ISOPRENOID METABOLISM IN HIGHER PLANTS

Plants contain an enormous range of isoprenoid compounds with a wide variety of structures and functions. Some are primary metabolites, e.g., the steroids, but the majority are synthesized as secondary metabolites that are uniquely plant products.
Isoprenoids have been known since antiquity as ingredients of perfumes, soaps, flavoring and as food colorants.

The isoprenoids are built up of C_5 isoprene units and the nomenclature of the main classes reflects the number of isoprene units present (Table 1).

Table 1. Main Classes of Isoprenoids found in Plants

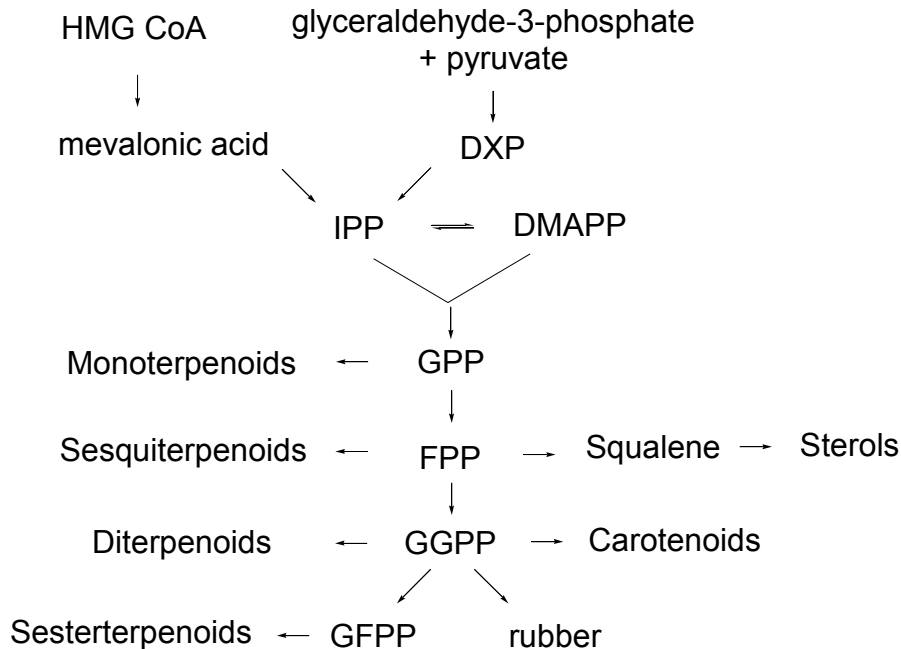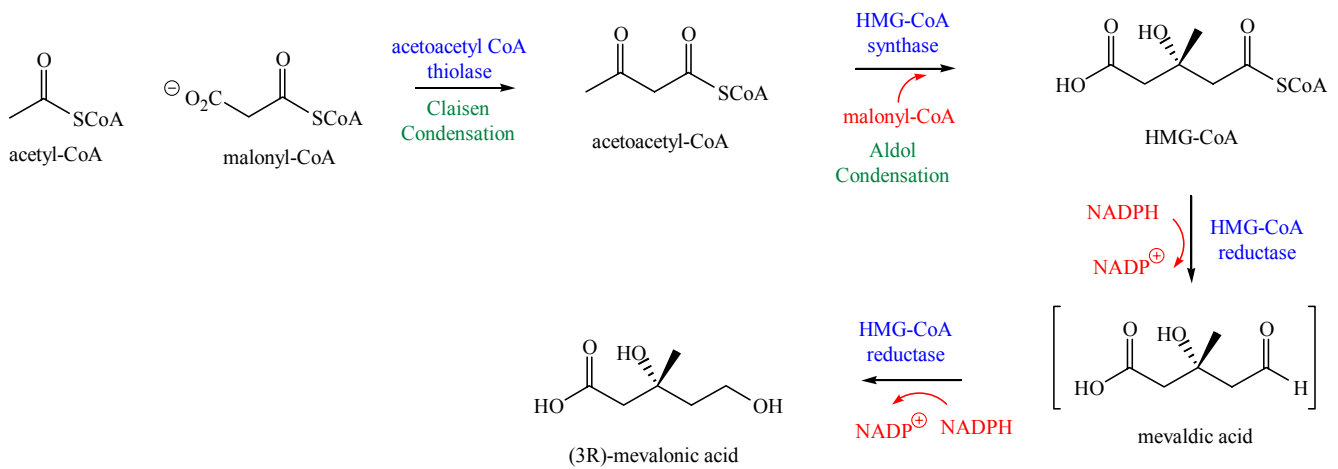| Carbon atoms	Name	Parent Isoprenoid
10	Monoterpenoids	GPP
15	Sesquiterpenoids	FPP
20	Diterpenoids	GGPP
25	Sesterterpenoids	GFPP
30	Triterpenoids	Squalene
40	Tetraterpenoids	Phytoene
>40	Rubbers	GGPP + $(C_5)_n$

General Pathway of Terpenoid Biosynthesis
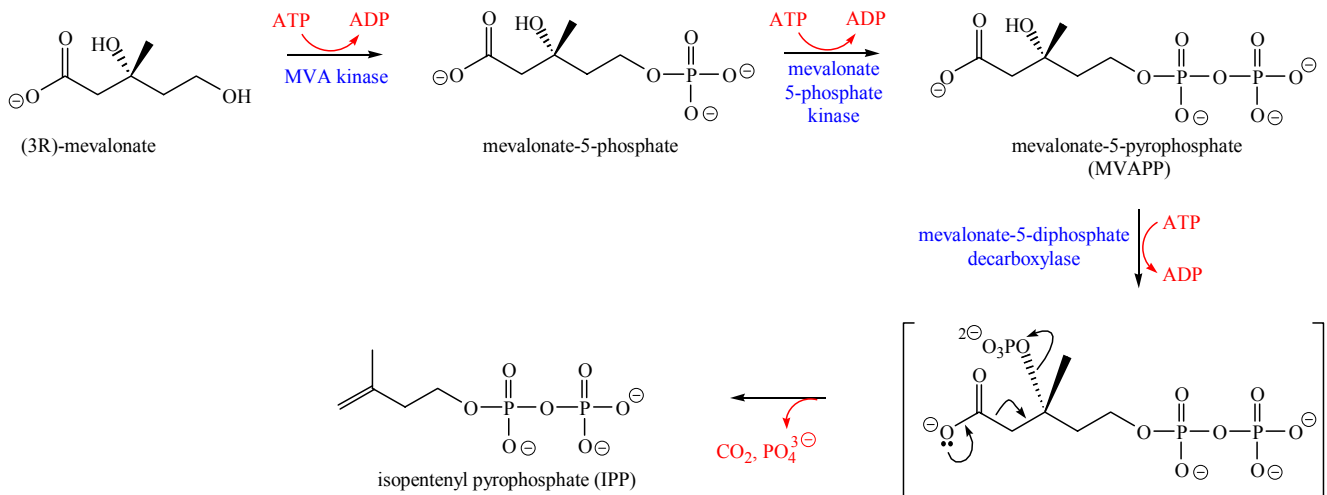

FIGURE 1.

