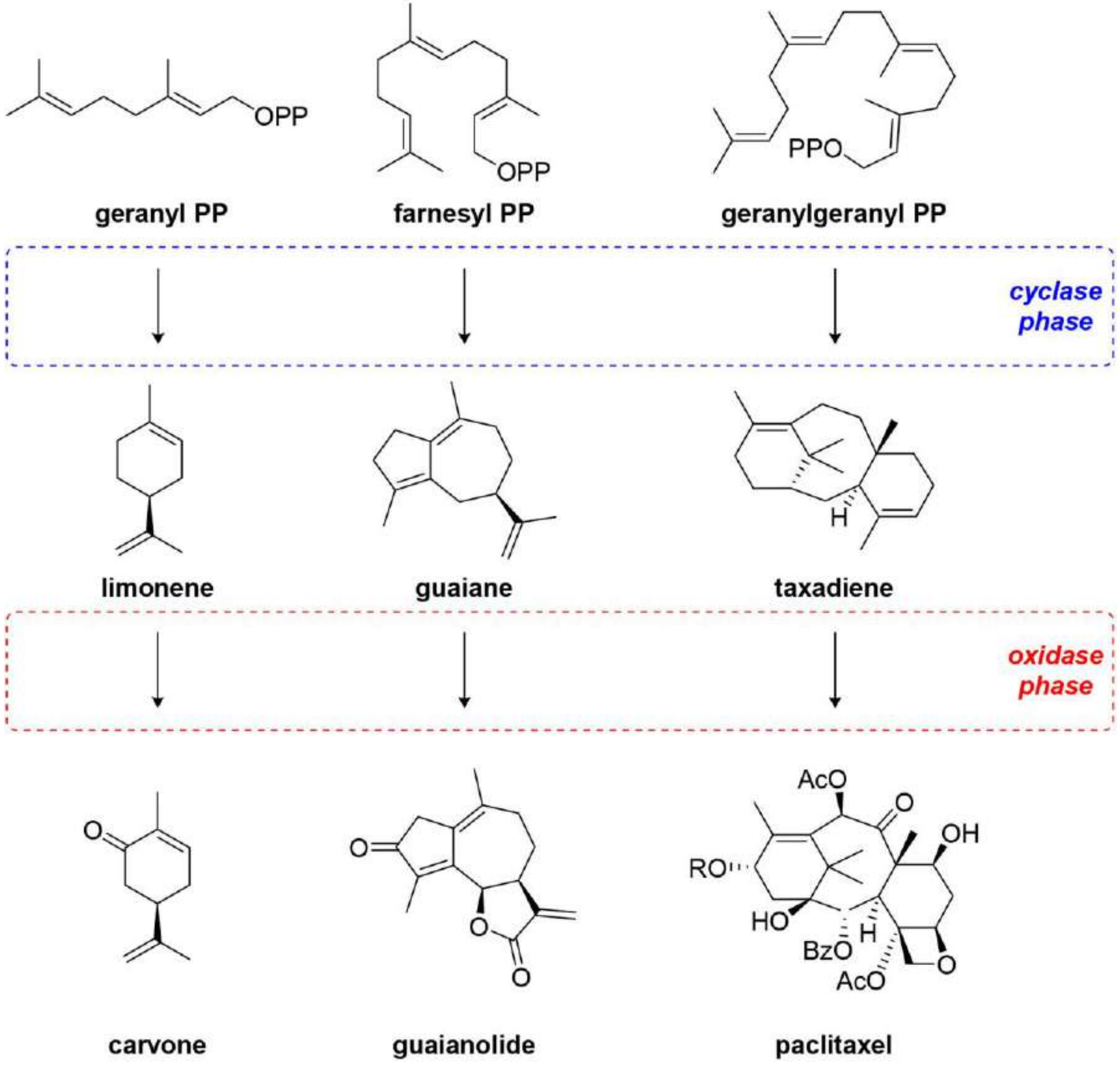
Terpenoids Metabolic Pathways



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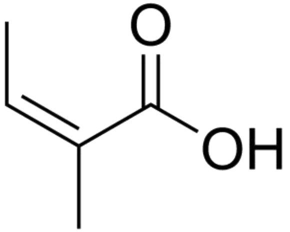
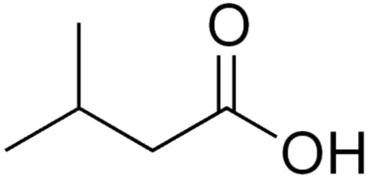
Terpenoids Metabolic Pathways

- Story start with two basic isoprenoid units, dimethylallyl-5-pyrophosphate (DMAPP) and isopentenyl-5-pyrophosphate (IPP), produced either by the mevalonate or the deoxyxylulose (methyl erythritol) routes. Then, synthase enzymes join these isoprenoid units into geranyl (C-10), farnesyl (C-15), geranyl-geranyl (C-20), geranyl-farnesyl (C-25) and bis-farnesyl (C-30) linear intermediates.
- These relatively simple molecules cyclize into the immensely diverse range of (poly)cyclic structures when the cyclase enzymes take over.
- Finally, oxidase enzymes transform these (poly)cyclic hydrocarbons into the individual examples of the final natural products (NPs).

Hemiterpenes (C_5)

- Are terpene formed from a **single** isoprene unit and having five carbon atoms.
- Relatively few true hemiterpenes are produced in nature.
- IPP and DMAPP are reactive hemiterpenes intermediates in the pathway.
- Used as alkylating agent in the formation of meroterpenoids.
- <u>Examples</u>: angelic acid, isovaleric acid





Monoterpenes (C_{10})

- **Monoterpenes** are a class of terpenes that consist of **two** isoprene units and have the molecular formula $C_{10}H_{16}$. Biochemical modifications such as oxidation or rearrangement produce the related monoterpen**oid**s.

Classification of Monoterpenoids

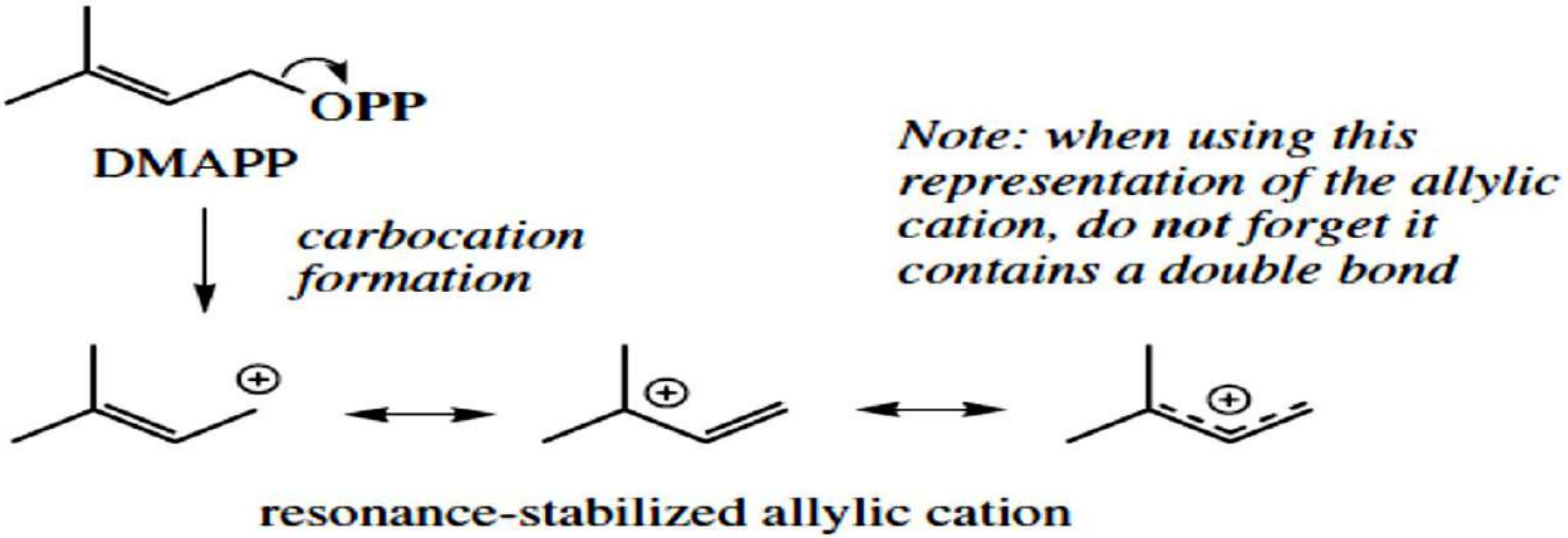
- Monoterpenoids can be classified as follows:

1- **Regular monoterpenoids**: those compounds fit the regular head-to-tail coupling mechanism in their construction. They can be further divided to acyclic, cyclic monoterpenoids and iridoids.

2- **Irregular monoterpenoids**: those compounds don`t fit the regular head-to-tail coupling mechanism in their construction.

Biosynthesis of Geranyl Pyrophosphate (GPP)

- The conversion of IPP into DMAPP generates a reactive electrophile and, therefore, a good alkylating agent. DMAPP possesses a good leaving group, the diphosphate, and can ionize readily to yield an allylic carbocation which is stabilized by charge d



Note: when using this representation of the allylic cation, do not forget it contains a double bond

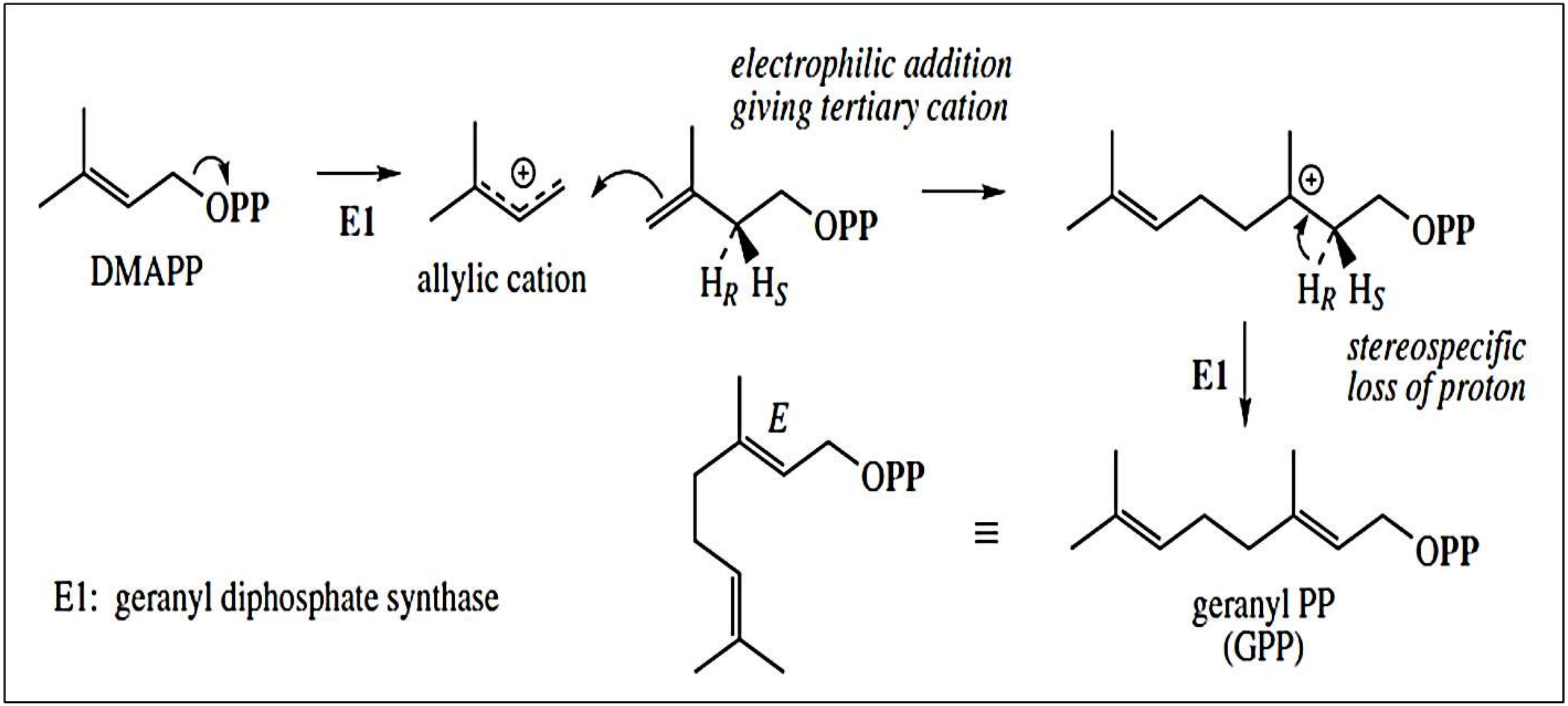
resonance-stabilized allylic cation

Biosynthesis of Geranyl Pyrophosphate (GPP)

- In contrast, IPP with its terminal double bond is more likely to act as a nucleophile, especially towards the electrophilic DMAPP. These differing reactivities are the basis of terpenoid biosynthesis, and carbocations feature strongly in mechanistic rationalizations of the pathways.

