Secondary Metabolism Part 3: Terpenes and Steroids

Lecture 10
Biofuels and Bioproducts

Bronx Community College - 2017
Chemistry and BioEnergy Technology for Sustainability NSF ATE 1601636

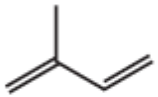
Outline

- Terpene Biosynthesis
  - Basics
  - Terpenoid nomenclature
  - MVA Pathway
  - MEP Pathway
  - Head-to-Tail coupling reaction (terpene polymerization)
  - Practical approaches and ^{13}C labeling
  - Introduction to Terpene Diversity
  - Introduction to Steroids

Slide Images modified from: Paul Dewick *Medicinal Natural Products: A Biosynthetic Approach* (3rd Ed. Wiley, 2009)

Terpene Basics

C_5 isoprene unit

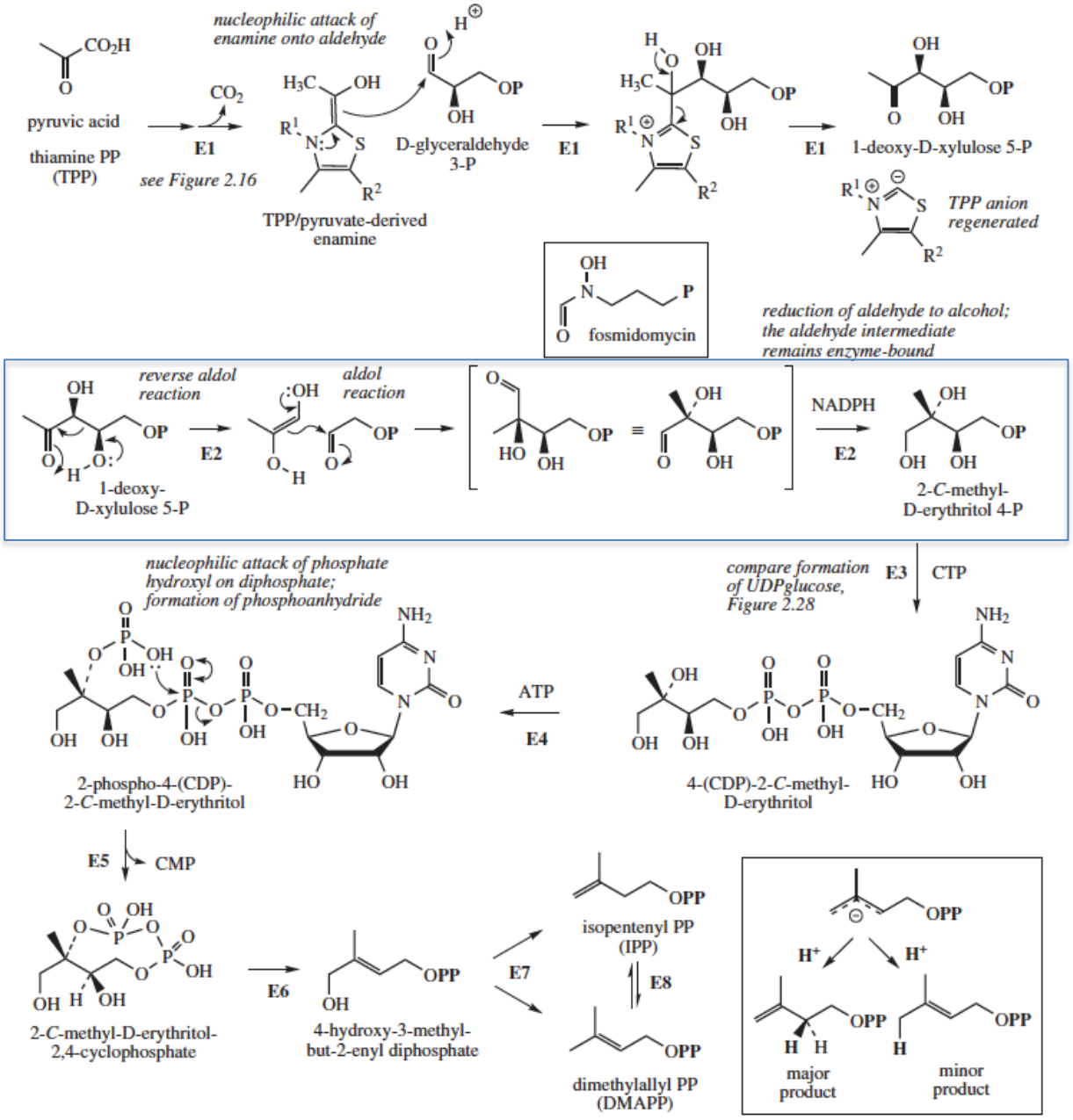


isoprene

- Largest group of natural products (35K terpenes identified to date)
- Head-to-tail joining of C_5 (isoprene, aka 'prenyl' unit) to give C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30}… structures.
- Polymerization chemistry is made possible by enzyme production of DMAPP and IPP, and a good LG (PP)
- Cyclizations and rearrangements are common
- Many examples of terpenes in 'mixed biosynthesis' e.g. alkaloids, phenolics, vitamins
- Some proteins (typically at CYS residues) have farnesyl (C_{15}) or geranyl-geranyl (C_{20}) groups attached to increase lipophilicity and/or association with cell membranes
- ***Two pathways*** can produce the isoprene starter unit:
  - **Mevalonic Acid pathway (MVA)**
  - **Methylerythritol Phosphate pathway (MEP)**