MEVALONIC ACID PATHWAY

Biosynthesis of Mevalonic Acid
A molecule of acetyl-CoA undergoes a Claisen condensation with a molecule of malonyl-CoA (as in fatty acid biosynthesis; see Biochemistry of Yeast Fermentation – Fatty Acids) to form acetoacetyl-CoA which reacts with a third molecule of manonyl-CoA in an Aldol Condensation to give 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). Reduction with HMG-CoA reductase yields mevaldic acid which is reduced again to mevalonic acid.

acetyl-CoA malonyl-CoA

acetoacetyl CoA thiolase
Claisen Condensation

acetoacetyl-CoA

HMG-CoA synthase
malonyl-CoA
Aldol Condensation

HMG-CoA

NADPH
NADP$^{\oplus}$ **HMG-CoA reductase**

HMG-CoA reductase
NADP$^{\oplus}$ NADPH

(3R)-mevalonic acid

mevaldic acid

Conversion of Mevalonic acid to Isopentenyl pyrophosphate (3-methylbut-3-enylpyrophosphate)

Mevalonate is converted into 3-phospho-5-pyrophosphomevalonate by three consecutive phosphorylations. This labile intermediate loses CO_2 and phosphate and yields 3-isopentenyl pyrophosphate (IPP).

ATP ADP
MVA kinase

(3R)-mevalonate

ATP ADP
mevalonate 5-phosphate kinase

mevalonate-5-phosphate

mevalonate-5-pyrophosphate (MVAPP)

mevalonate-5-diphosphate decarboxylase
ATP
ADP

isopentenyl pyrophosphate (IPP)

CO_2, $PO_4^{3\ominus}$

$^{2\ominus}O_3PO$

DXP PATHWAY

Recently a second pathway to isopentenyl pyrophosphate was discovered and in 2002 this pathway was shown to occur in grapes.

Biosynthesis of 1-deoxy-D-xylulose 5-phosphate (DXP)

The first step in this pathway is the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) from pyruvate and glyceraldehyde 3-phosphate, both glycolysis intermediates. See Biochemistry of Yeast Fermentation; Sugars; Glycolysis.

pyruvate glyceraldehyde 3-phosphate

1-deoxy-D-xylulose 5-phosphate DXP
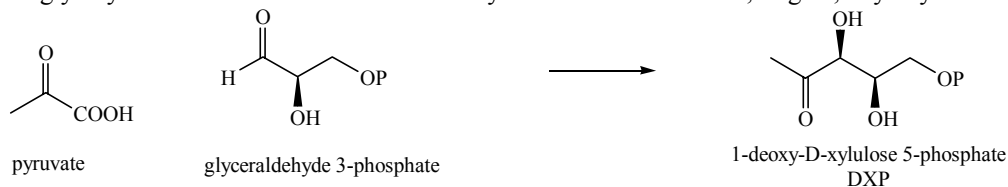
Pyruvate reacts with thiamine diphosphate (TPP) to form active acetaldehyde (TPP-C$_2$). See Biochemistry of Yeast Fermentation; Sugars; Alcoholic Fermentation. Active acetaldehyde then reacts with glyceraldehyde-3-phosphate to form DXP. See Figure 2.
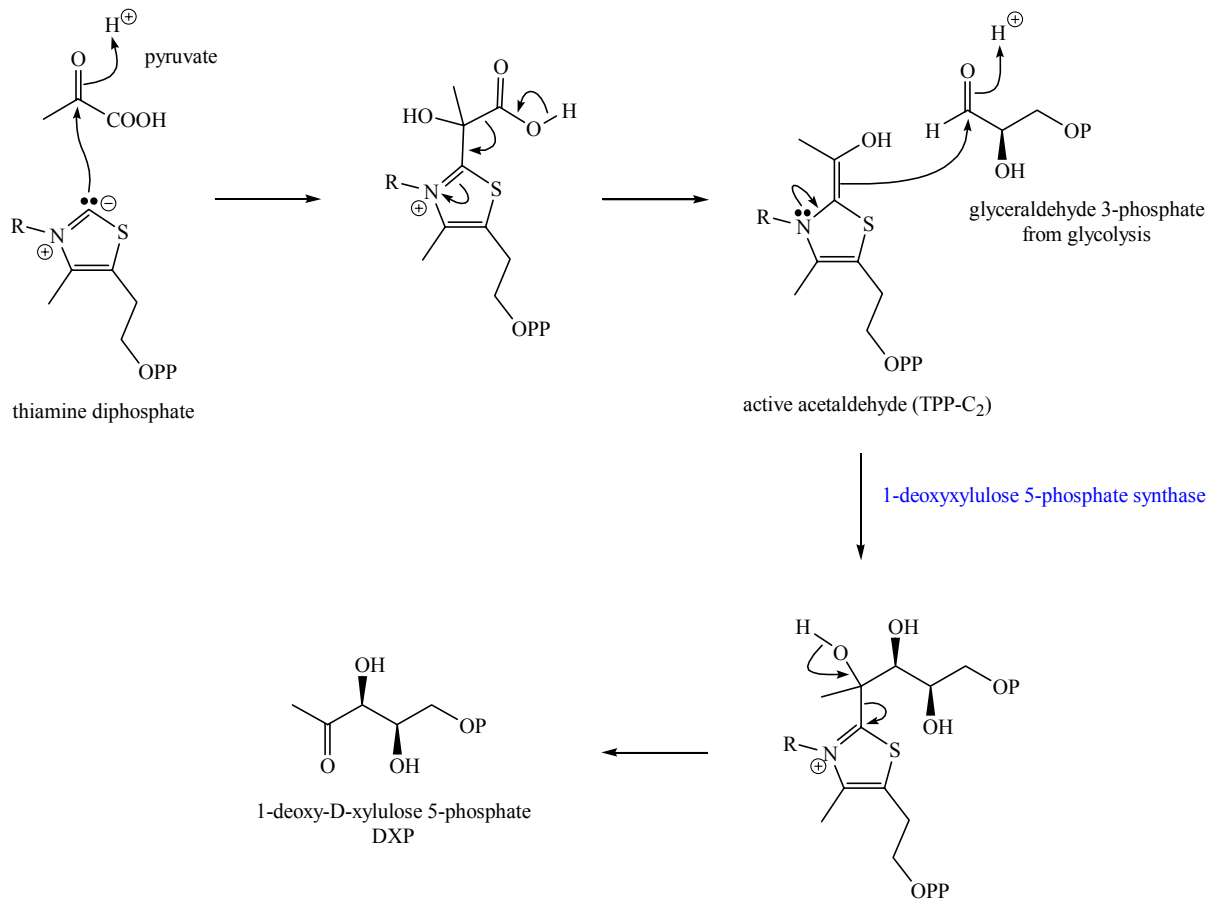
Biosynthesis of 1-deoxy-D-xylulose 5-phosphate (DXP)

FIGURE 2

Conversion of DXP to Isopentenyl pyrophosphate (3-methylbut-3-enylpyrophosphate)

DXP undergoes a pinacol-like rearrangement to form 2C-methyl-D-erythritol-4-phosphate, which reacts with cytidine triphosphate (CTP). After phosphorylation, an internal rearrangement takes place yielding 1,4-dihydroxy-3-methylbutan-2-one 1-phosphate. A series of steps yet to be elucidated produces ispentenyl diphosphate. See Figure 3.

