

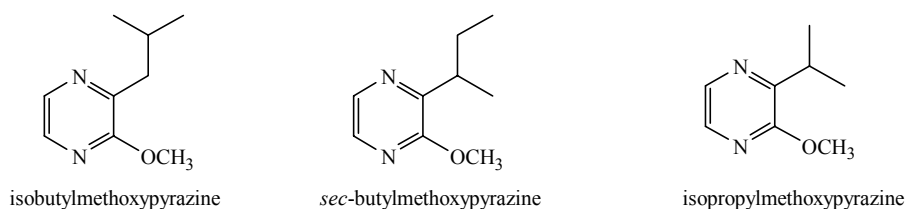
VARIETAL AROMA COMPOUNDS

We will now look at three classes of very powerful odorants that contribute to the varietal character of wines; these are the Methoxypyrazines, thiols with a pleasant odor and the C₁₃-norisoprenoids.

METHOXPYRAZINES

Climate is generally considered to have paramount importance to wine flavor. Sauvignon blanc grapes and wines have characteristic herbaceous or vegetative aroma. Cabernet sauvignon wines are characterized by berry and vegetative aromas. Winemakers have known for decades that young vines in cool climates and vigorous vines with dense canopies tend to yield very intensely vegetative wines.

Methoxypyrazines are grape-derived flavor compounds that contribute a very characteristic vegetative, herbaceous, bell pepper or earthy aroma to Cabernet sauvignon, Sauvignon blanc and Semillon wines. Three Methoxypyrazines, 2-methoxy-3-isobutylpyrazine, 2-methoxy-3-*sec*-butylpyrazine and 2-methoxy-3-isopropylpyrazine provide this aroma.



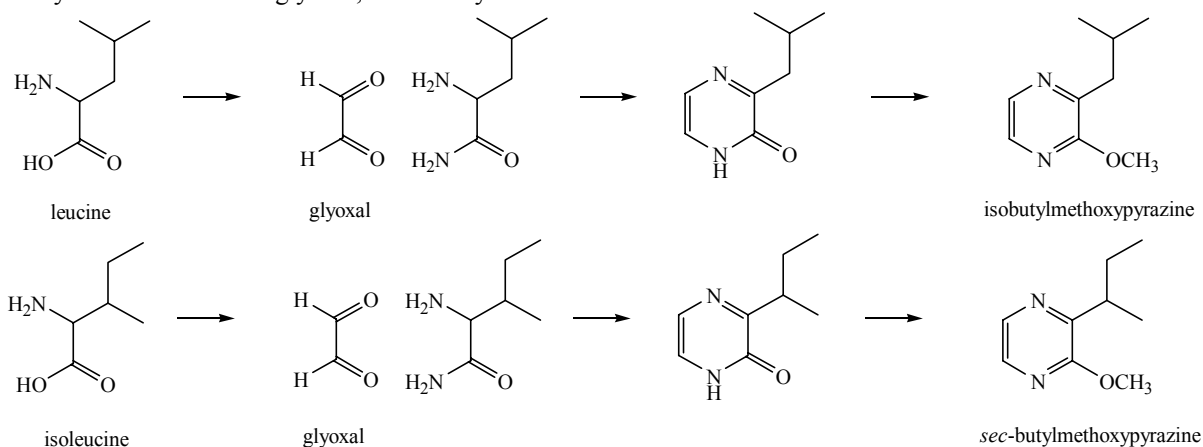
All three are very powerful odorants with extremely low thresholds in water as shown below.

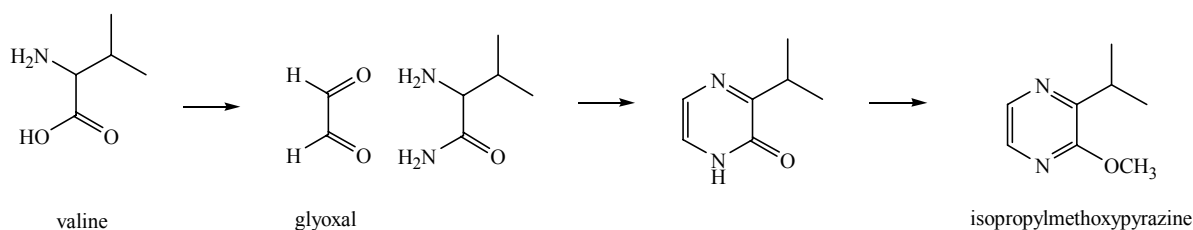
3-Alkyl-2-methoxypyrazine	Threshold (ng/L)
3-isobutyl-2-methoxypyrazine	2
3- <i>sec</i> -butyl-2-methoxypyrazine	1
3-isopropyl-2-methoxypyrazine	2

In Cabernet sauvignon, Sauvignon blanc and Semillon wines the normal concentration of isobutylmethoxypyrazine is about 15-20 ng/L or ten times its threshold concentration, whereas the *sec*-butylmethoxypyrazine and isopropylmethoxypyrazine concentrations are at or near their thresholds.

Biosynthesis

A proposed pathway for the biosynthesis of the alkylmethoxypyrazines involves amidation of the appropriate amino acid, followed by condensation with glyoxal, then methylation.





Viticultural Factors that Determine the Concentrations of Methoxypyrazines

Studies of the occurrence of isobutylmethoxypyrazine have shown that it has a consistent and systematic relationship to the grape variety and the vine growing conditions. While Cabernet Sauvignon, Sauvignon blanc and Semillon produce significant isobutyl-methoxypyrazine levels, some other cultivars do not seem to produce this compound at all. In a comparative study of different grape varieties, using vines within the same vineyard, high levels of isobutylmethoxypyrazine were evident in Cabernet Sauvignon and Sauvignon blanc grapes, but Pinot noir showed no detectable methoxypyrazine at either véraison or normal harvesting maturity.

With increasing grape berry maturity there is a profound decrease in the concentration of isobutylmethoxypyrazine. Comparatively high levels, often over 100 ng/L, are present at véraison in the fruit of Cabernet Sauvignon and Sauvignon blanc grape varieties. However, these levels fall very rapidly in the early stages of ripening, and they can be less than 1% of the véraison concentration by the time of harvesting.

Ripening temperature also has an impact. At comparable stages of fruit ripeness, substantially higher methoxypyrazine levels occur in cool regions by comparison with warm regions. In warm areas, the level of isobutylmethoxypyrazine can fall well below its sensory detection threshold by the time of fruit harvesting, whereas in cool areas it can be 20-30 ng/L.