- Geranyl diphosphate (GPP) biosynthesis is believed to involve ionization of DMAPP to the allylic cation, addition to the double bond of IPP, followed by loss of a proton.

Biosynthesis of Geranyl Pyrophosphate (GPP)



E1: geranyl diphosphate synthase

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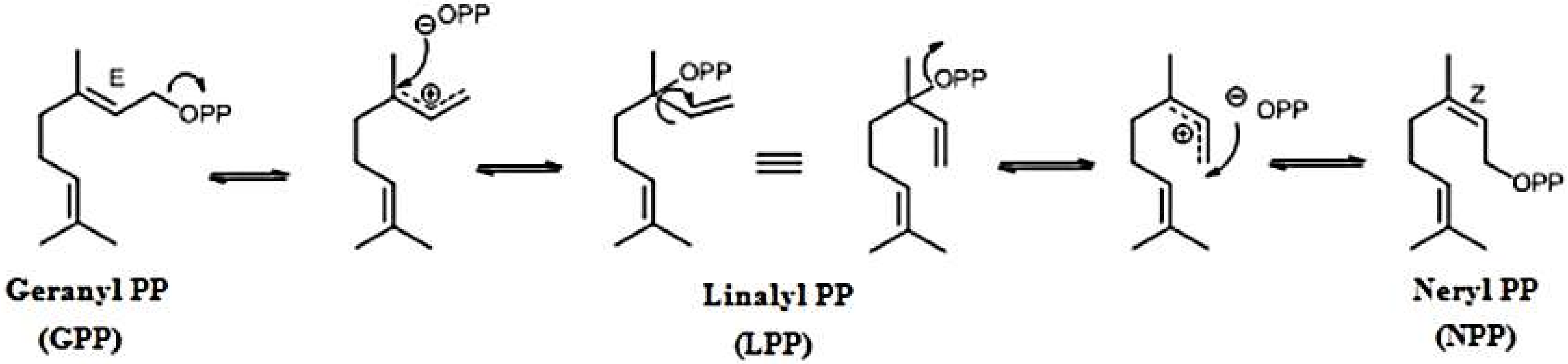
Isomers of GPP

- **Linalyl PP** and **neryl PP** are isomers of GPP and are likely to be formed from GPP by ionization to the allylic cation, allowing a change in attachment of the diphosphate group (to the tertiary carbon in linalyl PP) or a change in stereochemistry at the double bond (to Z in neryl PP).



Acyclic Monoterpenoids

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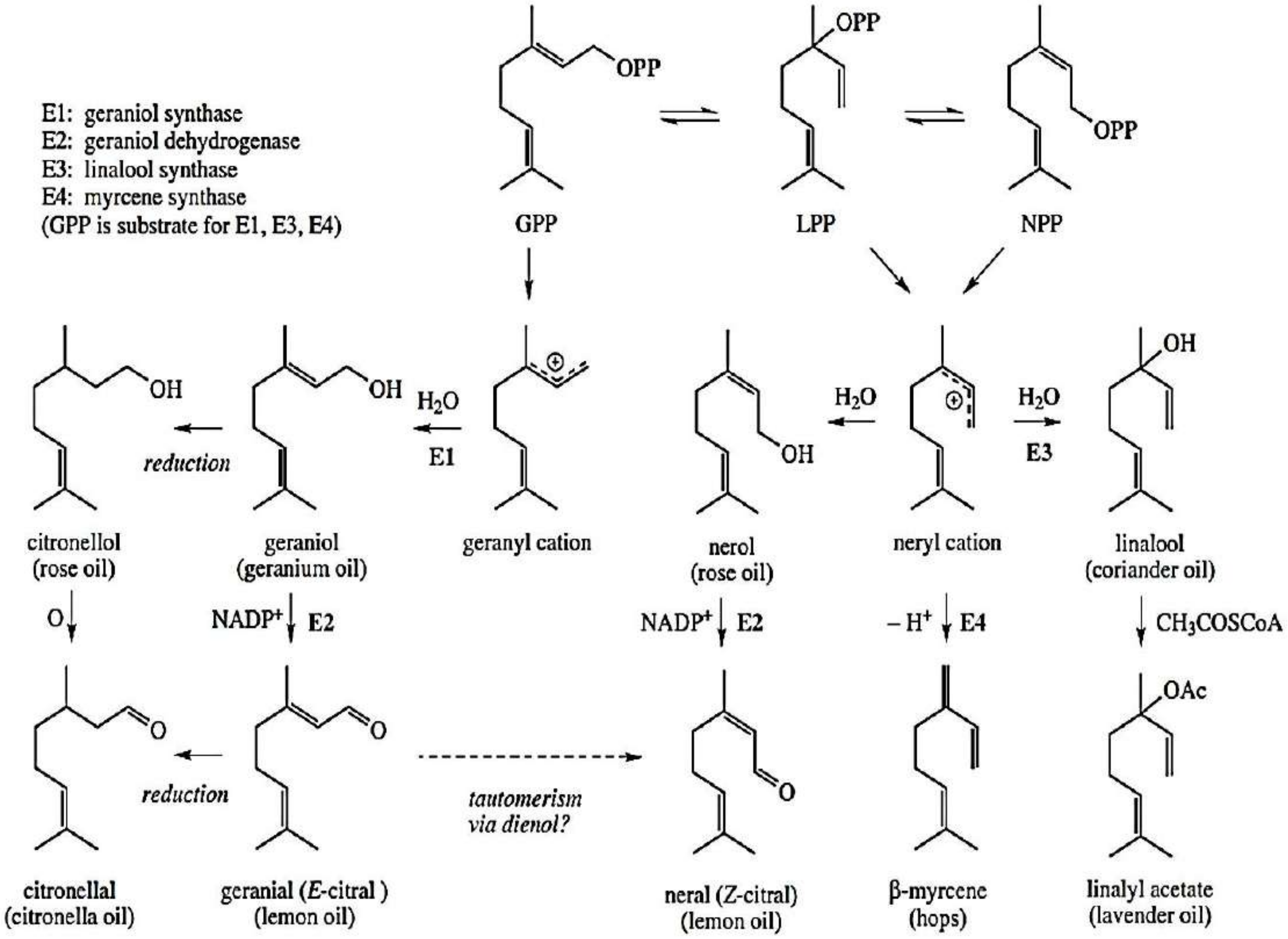
Biosynthesis of Acyclic Monoterpenoids

- **Geranyl PP** and its isomers **Linalyl PP** and **neryl PP** can give rise to a range of acyclic (linear) monoterpenoids by relatively modest changes.
- Acyclic Monoterpenoids found as components of **volatile oils** used in flavoring and perfumery.
- The resulting compounds may be hydrocarbons, alcohols, aldehydes, or perhaps esters, especially acetates by reaction with acetyl-CoA.
- Where enzymes have been characterized, it has been demonstrated that the reactions proceed through the carbocation intermediates. Thus, geraniol is the result of addition of water to the geranyl cation and is not formed by hydrolysis of GPP.

Biosynthesis of Acyclic Monoterpenoids



E1: geraniol synthase
E2: geraniol dehydrogenase
E3: linalool synthase
E4: myrcene synthase
(GPP is substrate for E1, E3, E4)

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Cyclic Monoterpenoids

Monocyclic

Bicyclic

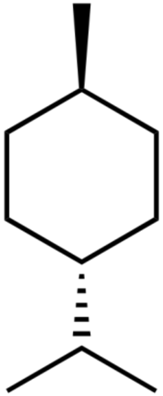
- **Outlines**
  - **Biosynthesis**
  - **Stereochemistry**
  - **Terpenoid containing drugs**
    - **Scientific name and part used**
    - **Chemical composition**
    - **Indication, therapeutic, and medicinal use**

Cyclic Monoterpenoids

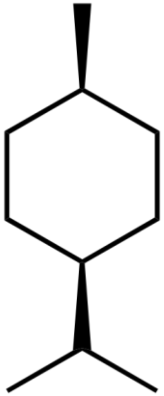
- The range of monoterpenoids chemical diversity is extended considerably by **cyclization reactions**, and monocyclic or bicyclic systems can be created.
- The basic structures of cyclic monoterpenoids are determined by highly specific synthases. Terpenoid synthases that produce cyclic products are also referred to as "**cyclases**".

Monocyclic Monoterpenes

- The parent molecule is **para-menthane** (methyl-isopropyl cyclohexane)
- It is the product of the hydrogenation or hydrogenolysis of various terpenoids, including *p*-cymene, terpinolene, phellandrene, and limonene.
- It is a colorless liquid with a fragrant fennel-like odor.
- It occurs naturally, especially in exudates of Eucalyptus fruits.
- The compound is generally encountered as a mixture of the *cis* and *trans* isomers, which have very similar properties.

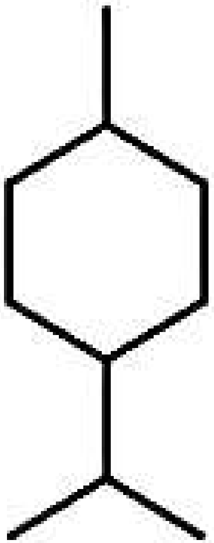


***trans-p*-menthane**

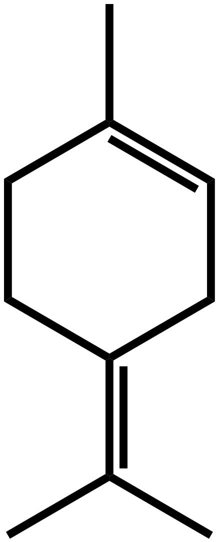


***cis-p*-menthane**

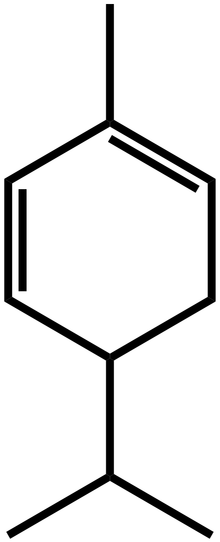
Monocyclic Monoterpenoids



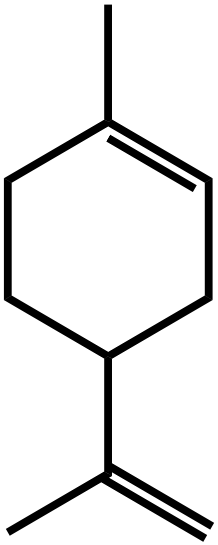
menthane
type



Terpinolene

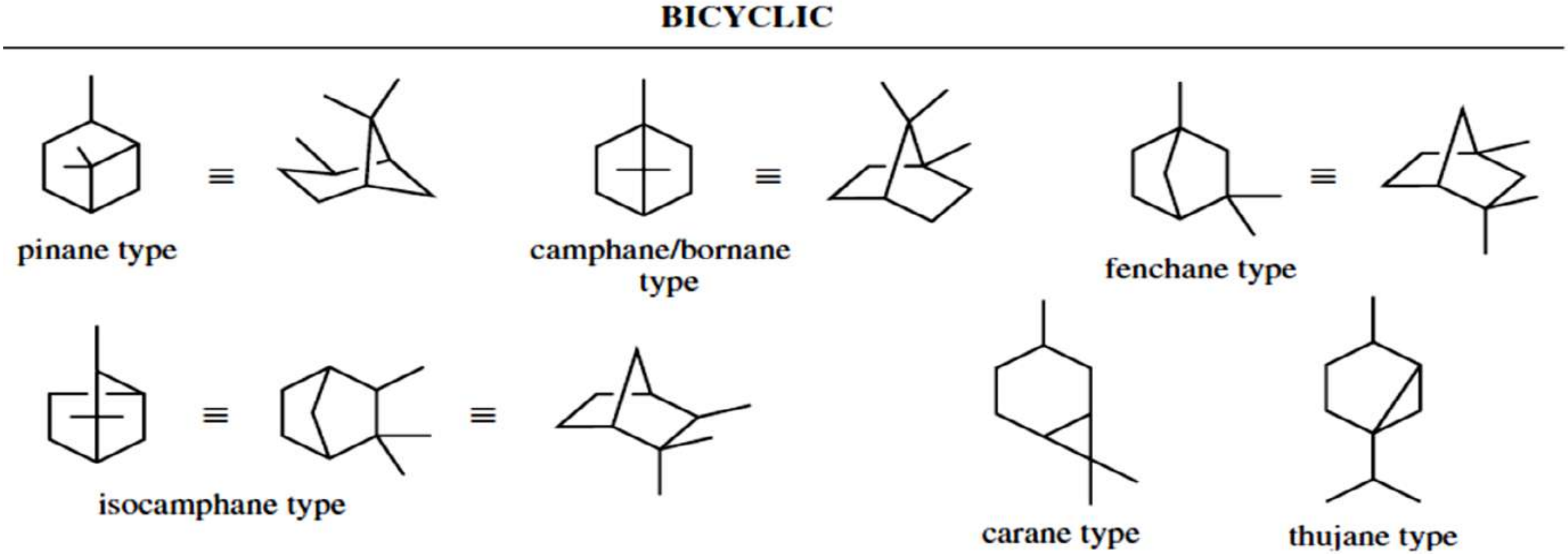


Phellandrene



Limonene

Bicyclic Monoterpenoids



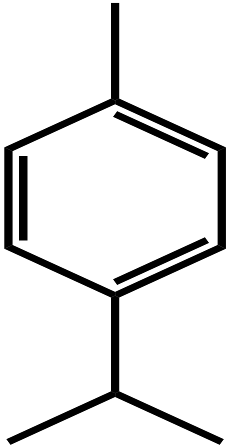
Gem Dialkyl Rule

- Ingold (1921) pointed that a gem alkyl group affects the stability of terpenoids.