1-deoxyxylulose
5-phosphate
DXP

2-C-methyl-D-erythritol
4-phosphate
MEP

1,4-dihydroxy-3-methylbutan-2-one
1-phosphate

isopentenyl diphosphate
IPP
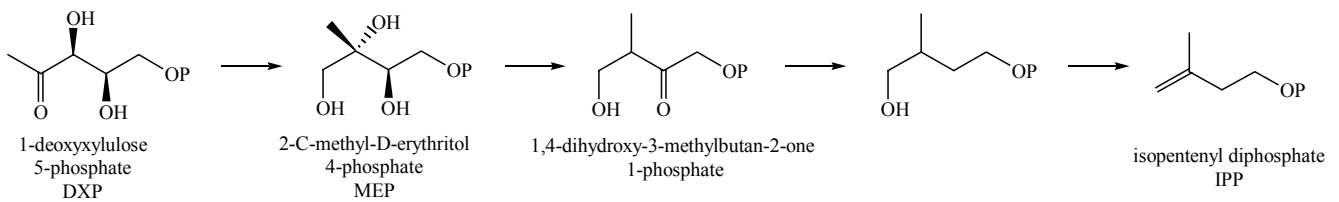
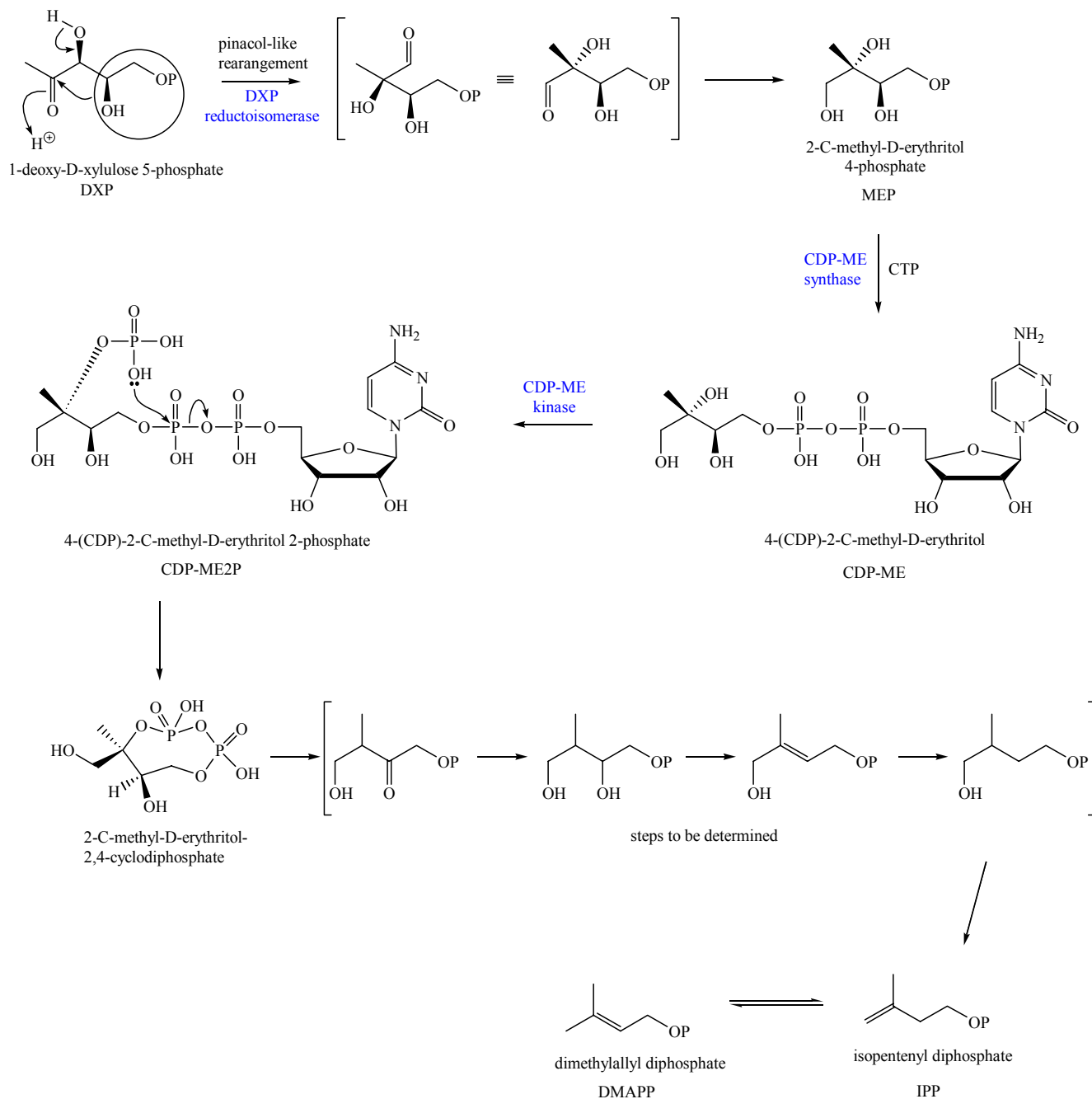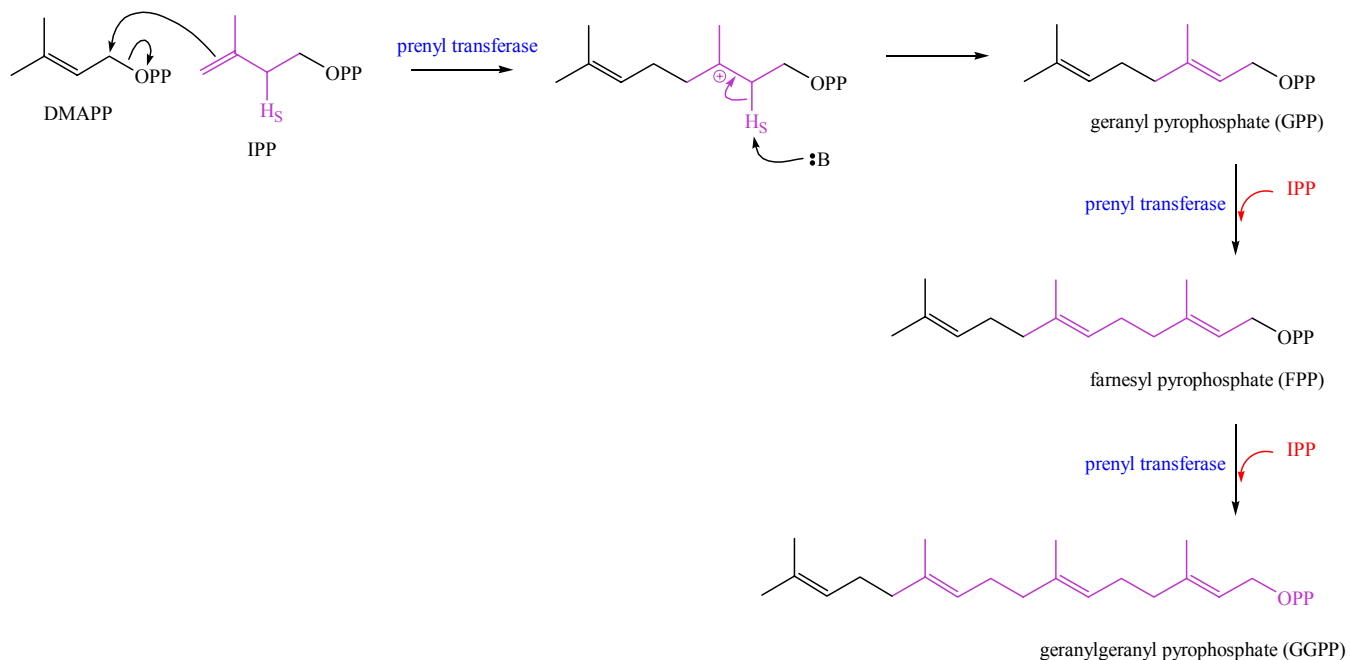# Conversion of DXP to Isopentenyl pyrophosphate