The influence of the vine canopy and the pruning and training system can also be very important. Particularly as fruit exposure to light influences the methoxypyrazine level significantly. Within the vine canopy, the more exposed fruit provides a consistently lower level of methoxypyrazines than the more shaded fruit, typically half or less of the level of that in the most shaded fruit within the canopy.

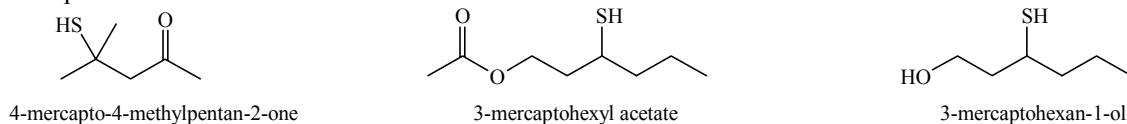
Concentration in Wine.

Recognition of the character of methoxypyrazine aroma as herbaceous or vegetative occurred at 4-8 ng/L in white wine, but it is clear from work with Sauvignon blanc wines that 30 ng/L is often considered to be overpowering and out of balance. This indicates a surprisingly narrow concentration window for methoxypyrazines if they are to contribute usefully to wine flavor. The desirable range in Sauvignon blanc wines appears to be 8-15 ng/L. A similar situation is indicated for Cabernet Sauvignon wines. Analysis of a range of Cabernet Sauvignon-based red wines has indicated a concentration range of isobutyl-methoxypyrazine of 7-15 ng/L in Bordeaux wines with good flavor balance and 27-29 ng/L in some wines that were showing distinct methoxypyrazine aroma.

SULFUR COMPOUNDS

Humans are particularly sensitive to certain forms of sulfur. For example, thiols are especially potent; some have the lowest aroma thresholds of any food odorant.

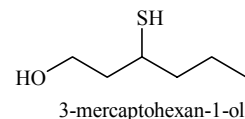
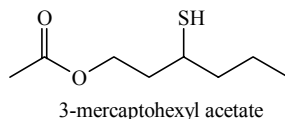
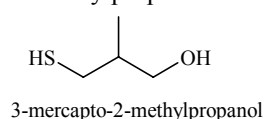
Sauvignon blanc is one of the most widely grown *Vitis vinifera* L. grape varieties in the world. Its wines have a characteristic aroma usually described as green pepper, box tree, broom, grapefruit, passion fruit and smoke. The compound responsible for the green pepper character, 3-isobutyl-2-methoxypyrazine is more marked if the grapes are under-ripe. Three sulfur compounds are involved in the varietal aroma as they are usually in concentrations greatly in excess of their respective perception thresholds. Two sulfur compounds reminiscent of box tree and broom have been identified, 4-methyl-4-mercaptopentan-2-one and 3-mercaptohexyl acetate; the third, 3-mercaptohexan-1-ol has an aroma of citrus zest, grapefruit and passion fruit.



Odorous Thiols in Sauvignon blanc

Thiol	Aqueous Odor Threshold (ng/L)	Concentration in Wines (ng/L)	OAV	Odor Description
4-methyl-4-mercaptopentan-2-one	0.8	4-25	5-30	box tree, broom
3-mercaptohexyl acetate	4	200-800	50-200	box tree, broom, passionfruit
3-mercaptohexan-1-ol	60	700-13,000	12-220	grapefruit

Cabernet sauvignon and Merlot, which are widespread varieties all over the world and notably in the region of Bordeaux give wines with fruity or floral notes. 3-Mercaptohexan-1-ol and 3-mercaptohexyl acetate along with another thiol, 3-mercapto-2-methylpropanol can contribute to this aroma.

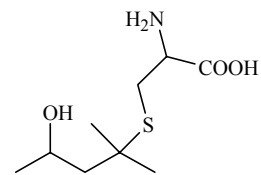
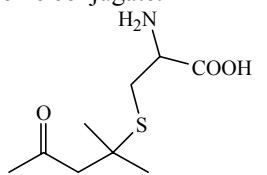
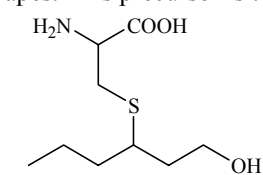


Odorous Thiols in Cabernet sauvignon

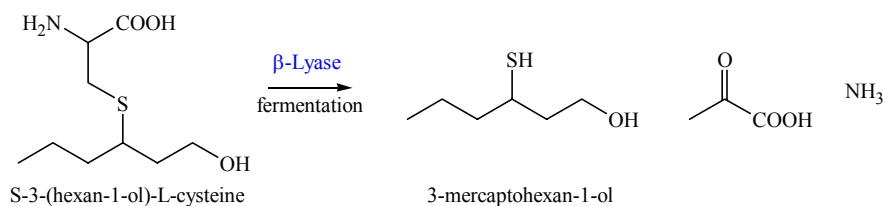
Thiol	Aqueous Odor Threshold (ng/L)	Concentration in Wines (ng/L)	OAV	Odor Description
3-mercaptohexyl acetate	4	1-200	0.25-50	box tree, broom, passionfruit
3-mercaptohexan-1-ol	60	10-5000	0.2-80	grapefruit
3-mercapto-2-methylpropanol	3000	250-10,000	0.1-3	broth, sweat

The concentrations of these thiols in Sauvignon blanc are invariably much greater than their thresholds (OAV's) and they contribute significantly to the aroma of the wines. In the Bordeaux reds, they are less important, but can still play a significant role.

Sauvignon blanc musts, like those of many grape varieties are not highly odiferous. The characteristic aroma of the grape variety appears during alcoholic fermentation. The thiols are formed during alcoholic fermentation from odorless precursors present in the grapes. This precursor is the S-cysteine conjugate.