- Gem dialkyl group tends to render the cyclohexane ring unstable whereas it stabilizes the three, four and five member rings.

- This rule limits the number of possible structure in closing the open chain to ring structure.

- The monoterpenoid open chain give rise to **only** one possibility for a monocyclic monoterpenoid Ex: The *p*-cymene structure.



p-cymene

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Biosynthesis of Monocyclic Monoterpenoids

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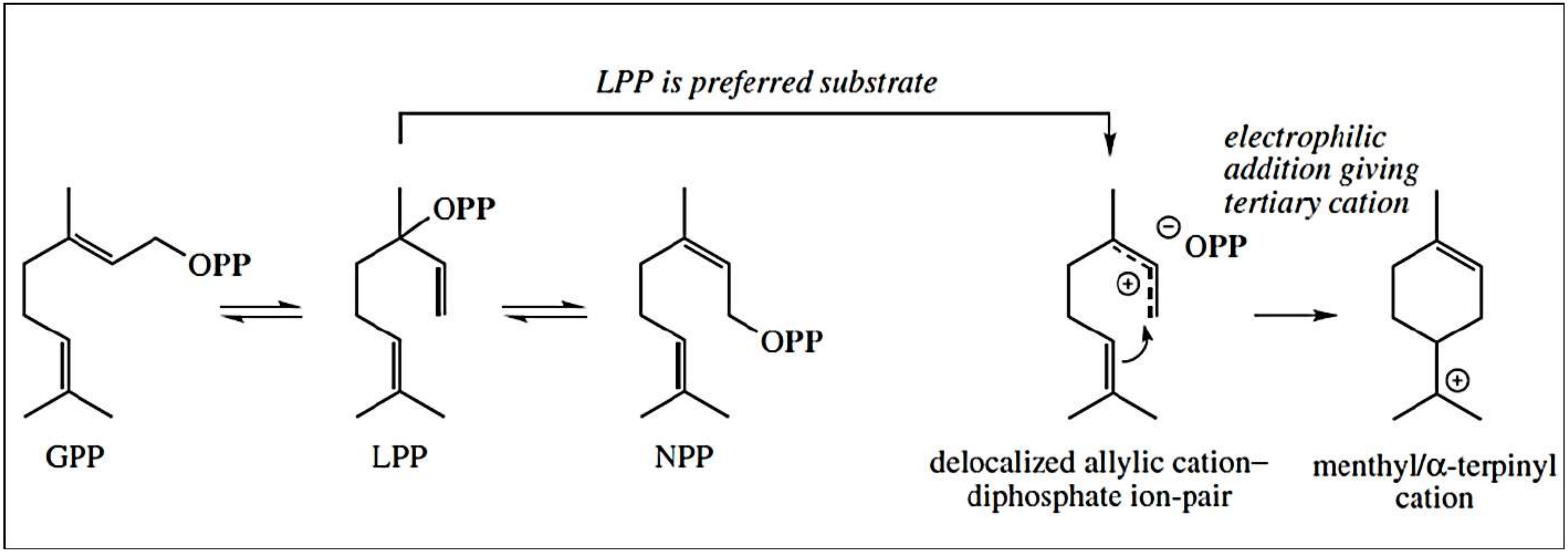
Biosynthesis of menthyl/α-terpiny cation

- Terpenoid cyclases use a common mechanism in which ionization of GPP leads initially to the tertiary allylic isomer Linalyl diphosphate (LPP) or Neryl diphosphate (NPP).
- This isomerization step is required because GPP **cannot** cyclize directly, given the presence of the trans-double bond, the E stereochemistry of this double bond being unfavorable for ring formation.
- Neryl PP or linalyl PP, however, do have favorable stereochemistry, with **linalyl PP** being the best substrate.

Biosynthesis of menthyl/α-terpiny cation

- Ionization of the enzyme-bound LPP or NPP intermediates promotes cyclization to a six membered ring carbocation (the menthyl or α-terpiny cation) tightly bound to the diphosphate anion, and bond formation follows due to the proximity of the π-electrons of the double bond.

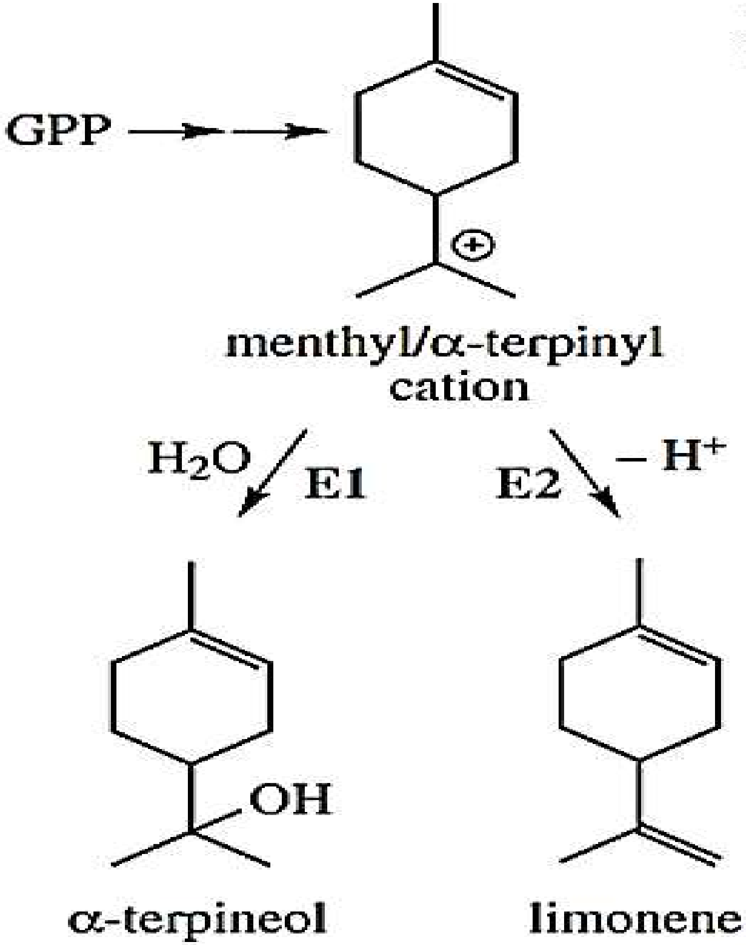


Biosynthesis of Cyclic Monoterpenoids

- The α-terpinyl/menthyl cation undergo additional changes including deprotonation of the carbocation or capture by a nucleophile (e.g., water) or further electrophilic cyclizations.
- Further terpenoid structural variability could be obtained by Rearrangements of the Wagner–Meerwein (W-M) type, in which carbon atoms or hydride migrate to achieve enhanced stability for the cation via tertiary against secondary character or to create a resonance stabilized allylic cation or decrease ring strain.

Biosynthesis of Monocyclic Monoterpenoids

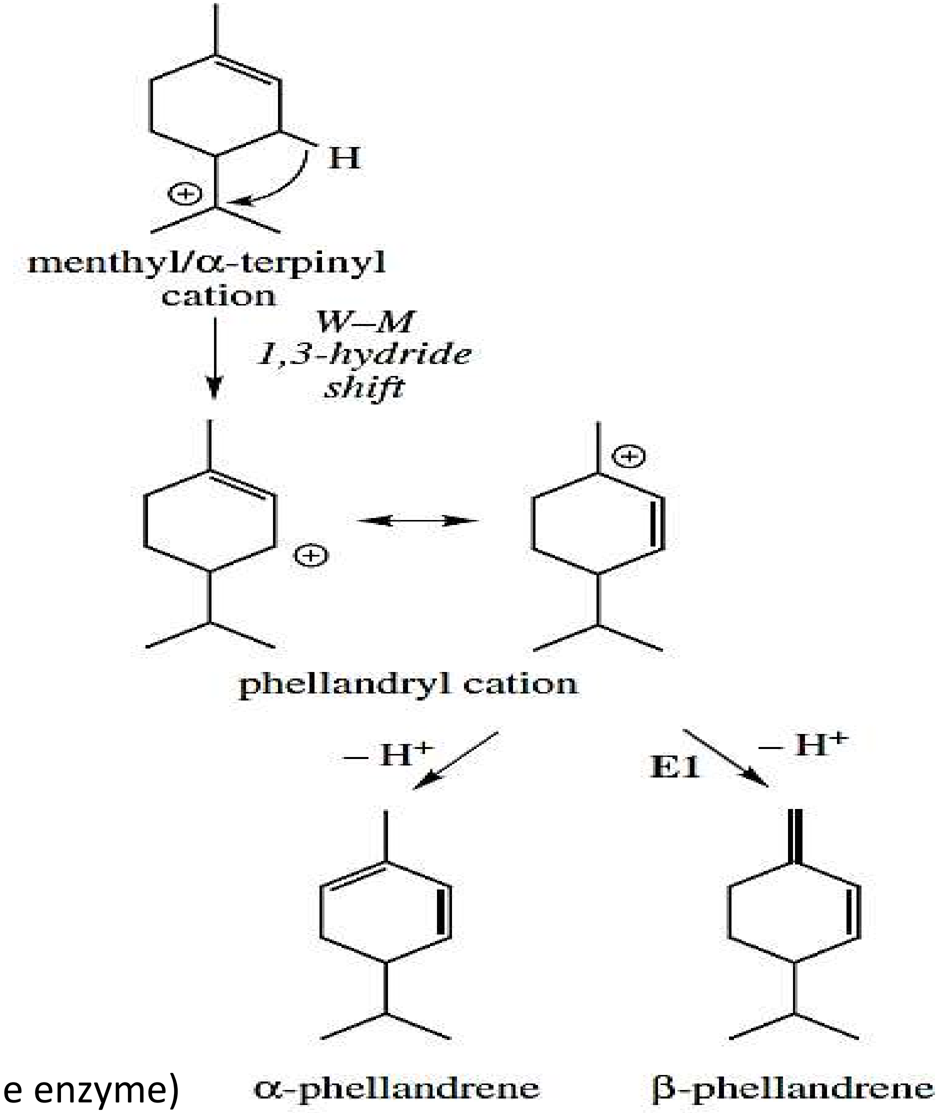
- The newly generated menthyl cation could be:
  - quenched by attack of water, in which case the alcohol **α-terpineol** would be formed
  - lose a proton to give **limonene**



E1: α-terpineol synthase
E2: limonene synthase
(GPP is substrate for all enzymes)

Biosynthesis of Monocyclic Monoterpenoids

- The menthyl cation, although it is a tertiary, may be converted by a 1,3-hydride shift into a favorable resonance-stabilized allylic cation (**phellandryl cation**).
- This allows the formation of **α-** and **β-phellandrenes** by loss of a proton from the phellandryl carbocation.