FIGURE 3

ISOMERIZATION OF ISOPENTENYL PYROPHOSPHATE AND PRENYL TRANSFERASE REACTIONS

IPP is isomerized to dimethylallyl pyrophosphate (DMAPP) with IPP isomerase. The isomerization involves addition of a proton to the sp^2 carbon of isopentenyl pyrophosphate and generation of the more stable tertiary carbocation. Elimination of a proton yields the most stable alkene (trisubstituted, Zaitev's Rule).

isopentenyl pyrophosphate (IPP)

most stable carbocation

dimethylallyl pyrophosphate (DMAPP)
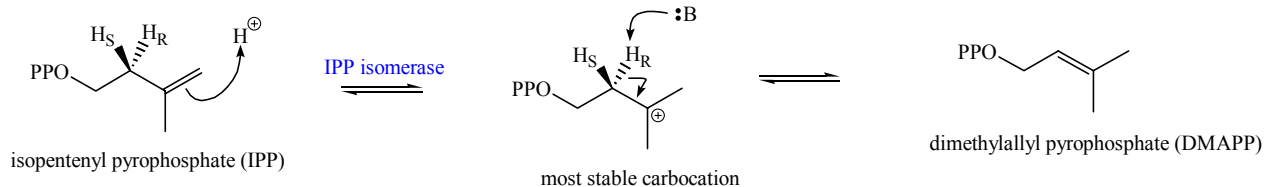
Next IPP reacts with DMAPP to give geranyl pyrophosphate (GPP). In the first step of the reaction, IPP acts as a nucleophile and displaces a pyrophosphate group from DMAPP. Pyrophosphate is an excellent leaving group. Its four OH groups have pKa values of 0.9, 2.0, 6.6, and 9.4. Therefore three of the four groups will be primarily in their basic forms at physiological pH (pH 7.3). A proton is removed in the next step, again yielding the most stable alkene. GPP rearranges to give the monoterpenes. GPP reacts with a second molecule of IPP to produce farnesyl pyrophosphate (FPP). FPP is the precursor to the sesquiterpenoids, and the steroids. FPP reacts with a third molecule of IPP to produce geranylgeranyl pyrophosphate (GGPP). GGPP is the precursor of the carotenoids.


DMAPP

IPP

prenyl transferase

geranyl pyrophosphate (GPP)

prenyl transferase   IPP

farnesyl pyrophosphate (FPP)

prenyl transferase   IPP
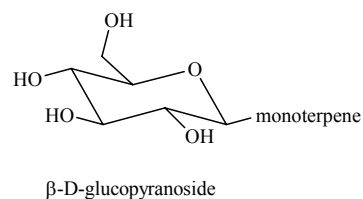
geranylgeranyl pyrophosphate (GGPP)
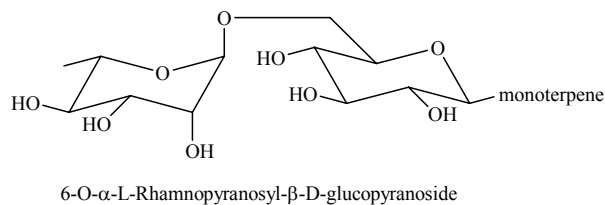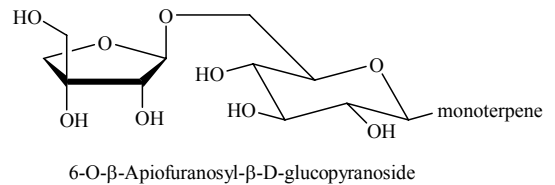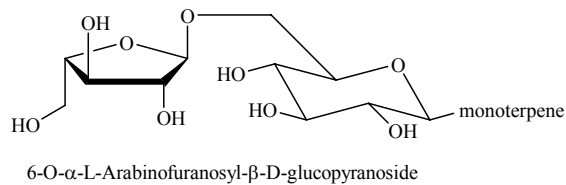
MONOTERPENOIDS

Interest in the monoterpenes originated due to their use in perfumes and as food flavors. About forty monoterpene compounds have been identified in grapes. Some of the monoterpene alcohols have a strong odor, especially geraniol, nerol, citronellol, linalol, α-terpineol, wine lactone, rose oxide and hotrienol, which has a floral aroma reminiscent of rose essence. The olfactory perception thresholds of these compounds are rather low, as little as a few hundred micrograms per liter. The most odoriferous are rose oxide and wine lactone. Furthermore, the olfactory impact of monoterpene compounds is synergistic. Geraniol and linalol play an important role in the aromas of grapes and wines from the Muscat family (Muscat á Petits Grains, Muscat of Alexandria, Muscat of Ottonel and Muscat d'Alsace), as concentrations are often well above the olfactory perception thresholds.

These compounds also play a role in the Muscat aroma of some Alsatian and German grape varieties: Gewürztraminer (rose oxide and wine lactone), Pinot Gris, Riesling, Auxerrois, Scheurebe, MuIler-Thurgau, etc. However, monoterpenes are only partially responsible for the varietal aromas of these wines and do not explain all of the nuances.

Figure 4 shows the biosynthesis of the important monoterpenes skeletons found in grapes.

geraniol nerol citronellol linalool

hotrienol α-terpineol wine lactone rose oxide
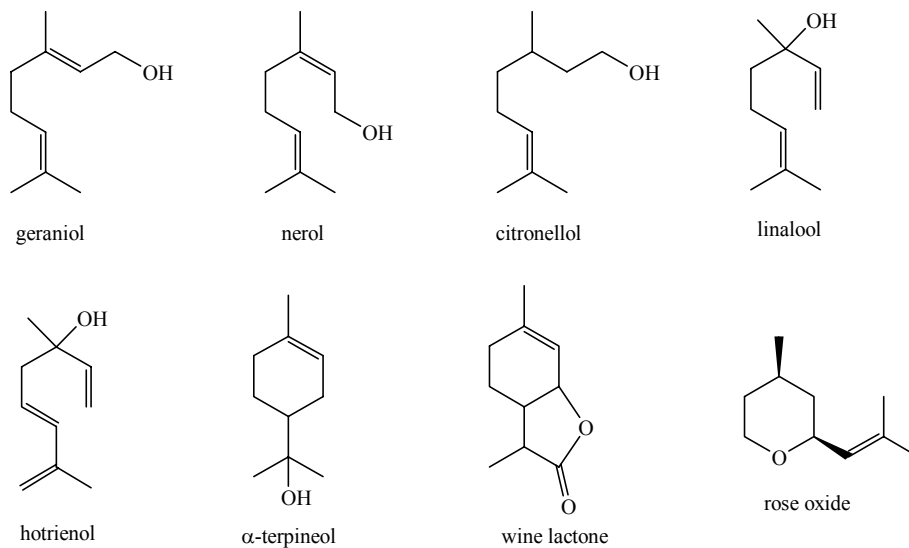
As with the anthocyanidins, most of the monoterpenes found in grape juice are present as glycosides. The main glycoside is the glucoside (i.e. β-D-glucopyranose attached to the monoterpene) but three disaccharides are also present: 6-O-α-L-arabinofuranosyl-β-D-glucopyranose, 6-O-α-rhamnosyl-β-D-glucopyranoside and 6-O-β-D-glucopyranoside.