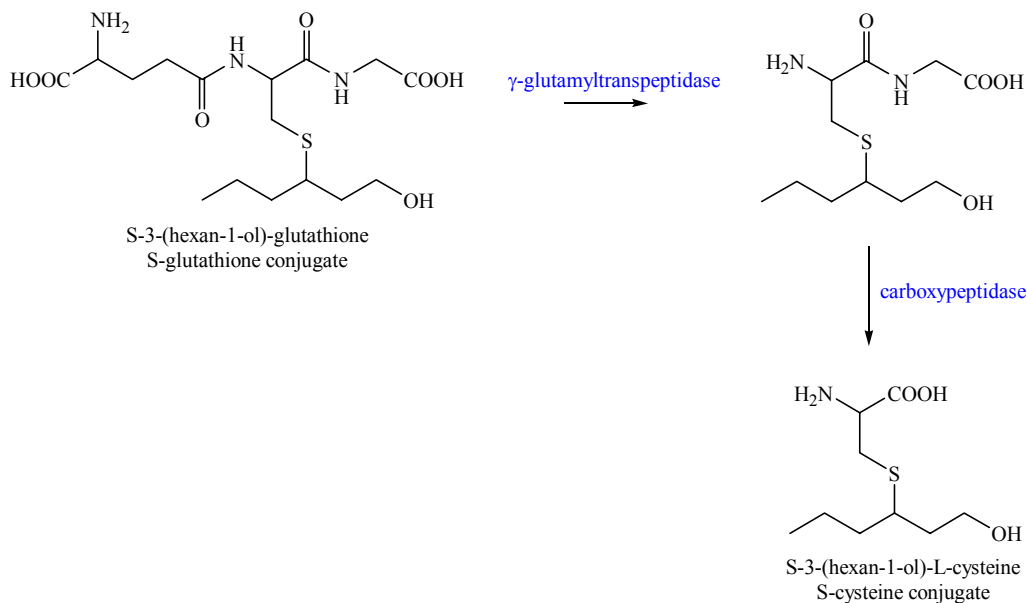


These aromas vary in intensity depending on the *Saccharomyces cerevisiae* yeast strain used for fermentation. The S-cysteine conjugate is hydrolyzed by β -lyase produced during fermentation to the thiol.

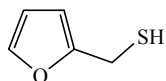


Synthesis

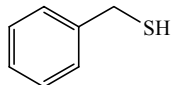
S-Glutathione conjugates are involved in the detoxification systems of living organisms and the S-cysteine conjugates can be produced by the breakdown of the corresponding S-glutathione conjugate. S-3-(hexan-1-ol)-glutathione has been identified in Sauvignon blanc musts.



Recently (2001) two thiols have been identified in wines, 2-furanmethanethiol with a roast coffee aroma and benzenemethanethiol with a strong empyreumatic aroma reminiscent of smoke. Empyreum refers to being in a fire that is usually smoky and hotter than the boiling point of water. Neither 2-furanmethanethiol nor benzenemethanethiol can be considered to be a varietal compound, but they are included here as they both are very powerful sulfur odorants.



2-Furanmethanethiol



Benzenemethanethiol

Both compounds have very low thresholds:

	Threshold (ng/L) in model wine	Aroma Description
2-furanmethanethiol	0.4	strong roast coffee
benzenemethanethiol	0.3	strong empyreumatic aroma

Synthesis

Furfurylthiol is formed during **alcoholic fermentation in the barrel** from furfural released by toasted staves (See Oak Barrel Aging of Red Wines; Volatile Compounds and Toasting). It has been found in red Bordeaux wines (Merlot, Cabernet franc and Cabernet sauvignon) in concentrations of 2 -25 ng/L and in Petit Manseng wines 31-62 ng/L.

Fermentation conditions favorable to an excess production of H₂S by the yeast promote the formation of this volatile thiol. A high sulfur content (cysteine, sulfates) and relatively low nitrogen levels favor the production of HS⁻ in excess of the amounts required for protein synthesis.

Benzenemethanethiol was found in white wines (Sauvignon blanc, Semillon, Chardonnay) with OAV's of 30-100. It is probably produced from benzaldehyde. Benzaldehyde is always present in wine and is produced by alcoholic fermentation in concentrations up to 0.5 mg/L.

ISOPRENOIDS

Monoterpenes

The monoterpenes are present in many grape varieties; the majority present as the conjugate glycoside. Usually the concentrations are below the thresholds, but sometimes in some varieties monoterpenes may contribute to the varietal bouquet, as exemplified in the table below for Muscat and Gewürztraminer. The monoterpenes with concentrations in green have OAV's > 1.

Monoterpenes	Odor Threshold (µg/L)	Muscat (µg/L)	Gewürztraminer (µg/L)	Riesling (µg/L)	Sauvignon blanc (µg/L)
linalool	50	453	6	40	17
α-terpineol	400	78	3	25	9
citronellol	18	ND	12	4	2
nerol	400	94	43	23	5
geraniol	130	506	218	35	5
hotrienol	110	ND	ND	25	ND
rose oxide	0.2		21		
wine lactone	0.01		0.10		

See Isoprenoid Metabolism in Higher Plants; Monoterpenoids; Figure 4.

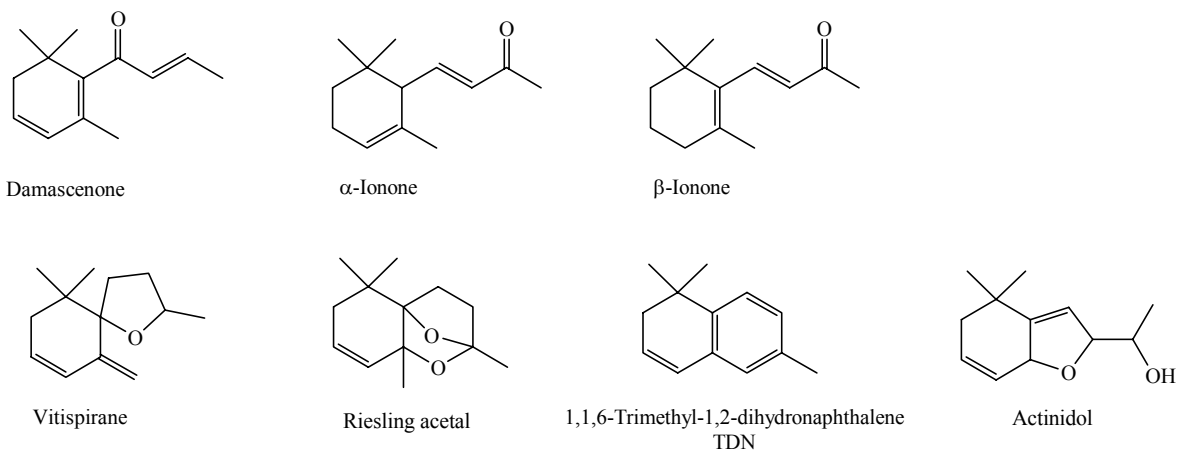
C-13 Norisoprenoids

The norisoprenoids are formed by oxidation of carotenoids with dioxygenase enzyme systems, and are stored as glycosides. This occurs during the véraison to maturity stage of the grapes development. See Isoprenoid Metabolism in Higher Plants; Carotenoids & C-13 Norisoprenoids.