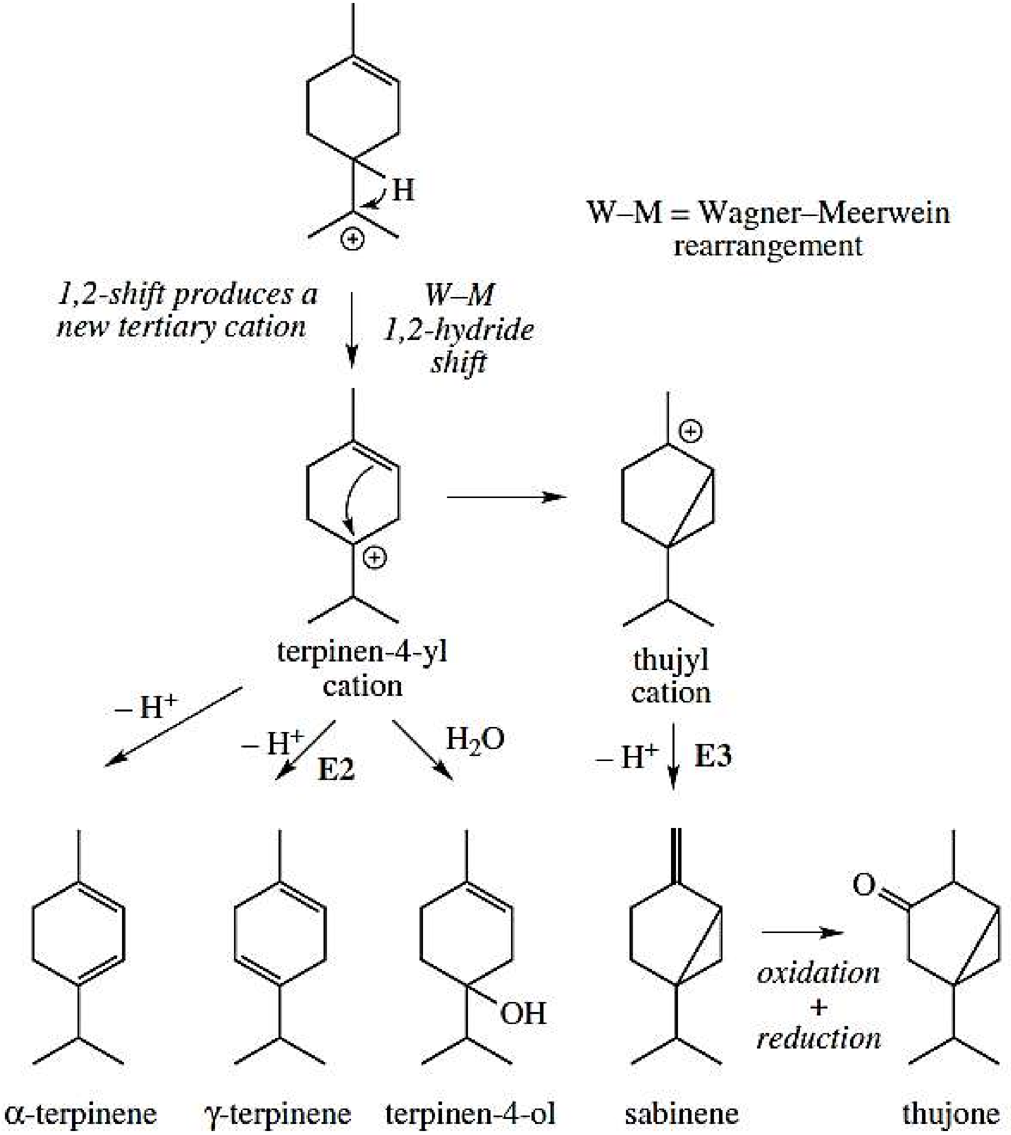


E1: β-phellandrene synthase (GPP is substrate for the enzyme)

Biosynthesis of Monocyclic Monoterpenoids

- A 1,2-hydride shift converting the menthyl cation into the **terpinen-4-yl cation** only changes one tertiary carbocation system for another, but allows formation of **α-terpinene**, **γ-terpinene**, and the **α-terpineol** isomer terpinen-4-ol.
- A further cyclization reaction on the terpinen-4-yl cation generates the **thujane skeleton**, e.g., **sabinene** and **thujone**.

E2: γ-terpinene synthase. E3: sabinene synthase (GPP is substrate for all enzymes)

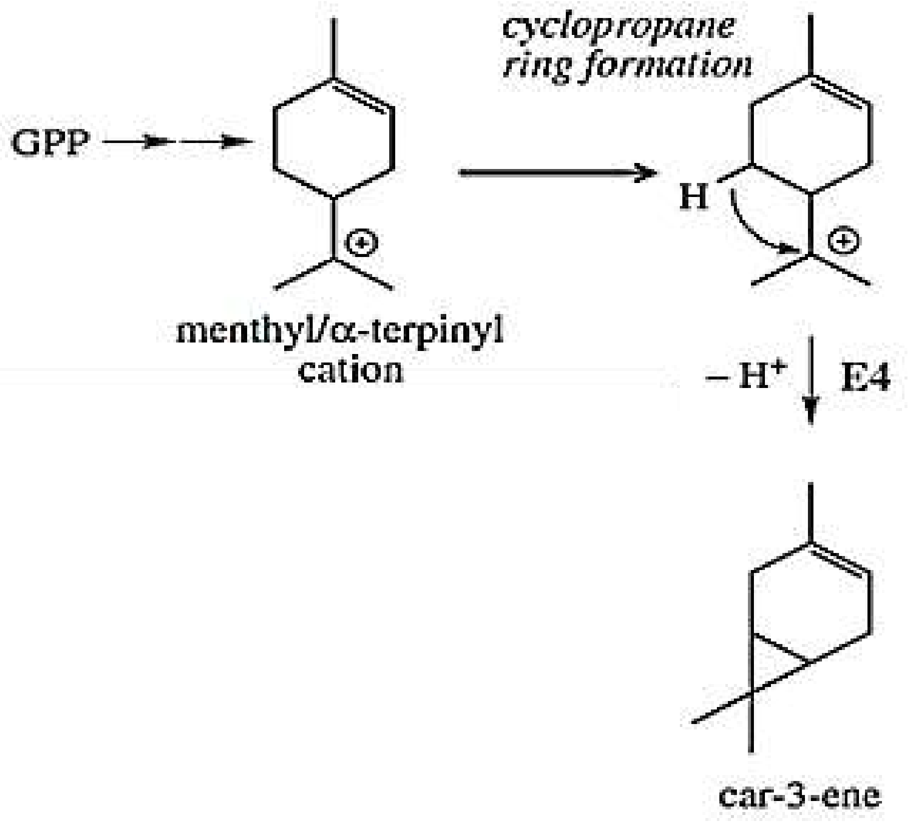


Biosynthesis of Bicyclic Monoterpenoids

Khaldoun S. Abdelwahed, Ph.D.

Biosynthesis of Bicyclic Monoterpenoids

- A less common bicyclic monoterpenoid synthesis involve loss of a proton from the menthyl cation. This result in the formation of a **cyclopropane ring** as exemplified by **3-carene** and generation of the **carane skeleton**.



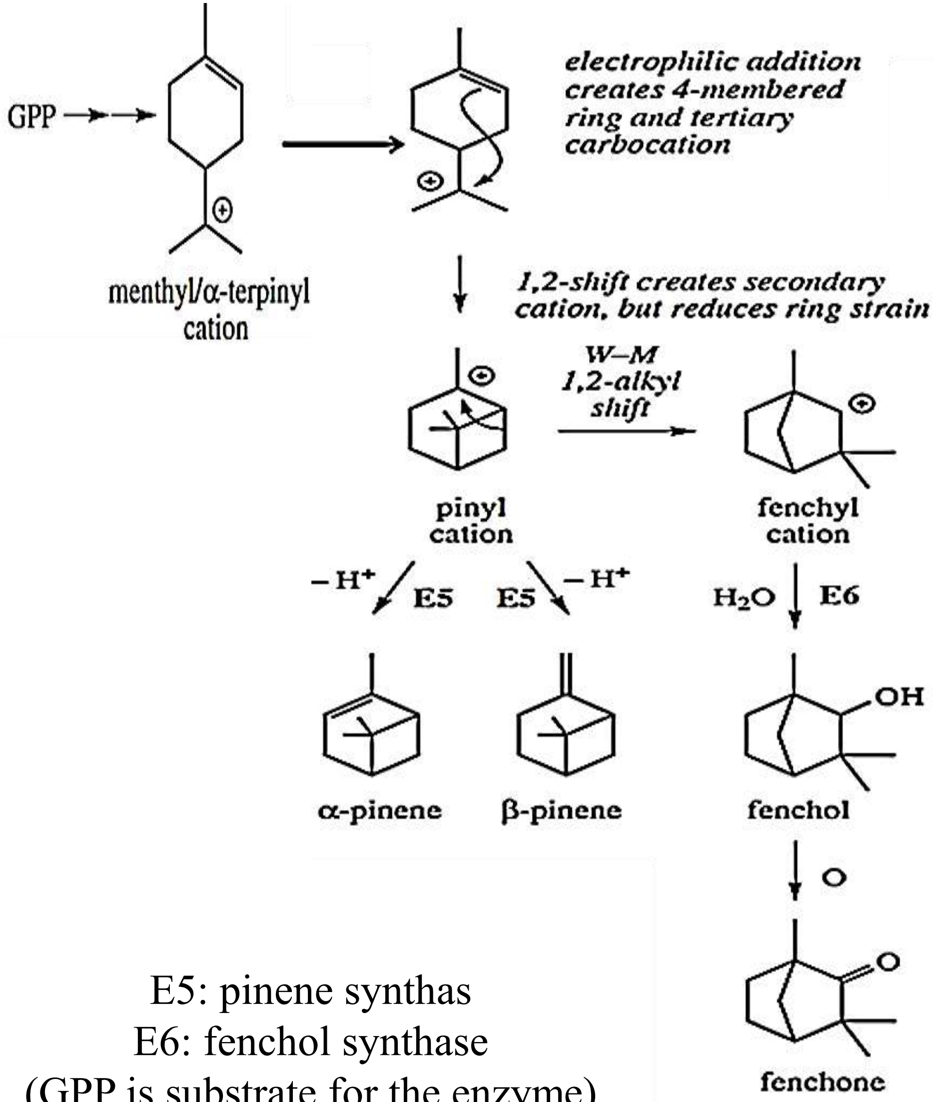
E4: 3-carene synthase
(GPP is substrate for the enzyme)

Biosynthesis of Monocyclic Monoterpenoids

- Most bicyclic monoterpenoids were formed by folding the cationic side-chain of menthyl cation towards the double bond (via the surface characteristics of the enzyme), thus allow a repeat of the cyclization mechanism and produce bicyclic **pinyl** and **bornyl cations**, according to which end of the double bond was involved in forming the new bonds.

Biosynthesis of Monocyclic Monoterpenoids

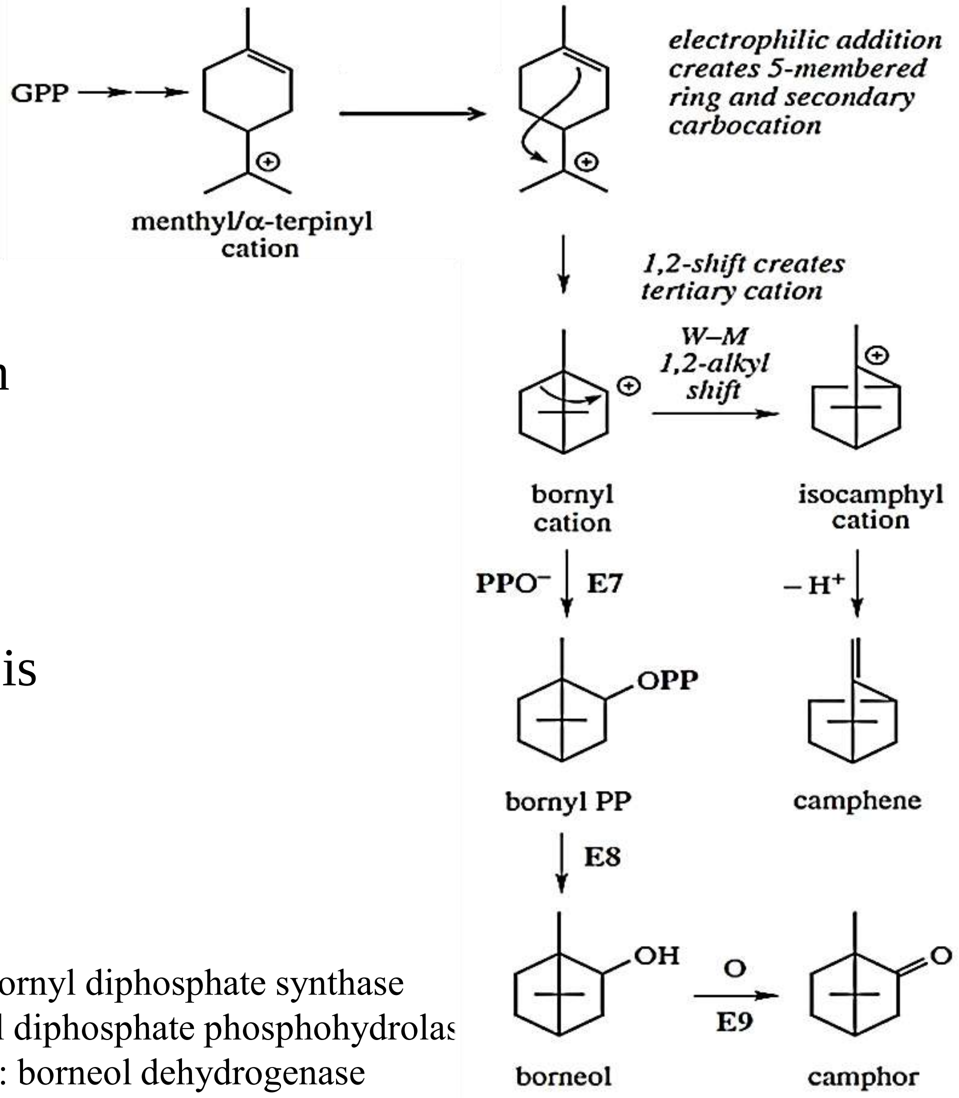
- Thus **α-pinene** and **β-pinene** arise by loss of different protons from the pinyl cation, producing the double bonds as cyclic or exocyclic respectively.
- The bicyclic pinyl cation, rearranges to the less-strained five-membered **fenchyl cation**, This produces the **fenchane skeleton**, exemplified by **fenchol** and **fenchone**.



E5: pinene synthas
E6: fenchol synthase
(GPP is substrate for the enzyme)

Biosynthesis of Monocyclic Monoterpenoids

- **Borneol**; unusually, this alcohol is actually derived by hydrolysis of bornyl diphosphate.
- Oxidation of the secondary alcohol in borneol then generates the ketone **camphor**.
- The **isocamphyl** tertiary carbocation is formed from the bornyl secondary carbocation by a Wagner–Meerwein rearrangement, and so leads to **camphene**.



E7: bornyl diphosphate synthase
E8: bornyl diphosphate phosphohydrolas
E9: borneol dehydrogenase
(GPP is substrate for E7)

Important Notes

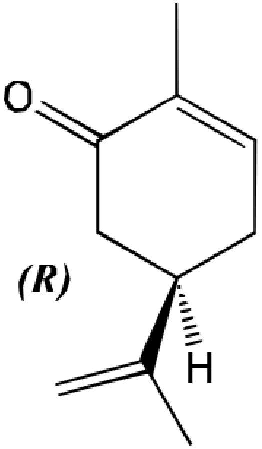
- Terpenoid cyclase enzymes, even highly pure proteins obtained by gene expression, rarely convert their substrate into a single product. Sometimes, multiple products in varying amounts, e.g., limonene, myrcene, α-pinene, and β-pinene, are synthesized by a single enzyme.
- This suggests that the enzyme is predominantly providing a suitable environment for the folding and cyclization of the substrate, whilst carbocation chemistry is responsible for product formation.
- Subsequent reactions, such as oxidation of an alcohol to a ketone or aldehyde, or reduction, or heterocyclic ring formation require additional enzyme systems.

Stereochemistry of Monoterpenoids

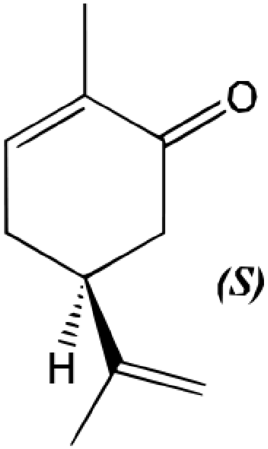
Khaldoun S. Abdelwahed, Ph.D.

Stereochemistry of Monoterpenoids

- Most monoterpenoids are optically active.
- Enantiomeric forms of the same compound can be isolated from different sources, e.g. **(+)-carvone** in caraway (*Carum carvi*: *Umbelliferae/Apiaceae*) and **(−)-carvone** in spearmint (*Mentha spicata; Labiatae/Lamiaceae*).



(*R*)-(-) carvone



(*S*)-(+) carvone

Stereochemistry of Monoterpenoids

- There are also examples of compounds found in both enantiomeric forms in the same organism, examples being **(+)-** and **(−)-limonene** in peppermint (*Mentha* × *piperita; Labiatae/Lamiaceae*) and (+)- and **(−)-α-pinene** in pine (*Pinus species; Pinaceae*).

- Where a single plant produces both enantiomers, it appears to contain two separate enzymes systems each capable of elaborating a single enantiomer.

Stereochemistry of Monoterpenoids

- The individual enantiomers can produce different biological responses, especially towards olfactory receptors in the nose.

- Thus, the characteristic odor of caraway is due to **(+)-carvone**, whereas **(−)-carvone** smells of mint.

- **(+)-Limonene** smells of oranges, whilst **(−)-limonene** resembles the smell of lemons.

Stereochemistry of Monoterpenoids

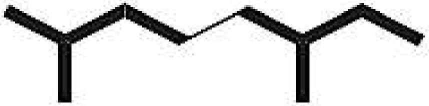
- In other systems, a particular structure may be found as a mixture of diastereomers.
- Peppermint (*Mentha* × *piperita; Labiatae/Lamiaceae*) typically produces **(−)-menthol**, with smaller amounts of the stereoisomers **(+)-neomenthol**, **(+)-isomenthol**, and **(+)-neoisomenthol**, covering four of the possible eight stereoisomers.
- Oils from various Mentha species also contain significant amounts of ketones, e.g. **(−)-menthone**, **(+)-isomenthone**, **(−)-piperitone**, or **(+)-pulegone**.

Irregular Monoterpenoids
Iridoids

Khaldoun S. Abdelwahed, Ph.D.

Irregular Monoterpenoids

- They are natural monoterpenoids that contain carbon skeletons derived from two isoprene C_5 units, but **do not** fit the regular head-to-tail coupling mechanism.
- They seem to be limited almost exclusively to members of the Compositae/*Asteraceae* plant family.



regular monoterpene skeleton

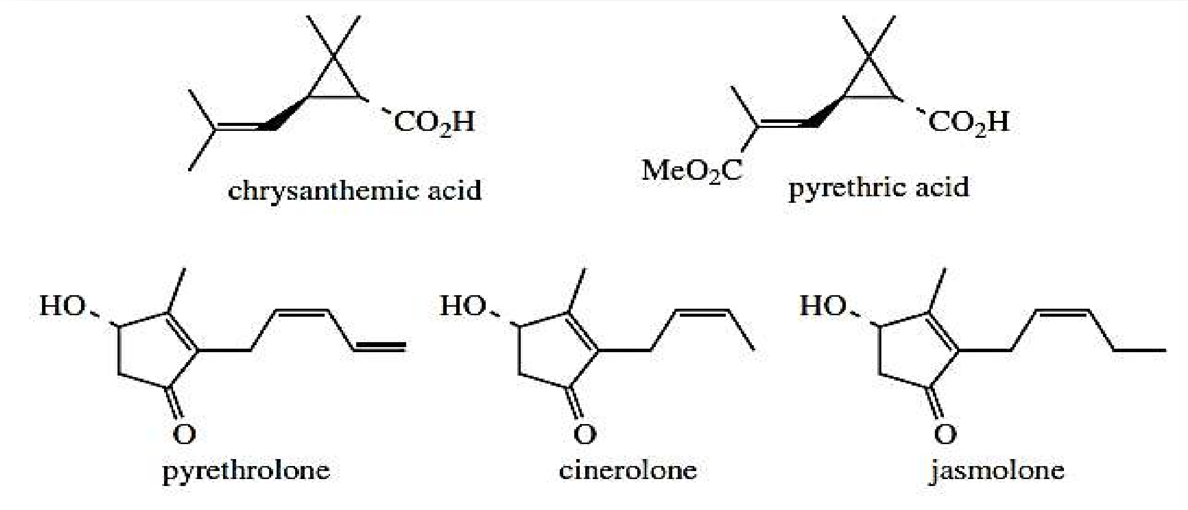
irregular monoterpene skeletons

Irregular Monoterpenoids

- Preeminent amongst these structures are **chrysanthemic acid** and **pyrethric acid**, found in ester form as the **pyrethrins** (pyrethrins, cinerins, and jasmolins), which are valuable insecticidal components in pyrethrum flowers, the flower head of *Chrysanthemum cinerariaefolium* (Compositae/*Asteraceae*)..
- These cyclopropane structures are readily recognizable as derived from two isoprene units, however, GPP and neryl PP do not appear to be involved.

Irregular Monoterpenoids

- **Pyrithrin I**, **cinerin I** and **jasmolin I** are esters of chrysanthemic acid with the corresponding alcohols.
- **Pyrithrin II**, **cinerin II** and **jasmolin II** are esters of pyrethric acid with the corresponding alcohols.



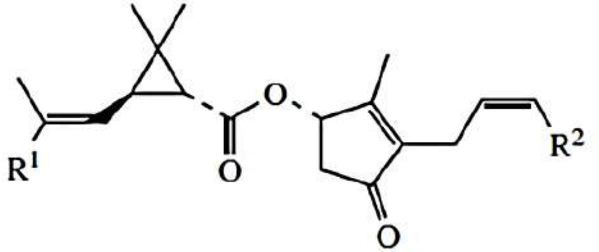
chrysanthemic acid

pyrethric acid

pyrethrolone

cinerolone

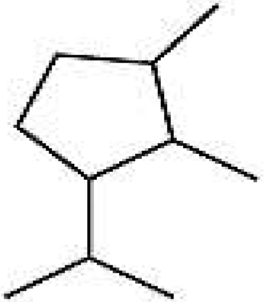
jasmolone



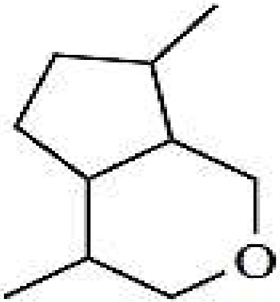
	R^1	R^2
pyrethrin I	Me	$CH=CH_2$
cinerin I	Me	Me
jasmolin I	Me	Et
pyrethrin II	CO_2Me	$CH=CH_2$
cinerin II	CO_2Me	Me
jasmolin II	CO_2Me	Et

Iridoids

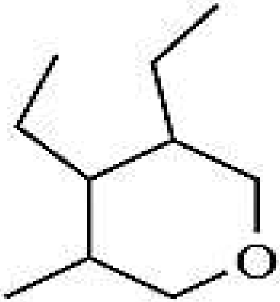
- The name derives from Iridomyrmex, a genus of ants which produces these compounds as a defensive secretion.
- The **iridane skeleton** found in iridoids is monoterpenoid in origin and contains a cyclopentane ring which is usually fused to a six-membered oxygen heterocycle.



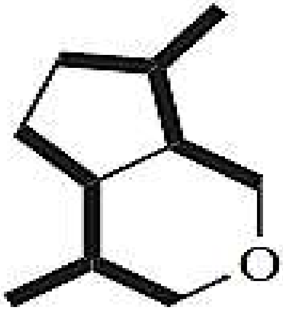
iridane



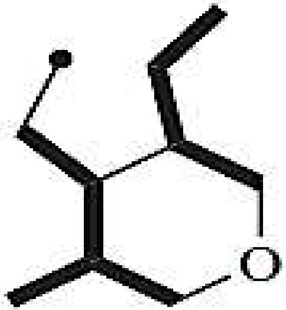
iridoid



secoiridoid



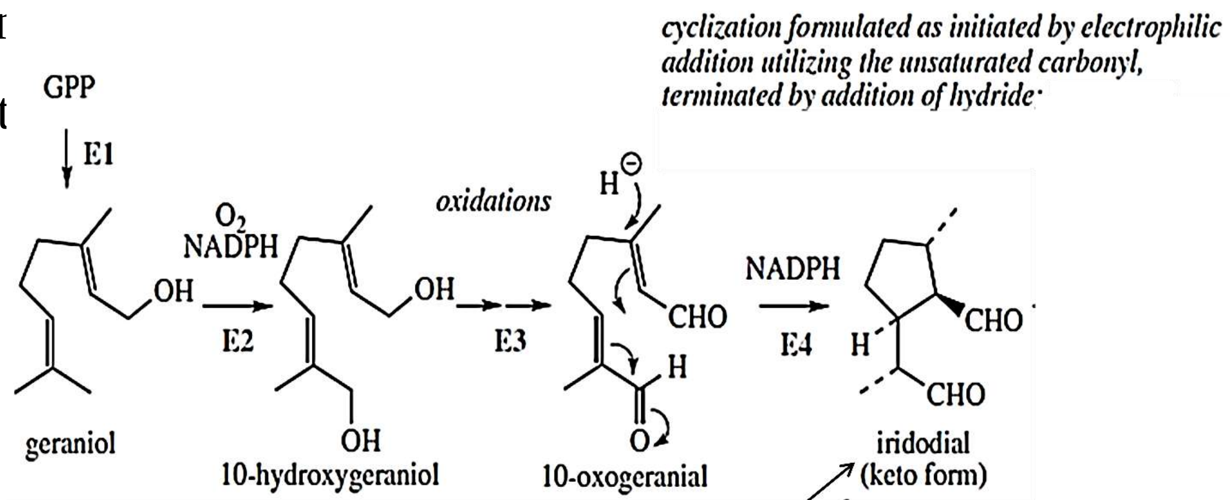
iridoid

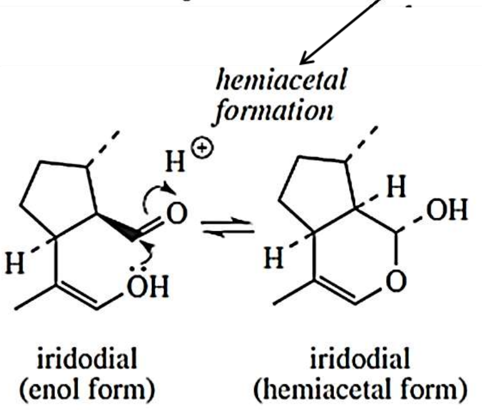


secoiridoid

Biosynthesis of Iridoids

- The iridoid system arises from **geraniol** by a type of foldi which is different from that already encountered with monoterpenoids.



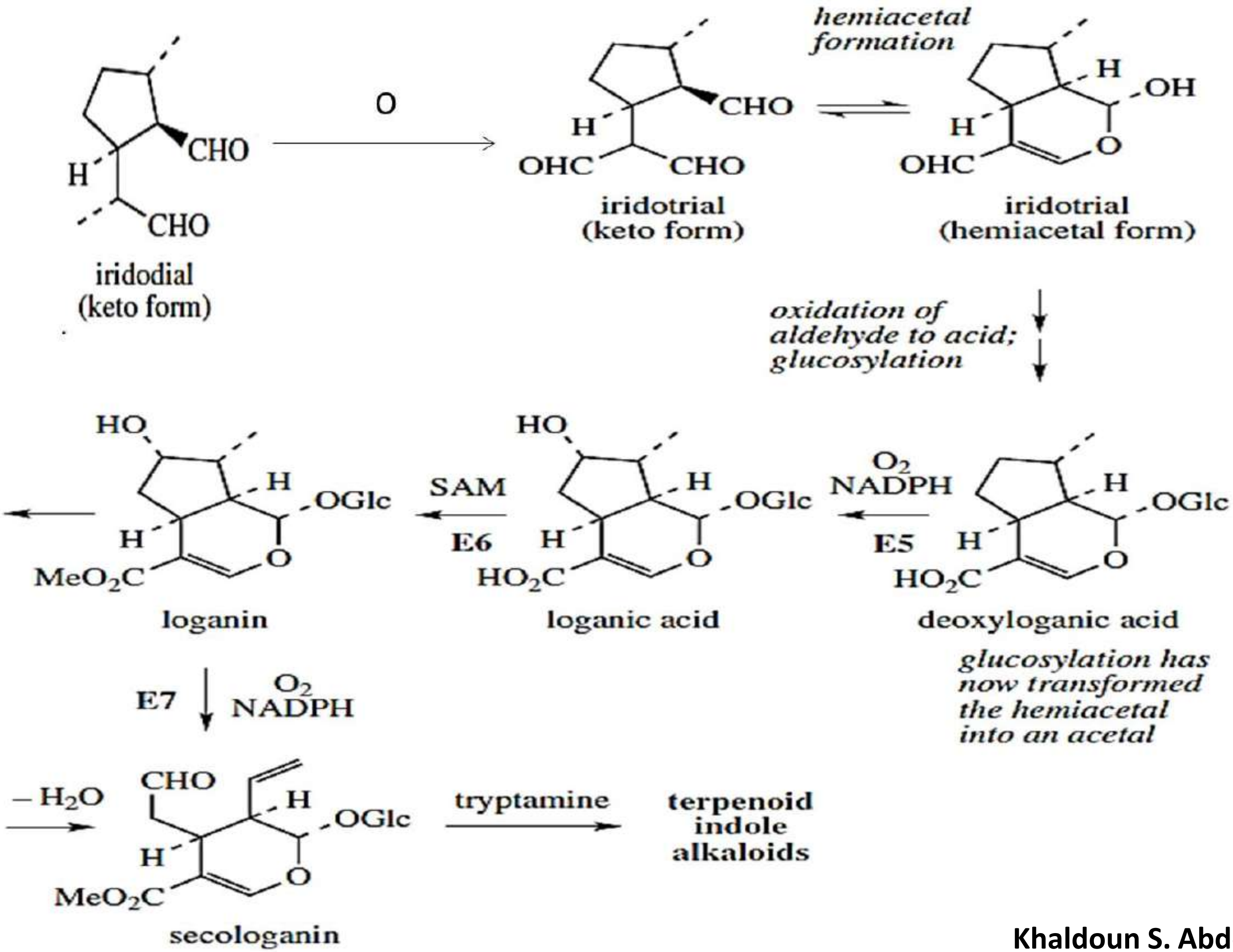


- Also markedly different is the fundamental cyclization to **iridodial** is formulated as attack of hydride on the dialdehyde, produced by a series of hydroxylation and oxidation reactions on geraniol.

Irregular Monoterpenoids

- Further oxidation gives **iridotrial**, in which hemiacetal formation then leads to production of the heterocyclic ring.
- A sequence of reactions then followed, and thousand different natural iridoids and secoiridoids are formed.
- **Loganin** is a key intermediate in the pathway to a range of complex terpenoid indole alkaloids.
- Fundamental in this further metabolism is cleavage of the simple monoterpene skeleton, which is still recognizable in loganin, to give **secologanin**, representative of the **secoiridoids**.

Irregular Monoterpenoids



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Monoterpenoids Containing Drugs

Essential Oils

Pyrethrins

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Essential Oils (Aetherola)

- An essential oil is defined by the International Organization for Standardization (ISO) as follows: "**Product obtained from vegetable raw material either by distillation with water or steam or from the epicarp of Citrus fruits by a mechanical process, or by dry distillation**".
- These oils typically contain a complex mixture of low boiling-point components.

Volatile Oil vs Fixed Oil

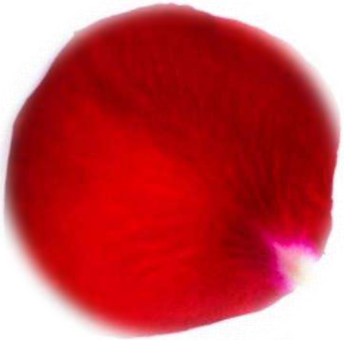
Volatile oil	Fixed oil
Also called as an essential oil.	Also called as natural non-volatile oil.
Volatile oil can evaporate when placed under room temperature	Fixed oils do not evaporate at room temperature
Have no permeant grease spot left after evaporation	Have permeant grease spot left after evaporation
Mixtures of cleoptenes & stearoptenes are termed as volatile oils	Esters of higher fatty acids & glycerin are called as fixed oils.
They are unable to undergo saponification	Can be saponified with alkaloid
Do not become rancid with light and air but can be oxidize and resinify	Become rancid with exposure to light or air
Their primary source is leaves, roots, in petals and bark.	Their major source is seeds of the plant.
Examples: Lemon, cinnamon, menthol, eucalyptus, sage	Examples: Coconut, olive, argan and jojoba

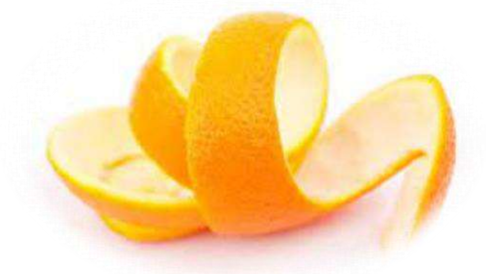
Volatile Oil

- Volatile oil may be present in:
  1. Free form with an aromatic characteristic odor.
  2. Combined with
     - Sugar as glycosides
     - Gums, resin, or both as oleo-gum, oleoresin, or oleo-gum-resin
- Volatile oil may be localized in:
  1. All plant tissue as Pinaceae, and Conifers.
  2. Specialized secretory structure usually on or near surface of plant.

Volatile Oil Occurrence

- Volatile oil may occur in
    - All tissue in the conifers
    - Petals in the rose
    - Bark and leaves in cinnamon
    - Pericarp in umbelliferous fruit
    - Glandular hair of stem and leaves in mint
    - Flower petal or rind in orange





Volatile Oil Localization

- Volatile oil present in specialized secretory structures:

1. **Glandular hairs**: leaves of *Lamiaceae* family: sage, thyme, peppermint, basil, lavender
2. **Modified parenchyma cells**: *Piperaceae* (black pepper) and Lauraceae (cinnamon, bay)
3. **Oil tubes (vittae):** Fruits of *umbelliferae f*amily: anise, cumin, caraway, coriander, fennel
4. **Oil cells:** *Zingiberaecea* family: ginger rhizome
5. **Lysigenous (Secretary) cavity**: eucalyptus, citrus, gossypium

Volatile Oil Formation

- Volatile oil can be formed:

1. Directly by protoplasm.
2. Decomposition of resinogenous layer of cell wall.
3. Hydrolysis of certain glycoside:
    - Amygdalin → benzaldehyde
    - Glycovanillin → vanillin
    - Sinalbin → isothiocynate
    - Singririn → allyl isothiocynate

Sources of Volatile Oil

Rosemary

Sage

Eucalyptus

Thyme

Basil

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Sources of Volatile Oil

Clove buds

Jasmine

Chamomile

Lavender

Rose

Sources of Volatile Oil

Coriander

Citrus

Caraway

Anise

Fennel

Sources of Volatile Oil

Coffee

Ginger

Nutmeg

Peppermint

Cinnamon

Physicochemical Prosperities

- **Odor**: Have very pleasant aromatic and characteristic odor.
- **Density**: Most are less than 1g/ml, except cinnamon oil and clove oil are heavier than water.
- **Color**: Colorless, but during extraction process mixed with other compounds and produce colors:
  - Chamomile oil: Blue
  - Cinnamon oil: Brown
  - Peppermint: Yellow

Physicochemical Prosperities

- During storing, their color may be changes as they undergo both oxidation and resinification. We must keep them in:

1. Well sealed container.
2. Well filler (to the rim) container.
3. Nitrogen gas that replace air.
4. Amber glass bottle or aluminum container.
5. Cool, dry, and dark place.

Why Plants Secret Volatile Oil?

1. Insect repulsion.
2. Increase disease resistance.
3. Allelopathic effect (germination inhibitors).
    - Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the germination, growth, survival, and reproduction of other organisms. These biochemicals are known as allelochemicals and can have beneficial or detrimental effects on the target organisms and the community.
4. Reproduction by attracting pollinators.

General Uses of Volatile Oils

1. Therapeutic uses: local stimulant, local analgesic, carminative, diuretic, expectorant, mild antiseptic, local irritant, and anthelmintic.
2. Pharmaceutical use: mouth washes and gargles.
3. Perfumery: oil of rose
4. Flavoring agent in food and pharmaceutics
5. Spices
6. Cosmetic trade.
7. Starting material for synthesis of other compounds

   Ex: citral → vitamin A

Preparation of Volatile Oil

- Factors influence the volatile oil preparation:

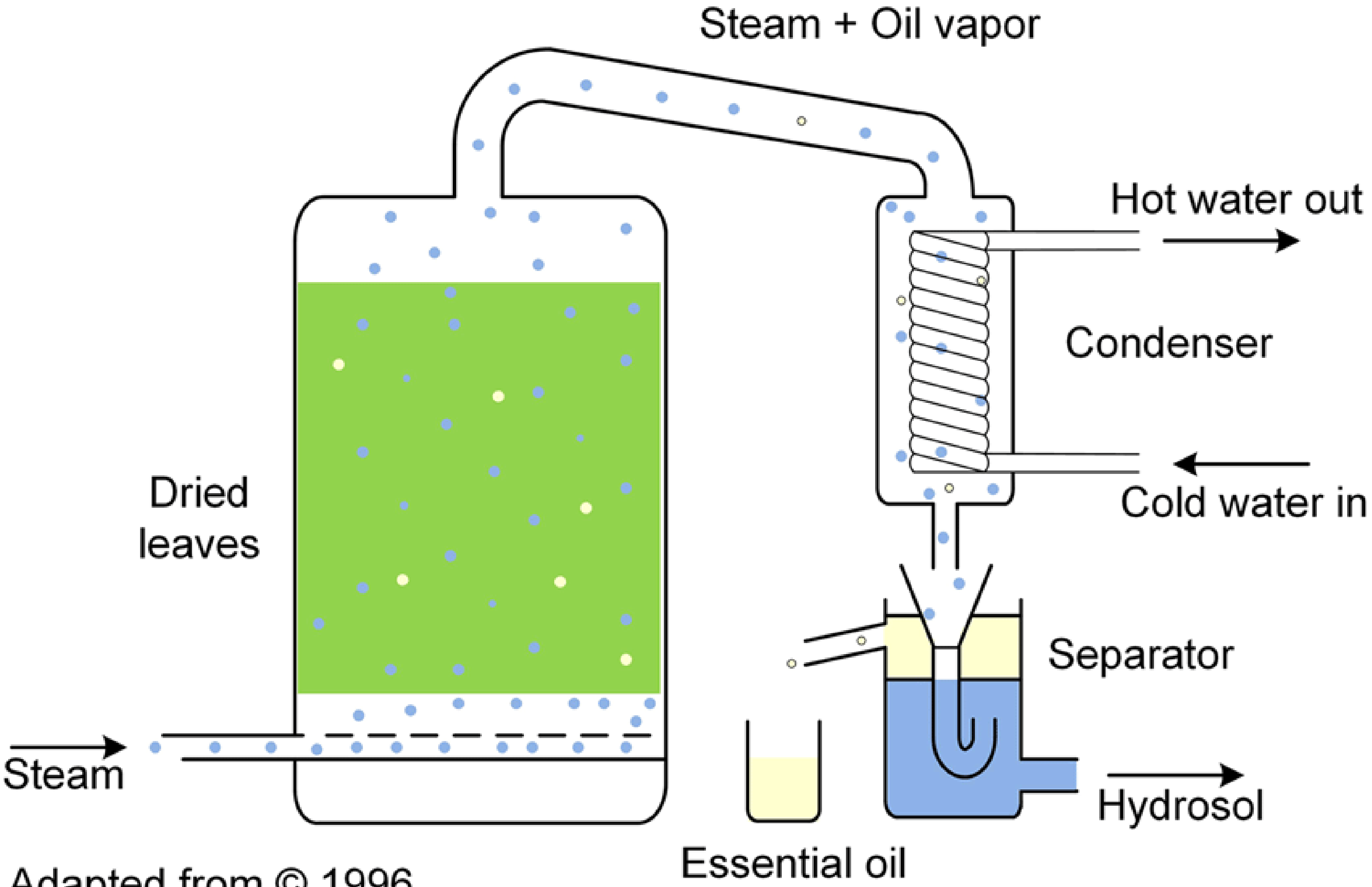
1. The condition of plant material (part used, fresh or dried, intact, crushed or powdered).
2. Location of oil in the plant (deep or superficial).
3. The quantity of the volatile oil in the plant.
4. The nature of chemical constituents of volatile oil.

Preparation of Volatile Oil

The following methods are commonly used in preparation of V.O

- **Distillation method**
  - Water distillation
  - Water & steam distillation
  - Direct steam distillation
- **Expression method**
  - Sponge method
  - Scarification
  - Rasping
  - Mechanical
- **Extraction method**
  - Extraction with volatile solvent
  - Extraction with non volatile solvent
    - Efleurage
    - Pneumatic
    - Maceration
- **Enzymatic hydrolysis method**

Distillation Principle



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Distillation Application

Thermostable oils

Oils present in large amount

Oils not rich in ester

Distillation Terminology

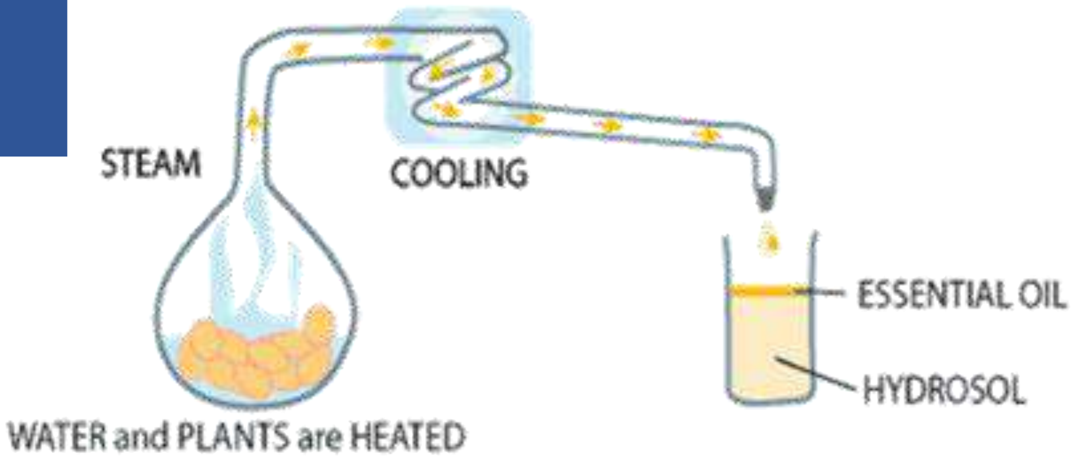
- **Hydro-diffusion:** process by which water or steam penetrates the plant tissues to take over the oil.

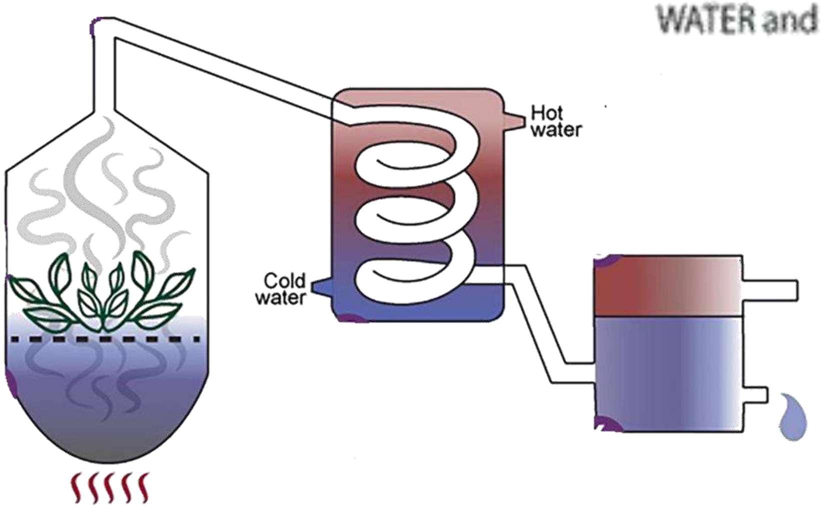
- **Aromatic water or Hydrosols:** distilled aqueous layer saturated with oil e.g., rose, orange flower & peppermint waters.

- **Cohobation:** return of aromatic water to the distillation chamber, in water distillation, in order to recover the dissolved oil.

Distillation Methods

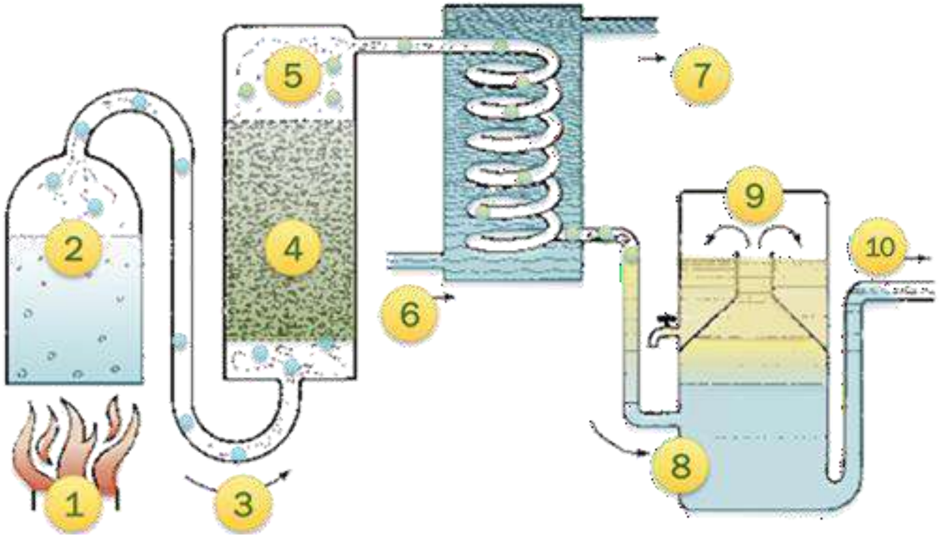
Water distillation





Water & steam distillation

Direct steam distillation



Water vs Water & Stem vs Direct Stem Distillation

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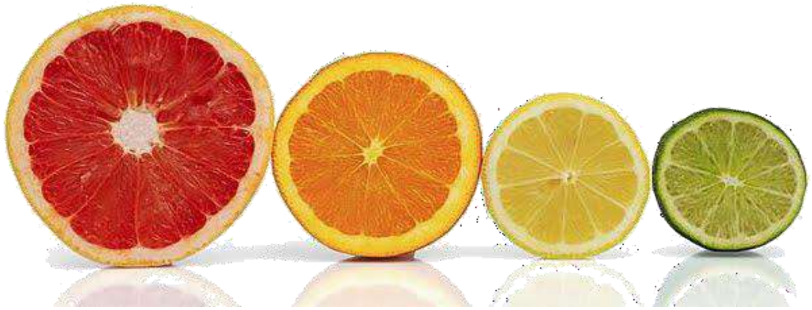
	Water	Water & steam	Direct steam
Process	Material is boiled with water	The plant material is packed and supported above the boiling water on a perforated grid, Steam is passed through the macerated crude drug	Plant material is supported on the grid and saturated steam from outside source injected through steam coil
Plant material	•Dried: powder, or Comminuted herbs • Flower petals •Constituents are not degraded by boiling up to 100°C	Fresh or dried especially plant material that their constituents undergo degradation by direct boiling	Water is completely absent as fresh material containing sufficient moisture (no maceration)
Mode of charging	Material completely covered with water	Water is present in the still, but steam alone is in contact with plant Note: dried material should be wet	Water is completely absent
Hydro diffusion	Better when material moves freely in water	Better when charging is even	Better when charging is even
Steam pressure	About atmospheric	About atmospheric	Can be modified

	Water	Water & steam	Direct steam
Temperature	About 100°C	About 100°C	Can be modified
Rate of distillation	Low	Good	High
Yield of oil	Relatively low	Better since hydrolysis is diminished	The best if no lumping or channeling
Advantage	•Low price •Portable still: could be carried near production area •Suitable for finely powdered material	•Low price •Portable still: could be carried near production area •Hydrolysis is prevented since no excessive wetting of material •Faster, lesser fuel	•Still are more durable •suitable for large scale production (commercial) •No thermal decomposition of oil constituents •Suitable for oil rich in esters
Disadvantage	•Burning of material •hydrolysis of some constituents as esters •Not suitable for large capacity/ commercial scale distillations • Poor quality oil	•Not suitable for powders efficient if material is entire or crushed •Hydrodiffusion may be reduced due to lumping or channeling	

Expression Method

- Some volatile oils can not be distilled without decomposition and usually obtained by expression of the plant material.
- Only used in the production of citrus oils (orange, lemon, grapefruit…etc)



- **Principle**
  - Mechanical process carried at room temperature based on puncturing and/or squeezing of plant material to release oil.

Expression Application

Heat sensitive oils

Oils present in large amount in outer peels of fruit

Expression Process

- The extraction process involves **three** steps:

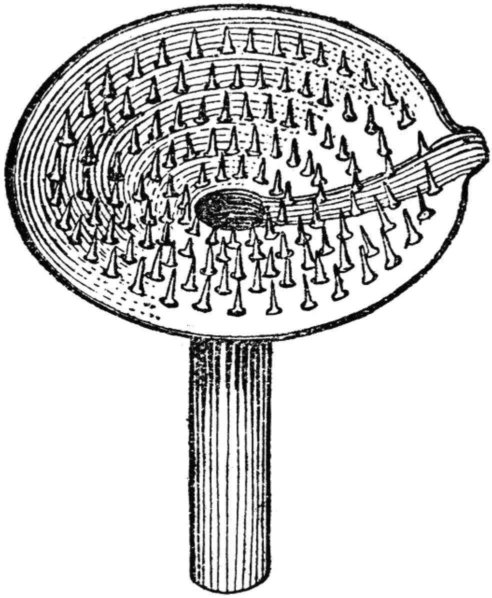
1. **Squeezing** of the peel under a stream of water which result an emulsion contain (volatile oil + water + pectin + cellulose + pigments + traces of waxes).
2. **Centrifugation** to remove water, pectin, and cellulose.
3. **Strong cooling** “ chilling” followed by filtration to remove waxes.

Expression-Sponge Method

- Sponge method based on **squeezing** the removed peels.
- The citrus fruit is washed, cut into halves & fleshy parts removed.
- The rind is turned inside out by hands (inverted) to rupture the oil cells and squeezed to release the volatile oil.
- A sponge collects the oozed oil, until the sponge becomes saturated with exuded water and oil.
- The sponge is then periodically squeezed in vessel and the upper oily layer in the vessel is separated.

Expression-Scarifying Method

- Based on **puncturing** (scarifying) the surface of whole citrus fruit.
- The instrument is funnel-shaped, formed of a shallow bowl with a tubular projection at the center.
- The bowl bears numerous metal needles are just long enough to penetrate the epidermis which scarify the oil glands to release the oil.



- The tubular part serves as:
  - ✓ Receiver to collect the oil.
  - ✓ A handle.

Expression-Rasping Method

- Based on removal of the outer layer of the peel with a grater.
- Collecting the rasping in special bags then strong pressing.
- The oil emulsion is collected in large vessels.

Expression-Machine Method

- Machine processes: whole fruits are charged into machines in which the peels are removed, sprayed with water, squeezed or pressed and the oil collected through wool.

Solvent Extraction Principle

- Based on extraction of the volatile oil from the plant material with a suitable solvent.
- According to the nature of the solvent used, **two** types are distinguished:

1. Volatile solvent extraction (Petroleum ether, hexane, benzene)
2. Non-volatile solvent extraction (tallow, lard, olive oil)

Solvent Extraction Application

Preparation of delicate flower oil

Oils present in small amount

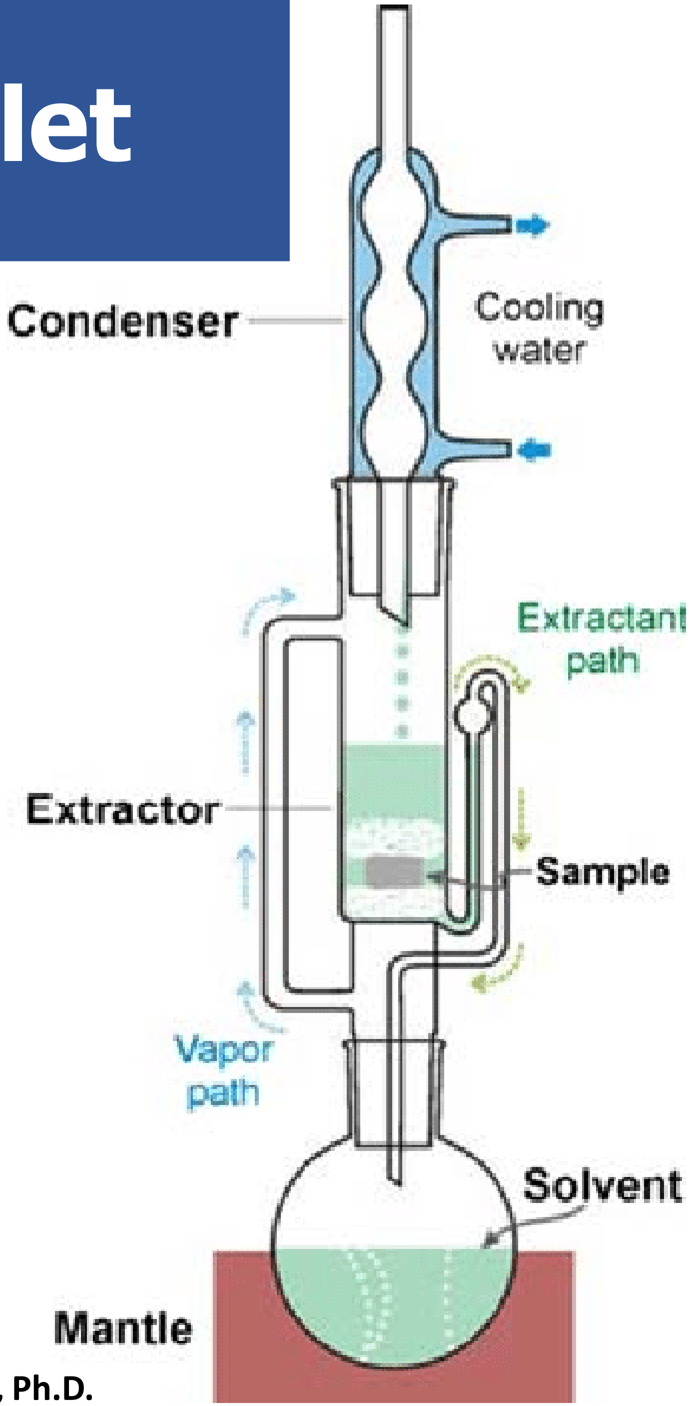
Oils formed of thermolabile constituents

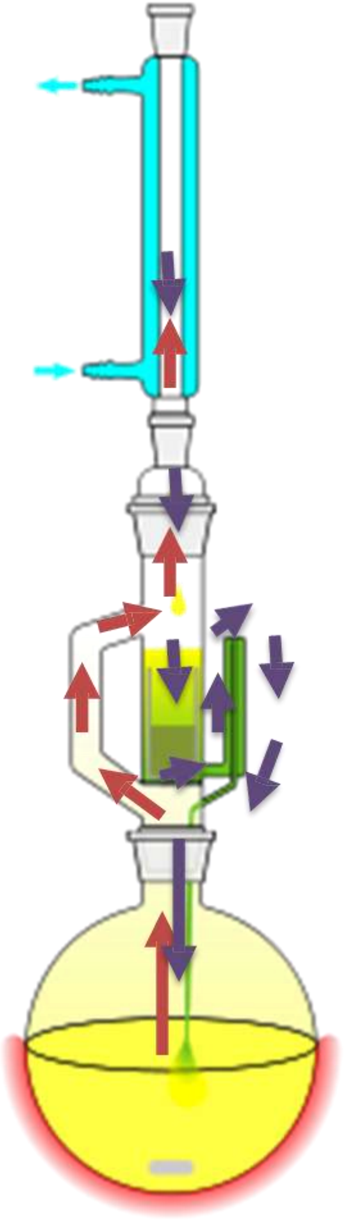
Extraction by Volatile Solvent

- The solvent should be:

  1. Dissolves the oil with the least amount of impurities.
  2. Dose not react with any of the oil constituents.
  3. Has a low boiling point.
  4. Leaves no residue on evaporation.

Soxhlet


Condenser
Cooling water
Extractant path
Extractor
Sample
Vapor path
Solvent
Mantle



Extraction by Non-Volatile Solvent

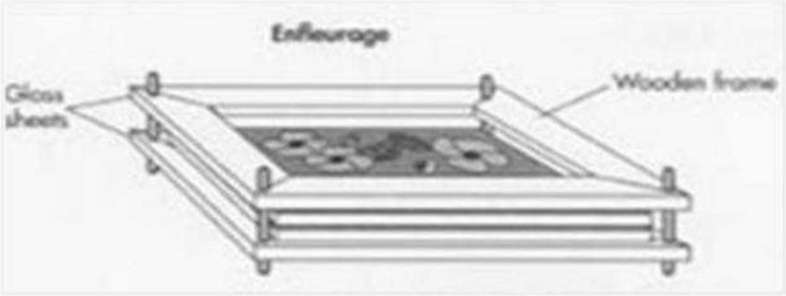
- Used for extraction of:
  1. Fixed oil
  2. Fats (lard and tallow)

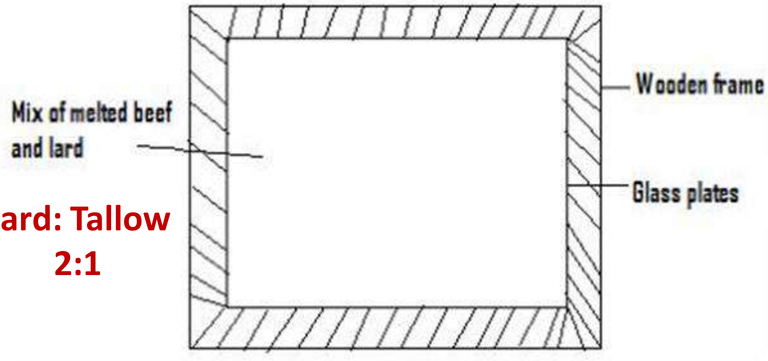
- Methods of extraction of non-volatile solvent:
  1. Enfleurage method
  2. Pneumatic method
  3. Maceration method

Enfleurage Method

- Enfleurage is a process that uses odorless fats that are solid at room temperature to capture the fragrant compounds exuded by plants.
- Equipment: Great number of glass plates closely arranged in

Enfleurage method

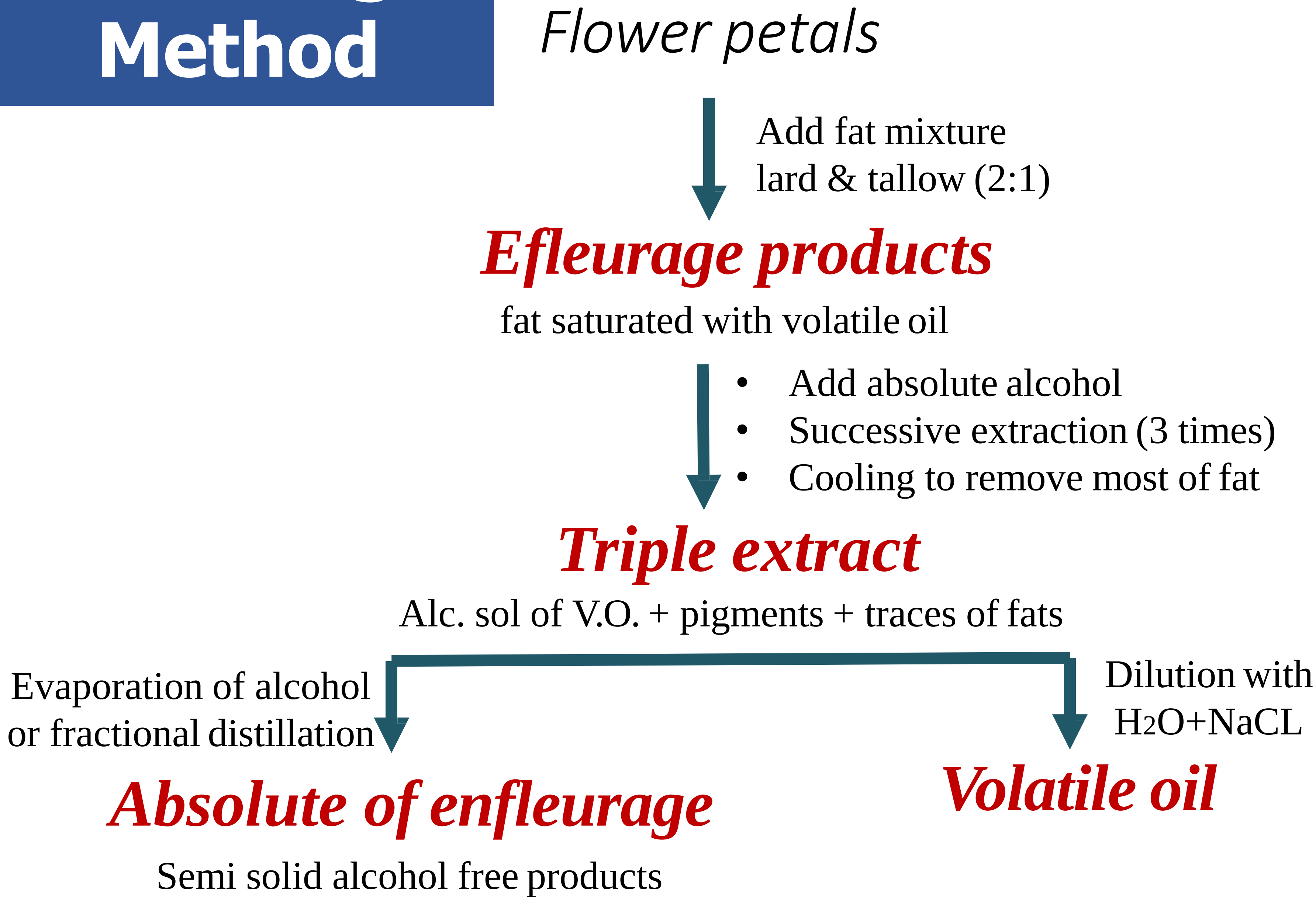




Lard: Tallow 2:1

- A mixture of melted lard is thickly smeared on both surfaces of each of the glass plates which are enclosed in a wooden frame.
- Each glass plate is sprinkled with flowers to cover its top.
- The plates are left for 24 hours.
- The flowers are then removed and replaced by a fresh supply.
- This is repeated until after some weeks, the fats becomes saturated with essential oil of the flowers.

Enfleurage Method



Flower petals

Add fat mixture
lard & tallow (2:1)

Efleurage products

fat saturated with volatile oil

- Add absolute alcohol
- Successive extraction (3 times)
- Cooling to remove most of fat

Triple extract

Alc. sol of V.O. + pigments + traces of fats

Evaporation of alcohol
or fractional distillation

Absolute of enfleurage

Semi solid alcohol free products

Dilution with
H_2O+NaCL

Volatile oil

Areej H. Musallam, MPharm

Pneumatic Method

- This process is similar in principle to the enfleurage method and involves the passage of a current of warm air through the flowers. The air laden with suspended volatile oil is then passed through melted fat in which the volatile is dissolved and absorbed.
- This process is applied to violets, roses, orange blossoms, lilies of the valley, etc, whereas jasmine, tuberose, and jonquil are not thus treated because of their small yield.

Maceration Method

- The flowers are gently heated in melted fat (lard or fixed oil) being incorporated and rotated in the fluid until exhausted, then they are strained out, squeezed and the exuded fluid is returned to the fat and so on until a special concentration is reached.

	Distillation	Scarification & Expression	Extraction
Applications	For dried & fresh material, rich in volatile oils with thermostable constituents	For preparation of oils present in large amounts in outer peels of fruits & rich in heat-sensitive constituents.	Suitable for fresh material with heat-sensitive oils present in small amounts
Advantages	Cheapest method (apparatus, solvent & source of heat)	•Carried at room temperature •Yields oils with more natural odors.	•Carried at room or low temperature •Yields oils with more natural odors
Disadvantages	High temperature & presence of water may affect the constituents.	Expensive due to need of high number of workers	Expensive due to use of solvent &/or high number of workers.

Enzymatic Hydrolysis Method

- Enzymatic hydrolysis is a process in which enzymes facilitate the cleavage of bonds in molecules with the addition of the elements of water and plays an important role in the human system for the digestion of food.
- This method is useful for specific oils, where the volatile components are present in a glucosidal combination in plants.
- Specific enzymes hydrolyze specific glycosides to release the volatile components.
- Later, steam distillation methods used to obtain volatile components.

Preparation of Oil After Enzymatic Hydrolysis

Plant name	**Non- volatile Glycoside**	**Volatile Glycoside**	**Other Hydrolytic Products**	**Hydrolytic Enzyme**
Gaultheria procumbens	Gaultherin &/or Monotropin	Methyl salicylate	Xylose + Glucose	Gaultherase
Geum urbanum	Gein	Eugenol	Glucose	β- Glucosidase
Brassica nigra	Sinigrin	Allyl isothiocyanate	Glucose + Potassium acid sulfate	Myrosin
Vanilla planifolia	Glucovanillin	Vanillin	Glucose	β- Glucosidase
Amygdala amara	Amygdalin	Benzaldehyde	Gentiobiose (2 glucose units) + HCN	Emulsin