6-O-α-L-Arabinofuranosyl-β-D-glucopyranoside

6-O-β-Apiofuranosyl-β-D-glucopyranoside

6-O-α-L-Rhamnopyranosyl-β-D-glucopyranoside

β-D-glucopyranoside

Formation of the Major Monoterpenes in Wine from Geranyl pyrophosphate

geraniol

citronellol

DMAPP

IPP

geranyl diphosphate

neryl diphosphate

nerol

linalyl cation

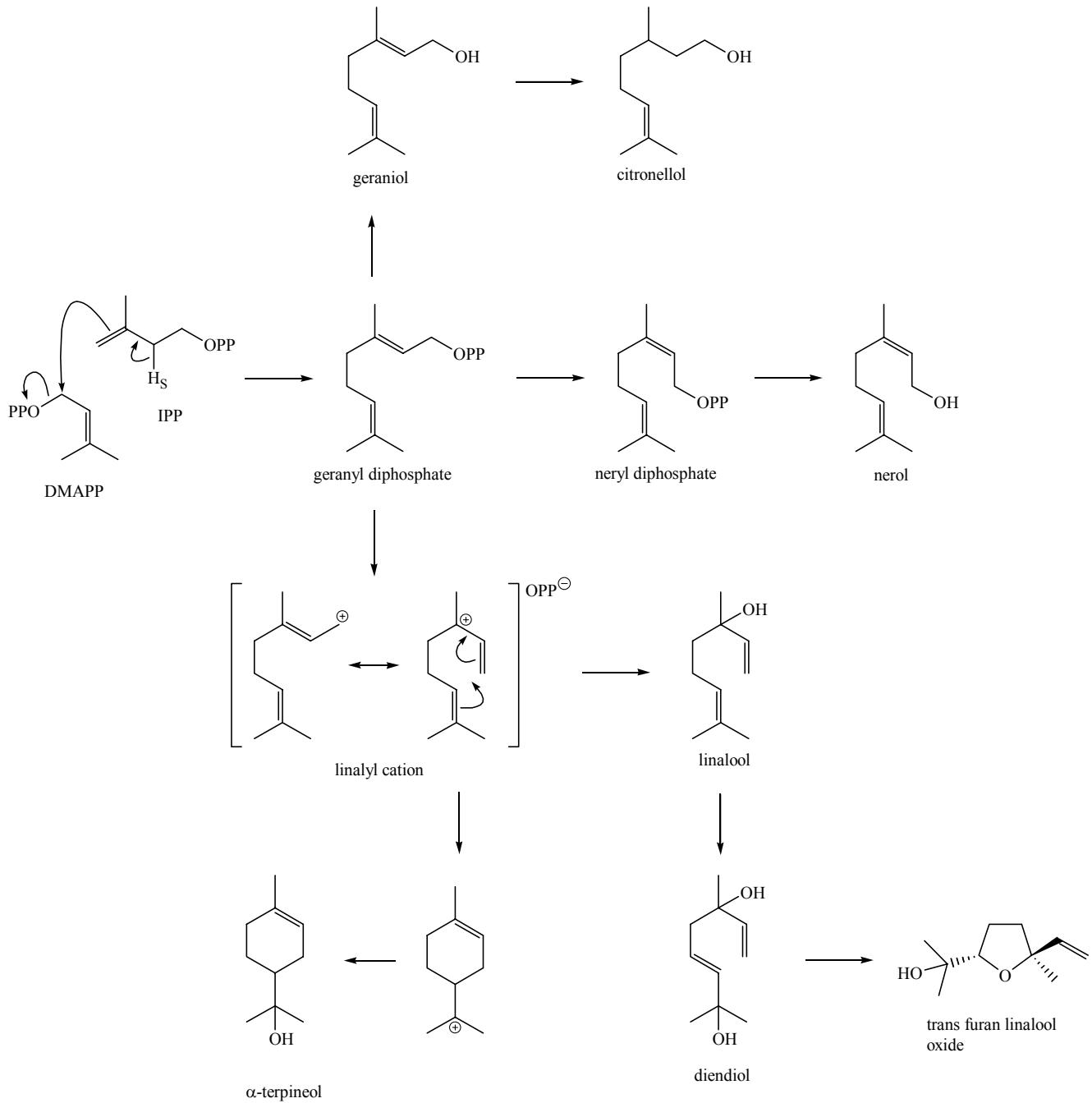linalool

α-terpineol

diendiol

trans furan linalool oxide

FIGURE 4.

CAROTENOIDS

The carotenoids are an abundant group of naturally occurring pigments, present in all green tissues, where they are constituents of the chloroplast, as well as being responsible for most of the yellow to red colors of flowers and fruits. Carotenoid hydrocarbons are called carotenes, whereas derivatives containing oxygen functions are the xanthophylls.

Unripe green fruit contains the same pigments as other photosynthetic tissues but upon ripening the chloroplasts differentiate into chromoplasts (storage organelles for pigments) and there is often, but not always, *de novo* synthesis of carotenoids.

In the grape, the concentrations of the carotenoids decrease steadily from bloom on, and at the same time the concentrations of the C_{13}-norisoprenoids steadily increase. Exposure of grapes to sunlight during ripening accelerates carotenoid breakdown and in accompanied by an increase in the glycosylated C_{13}-norisoprenoid derivative content (glycosides).

The most important carotenoids in grapes, in decreasing order, are lutein, β-carotene, neoxanthin and lutein-5,6-epoxide.

lutein

β-carotene

neoxanthin

lutein-5,6-epoxide
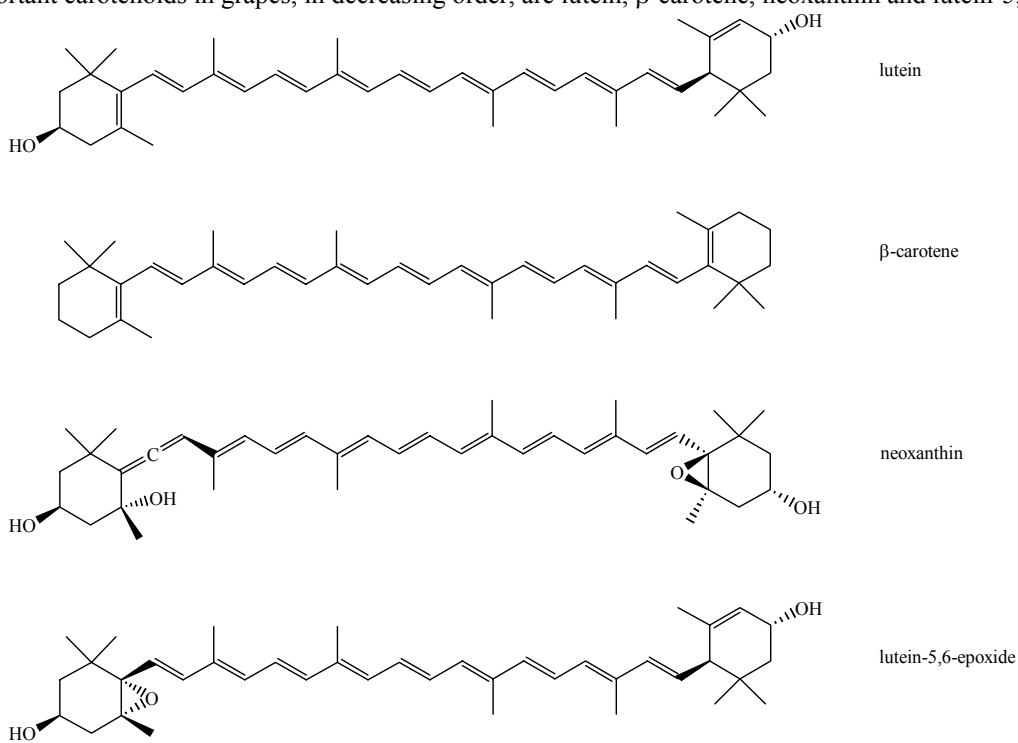
The first dedicated step in the formation of carotenoids is the head-to-head condensation of two molecules of all-*trans* geranylgeranyl pyrophosphate (GGPP) via the cyclopropyl carbonyl pyrophosphate, prephytoene pyrophosphate (PPPP) to form phytoene. See Figure 5.