Riesling wine has been found to have at least 40 norisoprenoids in glycoconjugated form demonstrating that these compounds are the most numerous and abundant terpenoids in Riesling and probably in most wines. The glycosides, on mild acid hydrolysis, are capable of generating many volatiles, among which are the flavor constituents damascenone, vitispirane, Riesling acetal and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN).

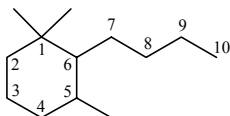
Many of the norisoprenoids are powerful odorants with pleasant odors, usually described as flowery and fruity, though some have camphoraceous notes. A few studies have separated enantiomers (when they exist) and each enantiomer has been found to have a different odor. Figure 2 provides examples of some of the strong C13-Norisoprenoid Odorants.

Figure 2: Some C13-Norisoprenoid Odorants



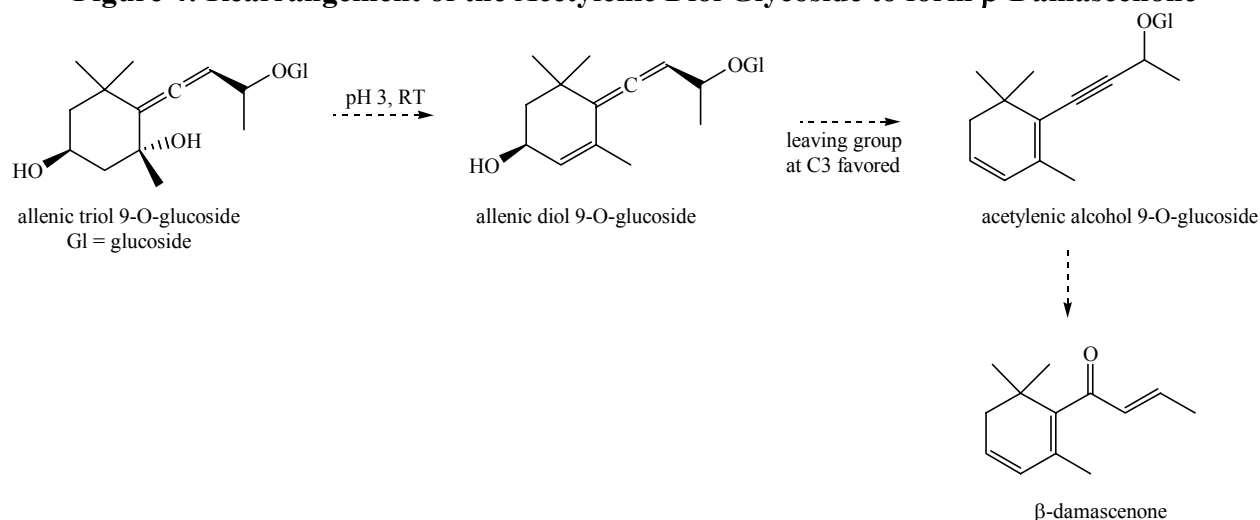
β-Damascenone

β-Damascenone is found in most wines, often above its threshold concentration of 2 ng/L. It has a narcotic scent reminiscent of exotic flowers with a heavy fruity undertone and is described as apple, rose and honey. It is considered to be derived from the carotenoid, neoxanthine and its odorless precursor, megastigm-6,7-dien-3,5,9-triol-9-O-β-D-glucopyranoside (allenic triol 9-O-glucoside) is stored in the grape (see Figure 3). After crushing grapes, the pH of the must is about 3.2 and these acidic conditions (which continue thru fermentation and aging of the wine) are sufficient to cause rearrangements to occur.

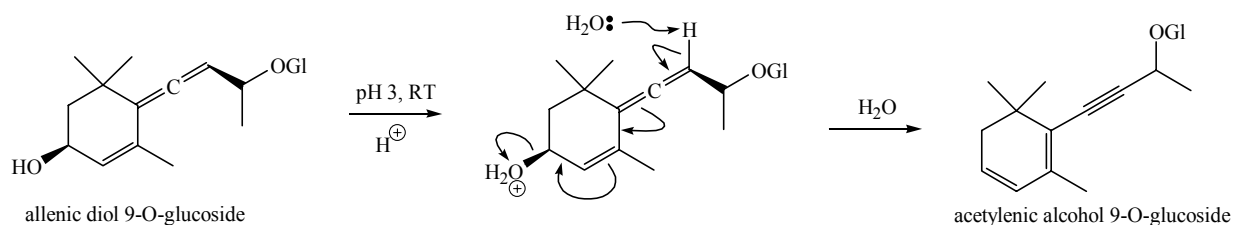


First the allenic triol 9-O-glucoside loses water, by elimination of the C4 hydrogen and C5 hydroxyl to form megastigm-4,6,7-trien-3,9-diol 9-O-glucoside (allenic diol 9-O-glucoside). See Figure 4.

Figure 4: Rearrangement of the Acetylenic Diol Glucoside to form β-Damascenone



If the C9 alcohol is protected (as a glucoside), then attack by a water molecule on the allenic diol 9-O-glucoside can occur at C8 with the C3 hydroxyl acting as a leaving group, yielding the acetylenic alcohol 9-O-glucoside.



The acetylenic alcohol has been synthesized and when added to a model wine, β-damascenone was recovered in greater than 90% yield.