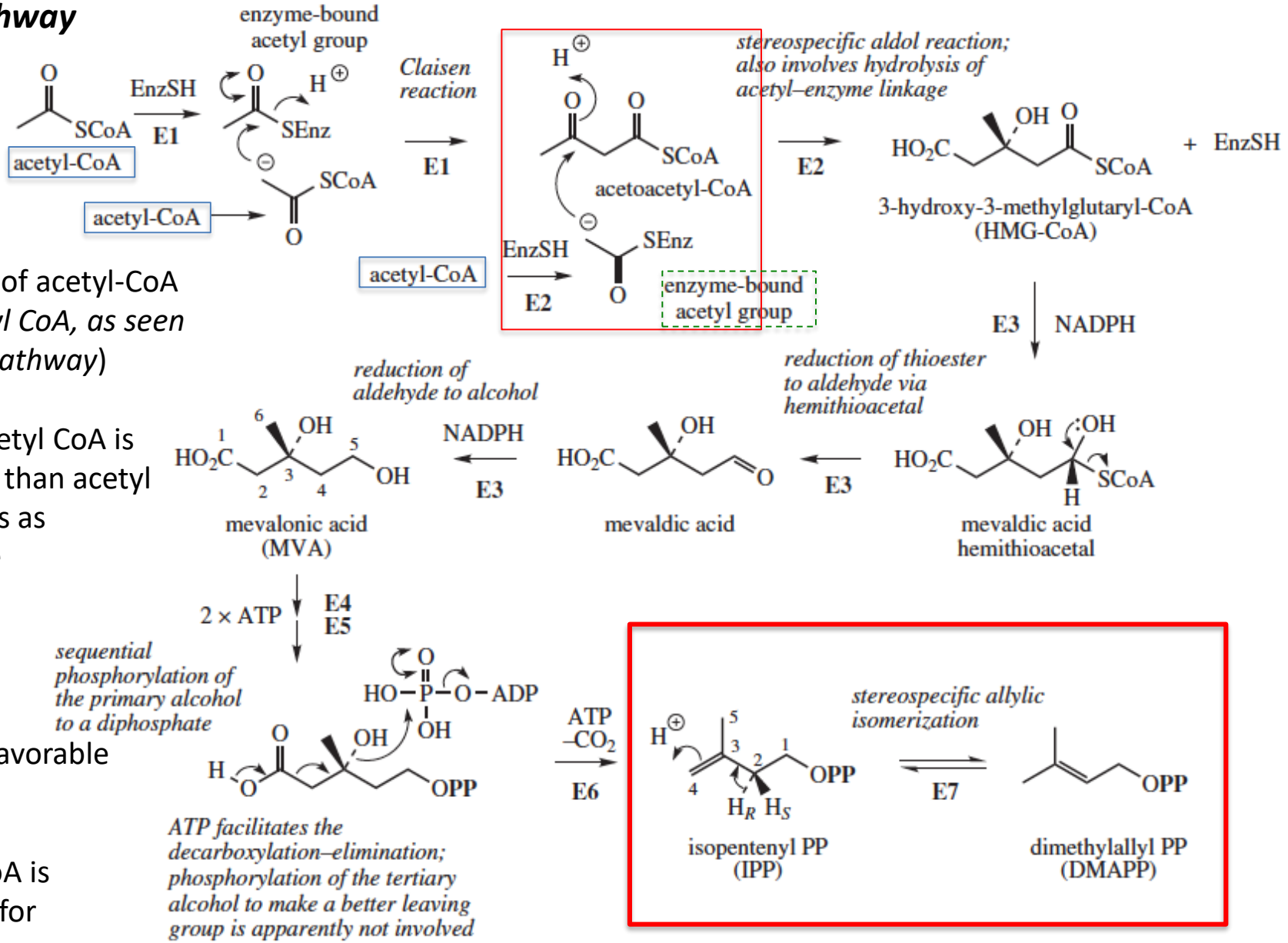
MEP Pathway

- Pyruvic acid and D-glyceraldehyde-3-P from glycolysis
- Sequence features reverse Aldol of deoxyxylulose phosphate/reduction occurring within the same enzyme (E2)
- Antibiotics such as fosmidomycin (from *Streptomyces lavendulae*) are attractive for treatment of e.g. malaria and tuberculosis since MEP pathway is not found in humans



E1: 1-deoxy-D-xylulose 5-phosphate synthase (DXP synthase)
E2: 2-C-methyl-D-erythritol 4-phosphate synthase; 1-deoxy-D-xylulose 5-phosphate reductoisomerase (IspC)
E3: 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase (IspD)
E4: 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (IspE)
E5: 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (IspF)
E6: 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (IspG)
E7: 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (IspH)
E8: isopentenyl diphosphate isomerase (IPP isomerase)

MVA Pathway



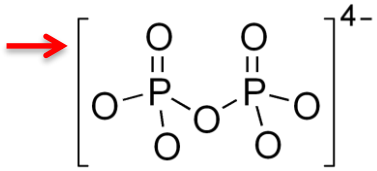
- 3 moles of acetyl-CoA (*not malonyl CoA, as seen in acetate pathway*)
- Acetoacetyl CoA is more acidic than acetyl CoA but acts as electrophile
- because enzyme can achieve unfavorable chemistry
- HMG-CoA is drug target for cholesterol lowering (statins)

E1: acetoacetyl-CoA synthase
E2: 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase)
E3: 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase)
E4: mevalonate kinase
E5: phosphomevalonate kinase
E6: mevalonate 5-diphosphate decarboxylase
E7: isopentenyl diphosphate isomerase (IPP isomerase)

The Hierarchy of Isoprene

- Starting Materials (DMAPP and IPP) ***derived from two independent pathways*** (MVA or MEP)

- Pyrophosphate (PP) is a good LG and thus polymerization occurs between terpenes

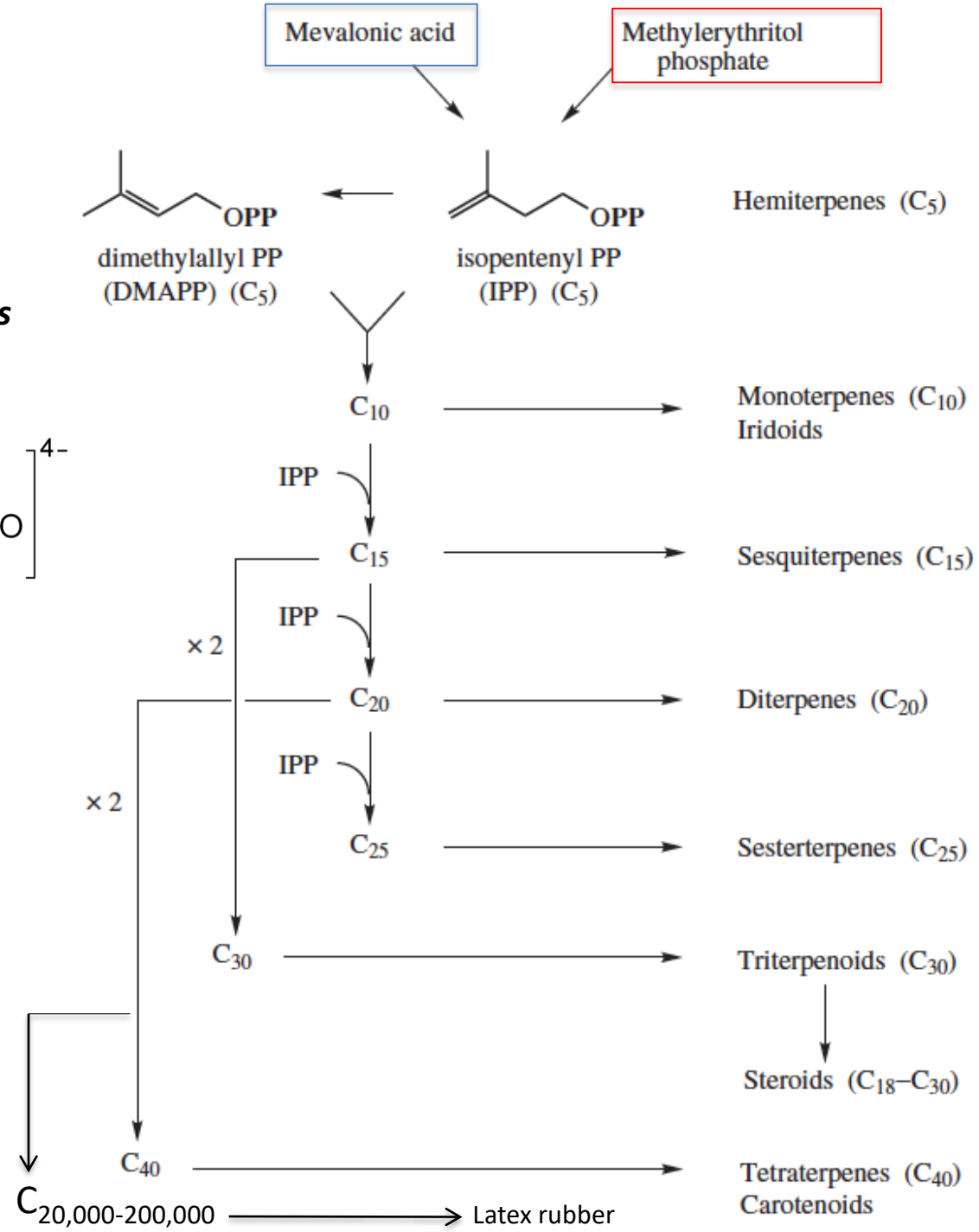


- ***MVA Pathway - Animals & Fungi***
- ***MEP Pathway – Plants, Algae, most Bacteria***

- Mevalonic Acid (MVA) produced via acetate (fatty acid) biosynthesis

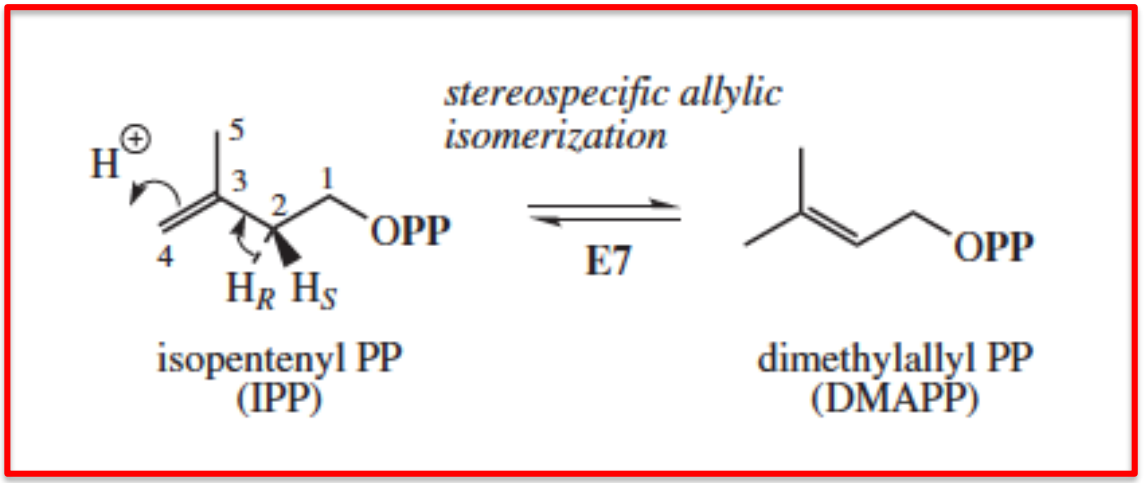
- Methylerithritol Phosphate (MEP) Pathway is more recently discovered and MEP is produced from pyruvate (glycolysis)

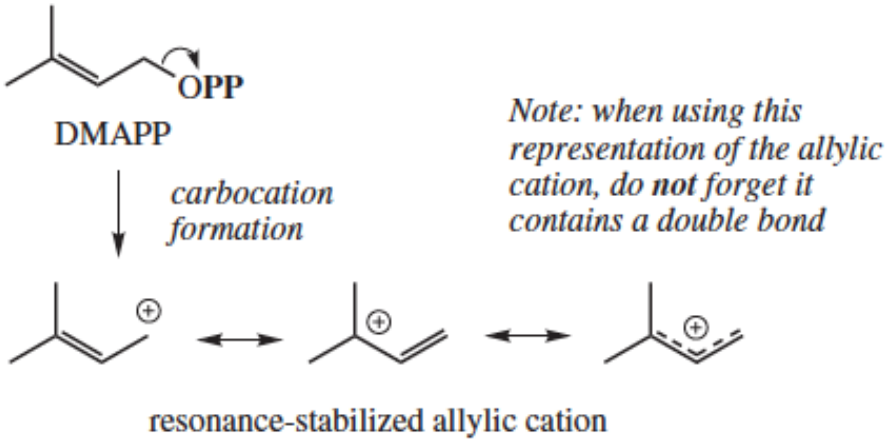
- IPP is typically the “extender unit”



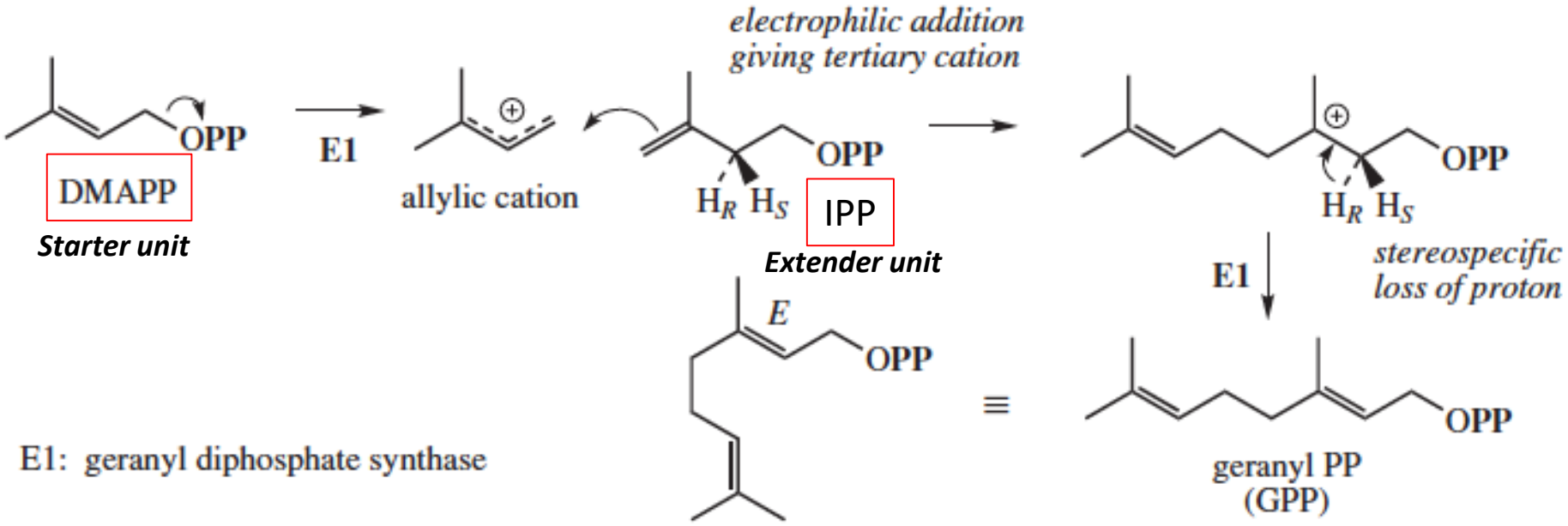
IPP and DMAPP *Made to Order*

- Isomerization of IPP and DMAPP performed in the amounts required for metabolism
- Resonance-stabilized allylic cation of DMAPP
- OPP is a good, stable LG
- Typically 4:1 ratio of IPP: DMAPP
- Why?
- Which is the “extender unit”?





Head to Tail Coupling: IPP to DMAPP

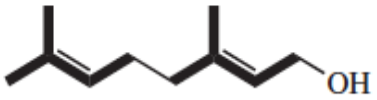


- Though 3° carbocation is most stable, nucleophilic attack occurs at the terminal, 1° carbocation
- Addition of a second mole of IPP gives the sesquiterpene (farnesyl PP)
- Addition of a third mole of IPP gives the diterpene (geranylgeranyl PP), etc…

- Coupling of e.g. two C_{15} sesqiterpenes gives C_{30} (triterpene). Can you draw this mechanism?

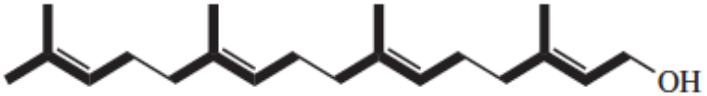
Some Common Terpenes of Various Sizes and Shapes

- Formed from 'Head-to-Tail' combination of DMAPP and IPP
- Cyclizations and rearrangements can give chiral compounds
- High-value compounds are extracted prior to biofuel/paper production e.g. pinenes 'terpentine' (1% yield)
- Bisabolene has been expressed[1] in yeast and *E.coli* (via MVA) and is considered "third generation biodiesel"



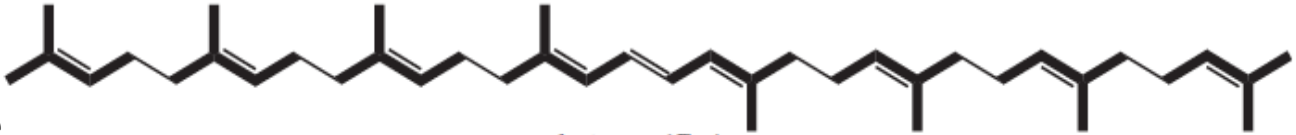
geraniol (C_{10}) ***monoterpene***

farnesol (C_{15}) ***sesqiterpene***

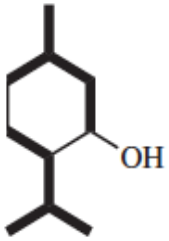


geranylgeraniol (C_{20}) ***diterpene***

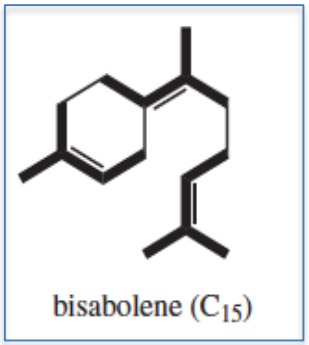
squalene (C_{30}) ***triterpene***



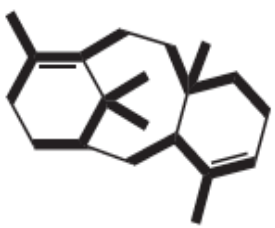
phytoene (C_{40}) ***tetraterpene***



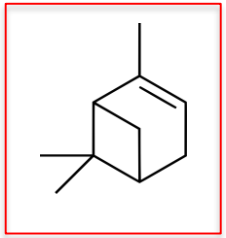
menthol (C_{10})



bisabolene (C_{15})



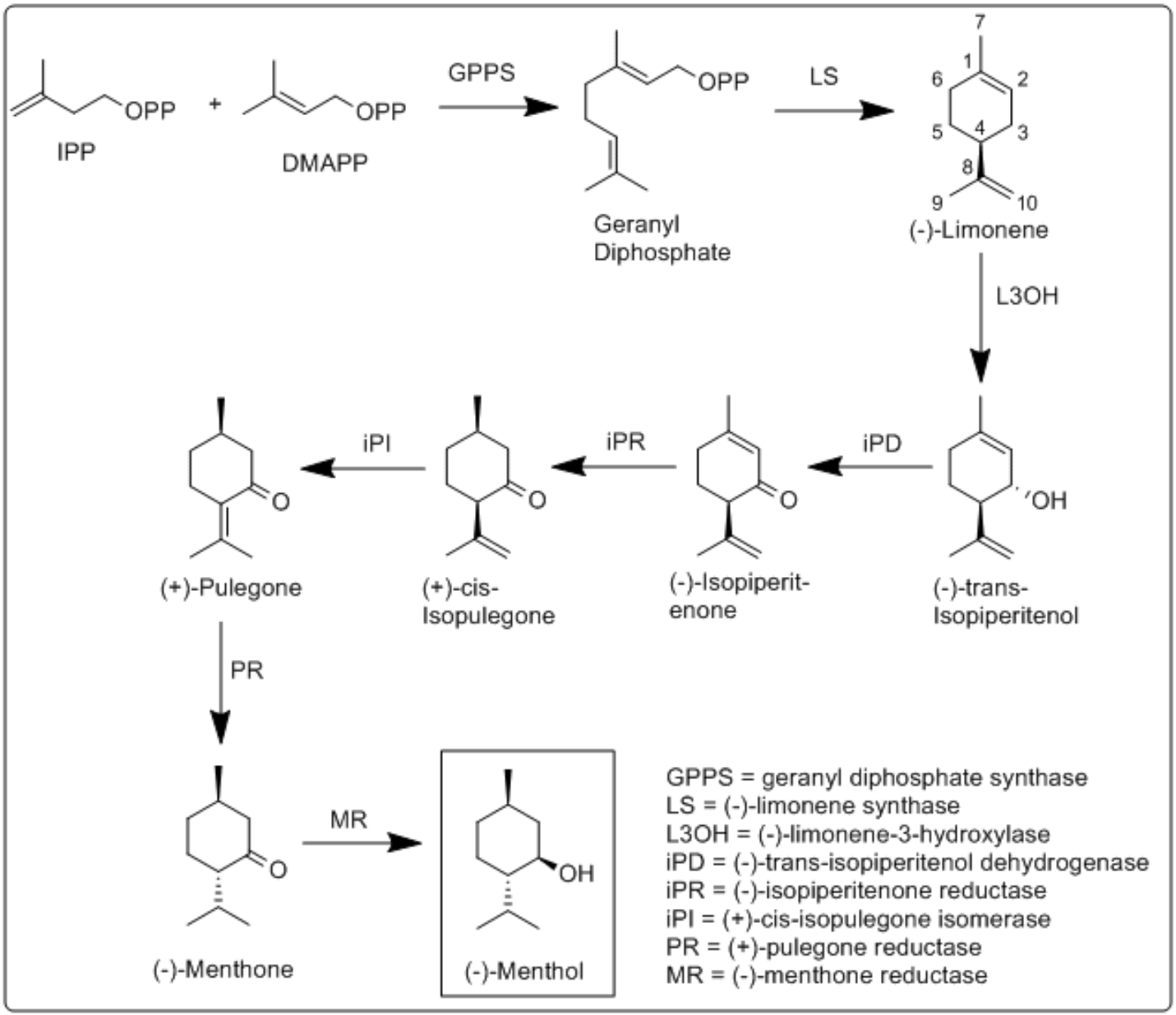
taxadiene (C_{20})



Pinene
(can you count the # of isoprene units and chiral centers?)

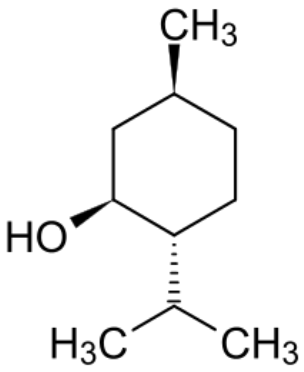
[1]Peralta-Yahya, et al *Nature Communications* 3, 483, 2011

The biosynthesis of (-)-menthol

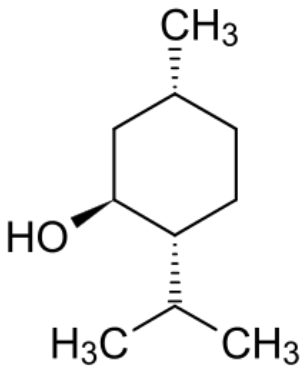


The many possible isomers of menthol

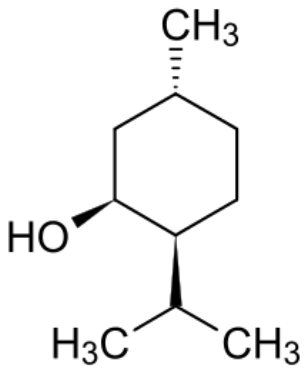
(+)-Menthol



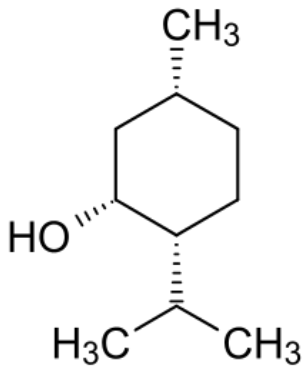
(+)-Isomenthol



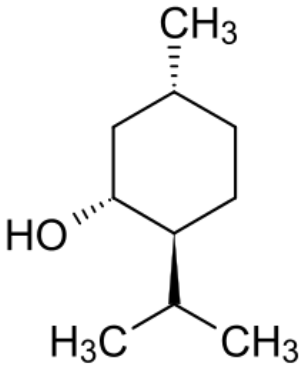
(+)-Neomenthol



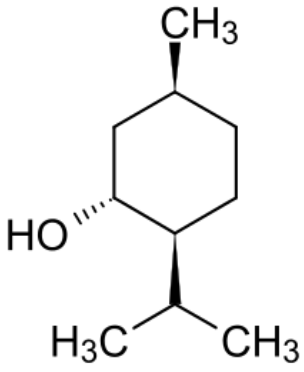
(+)-Neoisomenthol



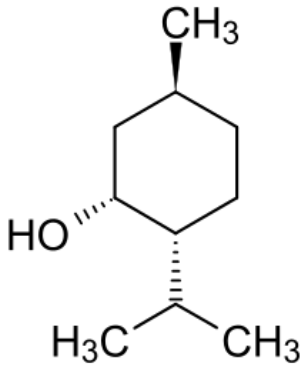
(-)-Menthol



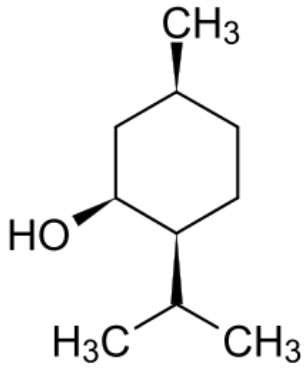
(-)-Isomenthol



(-)-Neomenthol



(-)-Neoisomenthol



Practical Applications and Interesting Facts about the MVA and MEP Pathways

- MEP is present in plants, algae and most bacteria
- MEP is not present in animals and fungi (they use MVA)

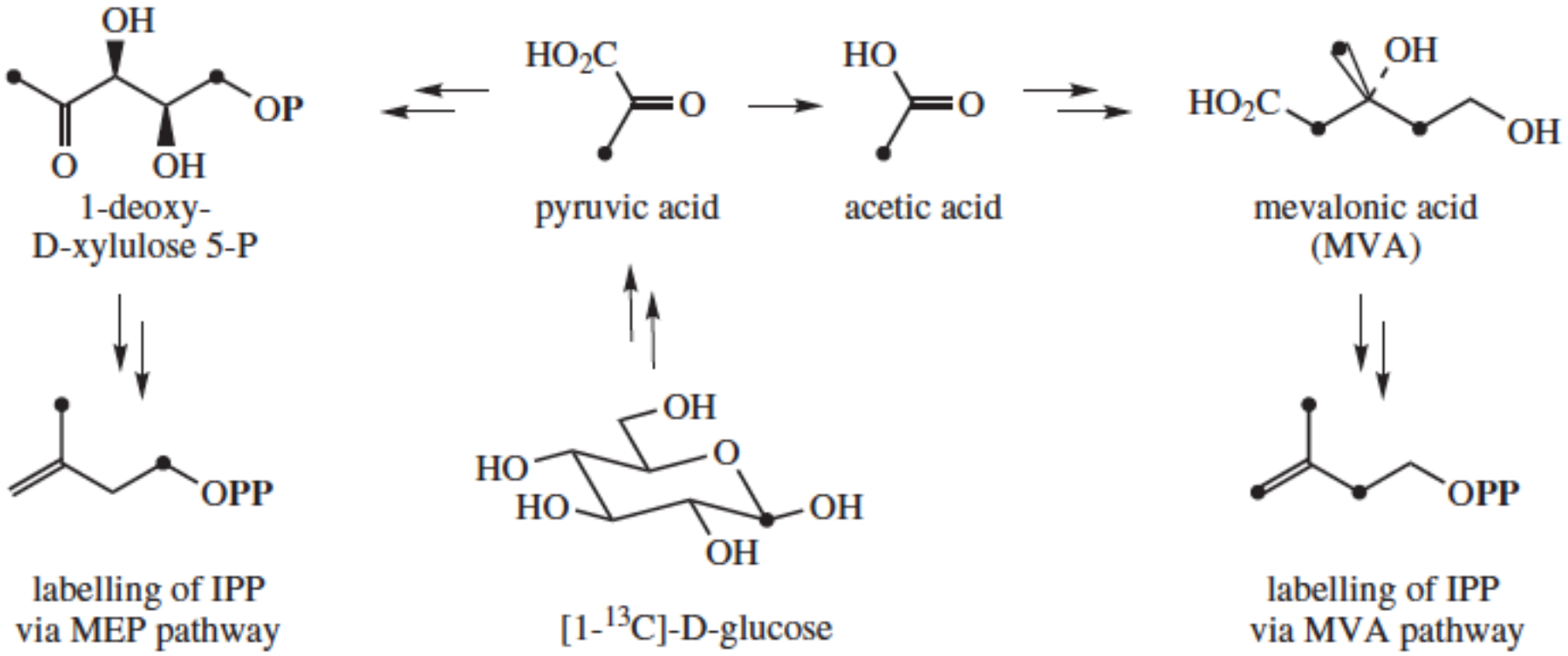
- MEP is an attractive target for microbial disease drugs since MEP pathway is utilized by pathogen but not found in humans

- Regulation of cholesterol (by statins) is achieved through inhibition of the HMG-CoA reductase enzyme found in the MVA pathway (animals)

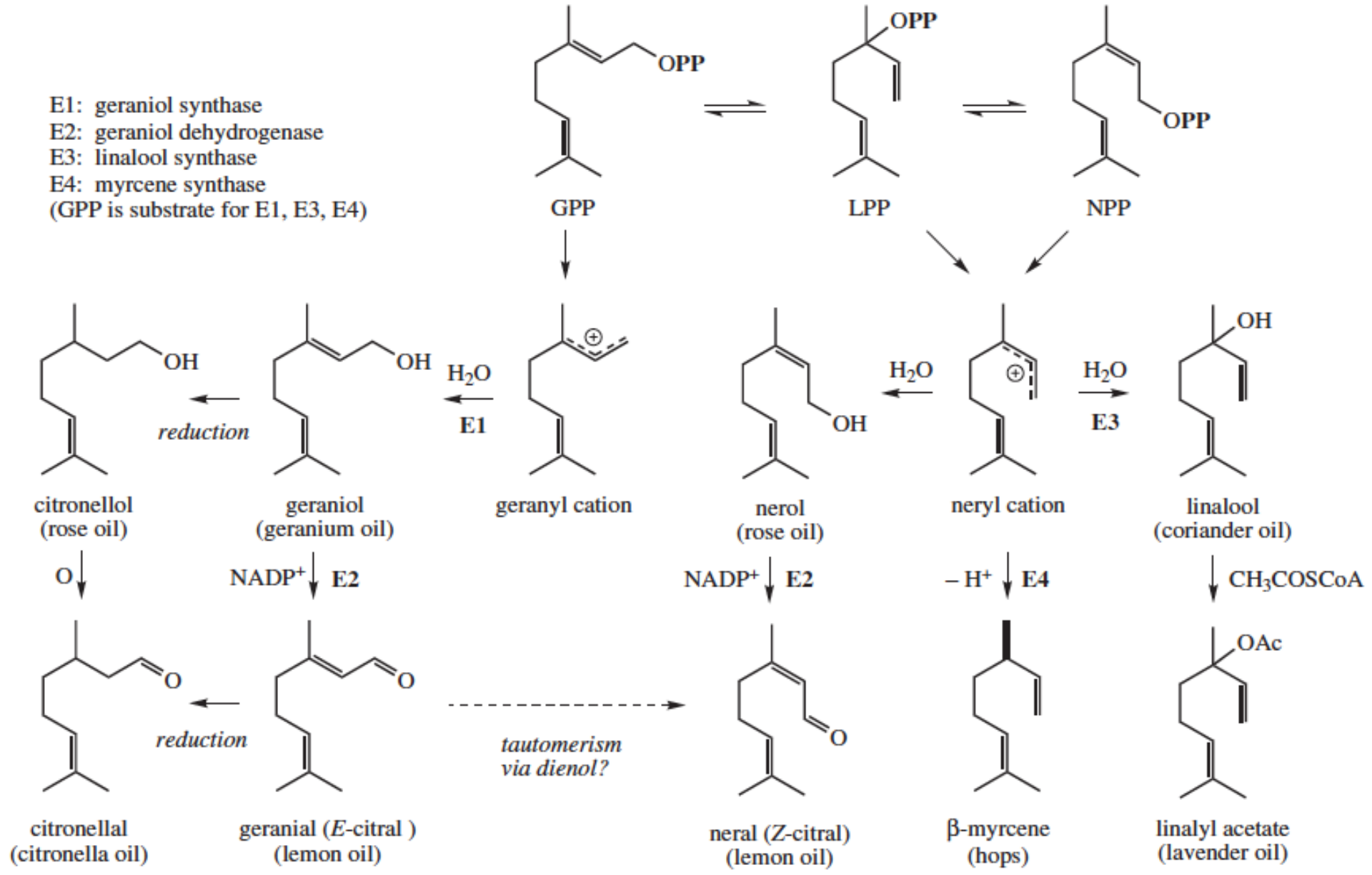
- Plants and some bacteria are equipped with, and employ both pathways, often concurrently

- Plants have compartmentalized production (MVA in cytosol, MEP in chloroplasts)

^{13}C Labeling used to Determine Which Pathway is Most Expressed (e.g. in Plants/Bacteria)



Different Mechanism, Different Smell



Steroids from Squalene (a triterpene)