The Formation of 15-*cis*-phytoene from GGPP
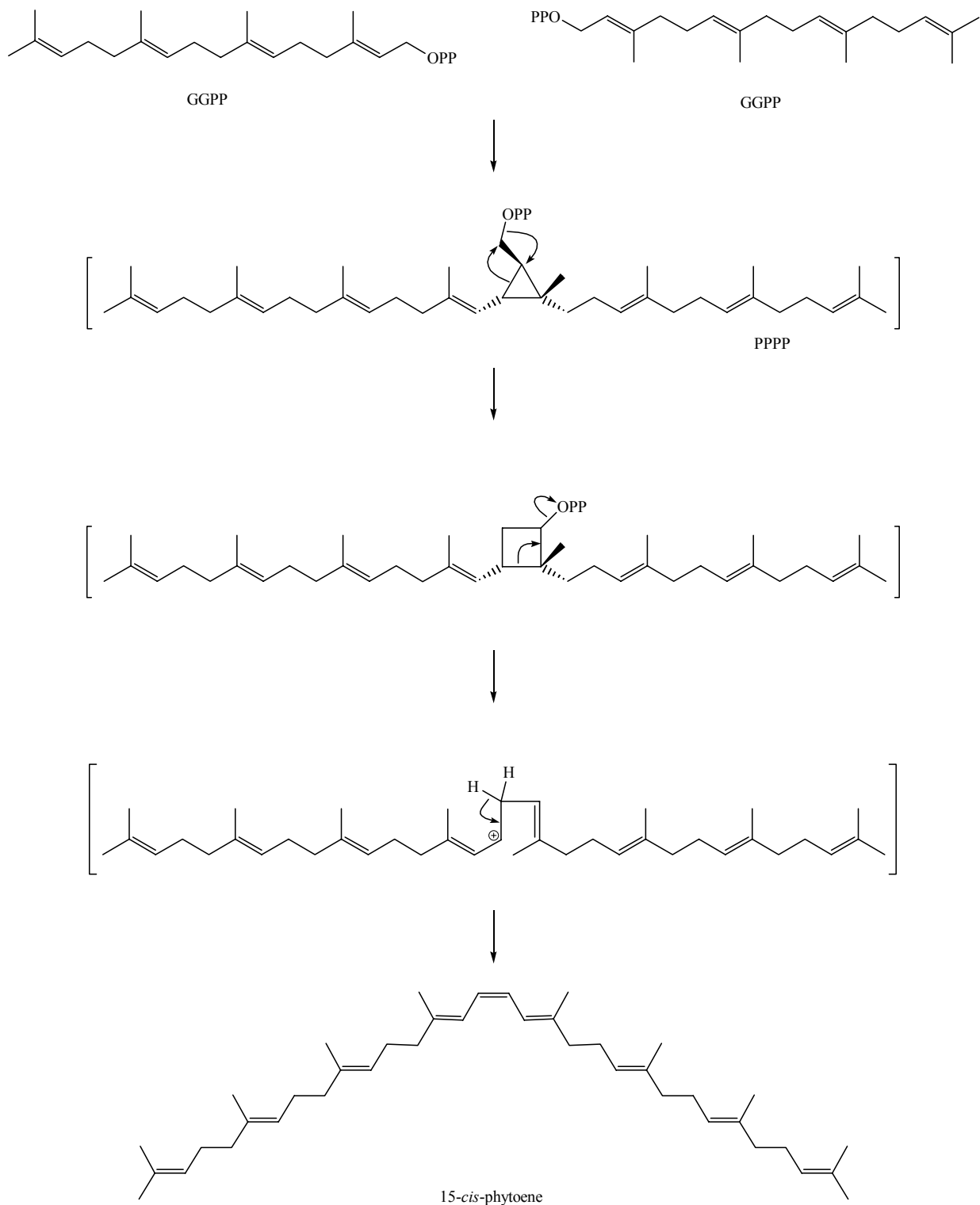
FIGURE 5.

The sequence from phytoene to lycopene involves four stepwise dehydrogenations, occurring alternatively to either side of the chromophore to form phytofluene, ξ-carotene, neurosporene and lycopene. In addition, in higher plants, the 15-15′ double bond is isomerized from the cid to the trans configuration. See Figure 6.

The Formation of Lycopene from 15-cis-Phytoene
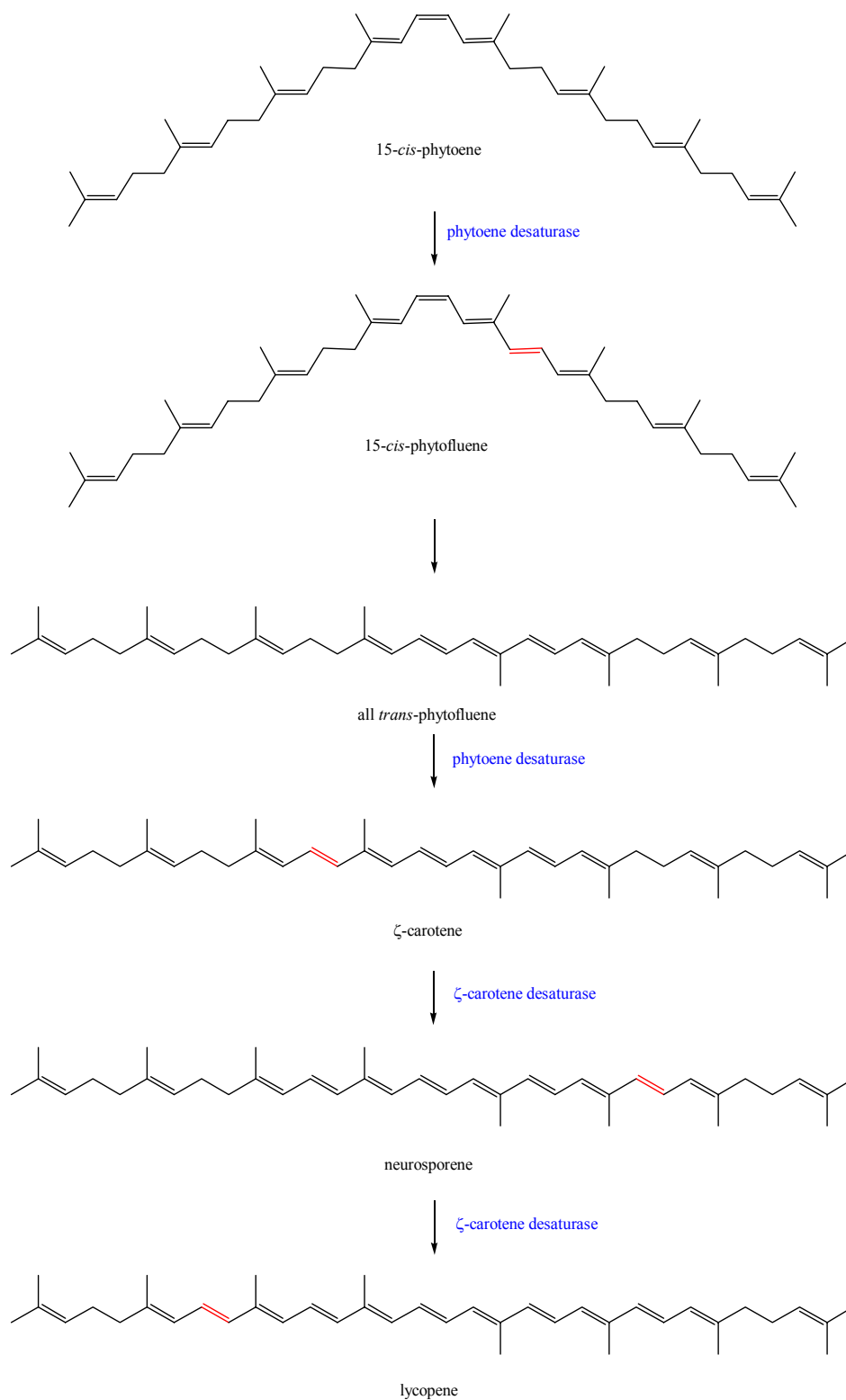

FIGURE 6.

The cyclohexane-ring end-groups on many of the carotenoids are formed independently.

The Formation of β-Carotene and the Xanthophylls
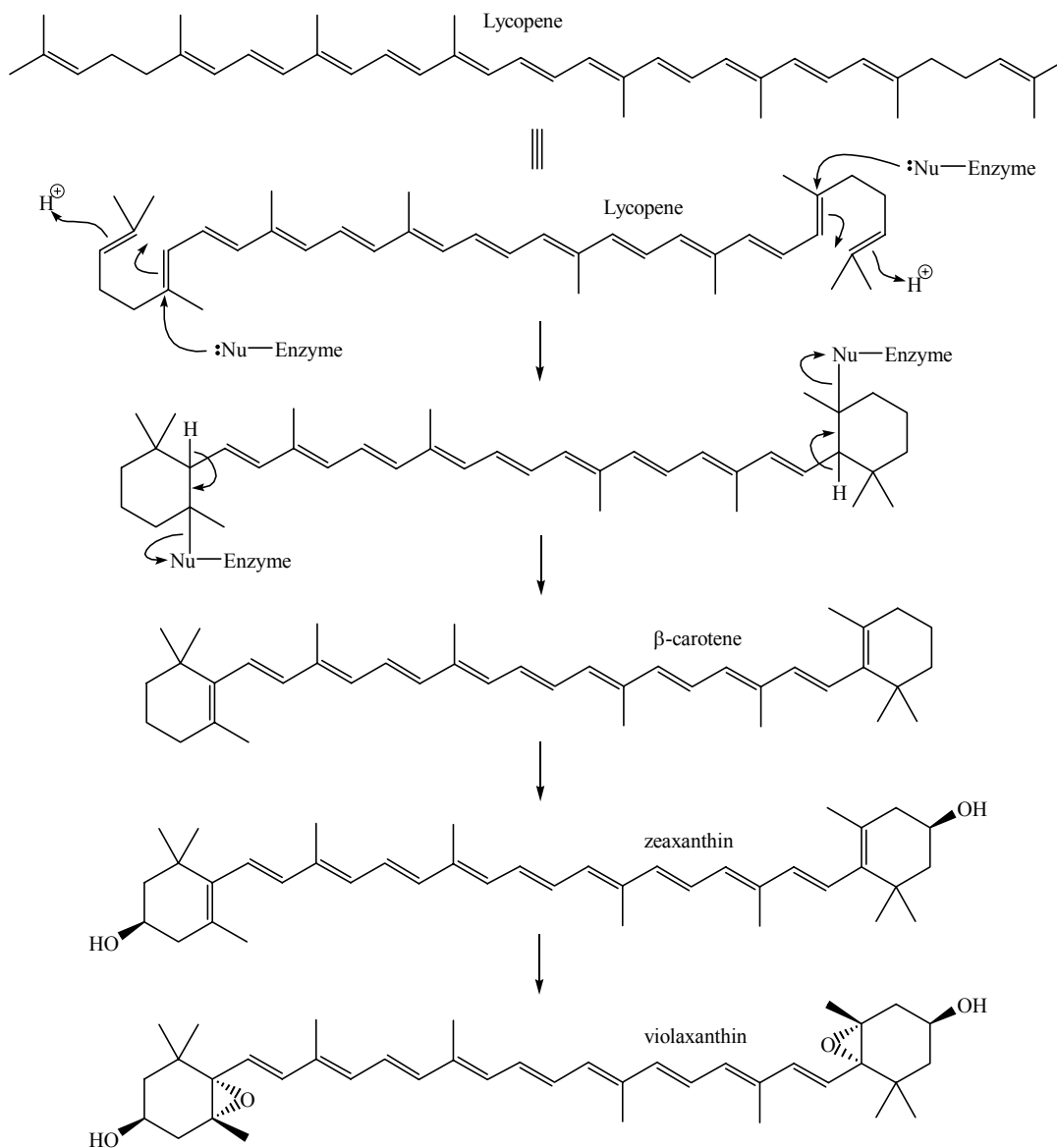

FIGURE 7.

α-Carotene can result from the dehydration and enzyme release step taking place at one of the cyclohexane end-groups via the removal of a proton on the other side of the carbon which has been attacked by the enzymic nucleophile.

The Formation of α-Carotene and the resulting Xanthophylls
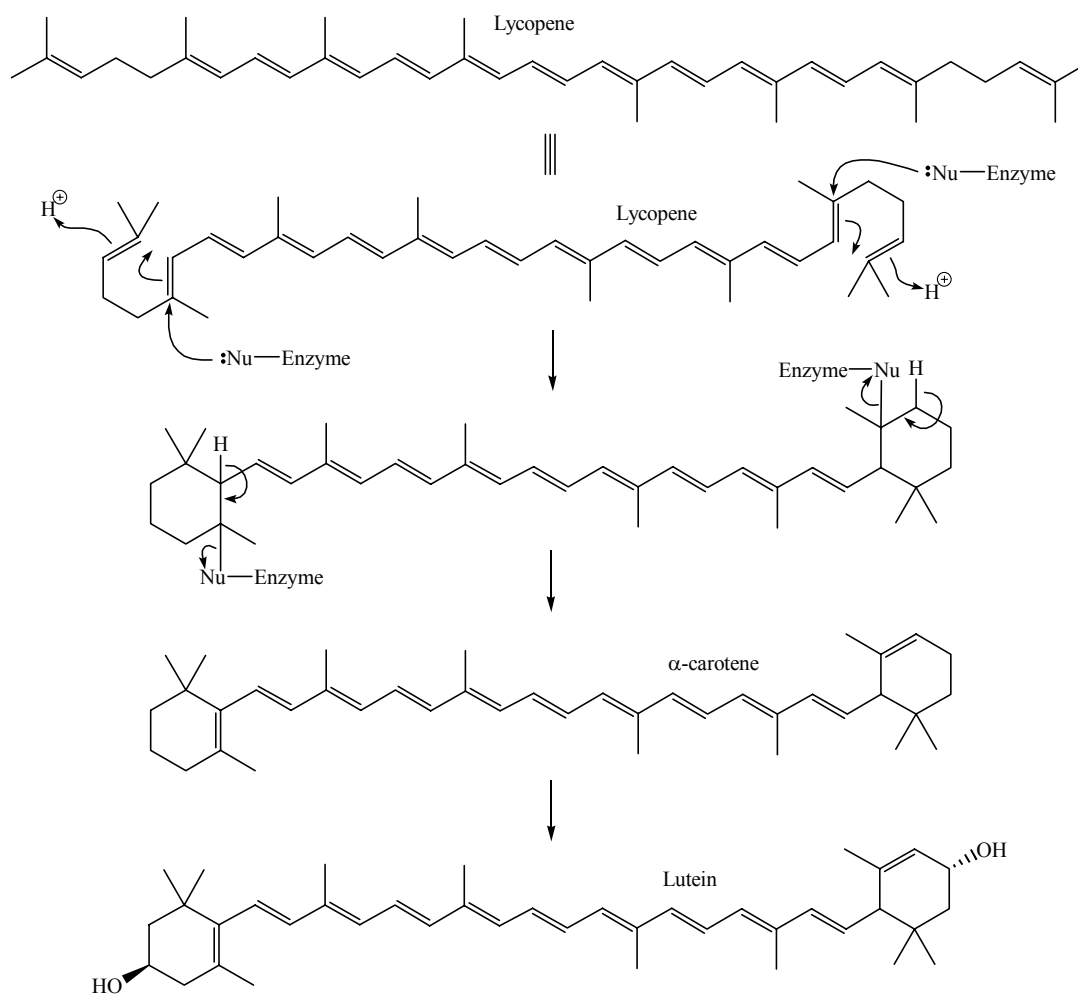
FIGURE 8.

C₁₃-NORISOPRENOIDS

A vast number of apparently carotenoid-derived substances have been identified in plant extracts, but knowledge of the biochemistry of carotenoid catabolism is still extremely limited. The *in vivo* cleavage of the carotenoid chain is generally considered to be catalyzed by dioxygenase systems. Although all the in-chain double-bonds seem to be vulnerable to enzymatic attack, thus resulting in the formation of major fragment classes with 10, 13, 15 or 20 carbon atoms, in fruit tissues a bio-oxidative cleavage of the 9,10 (9',10') double bond seems to be the most preferred. This last cleavage reaction creates a large number of aroma products.
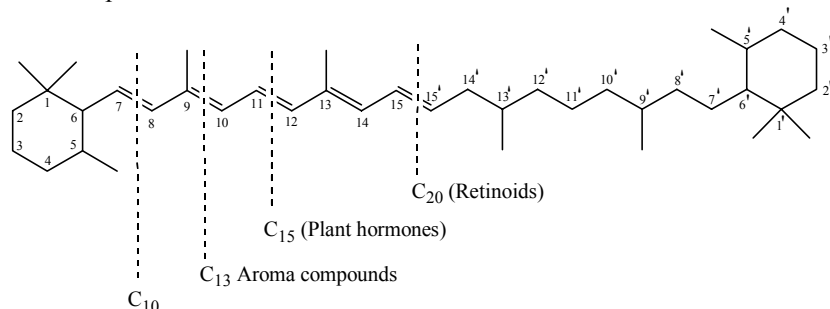
The three essential steps for the formation of C13-norisoprenoid aroma compounds can therefore be considered to consist of
(i) the initial dioxygenase cleavage,
(ii) the subsequent enzymatic transformation(s) of the primary degradation product in natural tissues, and
(iii) formation of the flavorless glycoside.
During winemaking, acid-catalyzed conversion of the glycoside into an aroma compound occurs. Figure 9 shows how the carotenoid, neoxanthin may yield two C₁₃-norisoprenoid glycosides in this way, which during the winemaking process produce the two powerful odorants, vitispirane and damascenone.

Glycosylation of any secondary metabolite will significantly increase its water solubility and may occur for a number of reasons. Some of these may be for
(i) accumulation and storage,
(ii) transport,
(iii) protect the plant from any toxicity of the liphophilic compounds (e.g., the cyanogenic glycosides are hydrolyzed by β-glucosidase releasing toxic cyanide as a defense mechanism),
(iv) stability of compound (the anthocyanidins are more stable as their glycosides, the anthocyanins).

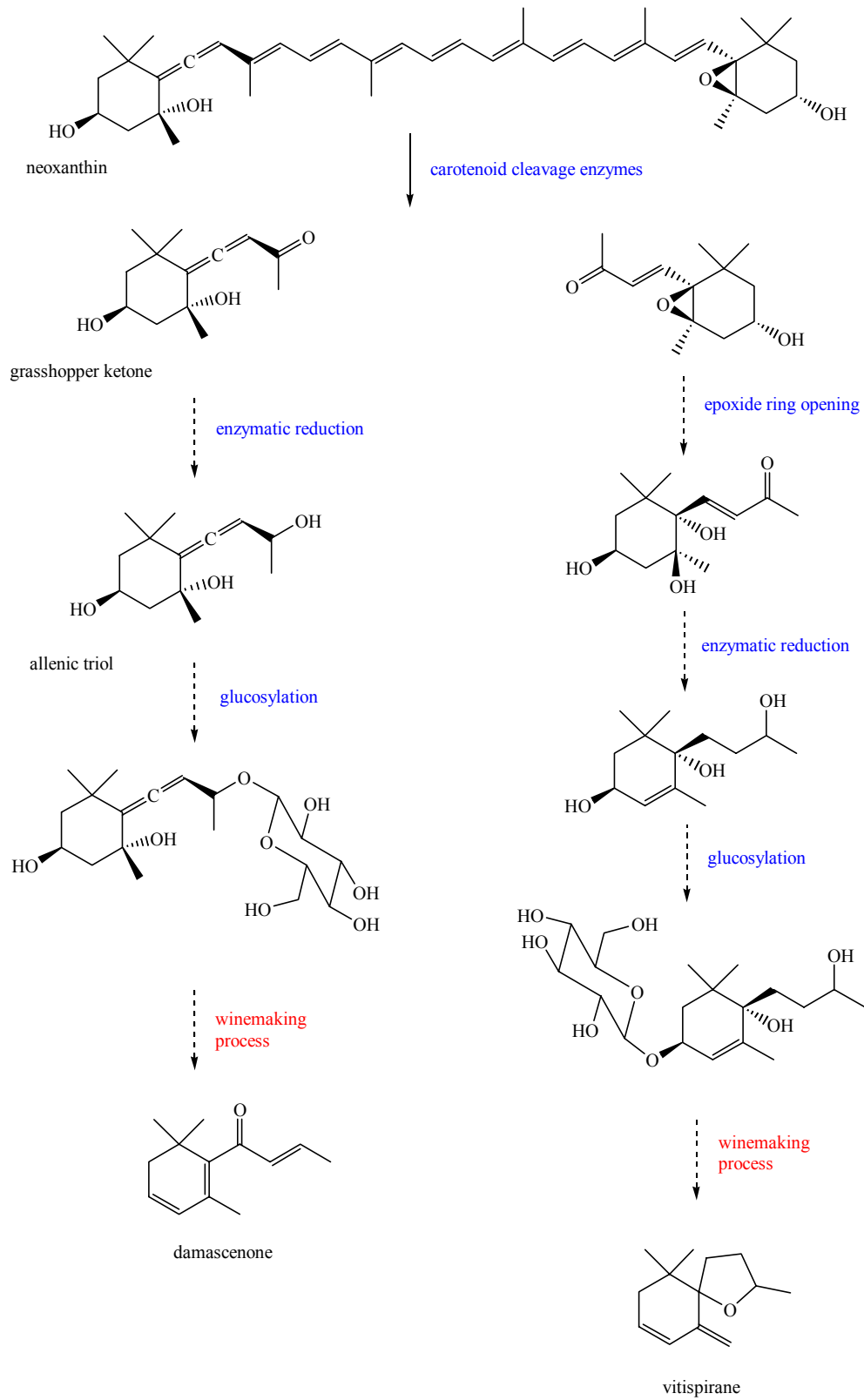# Formation of Two C₁₃-Norisoprenoid Glycosides from the Carotenoid Neoxanthin

FIGURE 9.