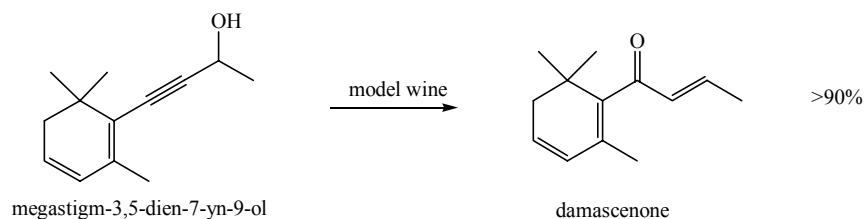
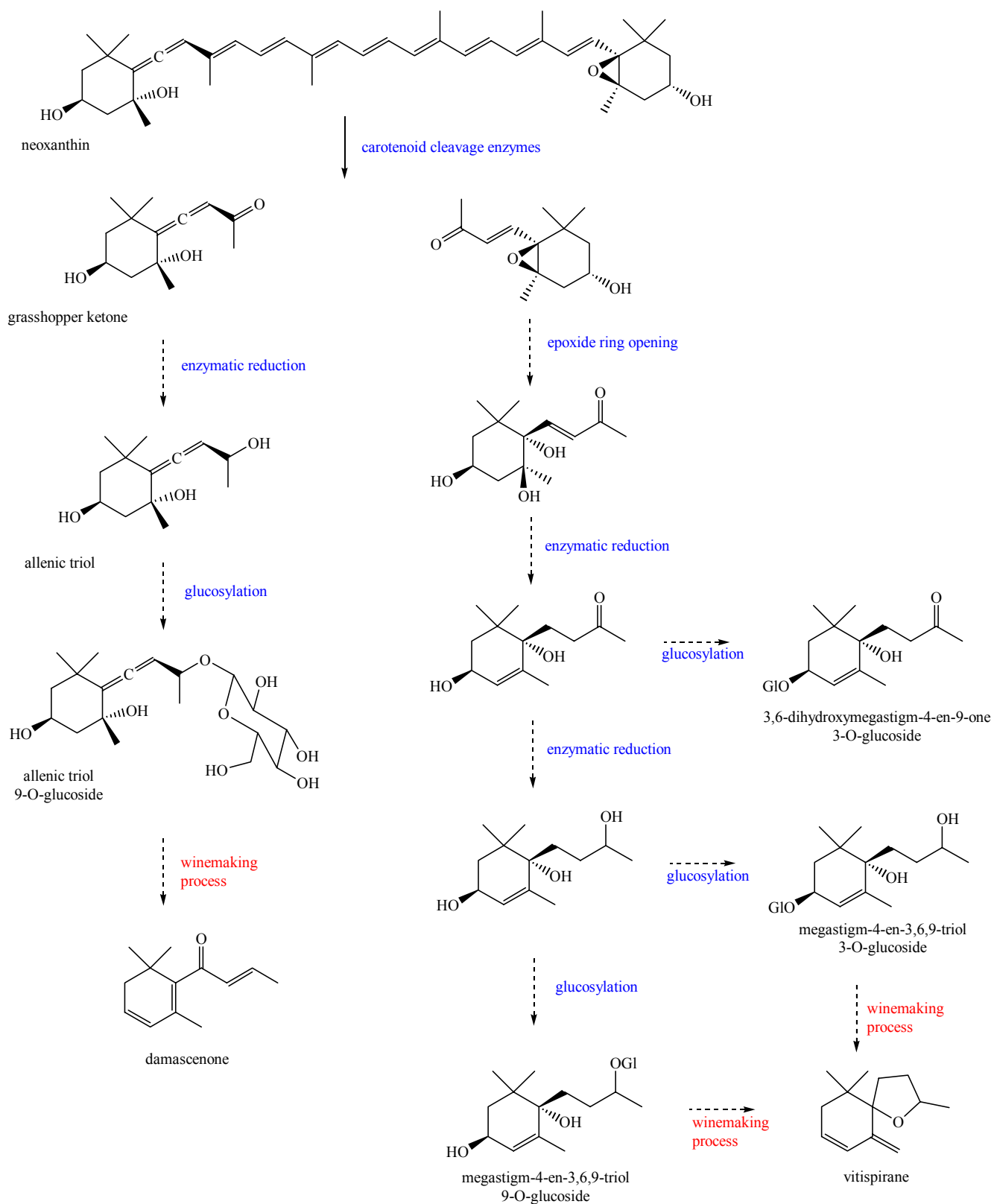
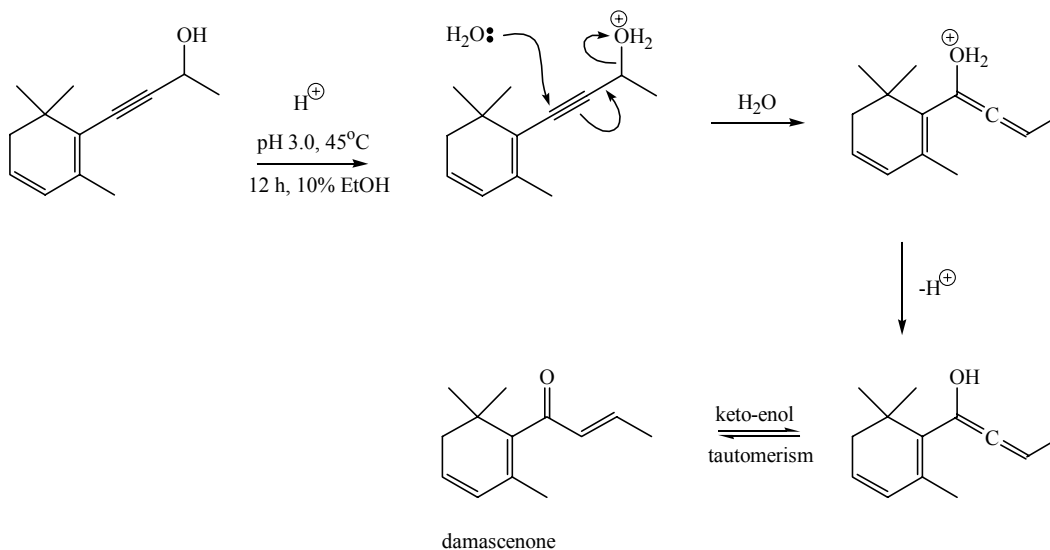


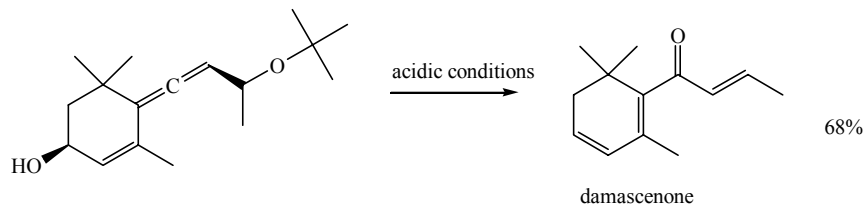
Figure 3: Formation of Two C₁₃-Norisoprenoid Glycosides from the Carotenoid Neoxanthin



Hydrolysis of the C9-glucoside and attack by water at C7 with the C9 hydroxyl as the leaving group yields β -damascenone.



