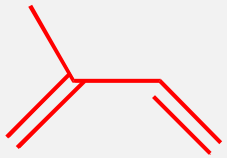
# Terpenos

# Terpenos e Terpenóides

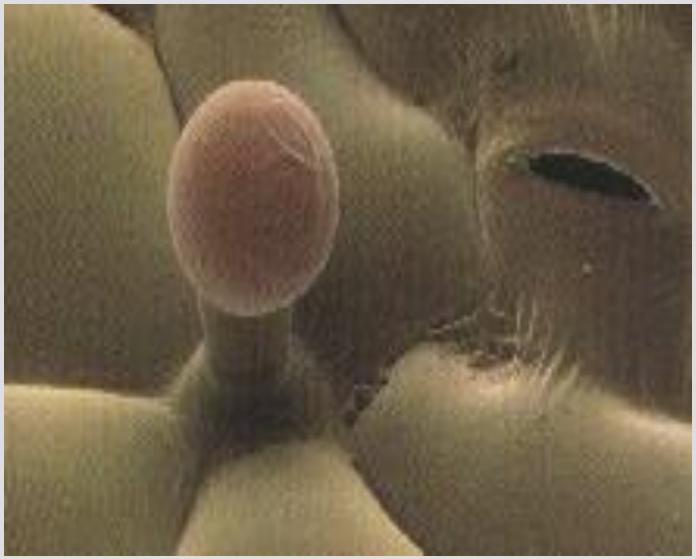
Regra do isopreno - Wallach (1887)

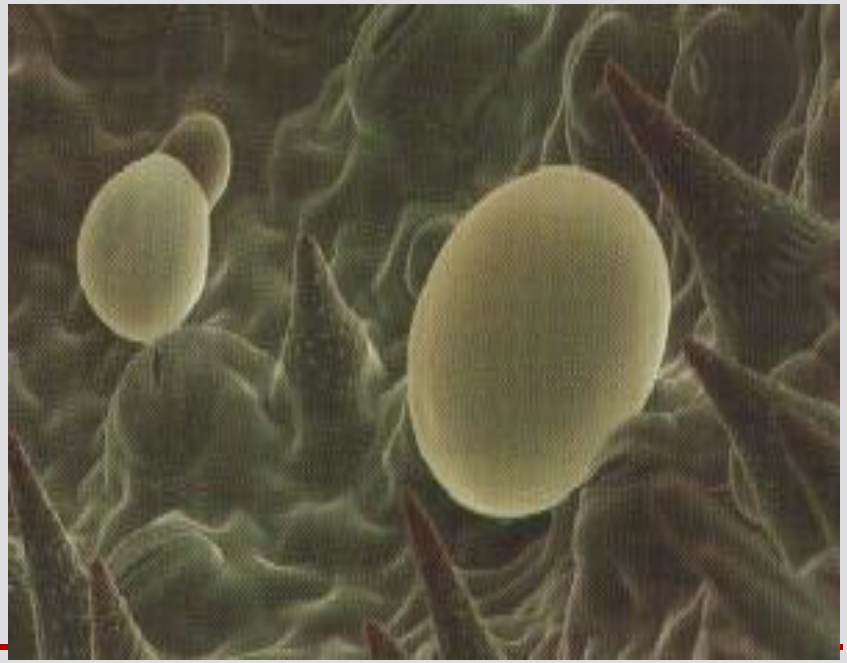


2-metilbuta-1,3-dieno
(isopreno)

**O nome terpeno deriva da terebentina (turpentine) de onde foram isolados a cânfora e o α-pineno. As estruturas foram elucidadas em torno de 1894.**

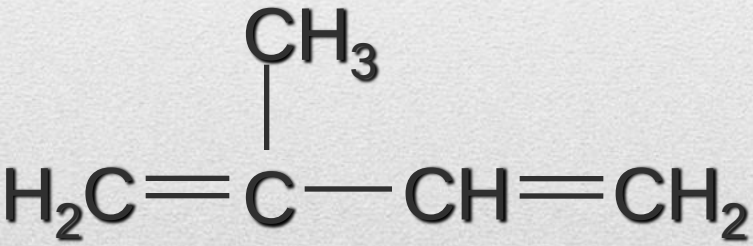
- ↑ Secretory glands ↑ oil yield



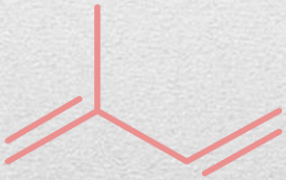


# Terpenes

- Terpenes are natural products that are structurally related to isoprene.



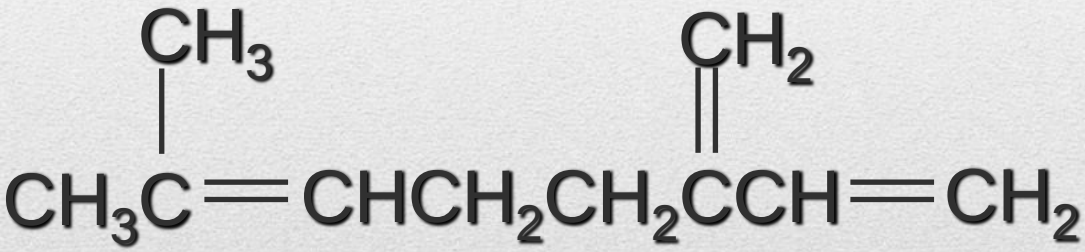

or



Isoprene
(2-methyl-1,3-butadiene)

# Terpenes

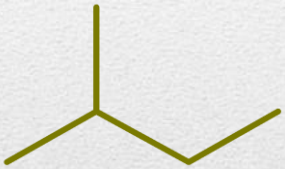
- *Myrcene* (isolated from oil of bayberry) is a typical terpene.



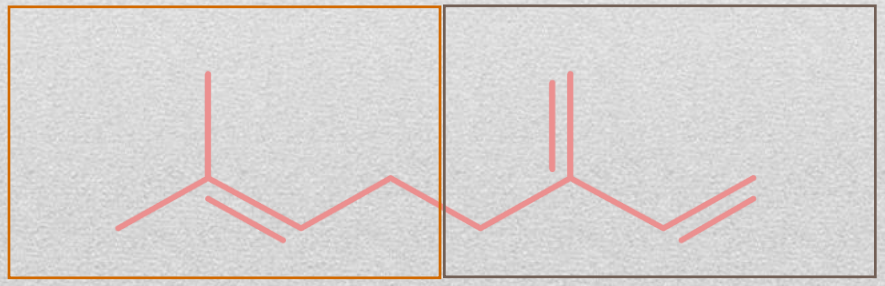

or

# The Isoprene Unit

- An isoprene unit is the carbon skeleton of isoprene (ignoring the double bonds)

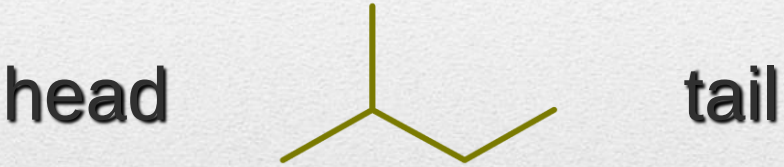


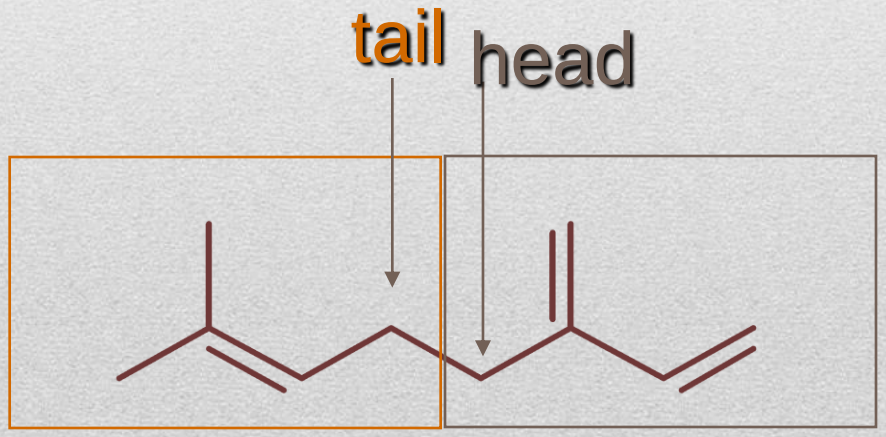
**Myrcene contains two isoprene units.**

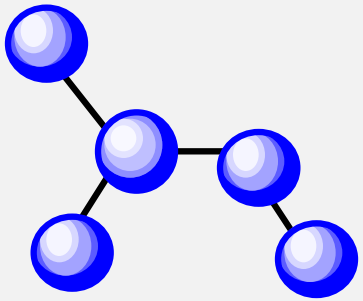


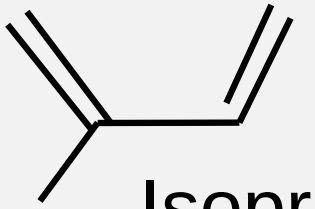
# The Isoprene Unit

- The isoprene units of myrcene are joined "head-to-tail."

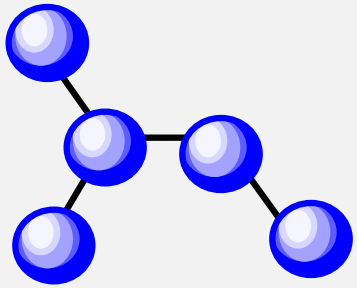


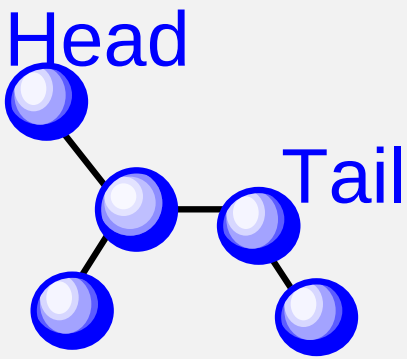



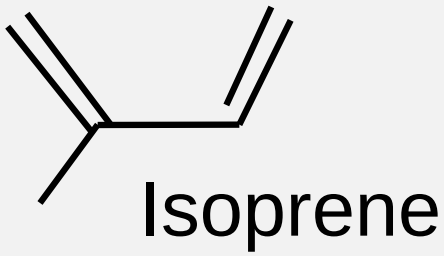


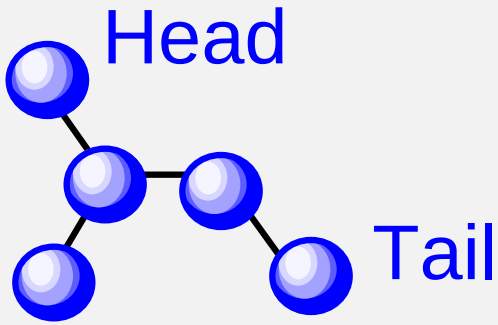


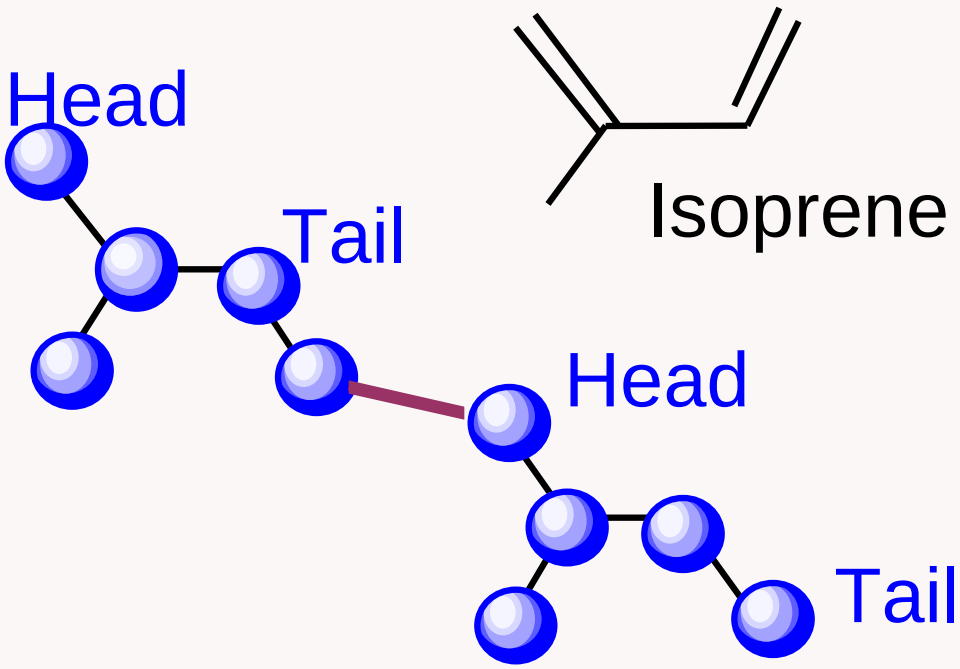
Isoprene




Head
Tail


Isoprene


Head
Tail


Head
Tail
Head
Tail


Isoprene

# Classificação dos Terpenos

| Classe | Número de átomos de Carbono |
|---|---|
| Monoterpeno | 10 |
| Sesquiterpeno | 15 |
| Diterpeno | 20 |
| Sesterpeno | 25 |
| Triterpeno | 30 |
| Tetraterpeno | 40 |

# Function of the Isoprenoid Compounds

- **C10 terpenoids (monoterpenes)**
  **components of volatile essences, essential oils**

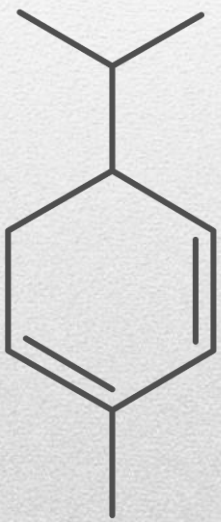
- **C15 (sesquiterpenes)**
  **components of essential oils, phytoalexins (self defense)**

- **C20 (diterpenes)**
  **gibberellins, resin acids, phytol (chlorophyll side chain)**

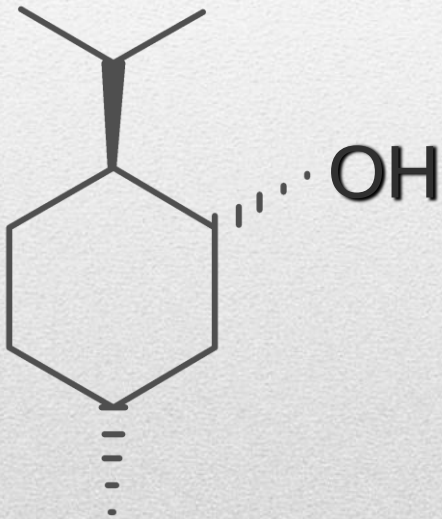
- **C30 (triterpenes)**
  **phytosterols, brassinosteroids**

- **C40 (tetraterpenes)**
  **carotenoids**

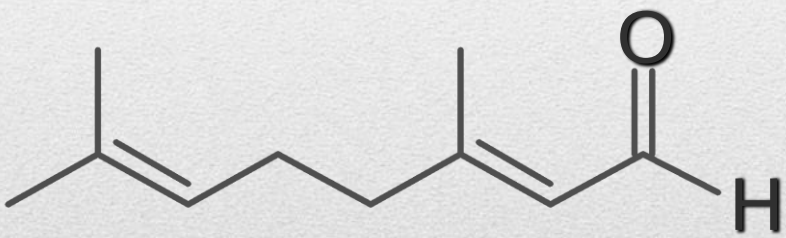
# Representative Monoterpenes



α-Phellandrene
(eucalyptus)

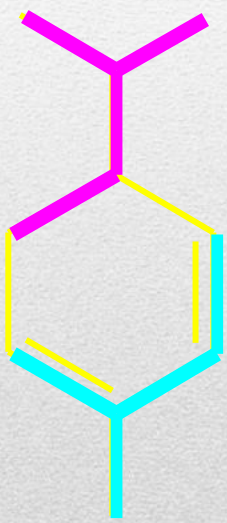


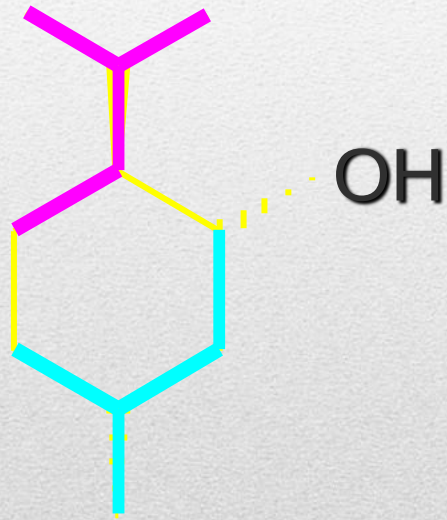

Menthol
(peppermint)

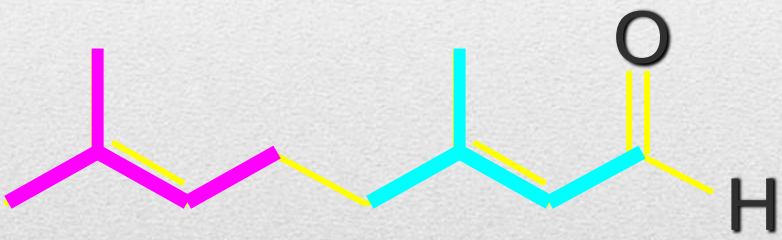



Citral
(lemon grass)

# Representative Monoterpenes






α-Phellandrene (eucalyptus)

Menthol (peppermint)

Citral (lemon grass)

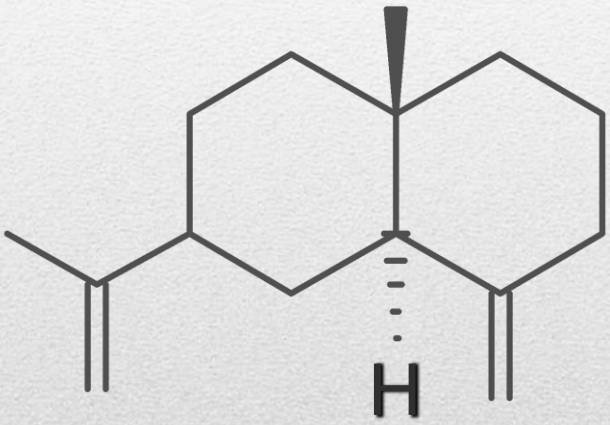
# Representative Monoterpenes

α-Phellandrene
(eucalyptus)

Menthol
(peppermint)

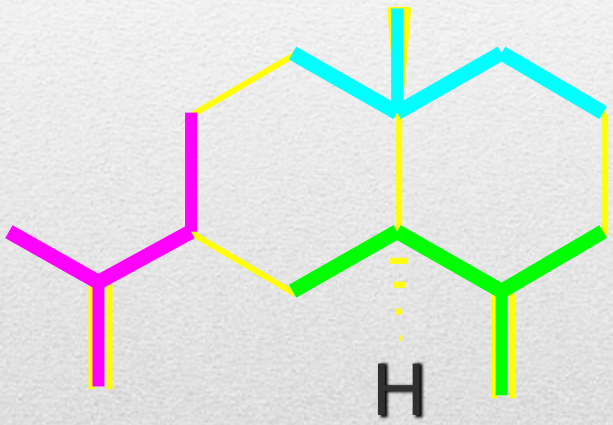
Citral
(lemon grass)

# Representative Sesquiterpenes



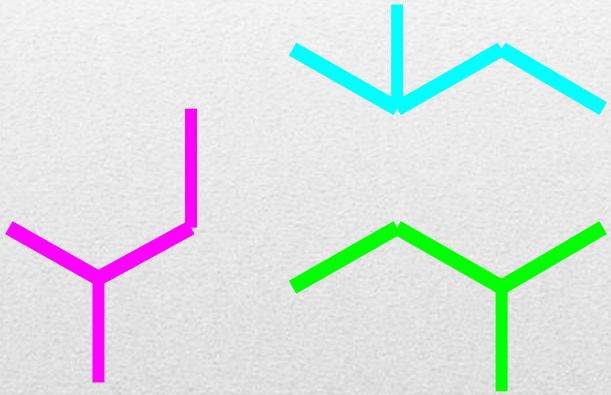

α-Selinene
(celery)

# Representative Sesquiterpenes



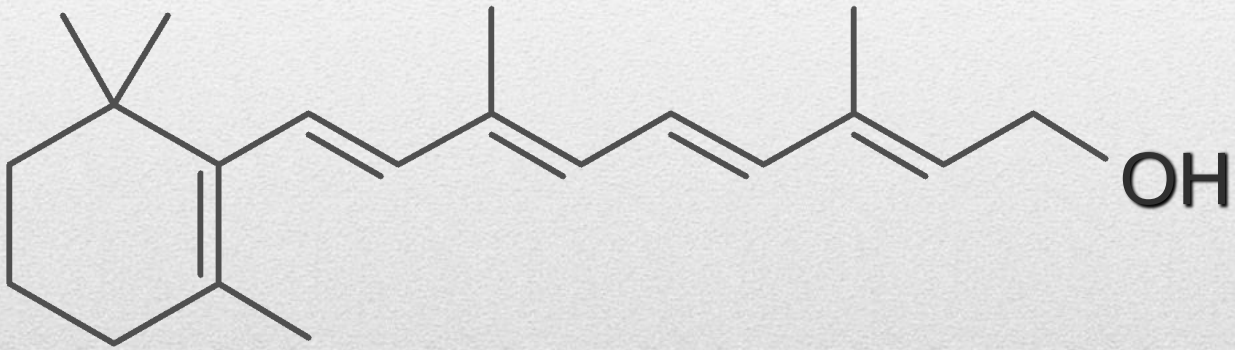

α-Selinene
(celery)

# Representative Sesquiterpenes



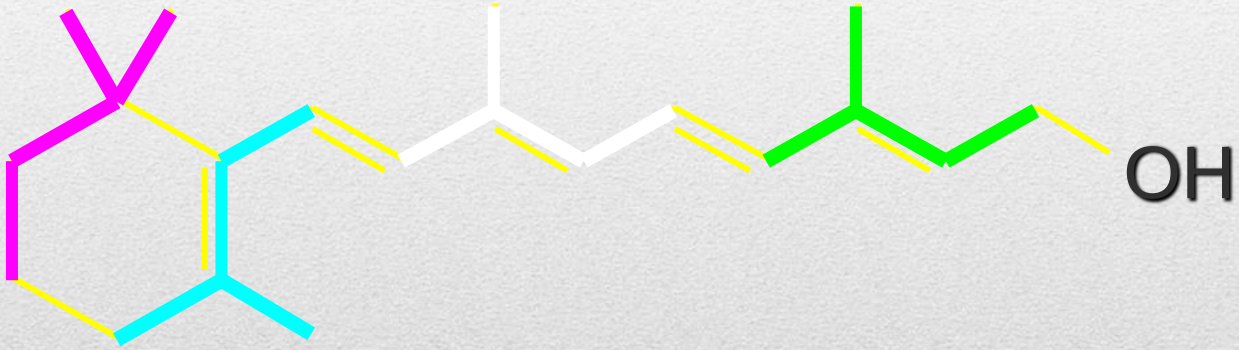
α-Selinene
(celery)

# Representative Diterpenes



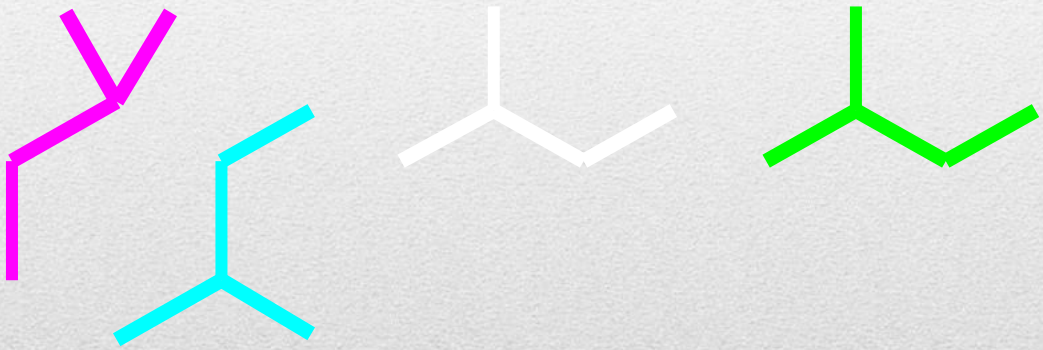

Vitamin A

# Representative Diterpenes




Vitamin A

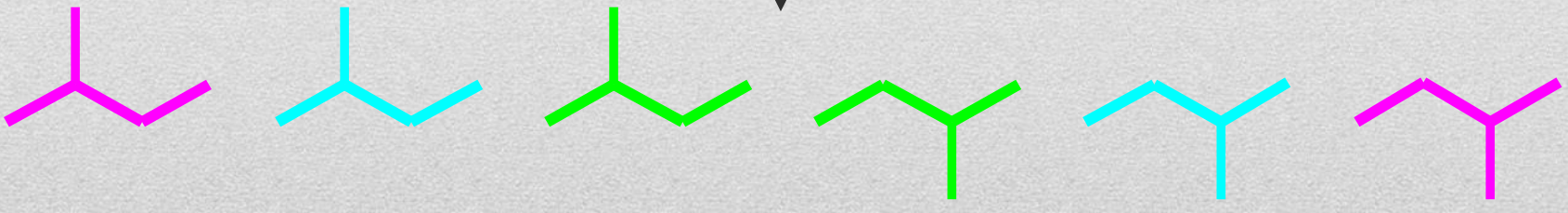
# Representative Diterpenes



Vitamin A

# Representative Triterpene

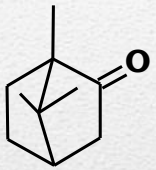
tail-to-tail linkage of isoprene units



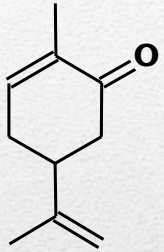
Squalene
(shark liver oil)

# Mono- e sesquiterpenos: Constituintes de óleos essenciais

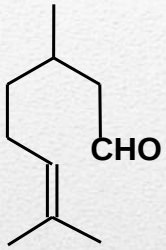
**MONOTERPENOS**



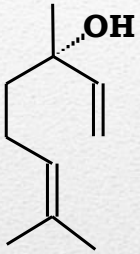

**cânfora**



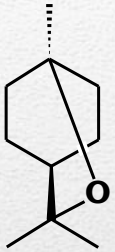

**carvona**




**citronelal**

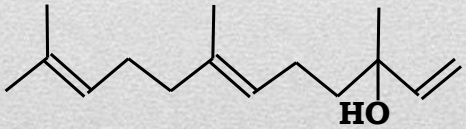



**linalol**
**(óleo de pau-rosa)**

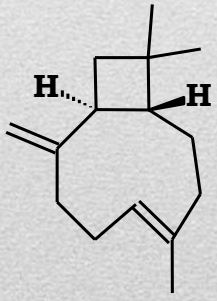



**eucaliptol**
**(1,8-cineol)**

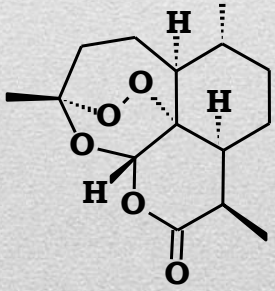
**SESQUITERPENOS**




**nerolidol**




**cariofileno**




**artemisinina**

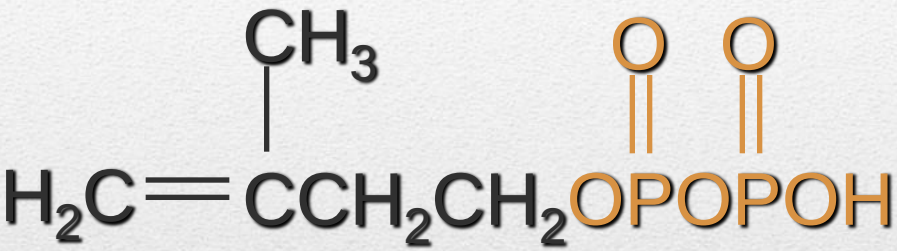
# ISOPENTENYL PYROPHOSPHATE: THE BIOLOGICAL ISOPRENE UNIT

# The Biological Isoprene Unit

- The isoprene units in terpenes ***do not*** come from isoprene.

- **They come from *isopentenyl pyrophosphate*.**

# Isopentenyl Pyrophosphate




or

Isopentenyl pyrophosphate

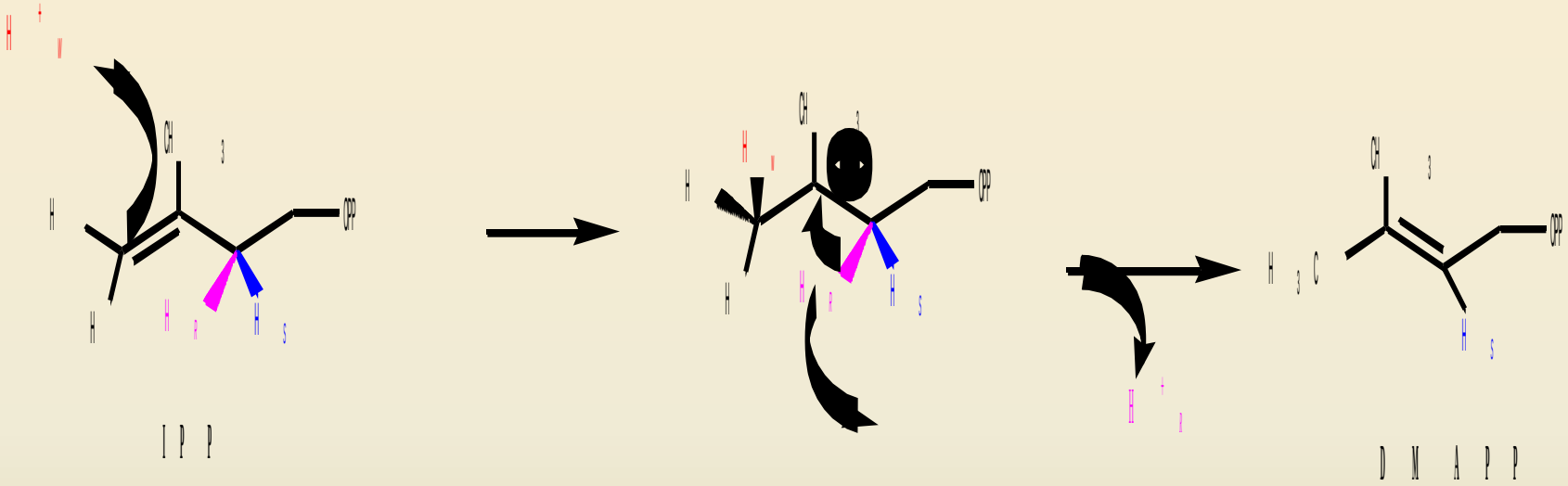
# Isopentenyl and Dimethylallyl Pyrophosphate

Isopentenyl pyrophosphate is interconvertible with 2-methylallyl pyrophosphate.

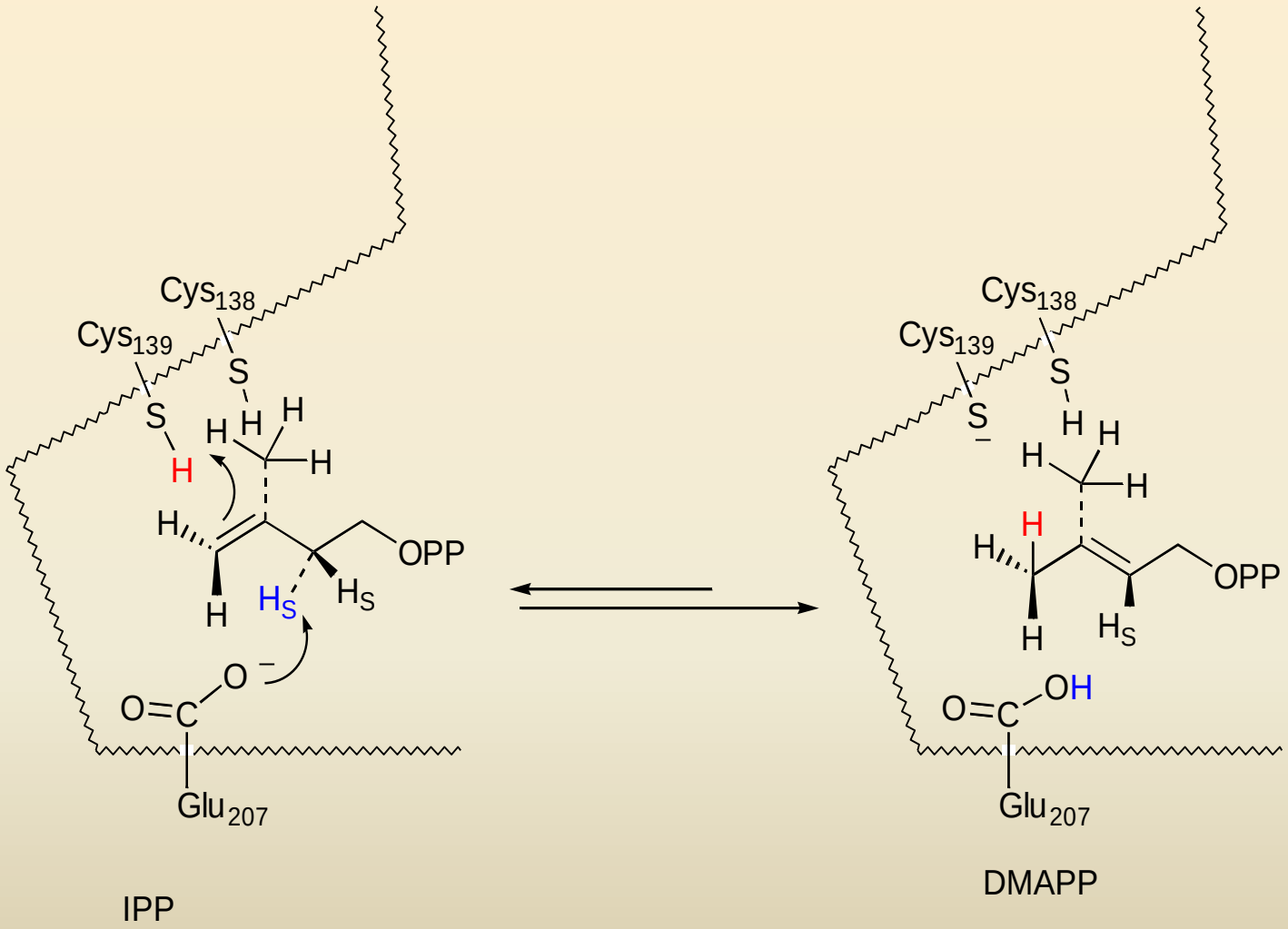
Isopentenyl pyrophosphate Dimethylallyl pyrophosphate

- Dimethylallyl pyrophosphate has a leaving group (pyrophosphate) at an allylic carbon; it is reactive toward nucleophilic substitution at this position.

# First specific step in the biosynthesis of terpenoids
## - isomerization of IPP into DMAPP-

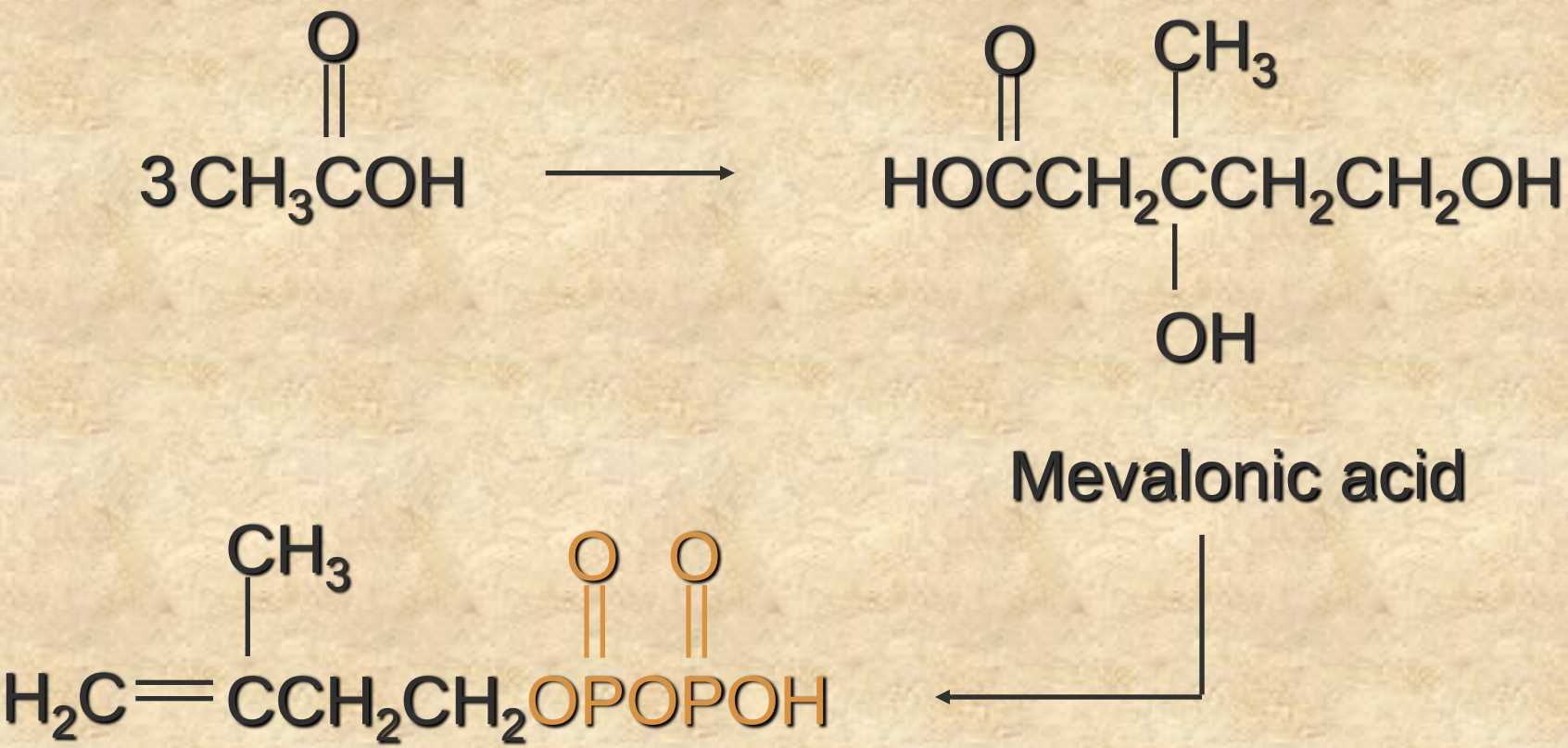


# IPP-Isomerase mechanism



# THE PATHWAY FROM ACETATE TO ISOPENTENYL PYROPHOSPHATE

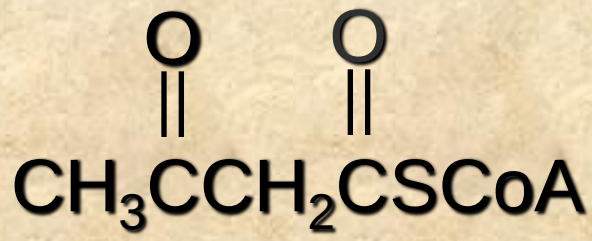
# The Biological Isoprene Unit




Isopentenyl pyrophosphate

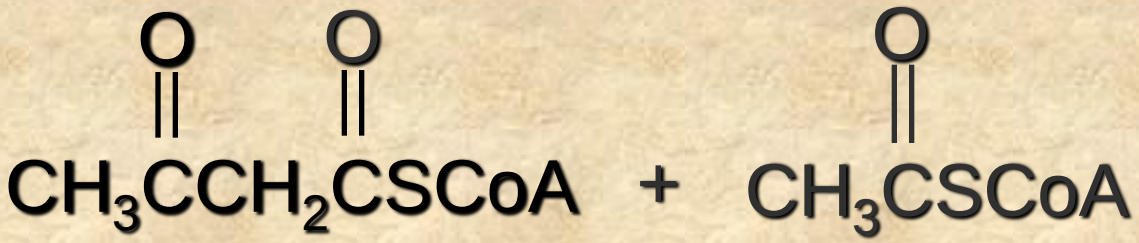
# Biosynthesis of Mevalonic Acid

- In a sequence analogous to the early steps of fatty acid biosynthesis, acetyl coenzyme A is converted to *S*-acetoacetyl coenzyme A.



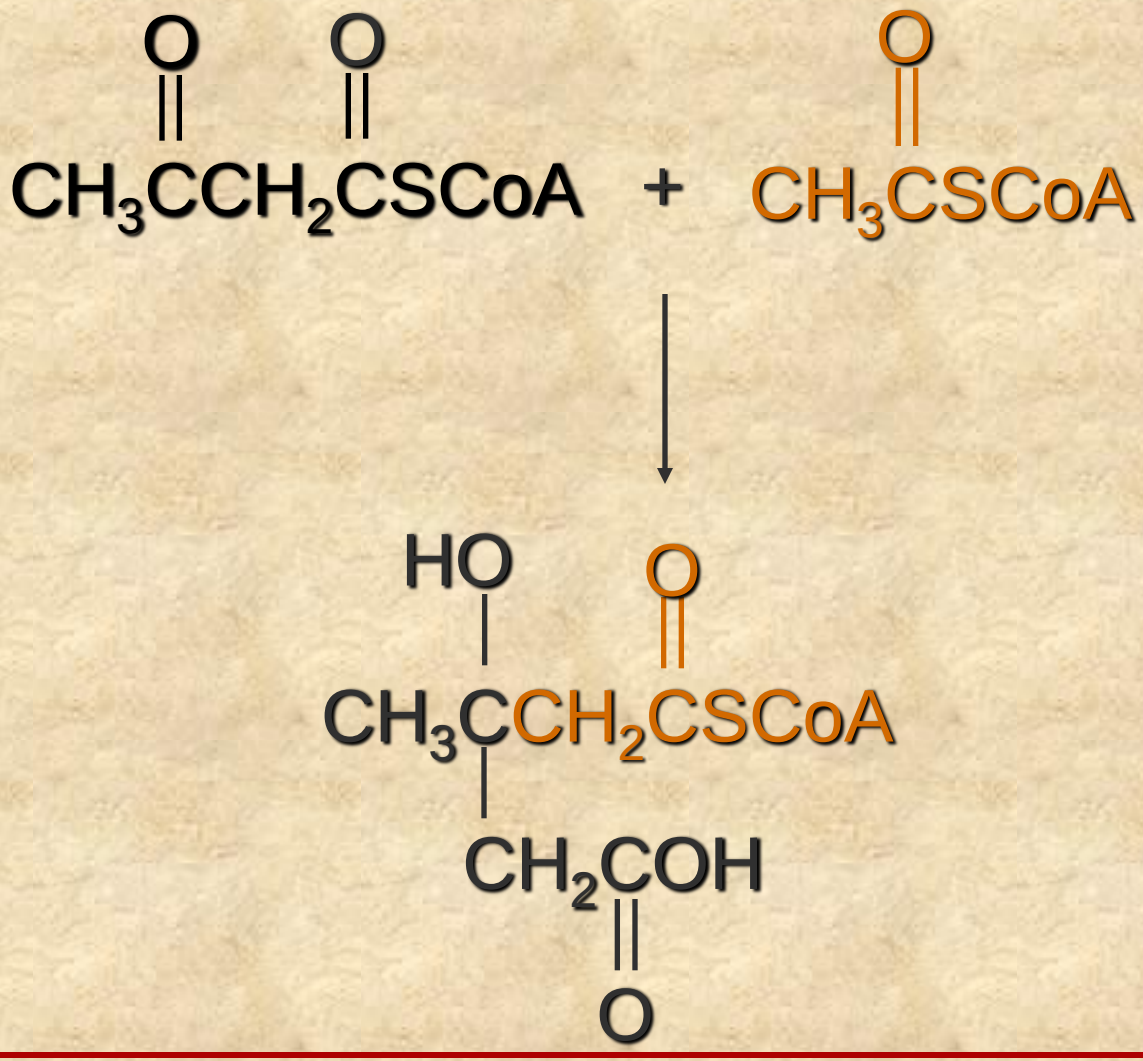

*S*-Acetoacetyl coenzyme A

# Biosynthesis of Mevalonic Acid



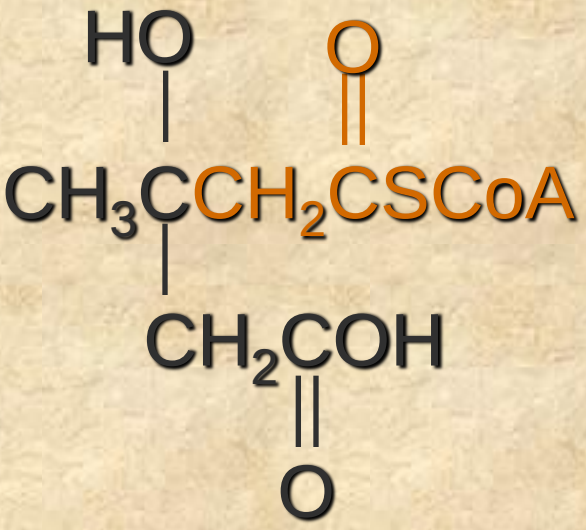

- In the next step, S-acetoacetyl coenzyme A reacts with acetyl coenzyme A.
- Nucleophilic addition of acetyl coenzyme A (probably via its enol) to the ketone carbonyl of *S*-acetoacetyl coenzyme A occurs.

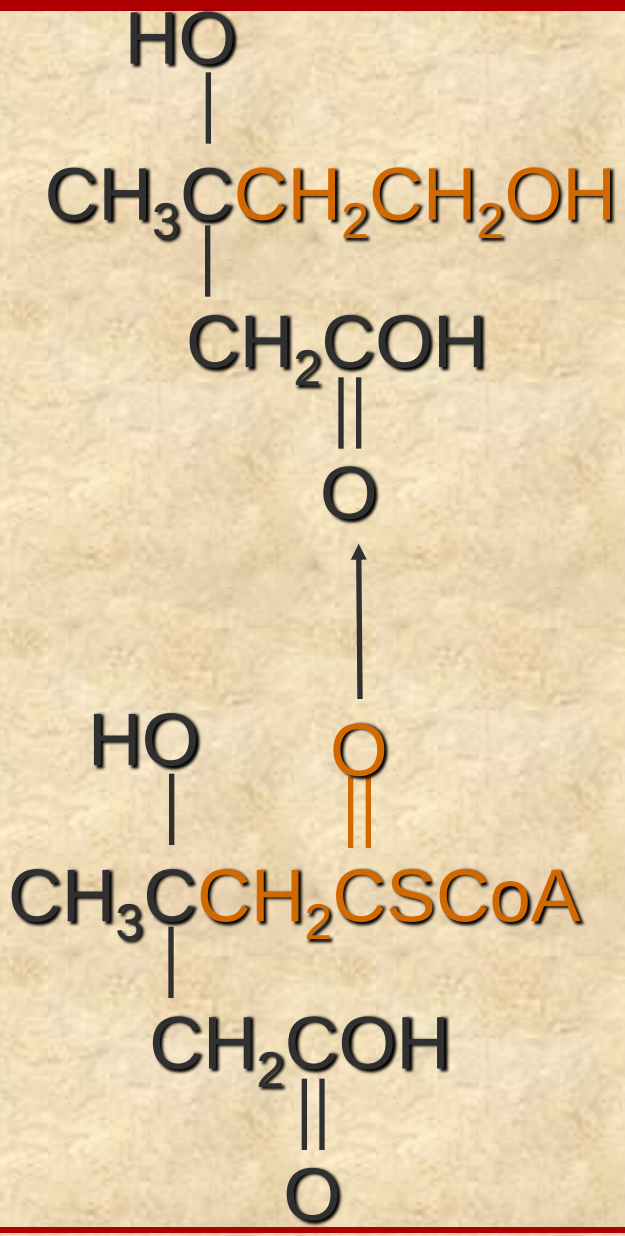
# Biosynthesis of Mevalonic Acid



# Biosynthesis of Mevalonic Acid

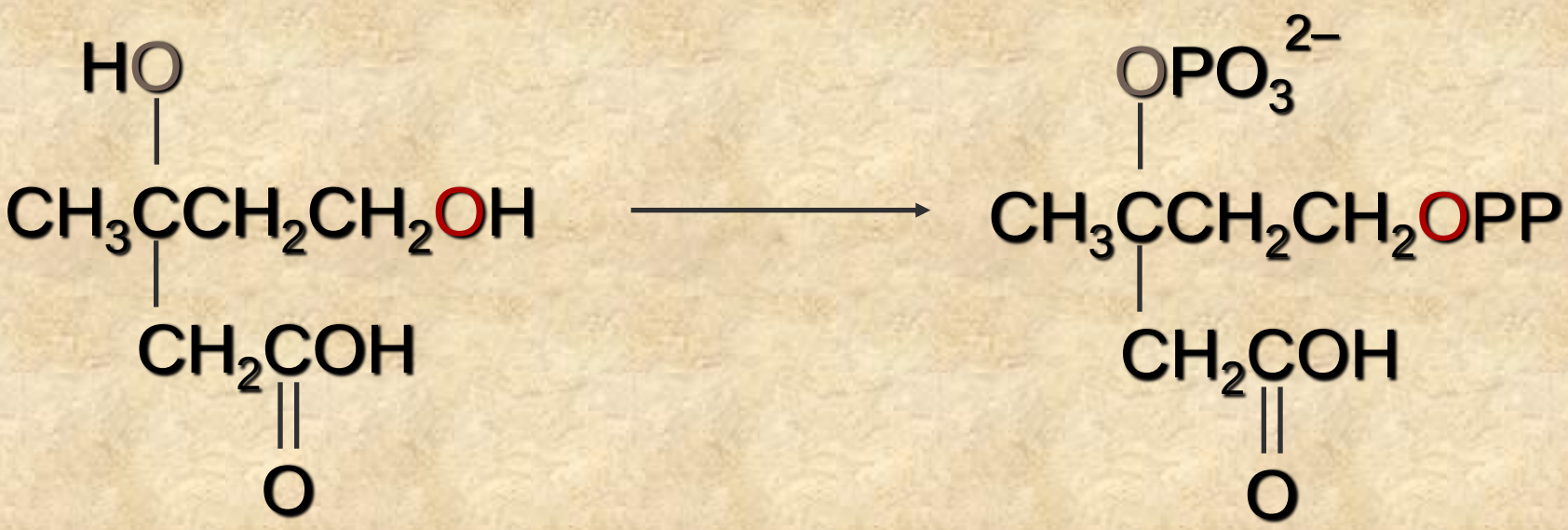
- Next, the acyl coenzyme A function is reduced.
- The product of this reduction is mevalonic acid.





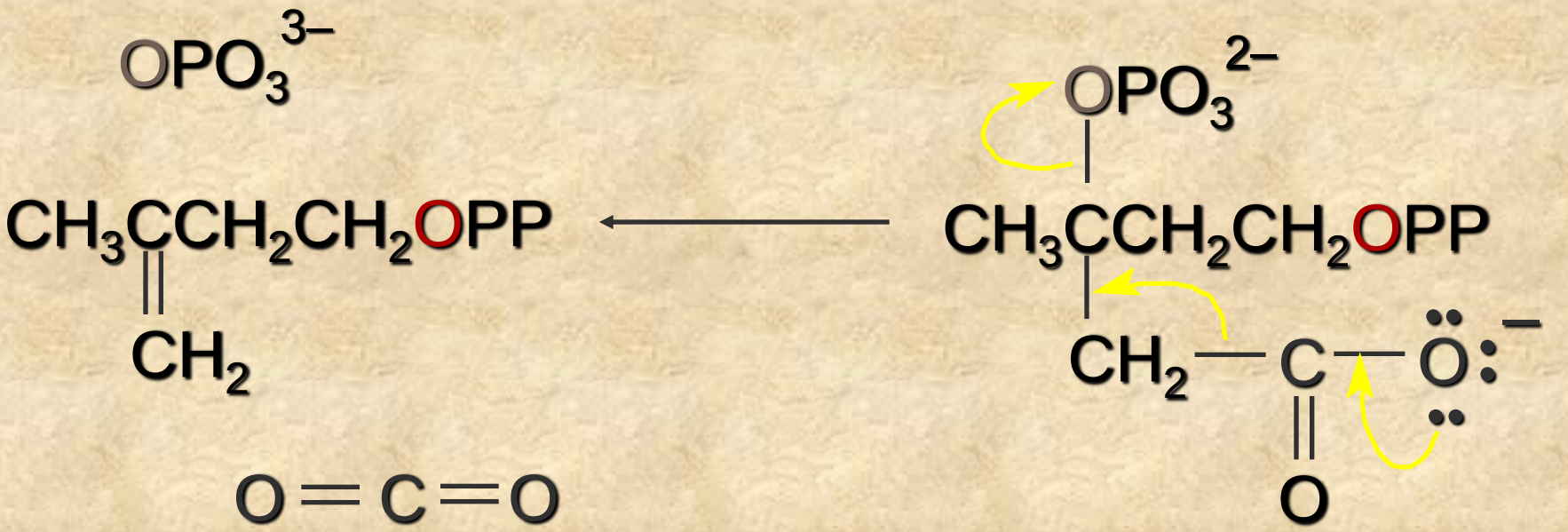

Mevalonic acid

# Conversion of Mevalonic Acid to Isopentenyl Pyrophosphate



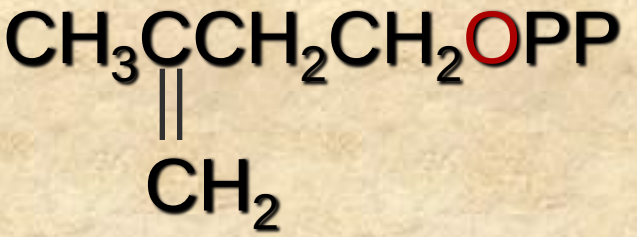

- The two hydroxyl groups of mevalonic acid undergo phosphorylation.

# Conversion of Mevalonic Acid to Isopentenyl Pyrophosphate



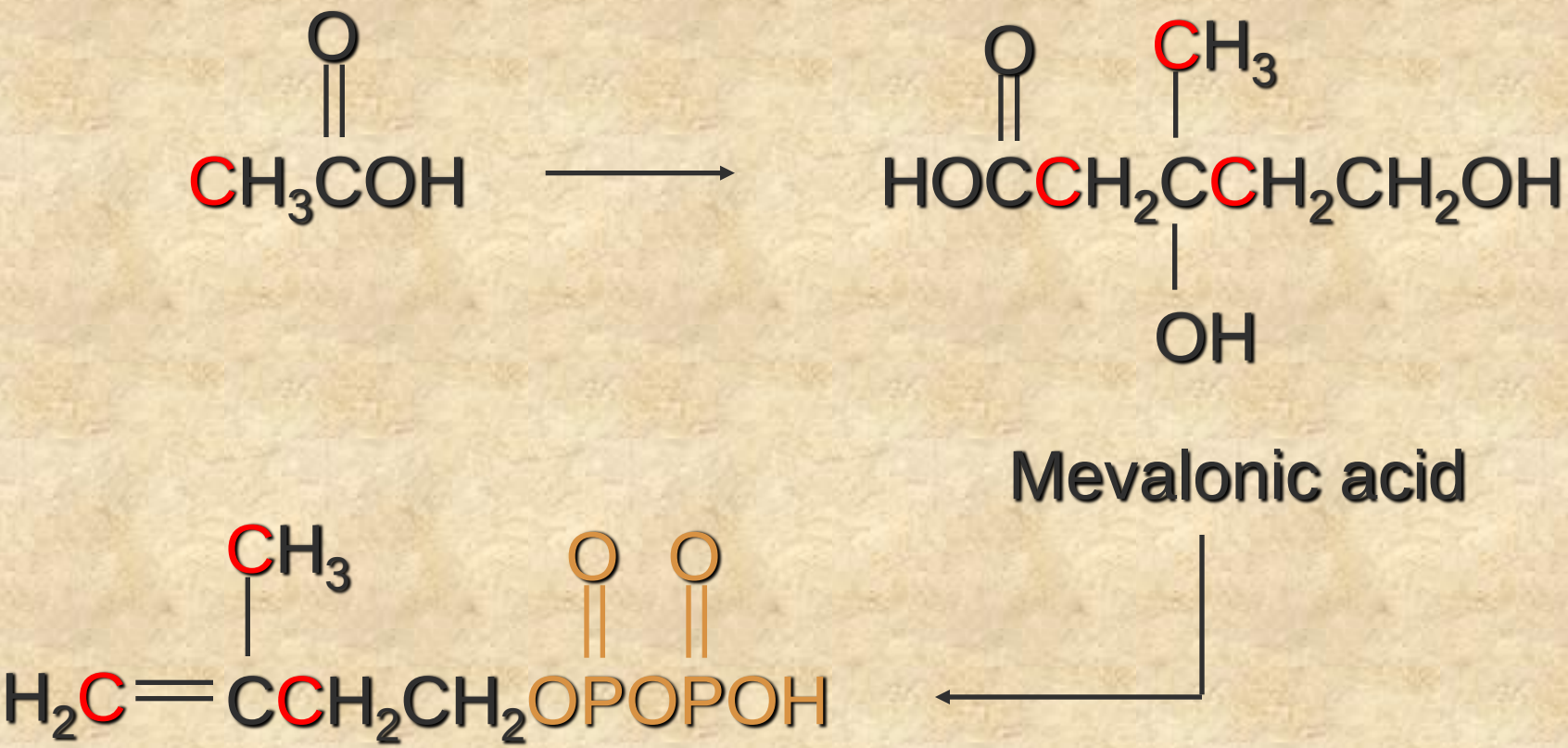

- Phosphorylation is followed by a novel elimination involving loss of $CO_2$ and $PO_4^{3-}$.

# Conversion of Mevalonic Acid to Isopentenyl Pyrophosphate



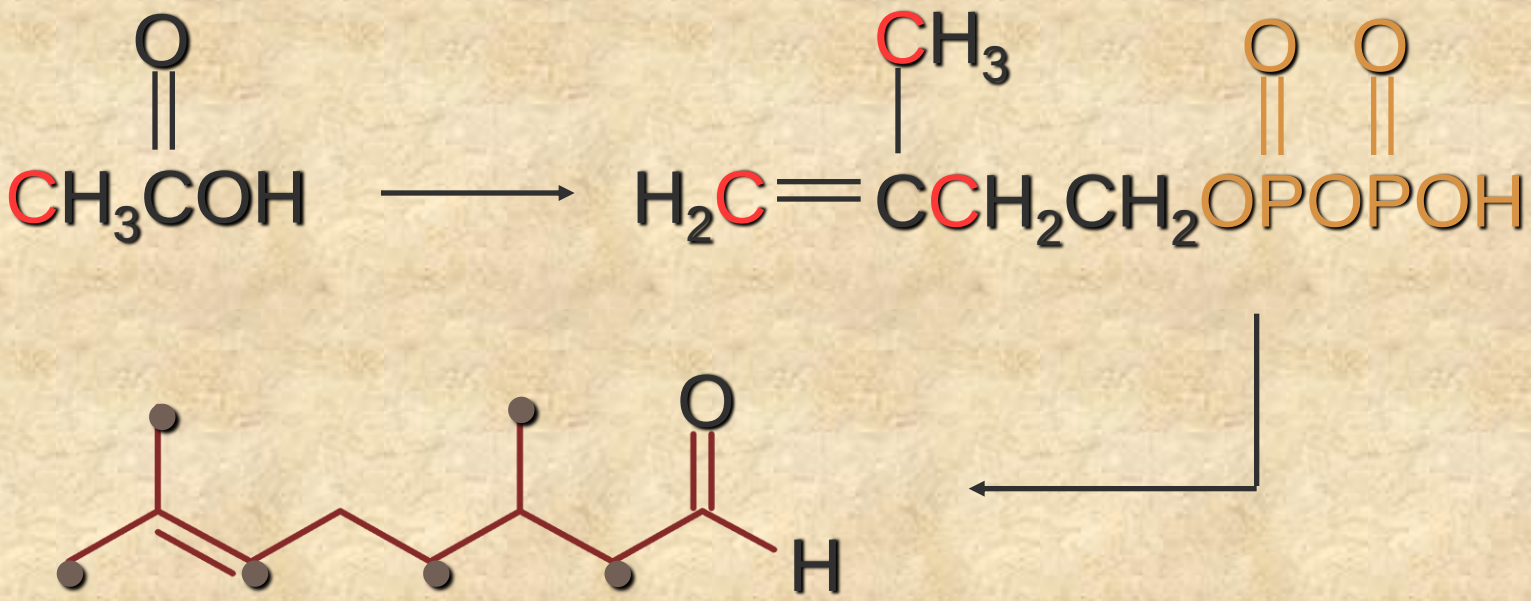

- The product of this elimination is isopentenyl pyrophosphate.

# Biosynthetic pathway is based on experiments with $^{14}C$-labeled acetate

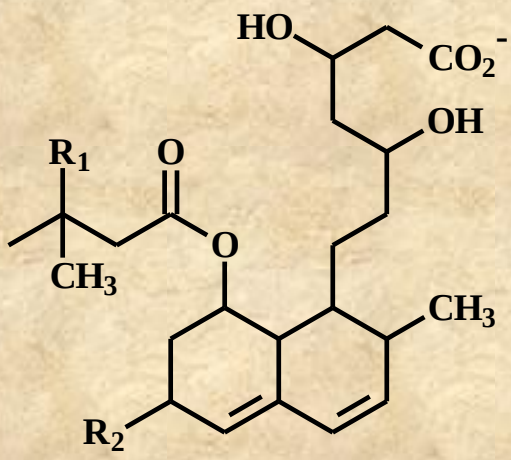


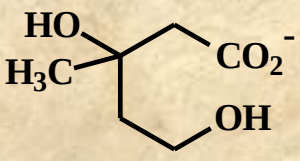
# Biosynthetic pathway is based on experiments with $^{14}C$-labeled acetate

- Citronellal biosynthesized using $^{14}C$-labeled acetate as the carbon source had the labeled carbons in the positions indicated.



# Inibidores da biossíntese de colesterol (produtos que mimetizam o ácido mevalônico)




mevalonate

| | | |
|---|---|---|
| $R_1 = H$ | $R_2 = H$ | Compactin |
| $R_1 = CH_3$ | $R_2 = CH_3$ | Simvastatin (Zocov) |
| $R_1 = H$ | $R_2 = OH$ | Pravastatin (Precavol) |
| $R_1 = H$ | $R_2 = CH_3$ | Lovastatin (Mevacor) |

# Mevalonate-Independent Pathway

Rohmer & coworkers, Braunschweig (1980s)

- eubacterial sterol (hopanoids) synthesis

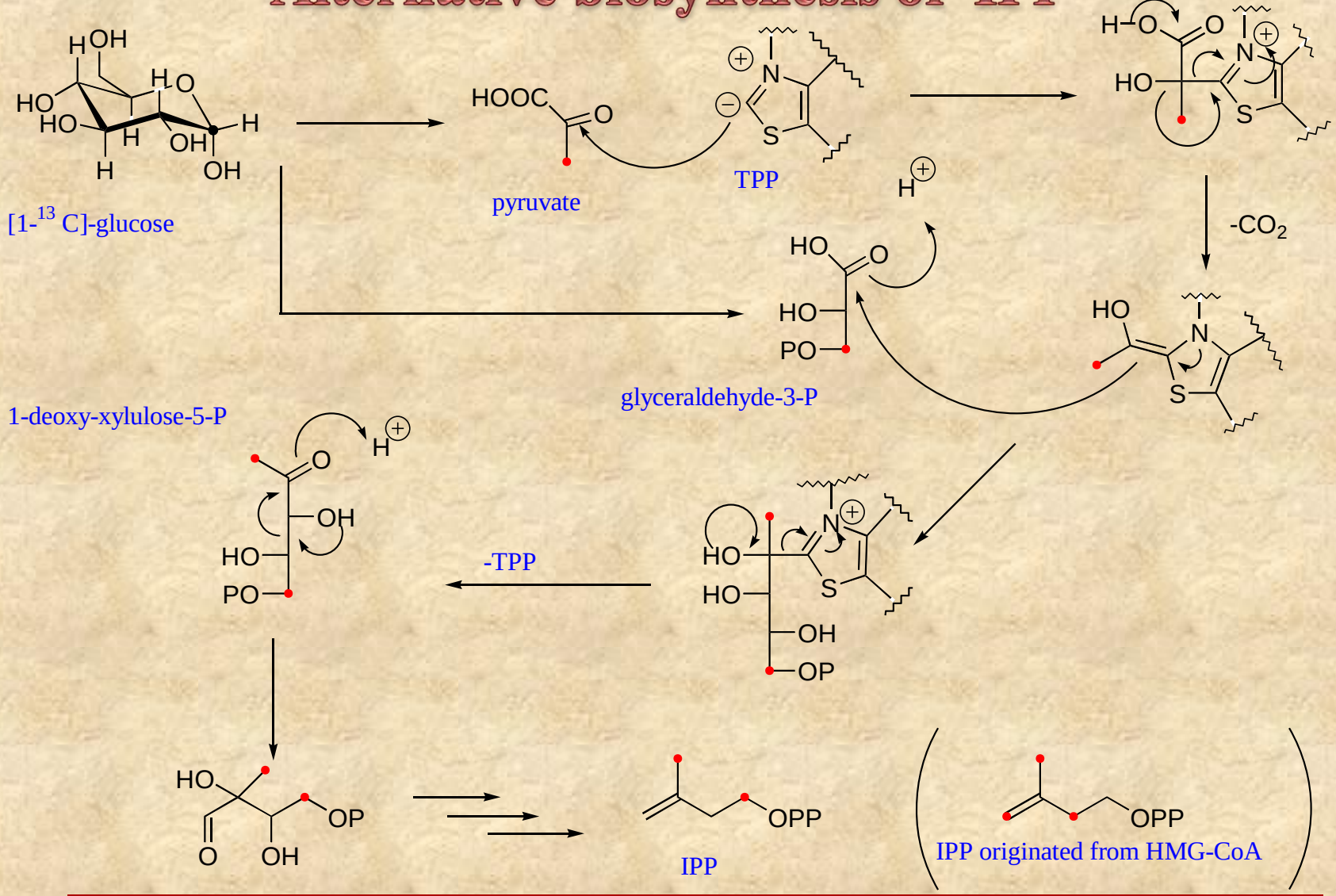
metabolic profiling with $^{13}$C-NMR

- fed [$^{13}$C]acetate and looked at label in endproducts and intermediates
- pattern did not match that of mevalonate pathway
- also mevilonin insensitive

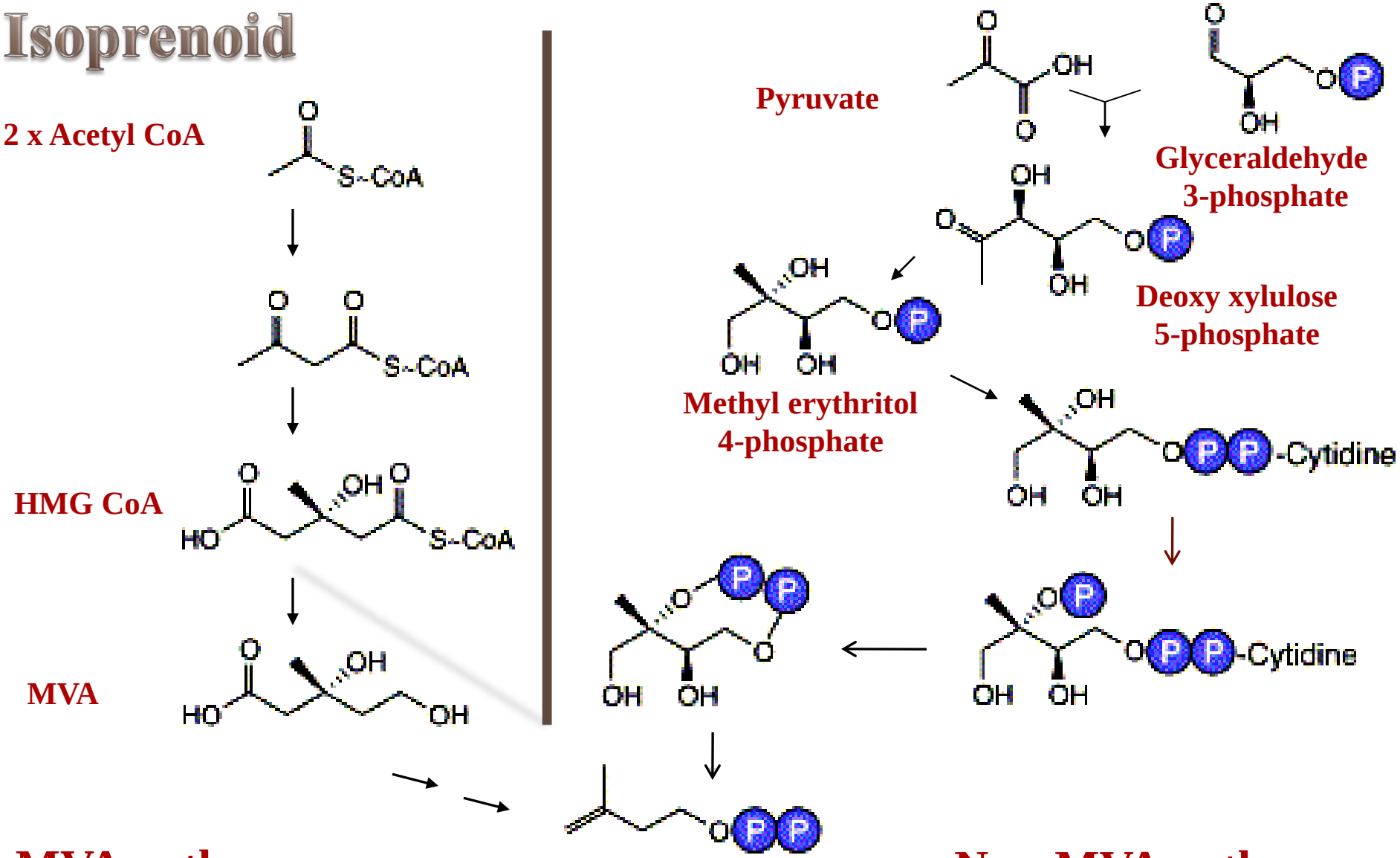
substrates

- glyceraldehyde 3P and pyruvate efficiently incorporated
- phosphorylated 5C sugar is intermediate
  - 1-deoxyxylulose-5P (DXP)
- The Non-mevalonate or DXP pathway

# Alternative biosynthesis of IPP



# Isoprenoid




**MVA pathway (cytoplasm)**

**IPP**

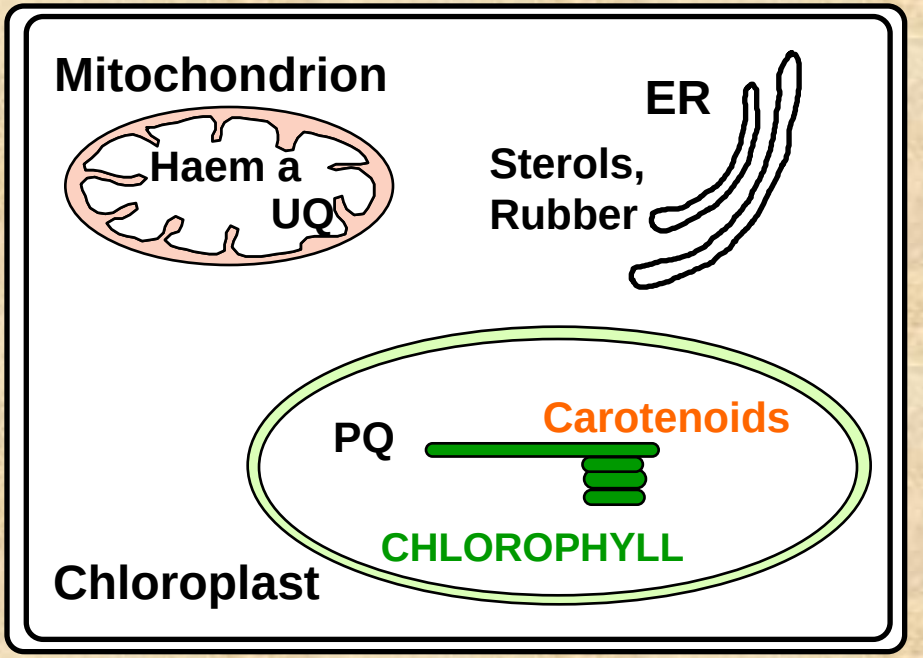
**Non-MVA pathway (plastids)**

# Sources of Isoprenoids in Higher Plants

- Higher plants like red (and brown) algae
- Cytosolic mevalonate pathway
  - **sterols ($C_{30}$), ubiquinone**
  - **certain sesquiterpenoid ($C_{15}$) phytoalexins**

- plastidic DXP pathway
  - carotenoids, phytol, PQ
  - mono- and di-terpenes ($C_{10}$ & $C_{20}$), and other sesquiterpenes

# Subcellular Location of Isoprenoid synthesis




Also

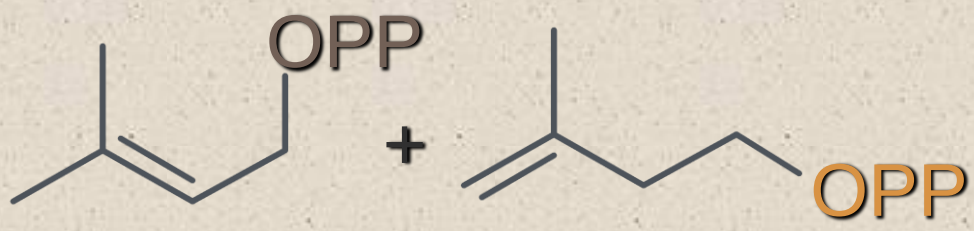
- Gibberellins, ABA, CKs
- tRNAs
- phytoalexins
- volatile oils

Feed IPP to cps, mitos or ER

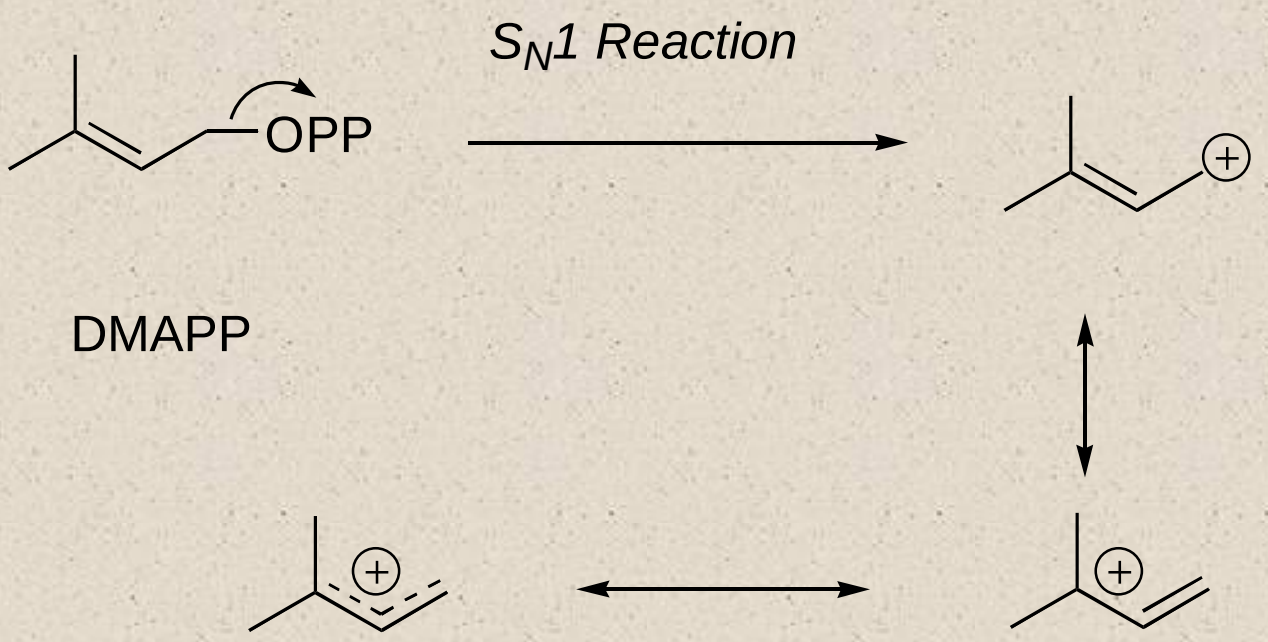
incorporated into organelle-specific isoprenoids

# CARBON-CARBON BOND FORMATION IN TERPENE BIOSYNTHESIS

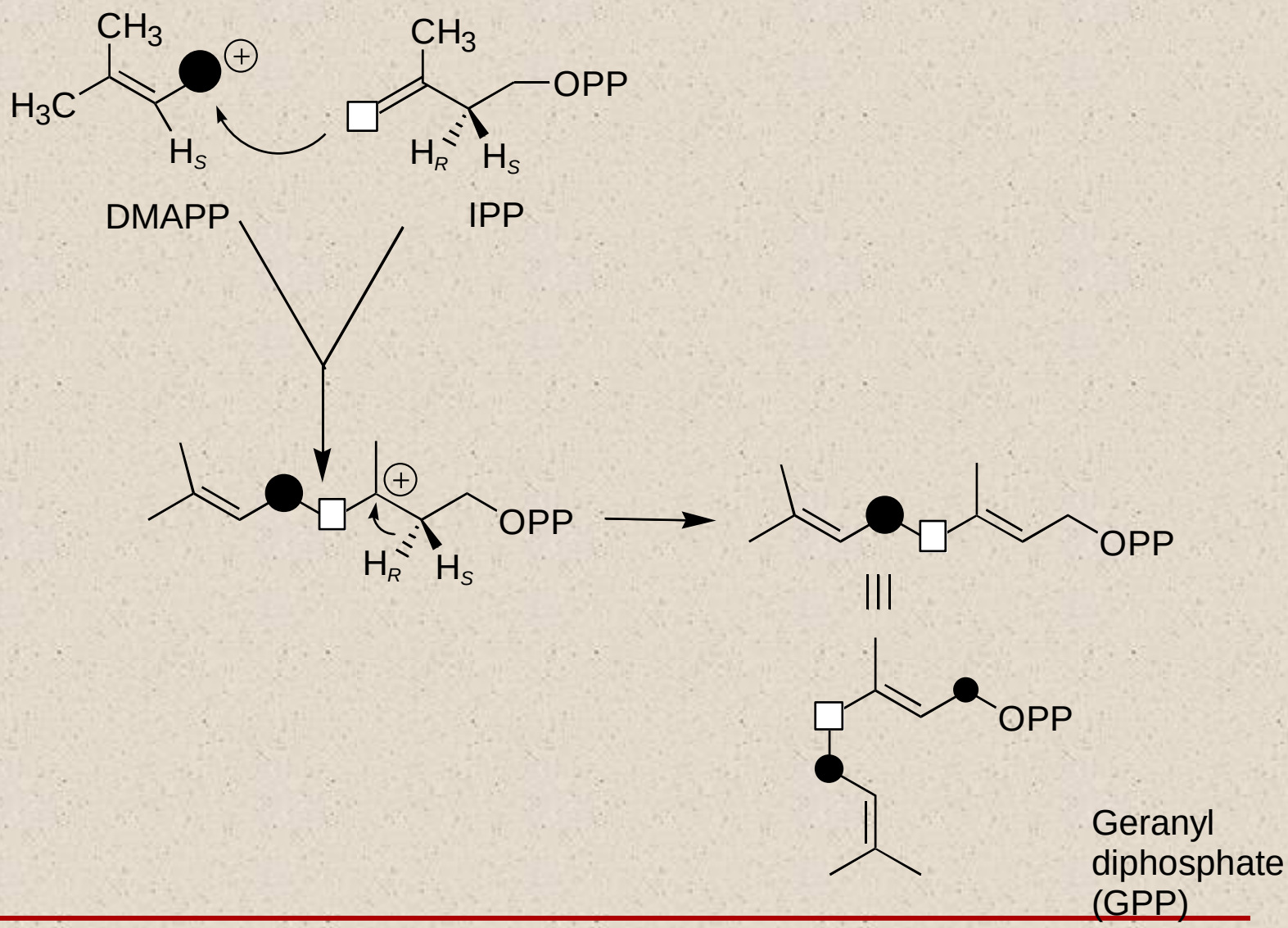
# Carbon-Carbon Bond Formation




- The key process involves the double bond of isopentenyl pyrophosphate acting as a nucleophile toward the allylic carbon of dimethylallyl pyrophosphate.

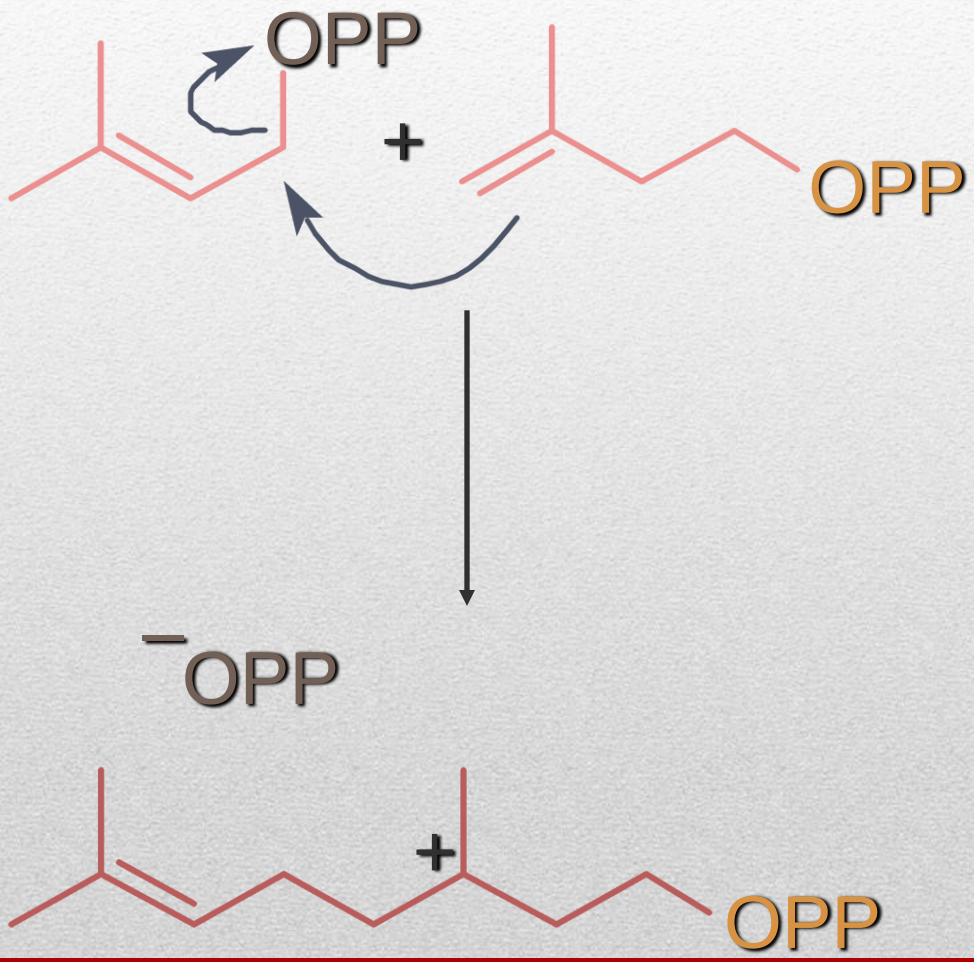

$S_N1$ Reaction
OPP
DMAPP

Head-tail condensation

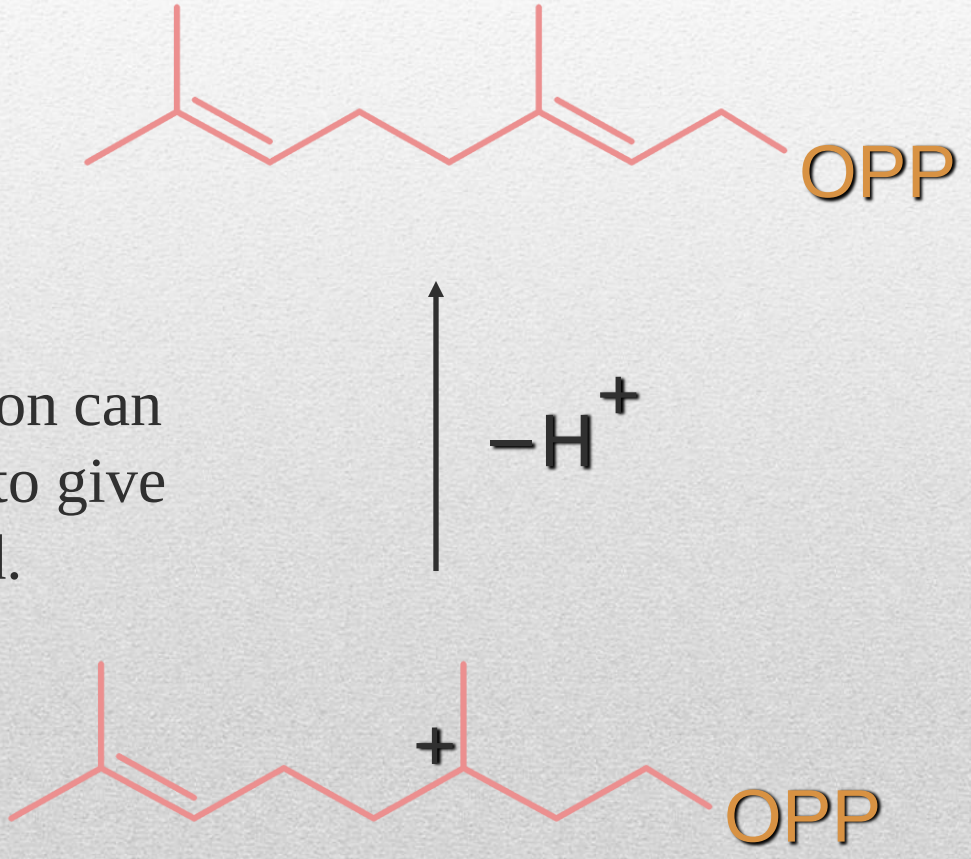


# FORMATION OF THE MONOTERPENE SKELETONS

# Carbon-Carbon Bond Formation

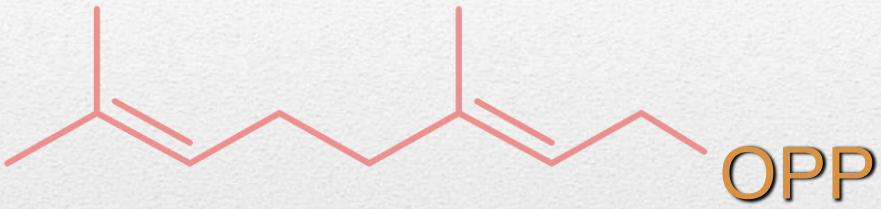


# After C—C Bond Formation…



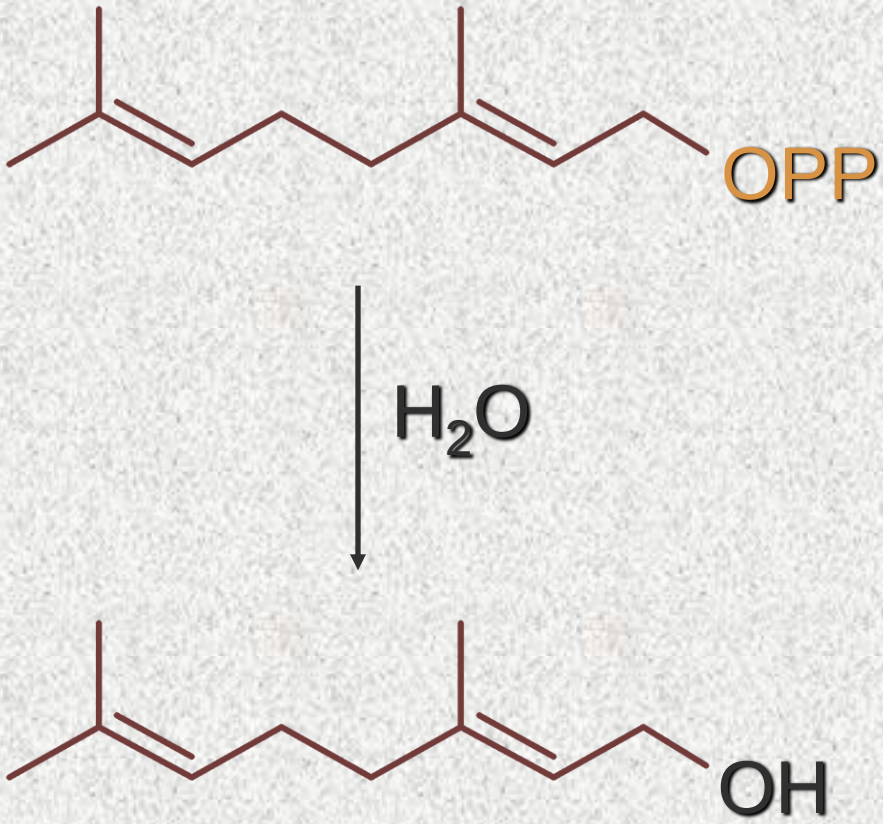

- The carbocation can lose a proton to give a double bond.

# After C—C Bond Formation…




- This compound is called geranyl pyrophosphate. It can undergo hydrolysis of its pyrophosphate to give geraniol (rose oil).

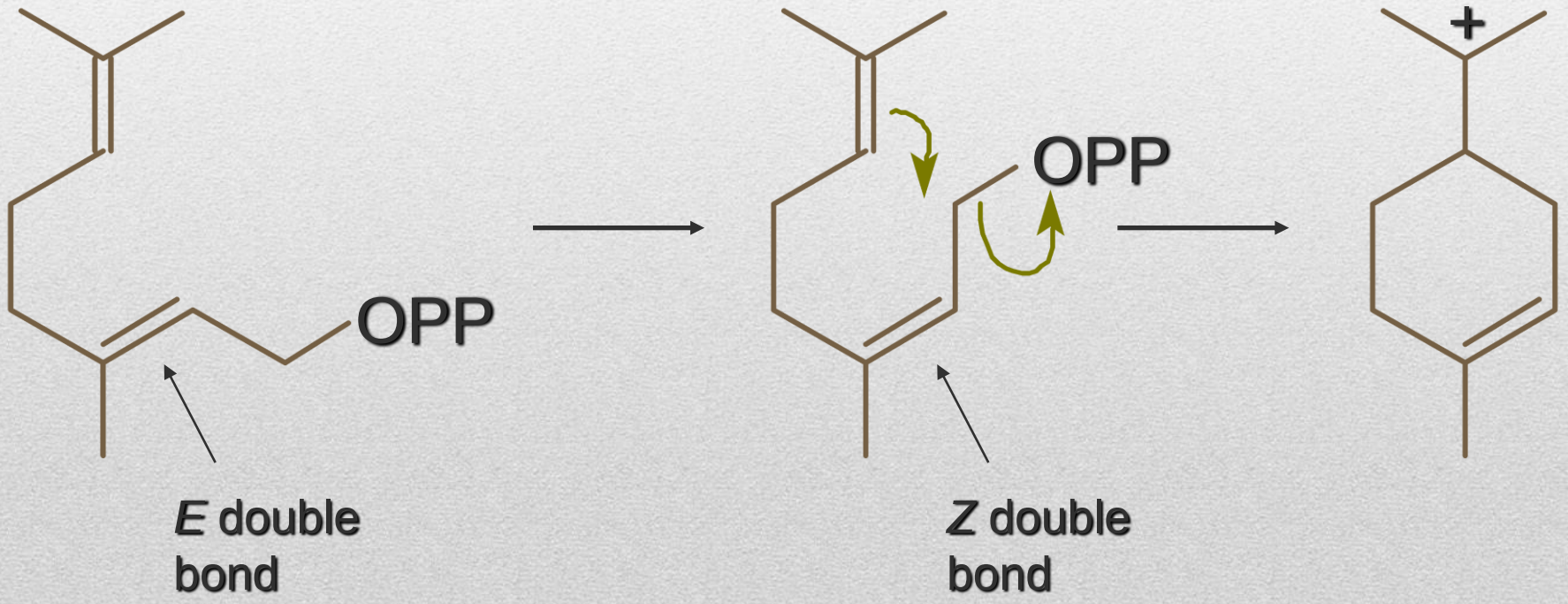
# After C—C Bond Formation…




Geraniol

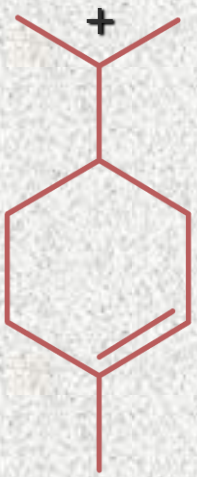
# Cyclization

- Rings form by intramolecular carbon-carbon bond formation.

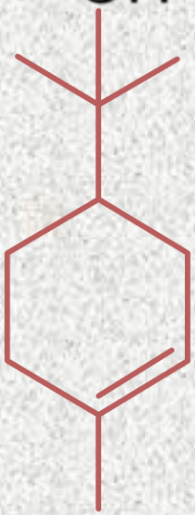


Limoneno

$-H^+$

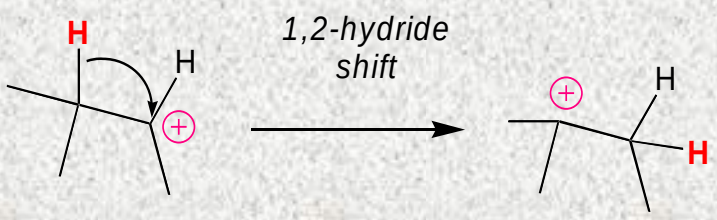


$H_2O$

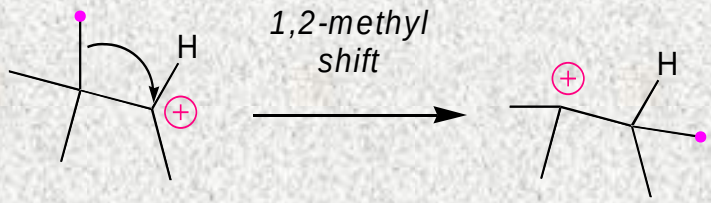


α-Terpineol

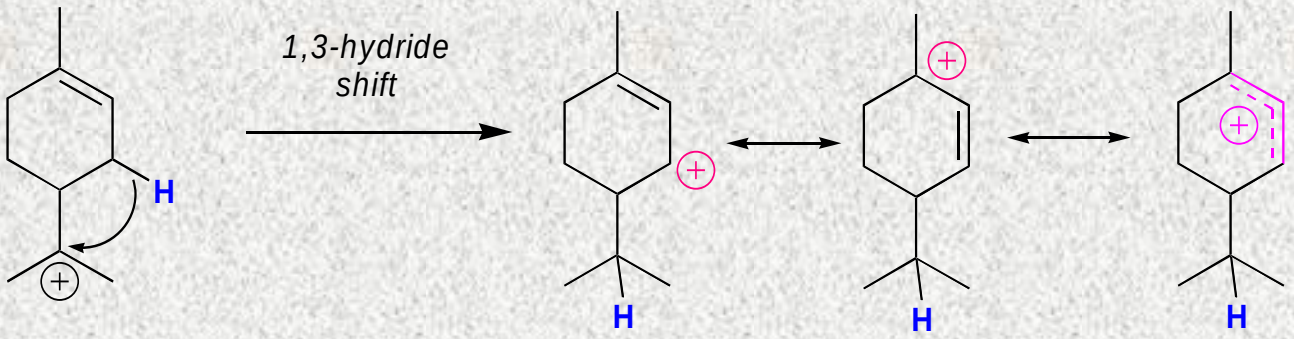
# Wagner-Meerwein Rearrangements




*secondary carbocation* *tertiary carbocation*



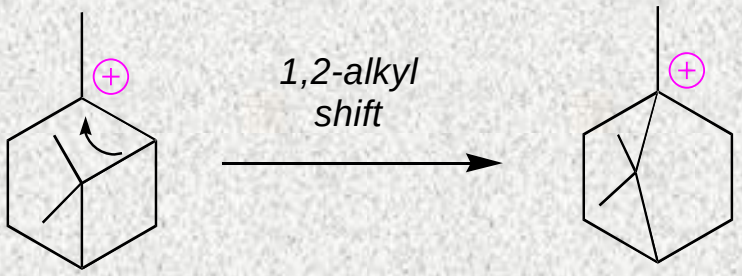

*secondary carbocation* *tertiary carbocation*




*tertiary carbocation*

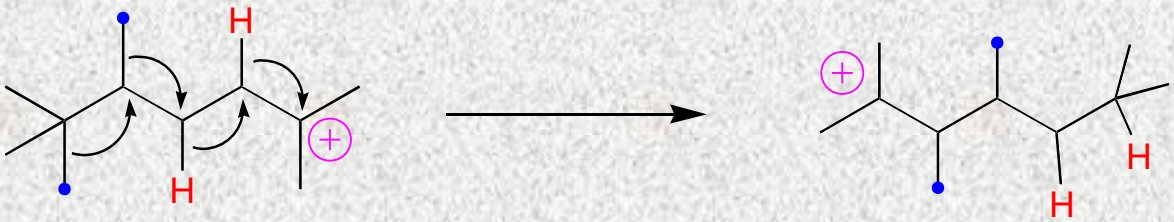
*ressonance-stabilized allylic cation*

# Wagner-Meerwein Rearrangements cont.



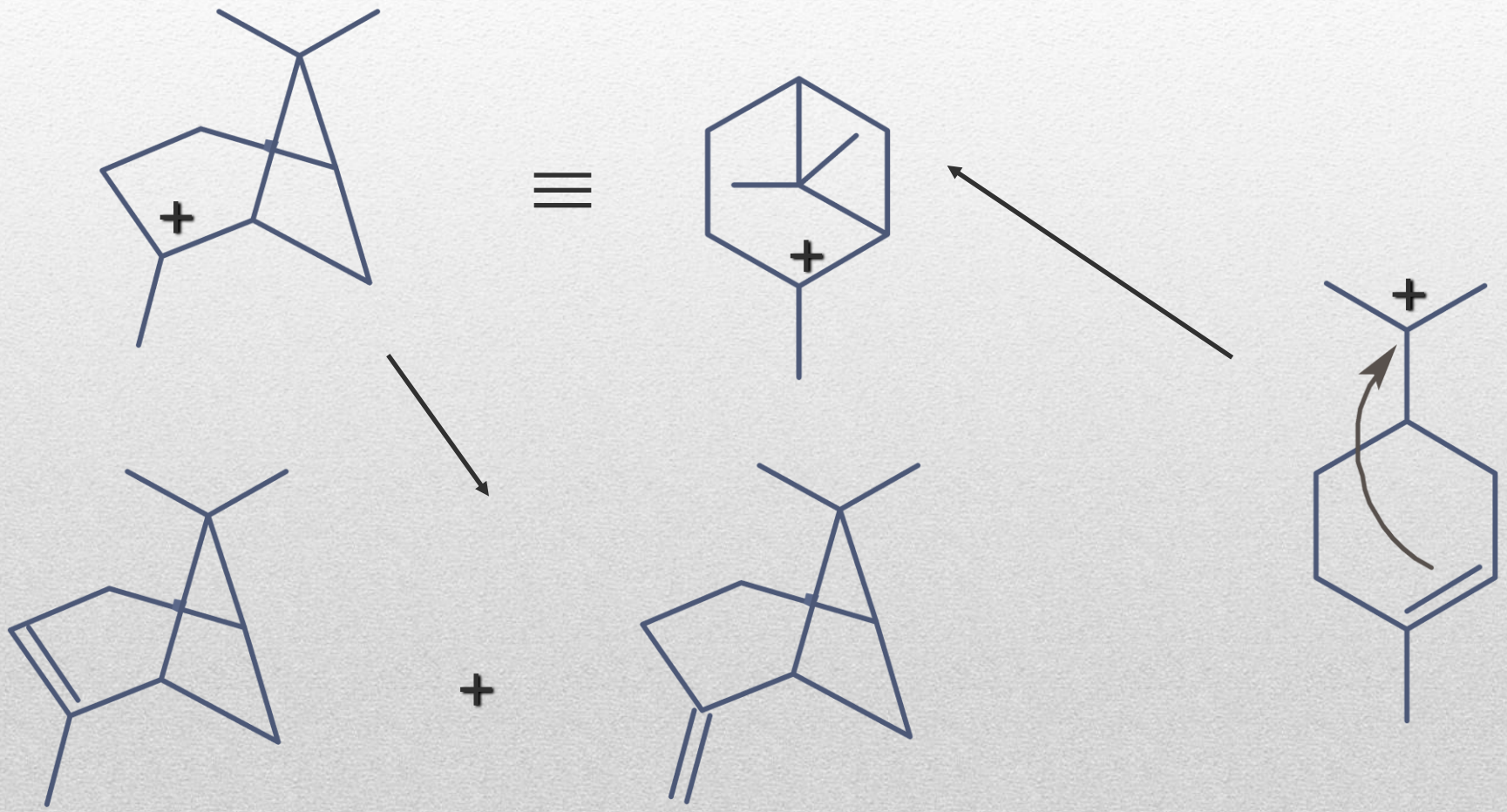

*tertiary carbocation, but strained 4-membered ring*

*secondary carbocation. but reduced ring strain in 5-membered ring*



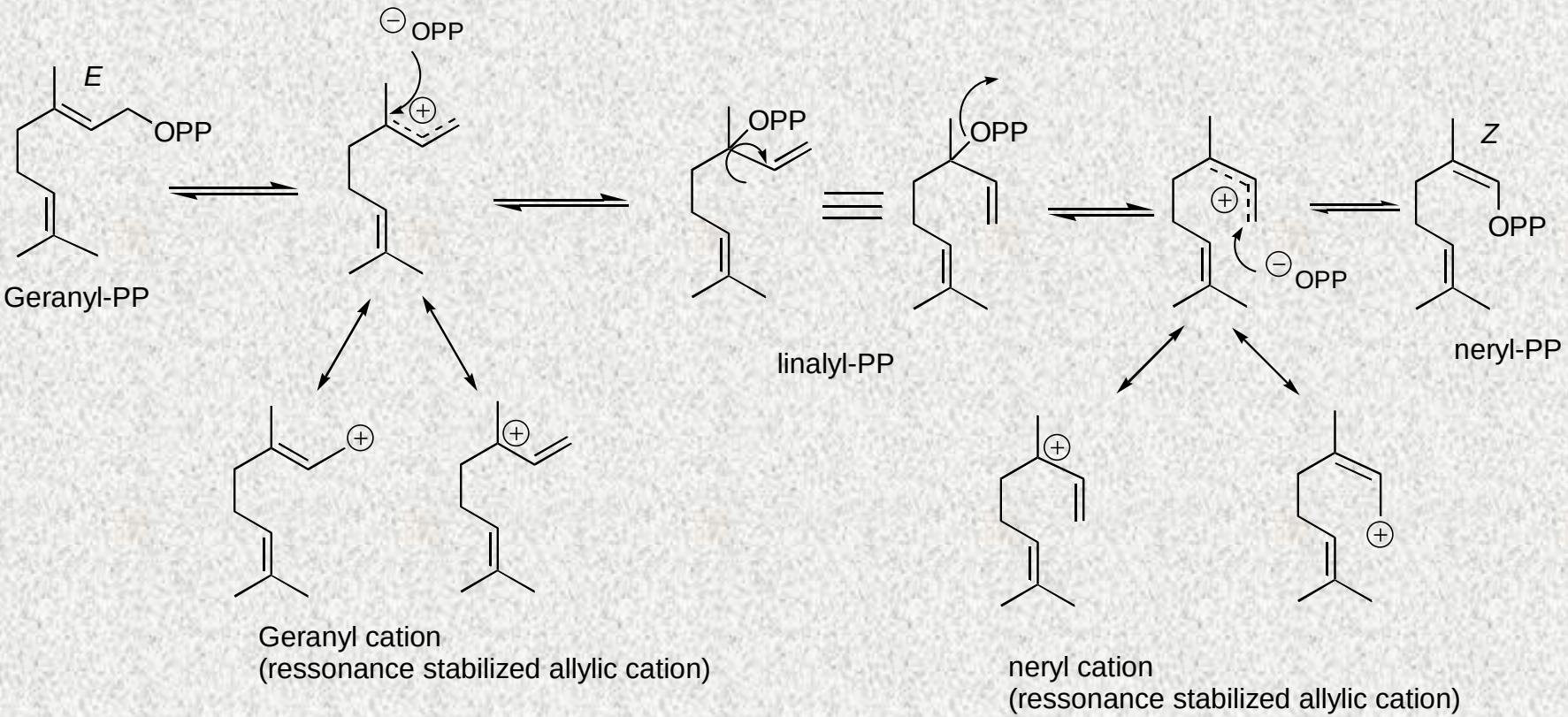

*a series of concerted 1,2 hydride and methyl shifts*

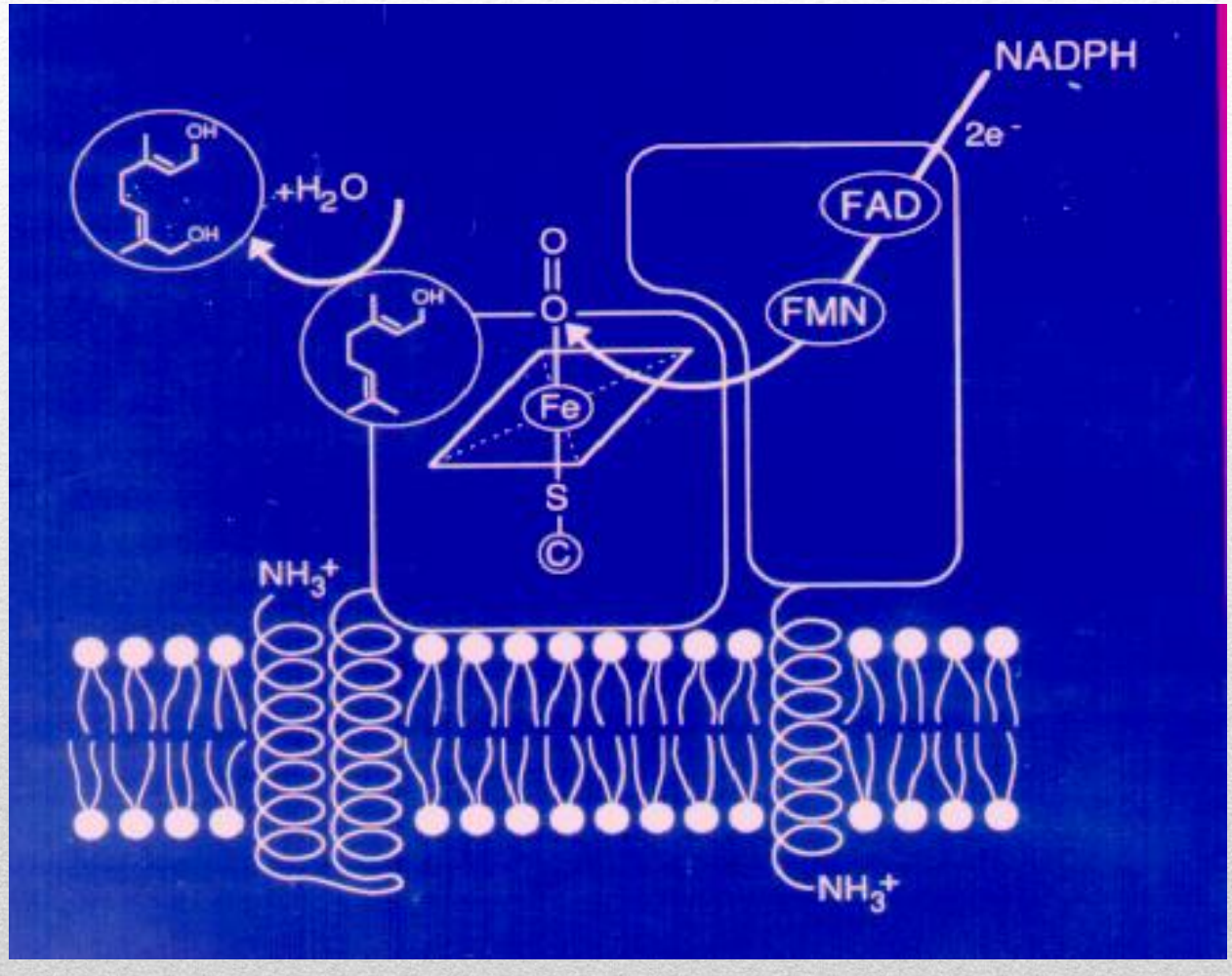
# Bicyclic Terpenes




α-Pineno β-Pineno

# Monoterpenes formation




NADPH
2e⁻
FAD
FMN
+H2O
OH
OH
OH
O
O
Fe
S
C
NH3+
NH3+

# FORMATION OF THE SESQUITERPENE SKELETONS

# Prenyltransferases

- Probably substrate specific
  - ie different ones to make GPP, FPP, GGPP

- GPP synthase in peppermint (Burke et al, 1999)
  - heterodimer of 28 & 37 kDa
  - plastid located

- GGPP synthases in *Arabidopsis* (Okada et al, 2000)
  - 1 & 3 go to cps, 2 & 4 go to ER and 6 is in mitos
  - 1 & 6 ubiquitously expressed
  - 2, 3 & 4 in flower, root and flower respectively

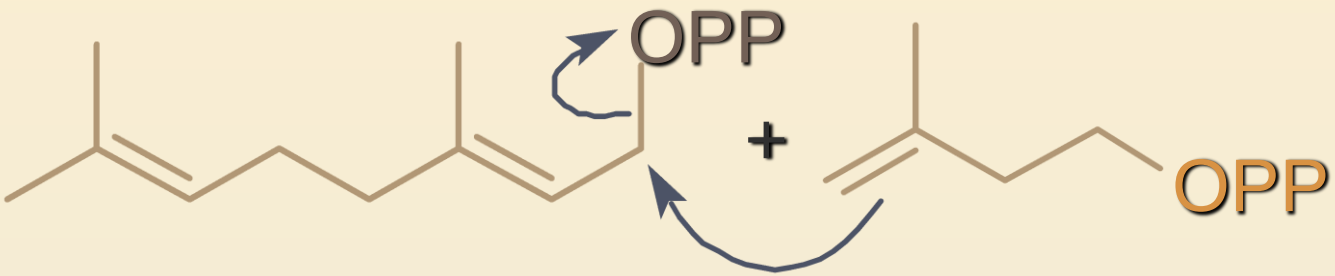
# Cyclases or Terpene Synthases

- Responsible for cyclization of isoprenoid
  - C10 to monoterpenes
  - C15 to sesquiterpenes
  - C20 to diterpenes

- Similar reaction to prenyltransferases
  - loss of PP group produces carbocation
  - this attacks a double bond
  - multiple double bonds mean alternative products

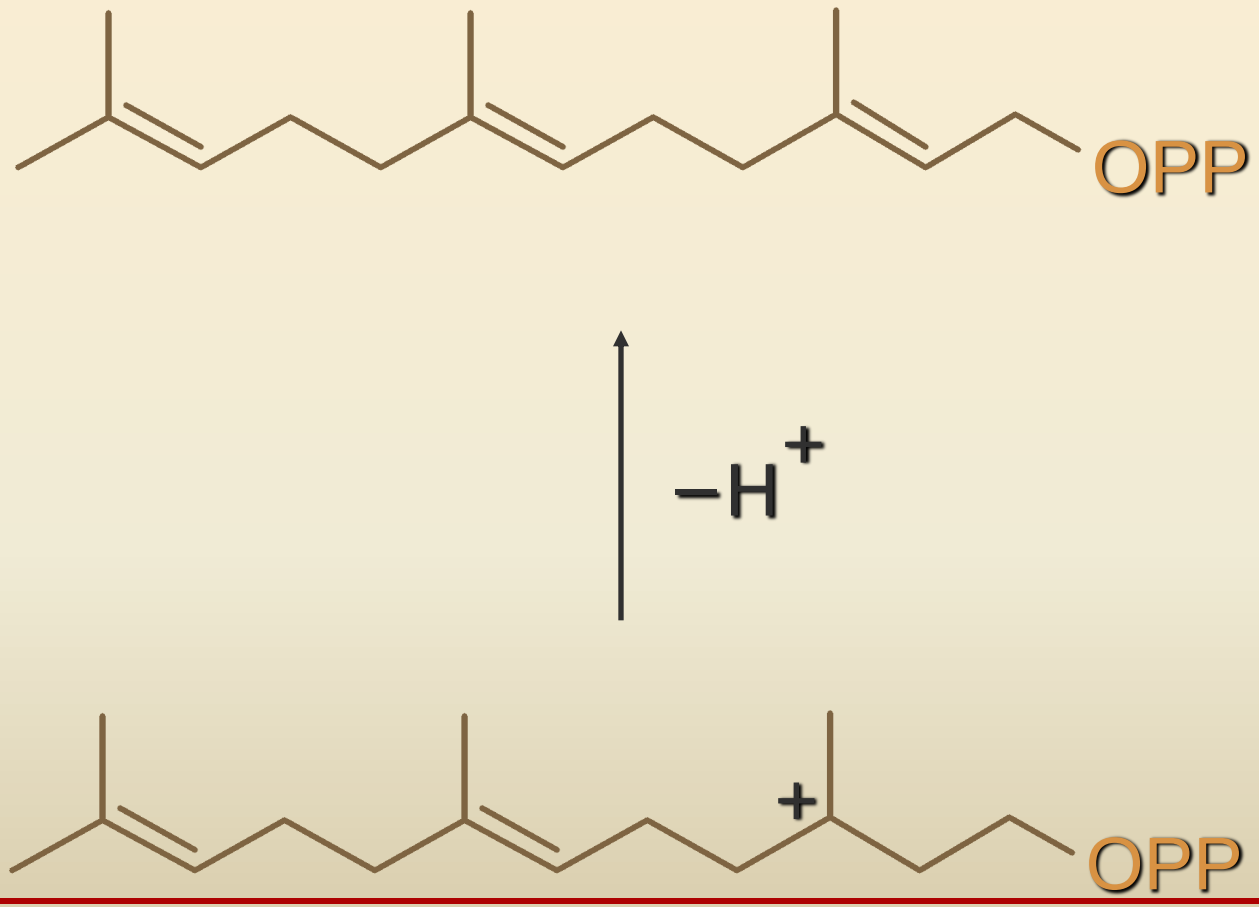
- Plant cyclases have sequence similarity
  - differ from fungal or animal enzymes

# From 10 Carbons to 15




Geranyl pyrophosphate

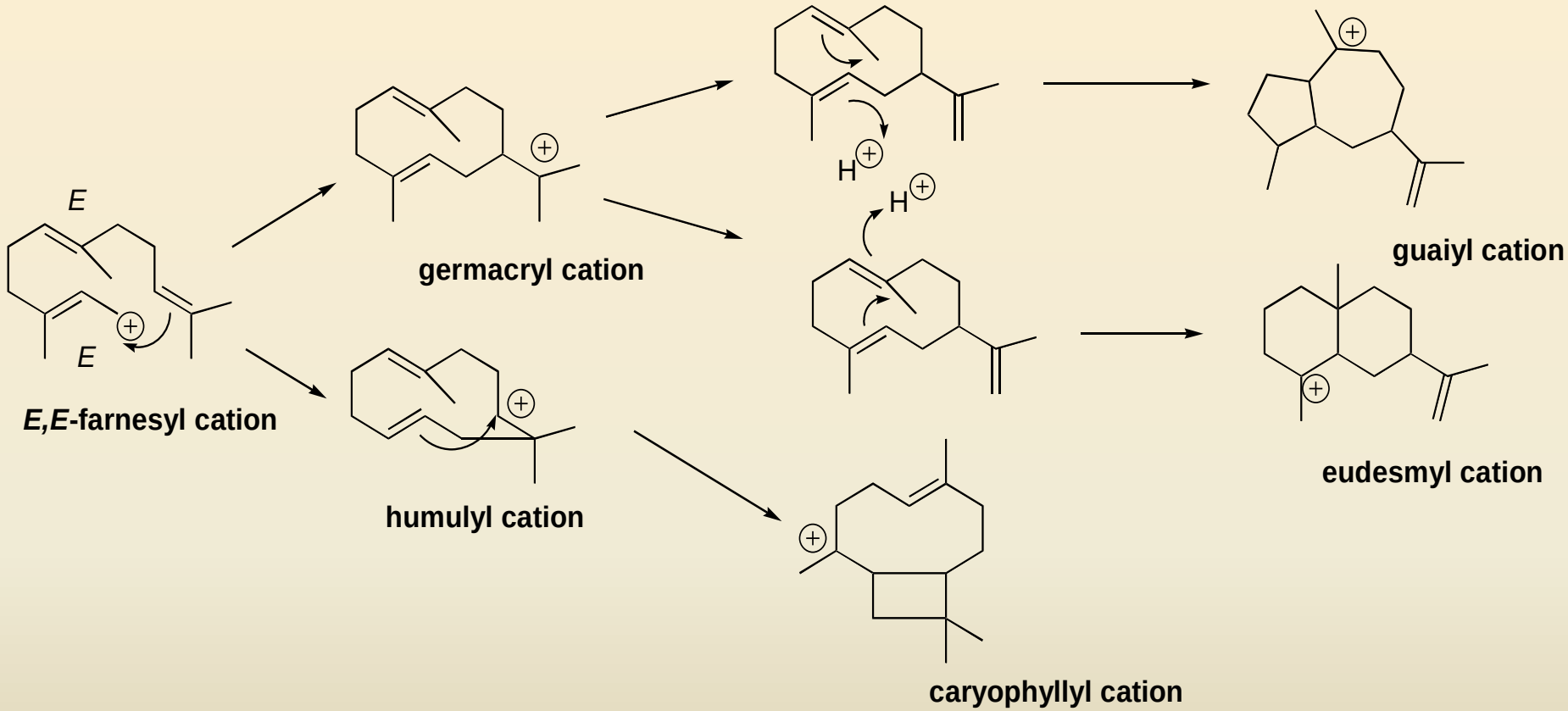
# From 10 Carbons to 15



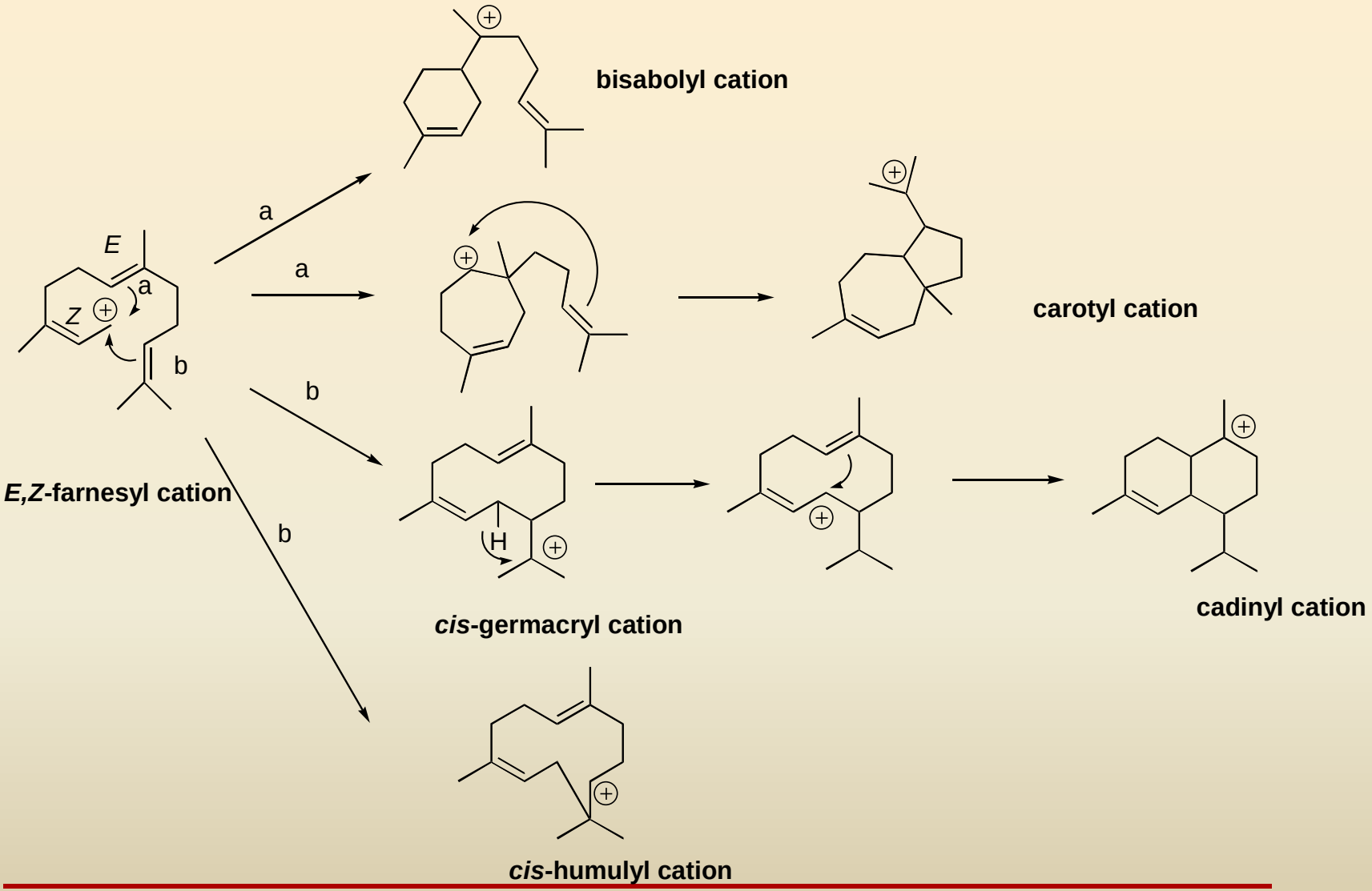
# From 10 Carbons to 15

- This compound is called farnesyl pyrophosphate.
- Hydrolysis of the pyrophosphate ester gives the alcohol farnesol

# Pathways for cyclization of farnesyl-PP

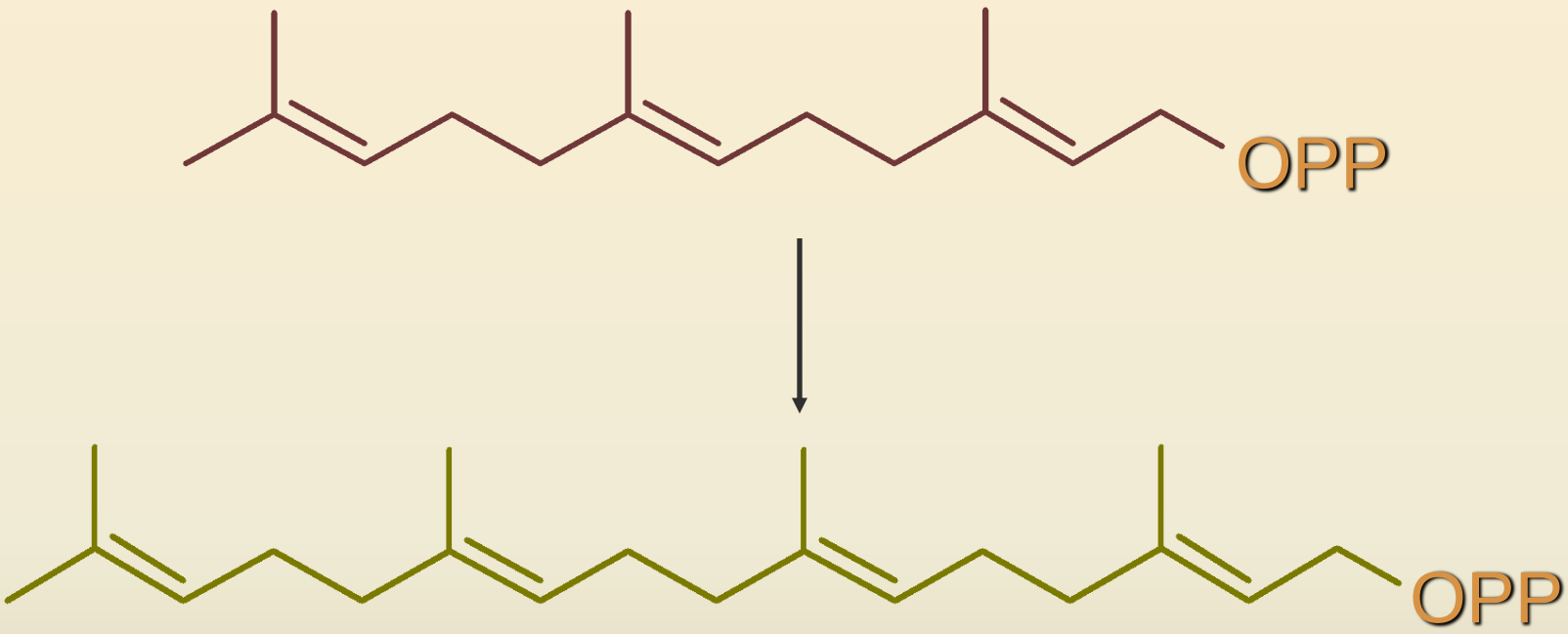


# Pathways for cyclization of farnesyl-PP



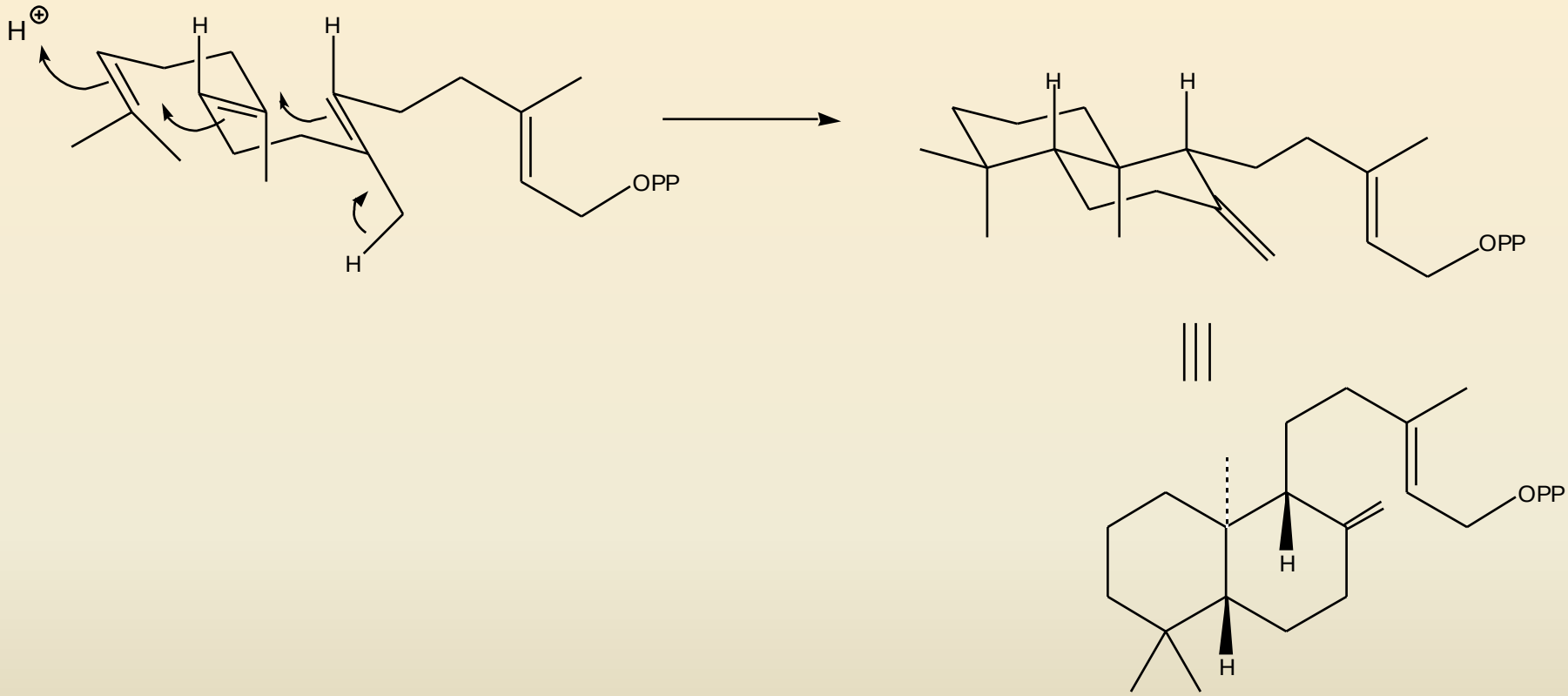
# Formation of the Diterpene Skeletons

# From 15 Carbons to 20



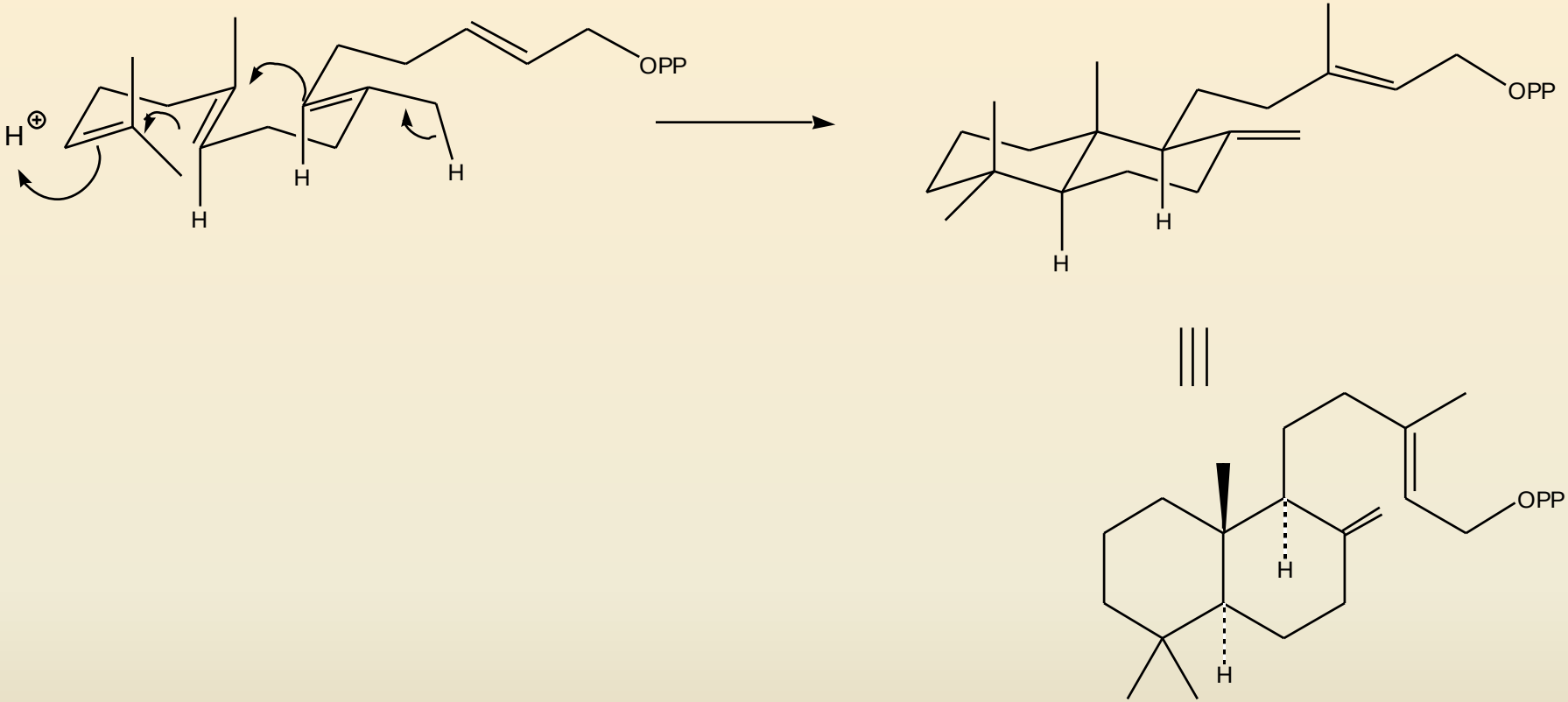

- Farnesyl pyrophosphate is extended by another isoprene forming the geranylgeranyl diphostate (GGPP)

# Ciclização dos Diterpenos




Copalil-pp

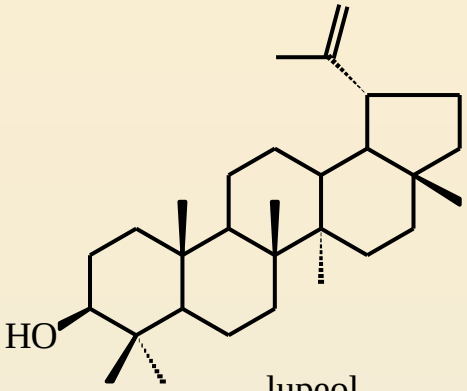
# Ciclização dos Diterpenos



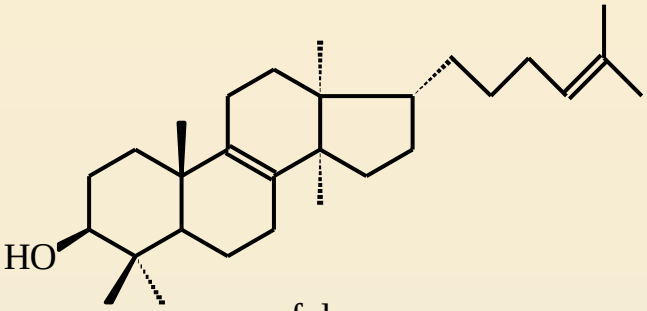

labdadienil-pp

# TRITERPENOS & ESTERÓIDES

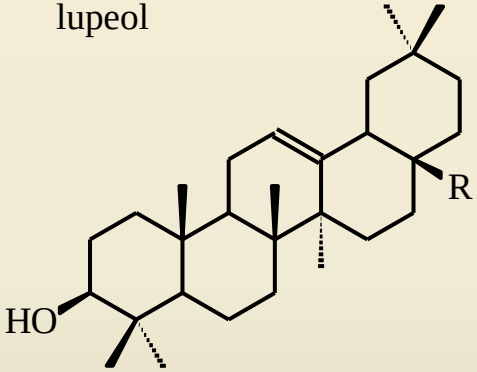
# Triterpenos



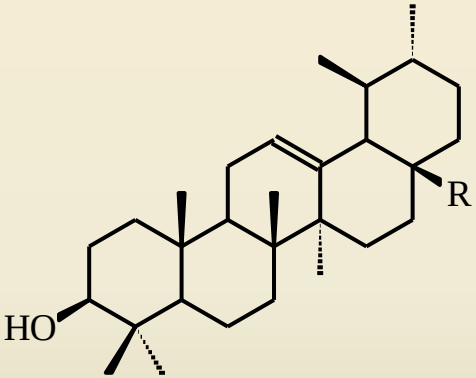

lupeol




eufol

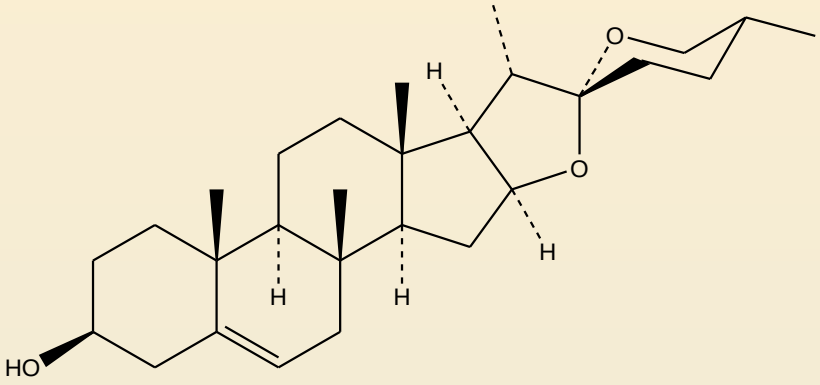



R=COOH (ácido oleanólico)
R=$CH_3$ (β-amirina)

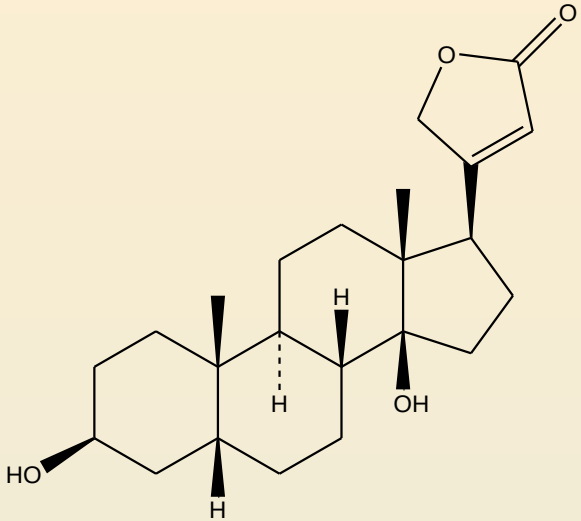



R=COOH (ácido ursólico)
R=$CH_3$ (α-amirina)

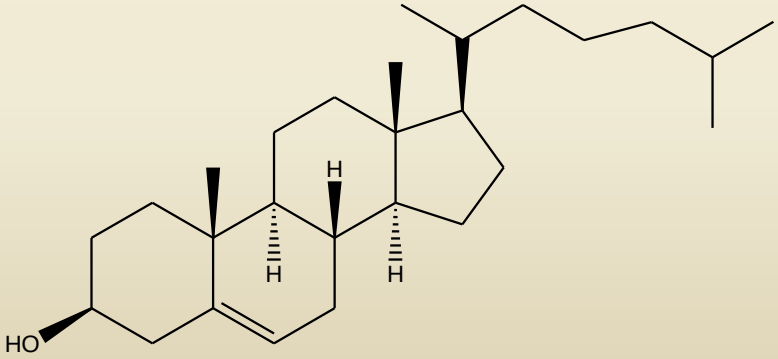
# Esteródes



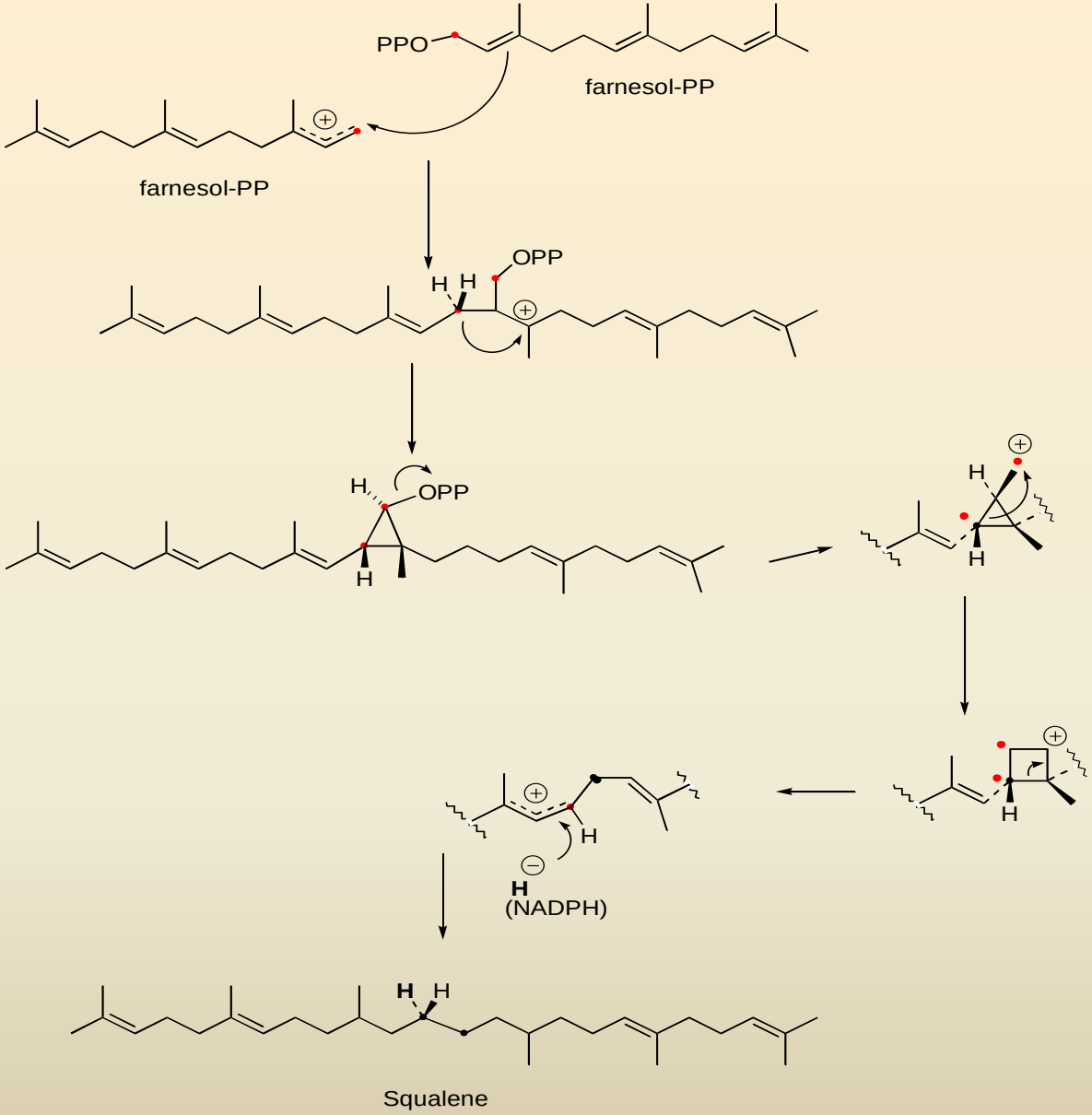

Diosgenina



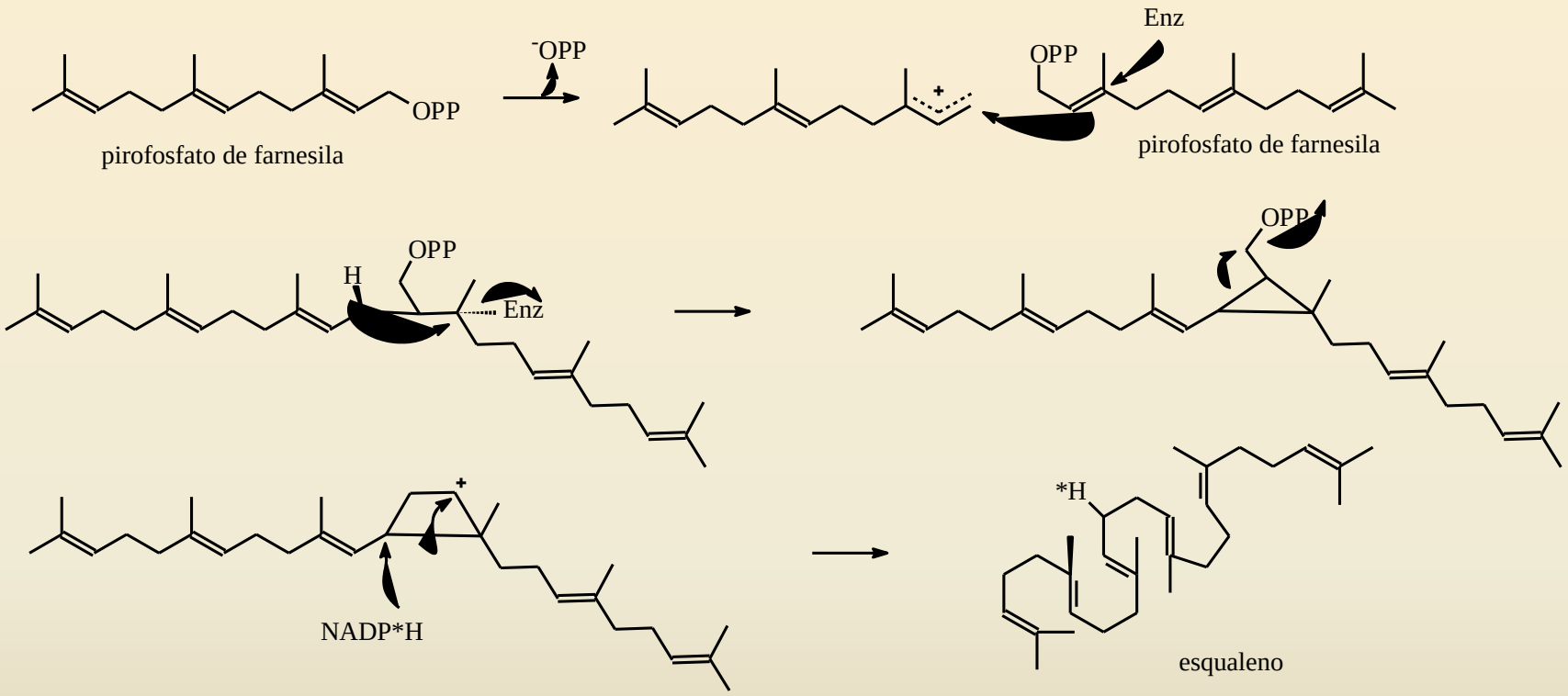

Digitoxigenina




Colesterol


PPO
farnesol-PP
farnesol-PP
OPP
H H
H
OPP
H
H
H
H
H
H
(NADPH)
H H
Squalene

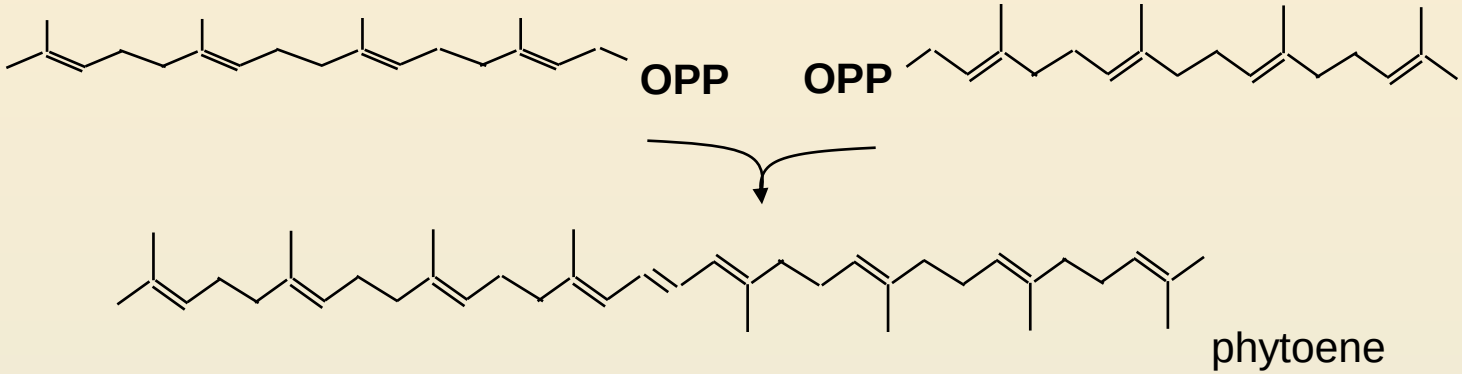
# Encadeamento cabeça-cabeça de sesquiterpenos para produzir triterpenos



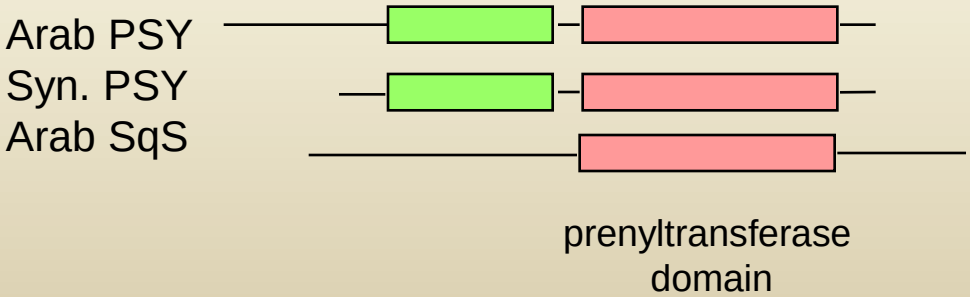

Um processo idêntico ocorre na formação de tetraterpenos a partir de diterpenos

# Coupling Enzymes

- Head-to-head condensation
  - Squalene synthase for sterols (C30), phytoene synthase for carotenoids (C40)

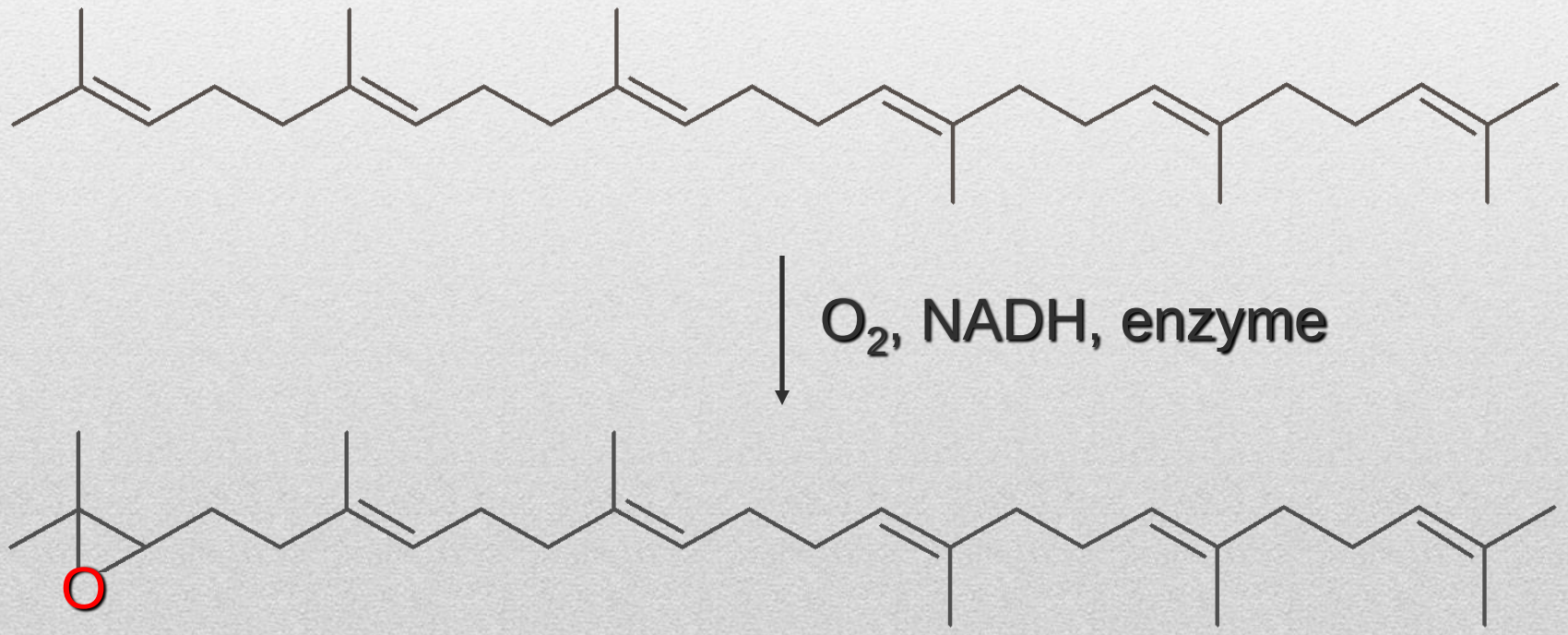



## PSY and SqS share domain in common

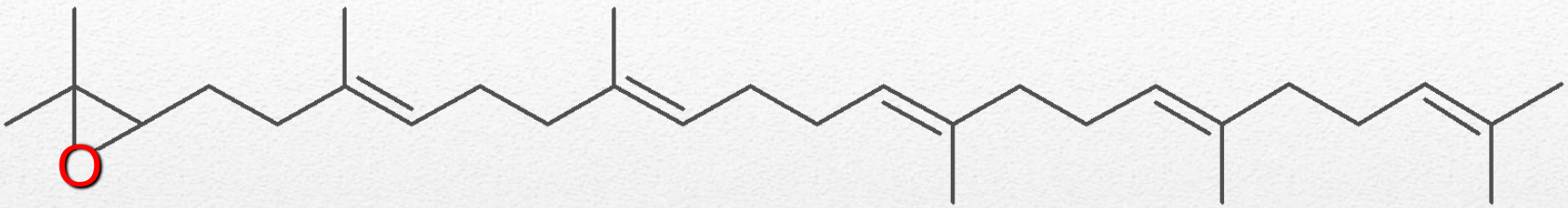


# Biosynthesis of Cholesterol

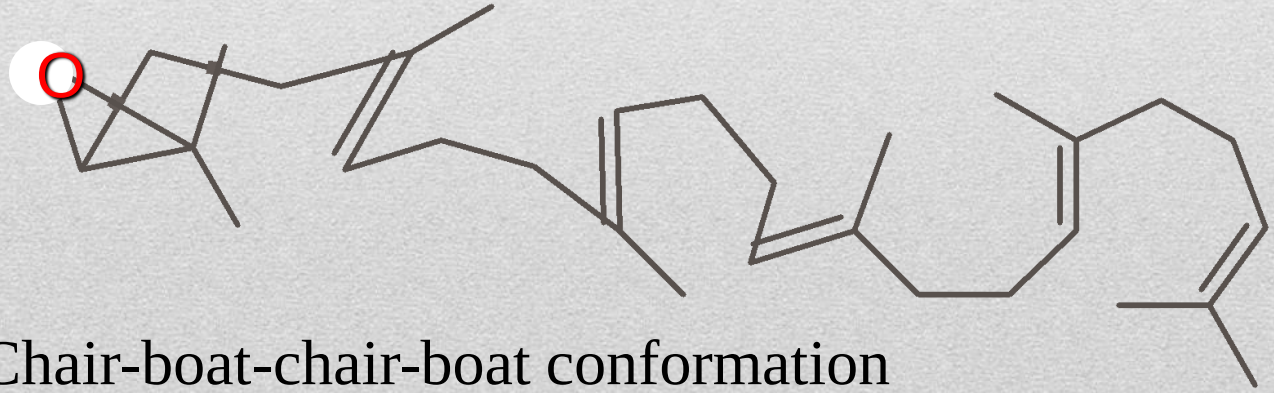
- Cholesterol is biosynthesized from the triterpene squalene. In the first step, squalene is converted to its 2,3-epoxide.



# Biosynthesis of Cholesterol

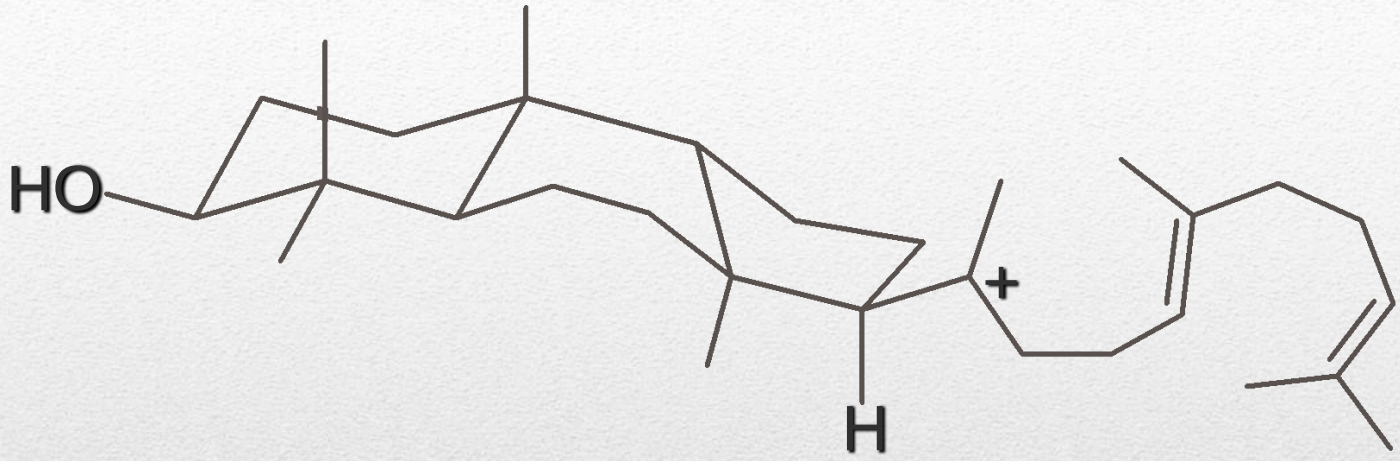



- To understand the second step, we need to look at squalene oxide in a different conformation, one that is in a geometry suitable for cyclization.

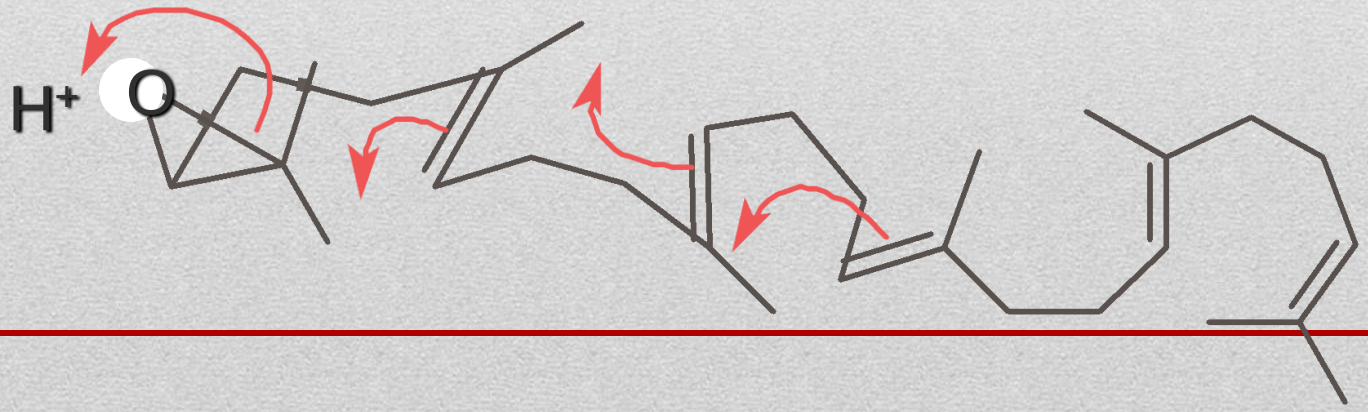



Chair-boat-chair-boat conformation

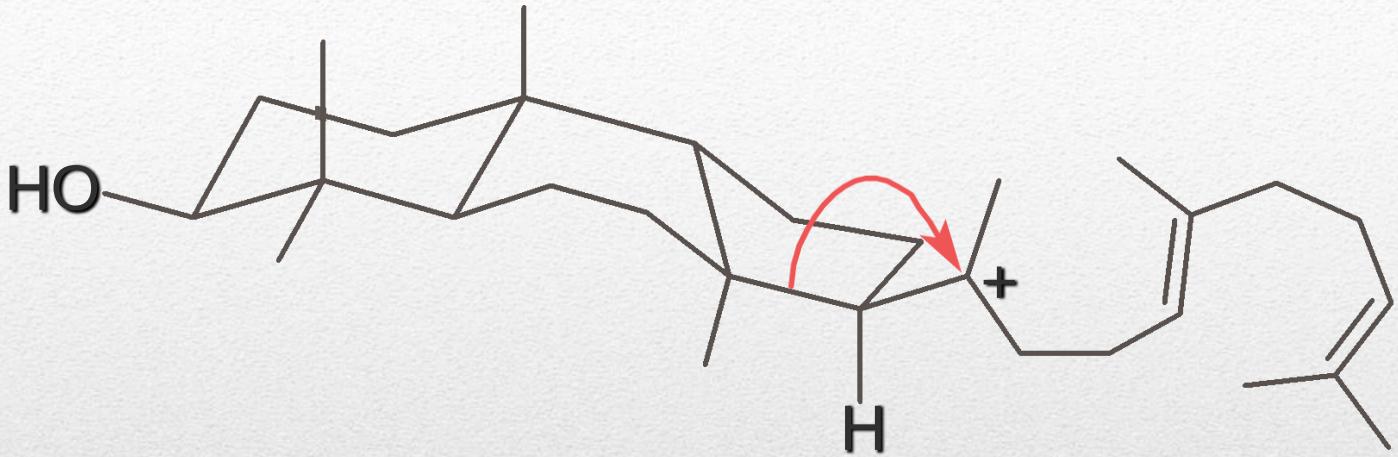
# Biosynthesis of Cholesterol



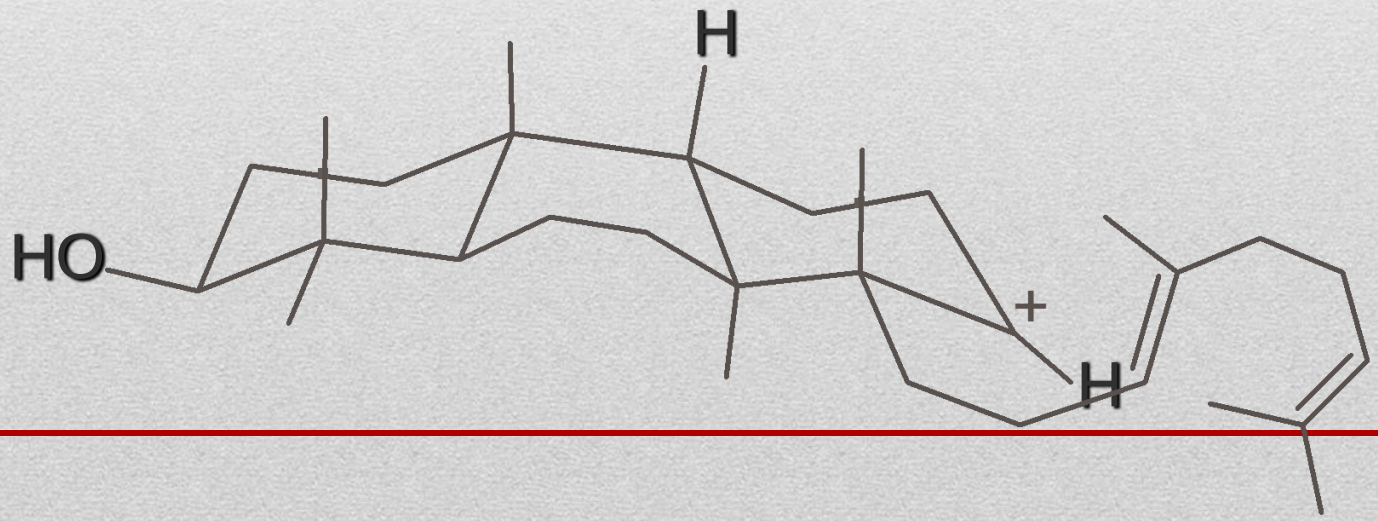

- Cyclization is triggered by epoxide ring opening.



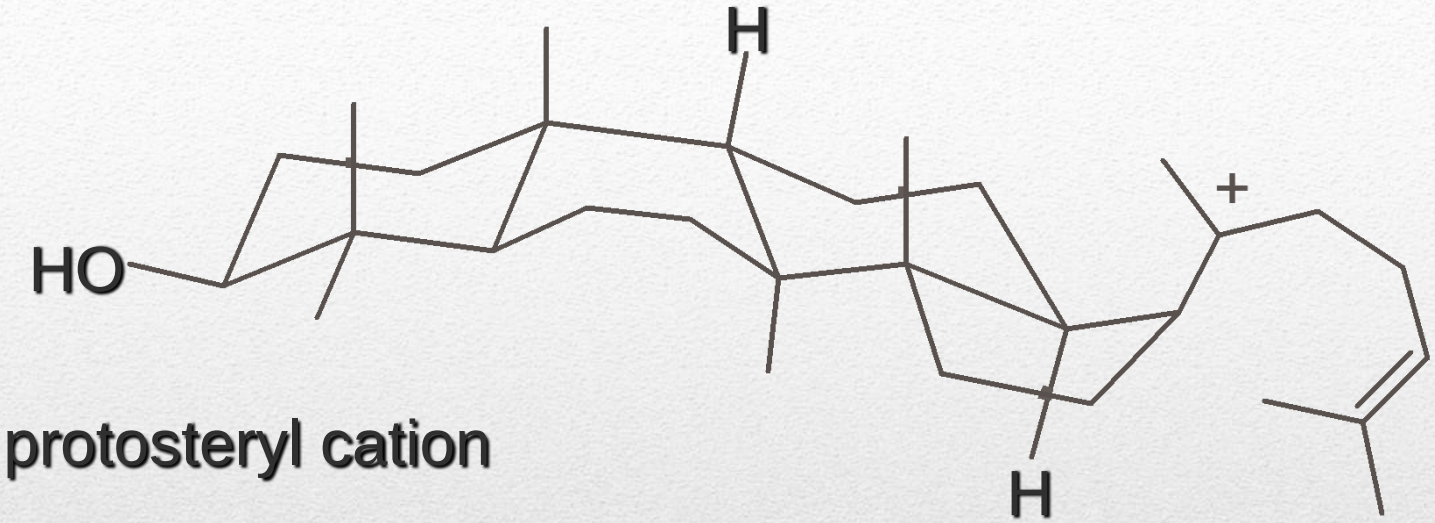
# Biosynthesis of Cholesterol




- The five-membered ring expands to a six-membered one.

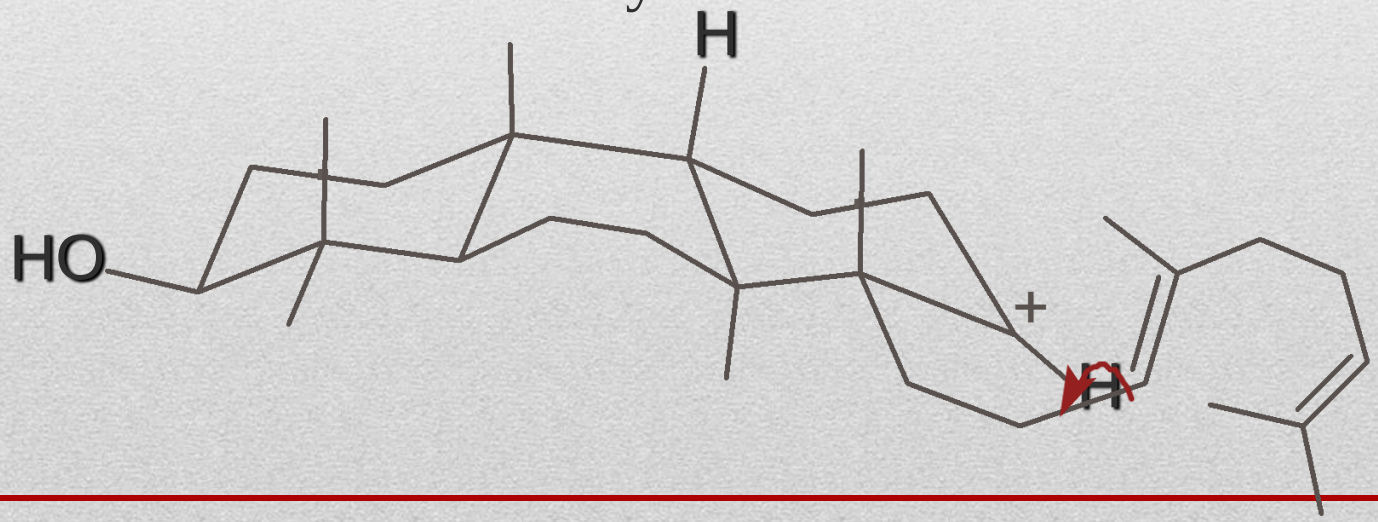


# Biosynthesis of Cholesterol

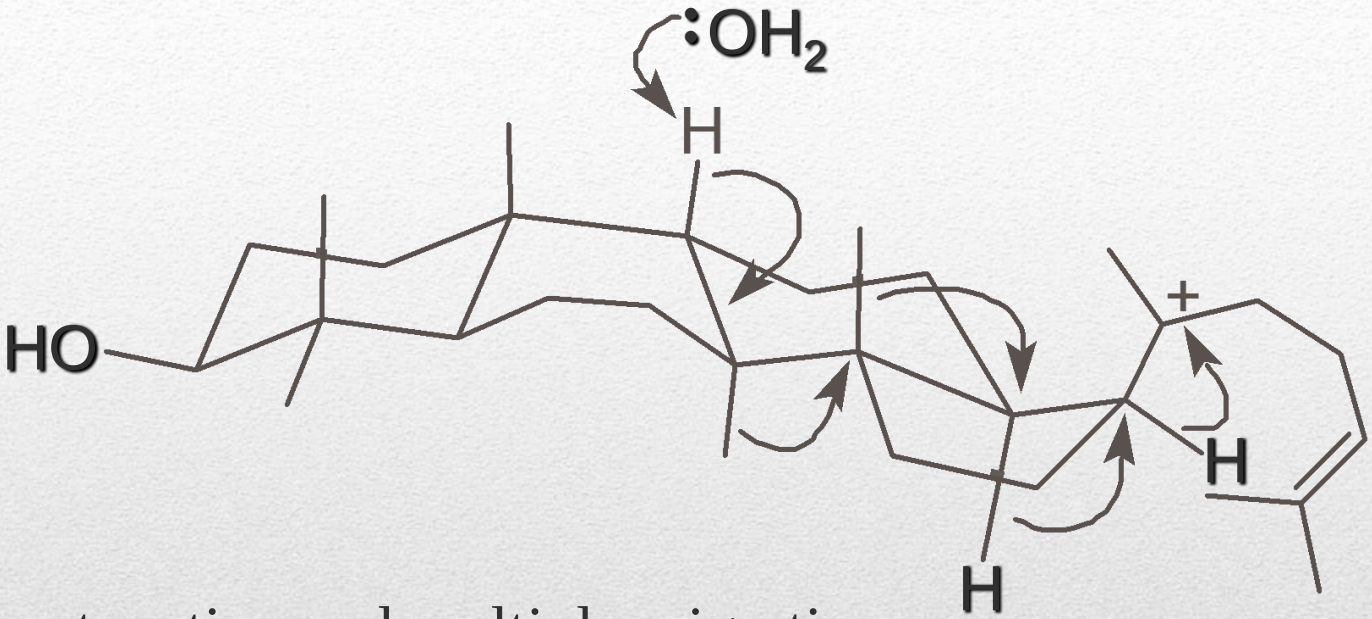



protosteryl cation

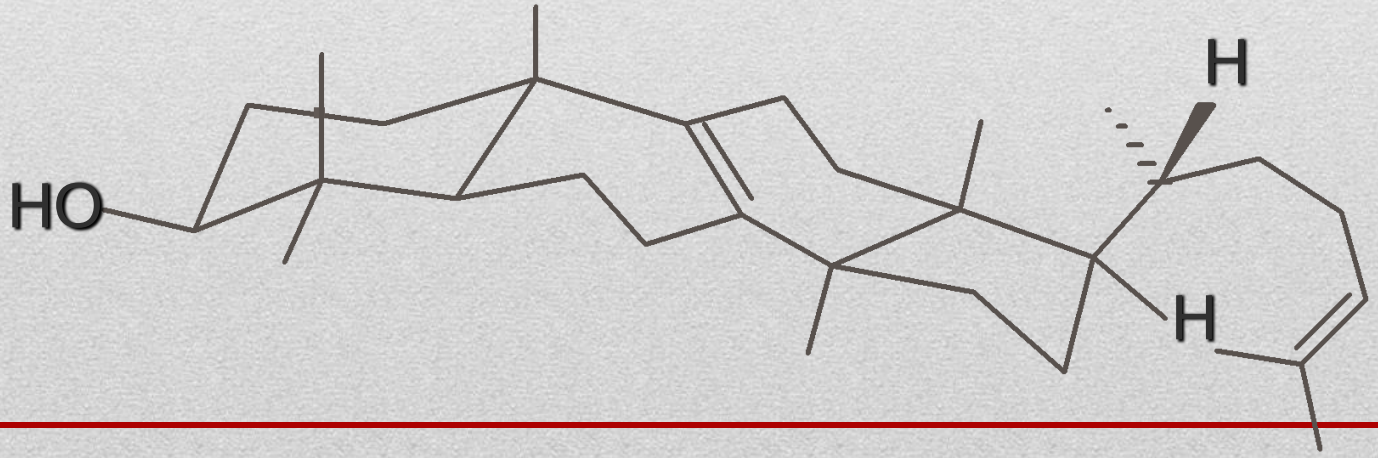
- Cyclization to form a tetracyclic carbocation.



# Biosynthesis of Cholesterol

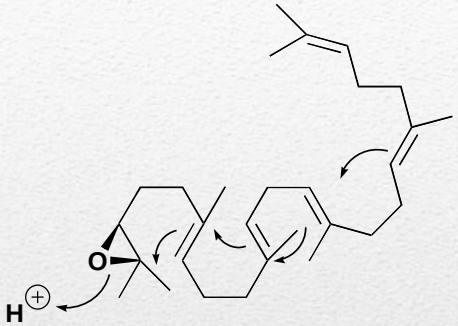



- Deprotonation and multiple migrations.

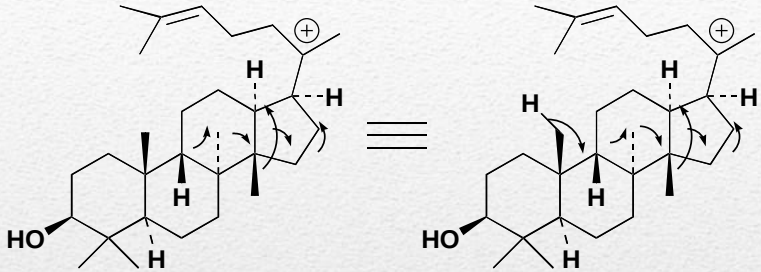


# Biossíntese de Esteróides

Sequence od W-M 1,2-hydride and
1,2 methyl shifts




squalene oxide

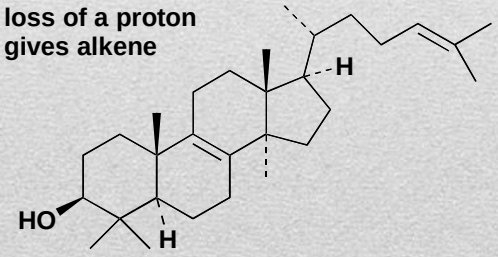



protosteryl cation

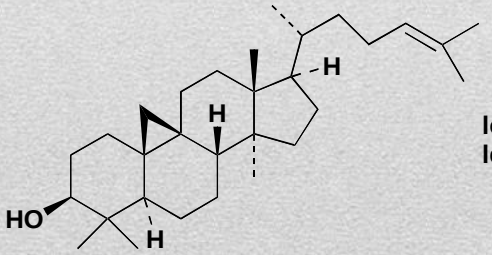
animals
fungi

plants

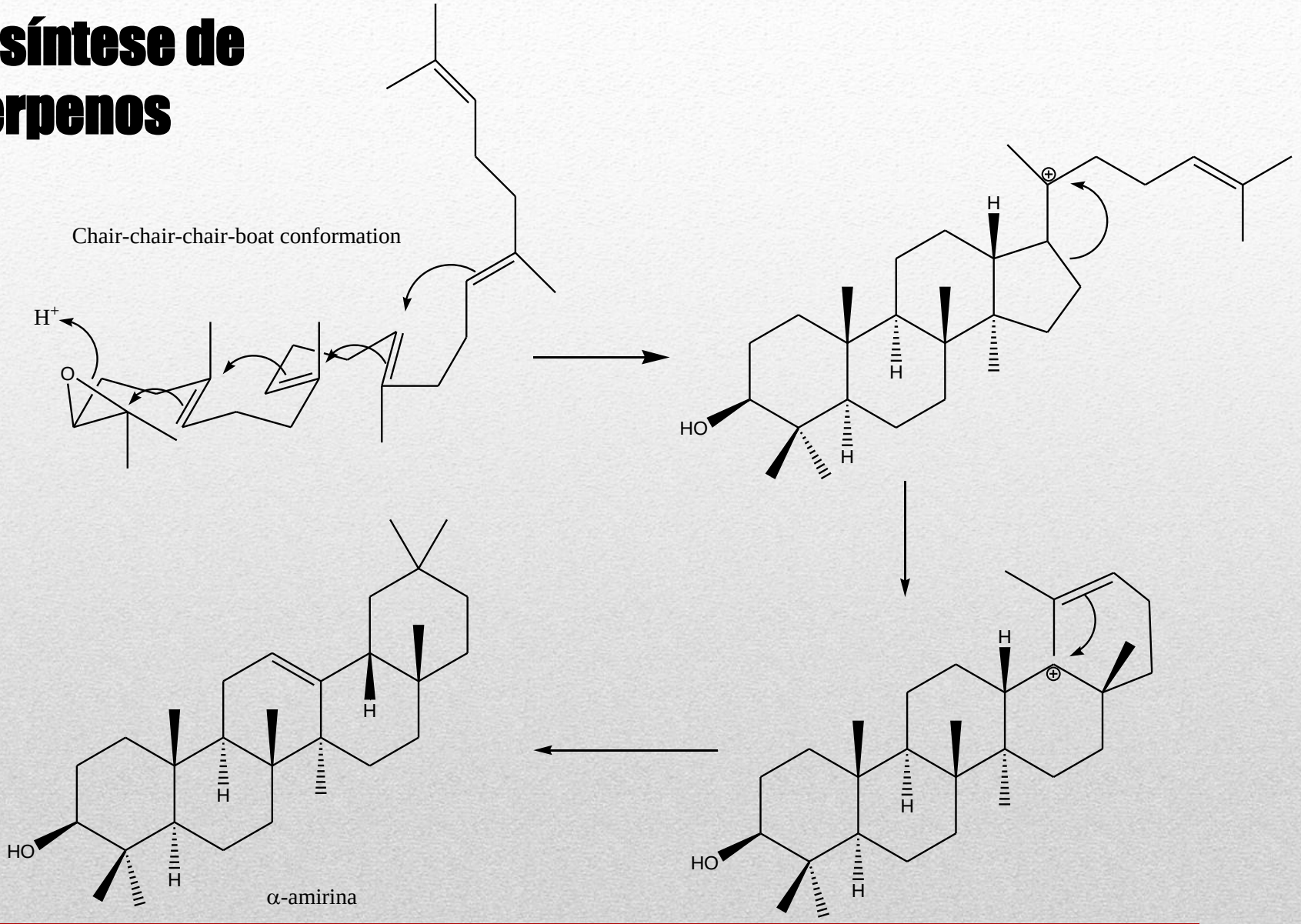
loss of a proton
gives alkene




lanosterol




cycloartenol

loss of proton
leads to cyclopropane

# Biossíntese de Triterpenos