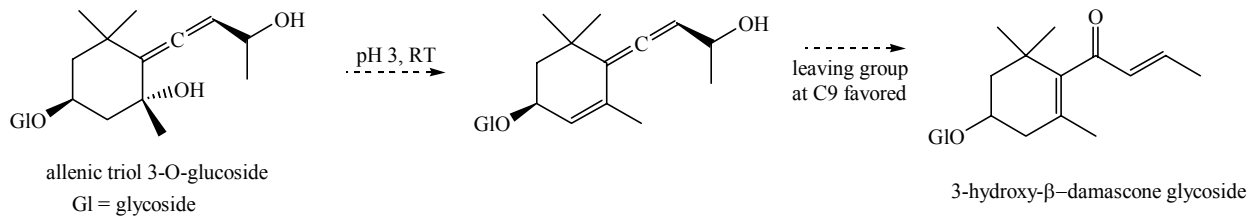
This mechanism has been tested with a model compound, using the *tert*-butyl ether as a protecting group (allenic diol 9-*O*-*tert*-butyl ether) instead of the allenic diol 9-*O*-glucoside and under acidic conditions, a 68% yield of β -damascenone was obtained.

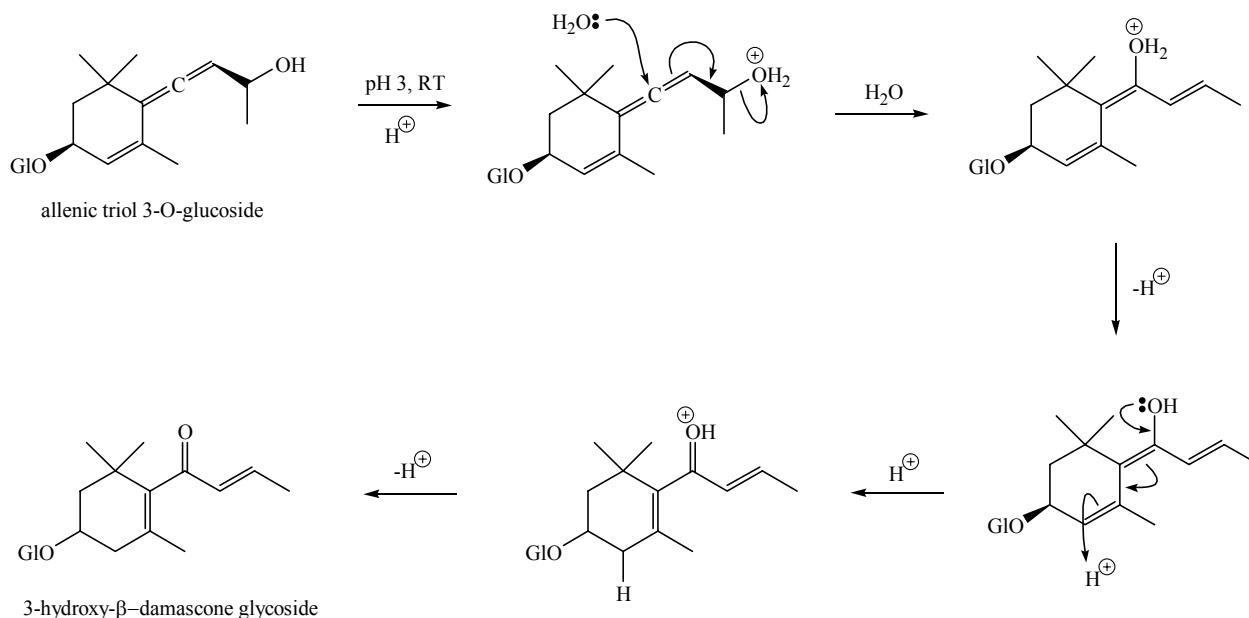


The allenic triol 9-*O*-glucoside rearranges under the acidic conditions in the grape juice (or wine) to form β -damascenone as outlined in Figure 4.

If the C3 alcohol of the allenic triol 3-*O*-glucoside is protected (instead of C9), then attack by a water molecule can occur at C7 with the C9 hydroxyl acting as a leaving group yielding 3-hydroxy- β -damascenone 3-*O*-glucoside. 3-Hydroxy- β -damascenone is odorless. See Figure 5.

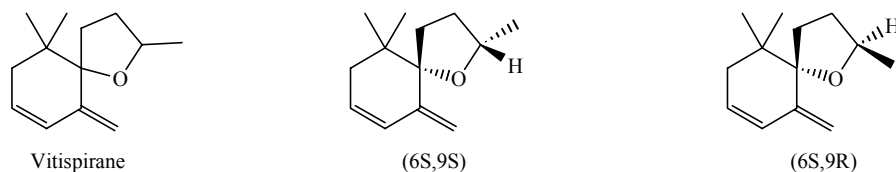
Figure 5: Rearrangement of the Acetylenic Diol C3-Glucoside to form 3-Hydroxy- β -damascenone



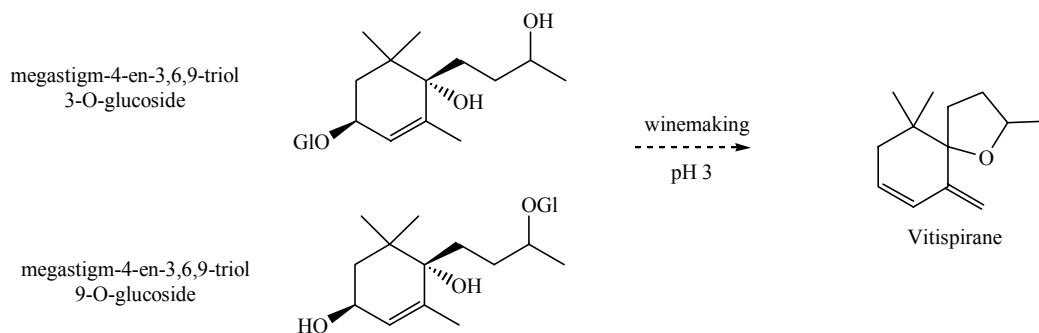


Vitispirane

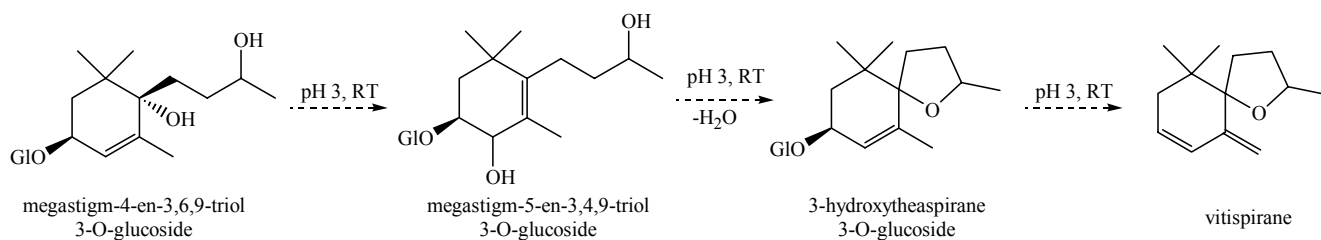
Vitispirane is an important norisoprenoid odorant that occurs in many wines. It has two chiral carbons and consequently four stereoisomers. Synthesis of two of the diastereoisomers provides us with their aromas: the (6*S*,9*S*) stereoisomer is unmistakably different than the (6*S*,9*R*) and is fresher and more intense. It is reminiscent of the green odor of chrysanthemum, and in addition, has a flowery-fruity note. The (6*S*,9*R*) stereoisomer is characterized by a heavy scent of exotic flowers with an earthy-woody undertone, and has a note of dry wines as in marc (a potent *eau de vie* distilled from pomace).



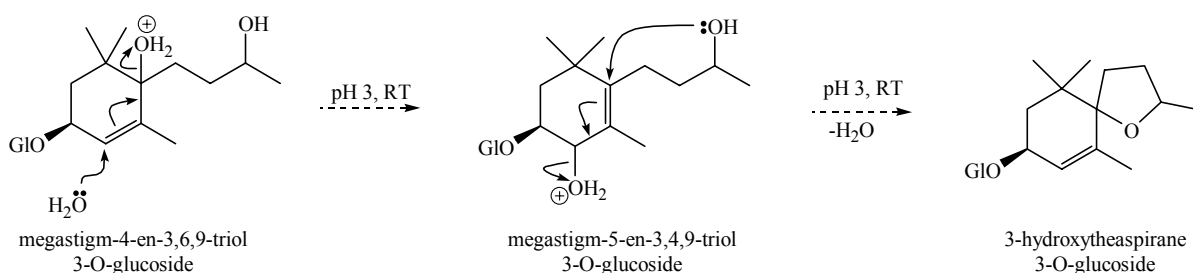
As with β-damascenone, vitispirane is considered to be derived from the carotenoid, neoxanthine. Vitispirane has two odorless precursors, megastigm-4-en-3,6,9-triol-3-O-β-D-glucopyranoside and megastigm-4-en-3,6,9-triol-9-O-β-D-glucopyranoside; both are stored in the grape (see Figure 3).



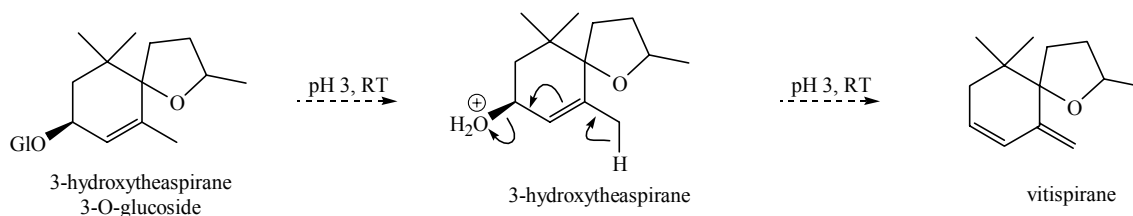
The first proposed pathway for the formation of vitispirane in wine is:



The first step involves a rearrangement of the allylic alcohol by attack of a water molecule at C4 and loss of the hydroxyl at C6 to give megastigm-5-en-3,4,9-triol 3-O-glucoside. Attack by the C9 hydroxyl at C6 and loss of the C4 hydroxyl gives 3-hydroxytheaspirane 3-O-glucoside.

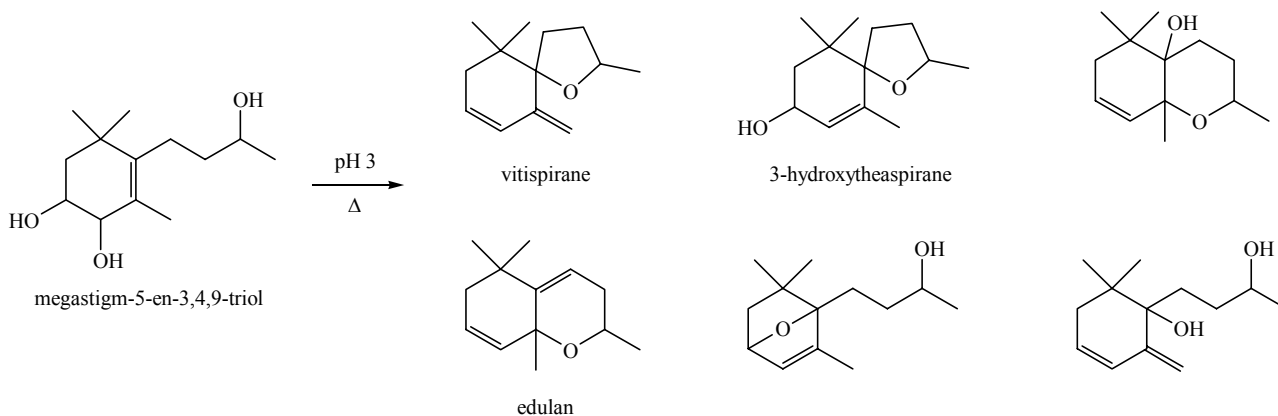


Hydrolysis of the glucoside and rearrangement of 3-hydroxytheaspirane provides vitispirane.



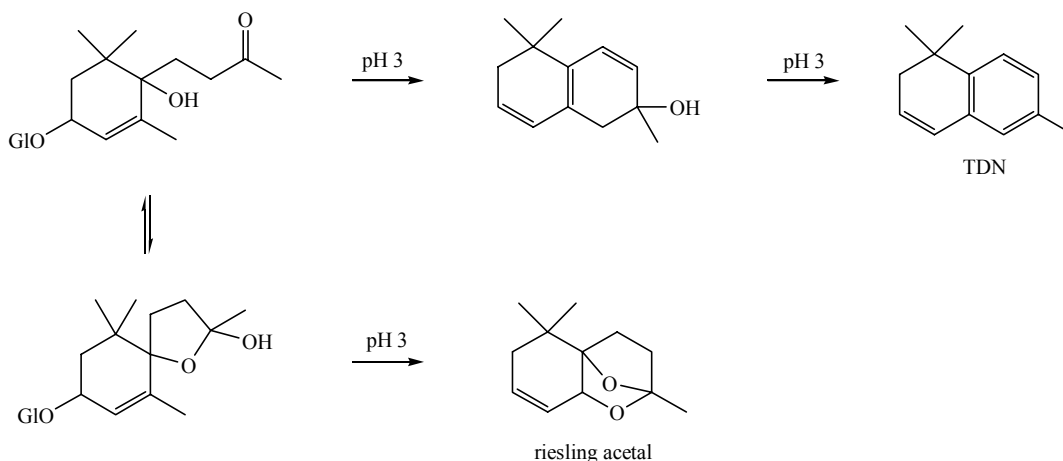
Evidence for this pathway.

The aglycone of second intermediate, megastigm-5-en-3,4,9-triol was synthesized and when heated at pH 3 a number of norisoprenoid products formed, including 3-hydroxytheaspirane, vitispirane and edulan.



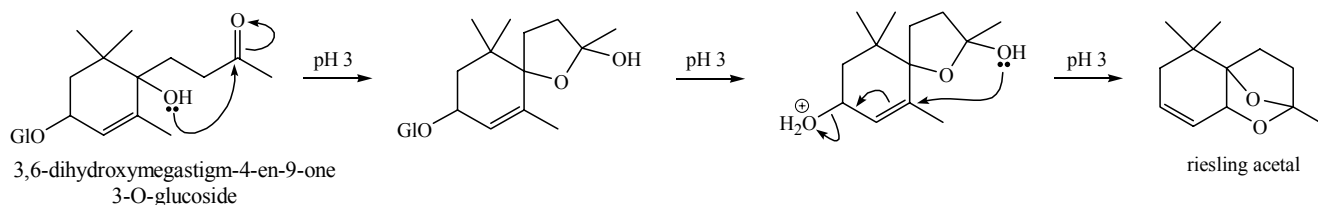
When the same experiment was carried with a protecting group at C3 (the 3-O-glucoside), vitispirane was formed in a yield greater than 90%.

3,6-dihydroxymegastigm-4-en-9-one
3-O-glucoside



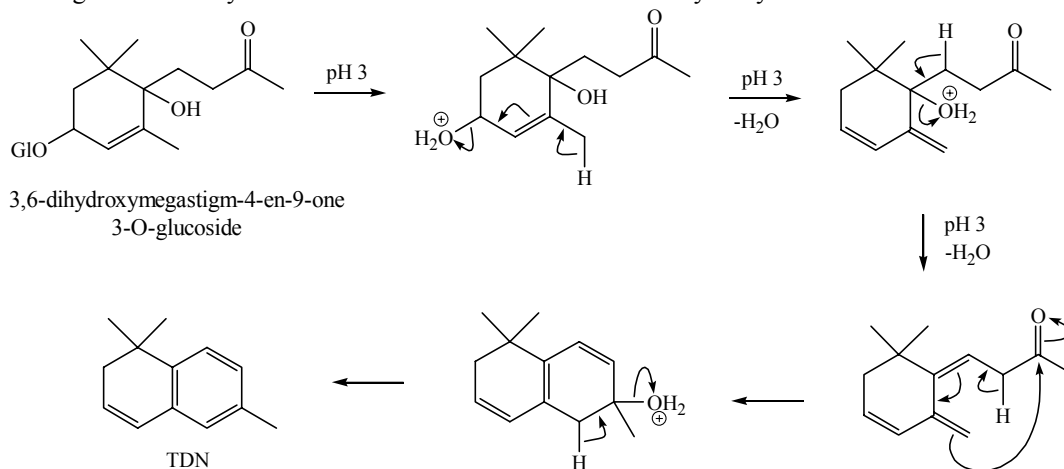
Riesling acetal

The proposed mechanism for the formation of Riesling acetal first involves attack of the C6 hydroxyl on the C9 ketone of 3,6-dihydroxymegastigm-4-en-9-one 3-O-glucoside to form the five membered ring hemiacetal. This step is followed by hydrolysis of the glycoside (either with acid or β -glucosidase released when the grape cells are broken). Attack of the C9 hemiacetal hydroxyl at C5 of the alkene and loss of the C3 hydroxyl as water provides Riesling acetal.



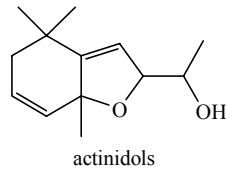
TDN

The first step in a suggested mechanism is hydrolysis of the glucoside of 3,6-dihydroxymegastigm-4-en-9-one 3-O-glucoside, which is followed by rearrangement of the double bond, loss of the C3 hydroxyl as water and formation of the exocyclic methylene. Loss of the C6 hydroxyl and formation of the C6-C7 double bond sets the molecule up for an interesting rearrangement. Finally aromatization occurs thru loss of the C9 hydroxyl and TDN is formed.

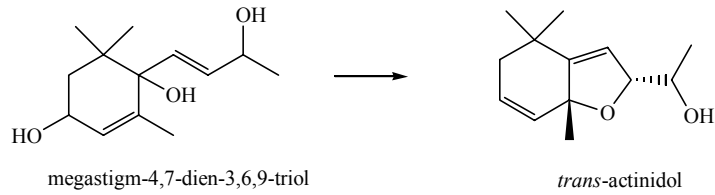


Actinidol

Little information is available concerning the formation of isomeric actinidols in wines as well as their sensorial contribution to wine aroma. Their odor has been described as camphoraceous or as woody and resinous.



A possible precursor is megastigm-4,7-dien-3,6,9-triol, as heating this compound at pH 3 gave the *trans*-actinidol as the major product.



A mechanism may be:

