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REVIEW

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Occurrence, Sensory Impact, Formation, and Fate of Damascenone in Grapes, Wines, and Other Foods and Beverages

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ABSTRACT: Among plant-derived odorants, damascenone is one of the most ubiquitous, sometimes occurring as an apparent natural product but more commonly occurring in processed foodstuffs and beverages. It has been widely reported as a component of alcoholic beverages, particularly of wines made from the grape *Vitis vinifera*. Although damascenone has one of the lowest ortho- and retronasal detection thresholds of any odorant, its contribution to the sensory properties of most products remains poorly understood. Damascenone can be formed by acid-catalyzed hydrolyses of plant-derived apocarotenoids, in both aglycon and glycoconjugated forms. These reactions can account for the formation of damascenone in some, but not all, products. In wine, damascenone can also be subject to degradation processes, particularly by reaction with sulfur dioxide.

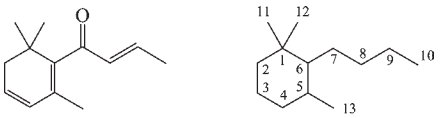
KEYWORDS: damascenone, wine, precursor, aroma, flavor, review

INTRODUCTION

The powerful odorant damascenone (**1**, Figure 1) was first isolated from the essential oil of *Rosa damascena*, and the structure was established by synthesis soon after.[1−4] It is one of a series of structurally related compounds known collectively as the "rose ketones" and is considered essential to the quality of rose oil.[1,3−5] It is now an important component in the international perfume industry and is produced in tonne quantities annually.[4] Damascenone is sometimes also referred to as β-damascenone to distinguish it from other double-bond isomers that have since been produced synthetically. The history of the discovery of damascenone and other rose ketones has been reviewed by Williams.[3]

Since the first isolation from rose oil, damascenone has been identified in a plethora of products of natural origin, beginning with Burley tobacco oil[6] and raspberry oil,[7] both in 1971. Although some studies indicate that damascenone is a natural product in various sources, it has been more commonly observed in essential oils and in processed foods and beverages, particularly those prepared by heating. In many such cases, the absence of damascenone from the raw plant materials from which such processed products have been manufactured has been specifically noted. This has suggested that much, if not all, of the damascenone found has been formed by the chemical transformation of one or more naturally occurring precursors. The studies of potential precursors and their hydrolysis products have therefore formed an important part of this review.

In the evaluation of the considerable literature on damascenone (or, for that matter, any other odorant) several caveats need to be borne in mind. Defining the sensory importance of damascenone and the processes leading to its formation and degradation requires, inter alia, reliable concentration data, and this, in turn, depends on the use of adequate analytical methods. A wide variety of methods for determining damascenone in various products have been reported and used. Most reports of damascenone identification and quantification have been based on gas chromatography−mass spectrometry (GC-MS) analysis. Sen et al.[8] and subsequently Kosteridis et al.[9] developed stable isotope dilution assays (SIDA) for damascenone using GC-MS and deuterium-labeled analogues as internal standards, methodologies that we regard as the most robust available. Yet even the use of the SIDA technique does not guarantee accurate analytical data as analytes can be both consumed or generated artifactually during sample preparation and chromatography.[10] We have previously observed that the simple expedient of freezing wine samples for storage and then thawing them prior to analysis can diminish the concentration of damascenone in the samples by up to 25% and that injection of samples of the damascenone precursor **4** (see below) into an improperly conditioned GC can result in decomposition to form damascenone and other products in the injector block (unpublished data). Among the many analytical methods used by the various groups whose works are described below, some are seemingly more robust than others, but in many of the papers reviewed we have found the information provided insufficient to be able to form a view regarding the reliability of the data presented. In most cases, we have reported such data, as it stands, without further comment. Nevertheless, and especially when contrary findings have been reported, it should be borne in mind that some findings might be based on analytical methodology that lacks a sufficient degree of robustness.



1 **2**

Figure 1. Structures of damascenone (**1**) and the megastigmane carbon skeleton (**2**).

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Caution is also required when one considers the apparent sensory impact of damascenone in various products. Although several comprehensive studies on the sensory impact of damascenone, including reconstitution and omission experiments, have been conducted and are described further in this review, the sensory impact of damascenone is most frequently assessed by two commonly used techniques: aroma extract dilution analysis (AEDA) and by calculation of the so-called odor activity value (OAV). These widely employed techniques are useful in determining which components in a mixture are more likely to contribute to the aroma and flavor of that mixture, but the limitations of such data, especially when applied to assessing the impact of damascenone in wine, the medium in which it is most frequently reported, have been well summarized by Pineau et al.[11] These relate, in part, to uncertainties, described by Lawless and Heyman,[13] surrounding the use of sensory detection thresholds. Furthermore, Buettner[12] has shown, in a study of odorants in human milk, that when stir bar sorptive extraction (SBSE) was coupled with GC-MS-O, damascenone, along with several other potent aroma compounds, could be detected at the olfactory detection port (but not by MS), even when blank runs, using preconditioned stir bars, were conducted. This was ascribed to the presence of traces of these compounds in the environment contaminating the bars after conditioning and prior to use.

There is now a considerable body of evidence that damascenone in plant-based products is formed as a result of acid-catalyzed hydrolysis of certain plant secondary metabolites (damascenone precursors). For some precursors, this can take place at room temperature, whereas for others, elevated temperatures are required. It should therefore be noted that the techniques used to prepare extracts for analysis could, in some instances, produce damascenone solely as an artifact of the preparation, particularly when high temperatures and low pH values are used. Furthermore, conditions sometimes used to liberate damascenone from so-called precursor fractions are not always appropriate to the product being studied. The generation of damascenone by boiling samples at 100 °C and low pH might be informative in the study of precursors to damascenone in, for example, stewed fruits or jams, but could be completely misleading in the investigation of the formation of damascenone in products that evolve at room temperature, such as beer and wine.

DAMASCENONE AS A NATURAL PRODUCT AND AS A COMPONENT OF FOODS, BEVERAGES, AND OTHER PLANT-DERIVED PRODUCTS

As well as occurring widely in grapes, wines, and other alcoholic beverages, which are described in separate sections, damascenone has been reported as a constituent of many other plant-based products (Tables 1 and 2). The first report of damascenone as an apparent natural product was in 1976 by Schreier et al.,[15] who observed the compound in extracts of wine grapes obtained under relatively mild conditions. Since this first report, damascenone has also been observed in extracts of grape juices prepared by liquid−liquid extraction with freon,[16−21] an isolation procedure that minimizes artifact formation,[18,22] and in the headspace above grape juice samples as determined by solid phase microextraction (SPME) sampling.[23−25]

Most reports of damascenone occurring as a possible natural product are as a component of various fruits (Table 1) which is consistent with the probable genesis of this compound by nonenzymatic transformations of plant-derived precursor forms, in an acidic environment, as described below. It was identified in a concentrate of African mango pulp obtained by crushing, freezing for storage, and then vacuum distillation.[26] The authors assumed that the damascenone observed was, along with several other compounds, an artifact formed during sample preparation or extraction, although other compounds they also assumed to be

Table 1. Damascenone in Fruits and Fruit Products[a]

fruit	sample	concentration (μg/kg)	refs
apple	fruit	nq[b]	43
	juice[c]	nq	27, 28, 46, 47
	jam	nq	44
	distillate	nq	45
apricot	pasteurized puree	nq	57, 58
Brazilian cherry	fruit	nq	62
babaco	fruit[d]	nq	67
blackberry	fruit	1.2−7.8	49, 50
		nq	51
	juice	nq	52
black currant	juice[c]	1.2−8.3	53
elderberry	heated juice	nq	73
		1−7.6	74
		mean 4.3	75
		max 37	
		mean 53	76
		max 200	
	fruit, juice, stewed fruit, wine	nq	72
grapefruit	concentrate[e]	nq	68
lychee	fruit	nq	36
	canned	nq	59
mandarin	juice[e]	nq	66
	juice	nq	64
mango	pulp[c]	nq	39
	pulp	nq	26, 48
nectarine	fruit[e]	1[f]	65
orange	reconstituted juice	nq	60
passion fruit	fruit	nq	37, 56
peach	juice[c]	nq	71
plum	fruit	nq	63
rambutan	fruit	nq	35
raspberry	fruit	nq	32−34
	essential oil	nq	7
starfruit	fruit	nq	55
strawberry	fruit	nq	38
	homogenate[c]	<0.1−5.4	38
	jam	nq	61
tomato	fresh	1−3	29, 30
		nq	31
	paste[c]	14	69
	sauce	14.5, 30	70

[a] Excluding grapes, wine, and spirits. [b] nq, not quantified or concentration not reported. [c] Concentration higher in heated sample than in fresh sample. [d] Observed is steam distillates but not solvent extracts. [e] Observed in heated, but not fresh, samples. [f] Semiquantitative data.

Table 2. Damascenone in Other Food Products

product	sample	concentration (μg/kg)	refs
celery	boiled[a]	nq[b]	104
cheese		nq	112
chilli	fried paste	nq	119
cloves	seed	nq	136
coffee	raw Arabica beans	0.8−55	113
	roasted Arabica beans	200−260	8, 114, 115
	roasted Robusta beans	200−293	8, 114
	brewed Robusta	3.8	8
dill seed	seed	nq	135
hazelnuts	raw	nq	117
	roasted	nq	117
honey	acacia	3.2	8
	linden	7.8	8
	goldenrod[c]	nq	132
	unpasteurized	nq	131
	other	0.8−74[d]	133
	other	nq	127−130
hops	hop	nq	164
	oil	nq	109
lobster	cooked tail meat	nq	111
malt		1100−1800	120
		nq	121, 122
mat rush		nq	126
milk	raw	nq	134
molasses		440	108
		nq	106, 107
popcorn		nq	110
potatoes	boiled	nq	116
rye bread		nq	118
soybean	fermented, paste	nq	124
spinach, cooked	SDE extracts	nq	103
tea	black, leaf	1.1, 1.7	8
		nq	99
	black, brewed	0.36	97
		nq	96, 99
	green, powdered	9	98
	green, leaf	nq	102
	green, brewed	0.01	98
		nq	100
	semifermented, leaf	nq	102
	rooibos, leaf	nq	101
yeast	paste	nq	125

[a] damascenone not observed in uncooked sample. [b] nq, not quantified or concentration not reported. [c] Damascenone observed in honey, but not in the flowers. [d] Semiquantitative data.

artifacts have been identified as natural products elsewhere. It was found in freon extracts[27] and the headspace[28] of fresh apple juices and was described as having the most intense odor of the volatile components of the freon extracts as determined by CHARM analysis.[27] Buttery et al.[29,30] reported damascenone as a component of vacuum distillates of fresh tomatoes. They measured a concentration of 1−3 ppb in fresh tomatoes using a purge and trap method. Mathieu et al.[31] also observed damascenone in the headspace of fresh tomatoes. It has been identified in the headspace of fresh raspberry fruits[32−34] and was described as the component with the largest odor potency in the headspace.[34] Ong et al. listed damascenone as among the most potent odorants in organic solvent extracts of rambutan[35] and lychee[36] fruits. In both studies, the fruit samples were sequentially extracted with Freon 113 and then ethyl acetate. In the latter study,[36] the damascenone was found only in the freon extract, whereas the solvent extract containing damascenone was not specified in the former case.[35] Traces of damascenone were observed in vacuum headspace isolates from samples of yellow passion fruits[37] and in organic extracts, handled at or below room temperature, of fresh strawberries.[38] Damascenone was found in low-pressure distillates of mango pulp but in higher concentration when the pulp was pasteurized.[39]

In these examples, damascenone has been identified under conditions that should prevent, or at least minimize, the formation of this compound as an artifact of the analytical procedure. The possibility remains, however, that damascenone formation could start as soon as whole plant components are processed for analysis, especially if damascenone precursors are stored in parts of a plant in a relatively nonacidic environment but come into contact with plant-derived organic acids once samples are homogenized. This being the case, it is still open to question as to whether damascenone should be considered a genuine natural product.

The number of products from which damascenone has been isolated under harsher conditions than those described above, or products that have been prepared at elevated temperatures or following various other manufacturing processes, is considerably greater than those discussed so far (Tables 1 and 2). In many of the earliest of such studies, extracts of samples were prepared for analysis using simultaneous distillation−extraction (SDE), a process that effectively employs steam distillation coupled with continuous extraction by a refluxing organic solvent. Although this form of extract preparation is not always appropriate for obtaining information on the composition of raw materials, it has been regarded as a useful tool to investigate compounds that could be formed during high-temperature processing such as canning.[40,41] Even in these cases, however, the composition of extracts obtained by SDE will not necessarily give a true reflection of the composition of processed products as, in SDE, some compounds can be formed in, and then removed from, the boiling aqueous matrix and isolated in the organic phase before they have a chance to degrade further.[42]

Among various fruits and fruit products (Table 1), damascenone was first reported as a component of raspberry essential oil.[7] Soon after, it was identified in SDE extracts of apple.[43] These authors suggested that the damascenone observed might have been present in the apple samples as a natural product because it was observed at highest concentration in the first extract (after 30 min of boiling), but this observation is also consistent with most of the damascenone being formed hydrolytically, but rapidly, from one or more precursors. Damascenone was also identified (by mass spectrum only) among the volatiles produced during apple jam manufacture,[44] in apple distillate,[45] and in apple juice samples allowed to stand in nonsterile conditions for 24 h.[46] It was considered to be among the most important odorants of a Golden Delicious apple juice as determined by OAV calculations, and its concentration increased >200-fold in two apple juice samples following pasteurization at 85 °C for 30 min.[47] It has been reported in SDE extracts of mango,[48] fresh blackberries (SBSE of the liquid phase, up to 7.8 μg/kg,[49,50] unheated (organic solvent extraction then silica chromatography[51]) and

heated blackberry juice (SDE extracts[52]), both an unheated (1.2 μg/L) and a heated (8.3 μg/L) sample of a black currant juice,[53] okra,[54] starfruit (SDE extracts),[55] passionfruit extracts,[56] pasteurized apricot puree,[57,58] canned lychees,[59] reconstituted orange juice,[60] and strawberry jam[61] as well as in supercritical CO_2 extracts of Brazilian cherry,[62] reduced-pressure SDE isolates of commercial plum cultivars,[63] mandarin juice,[64] and white-fleshed nectarines.[65] In this last study, damascenone was not reported among the components of the headspace of the intact fruit and was found at much higher concentration in SDE extracts obtained at atmospheric pressure. It was reported as a component of a heated, but not unheated, mandarin juice.[66] Similarly, it was observed in reduced pressure steam distillates of fresh babaco fruit, but not in diethyl ether extracts of fresh babaco pulp,[67] and in reconstituted grapefruit concentrate, but not in fresh grapefruit juice.[68] Damascenone was assessed as one of the most important volatile components of tomato paste samples in which it was found at an average concentration of 14 μg/kg, >10 times that in fresh tomatoes.[69] The concentration of damascenone in tomato paste sauce increased during storage or mild heating.[70] Similarly, the concentration of damascenone in strawberry homogenate heated to 100 °C for 30 min was >50 times higher than in the corresponding unheated sample,[38] and the flavor dilution factor for damascenone was considerably higher in a heated, compared to an unheated, peach juice.[71]

Several studies have suggested the importance of damascenone to the aroma of elderberry products. It was reported as a constituent of SDE extracts of elderberry berry, juice, stewed fruit, and wine samples[72] and in organic solvent extracts of heated juice samples, in which it was tentatively identified by GC-FID.[73] It was found at concentrations ranging from 1 to 7.6 μg/L in samples of elderberry juices heated to 70 °C for 20 min and was described as one of the two main contributors to elderberry character as assessed by GC−olfactometry.[74] A subsequent study,[75] also of heated juice samples, reported a mean concentration of 4.3 μg/L and a maximum concentration of 37 μg/L (concentrations were based on an assumed response factor of 1:1 for damascenone and the internal standard) and a correlation of the concentration of damascenone with elderberry aroma. Even higher concentrations (mean, 52.7 μg/L; maximum, 200 μg/L) were reported by the same group[76] for elderberry juices analyzed by static headspace sampling and GC-MS. In this last study, the samples were treated with a pectolytic enzyme and mild heating (63 °C) prior to analysis. The observation of higher concentrations of damascenone in this, compared to previous, studies was attributed to the possible effect of this enzyme/heat treatment.[76]

Damascenone has been reported as a constituent of a wide array of leaf products, mostly essential oils (for some of the earliest examples, see refs 6 and 77−94. Among these, it was a constituent of the essential oils of dried elder flowers[83] and of "summer savory" (*Satureja hortensis*)[93] but was not found in organic extracts or headspace samples, respectively, of the raw materials. It was the dominant constituent of the essential oil of the leaves of *Lycium halimifolium* Mil.[95] Damascenone has been identified, and sometimes quantified, in various tea leaf and brewed tea samples, including SDE extracts (Table 2).[8,96−102] Trace amounts were observed in SDE extracts of cooked spinach[103] and of boiled celery leaves and stalks.[104] In the latter case, no damascenone was observed in extracts of unheated celery samples. Damascenone was also reported as a component of enzyme (Pectinol C) hydrolysates of isolates from Virginia and Burley tobacco.[105]

The occurrence of damascenone in processed foodstuffs is not limited to fruit pulp and leaf products. It has also been reported as a component of organic solvent extracts of sugar cane molasses,[106−108] hop oil,[109] popcorn,[110] cooked lobster tail meat,[111] and (tentatively) British Farmhouse Cheddar cheese,[112] of raw[113] and roasted Arabica and Robusta coffee beans,[8,114,115] boiled potatoes,[116] and raw and roasted hazelnuts,[117] and among the most potent odorants of rye bread crust and crumb,[118] brewed Robusta coffee (3.8 μg/kg),[8] Thai fried chilli paste,[119] malt (1100−1800 μg/kg),[120−122] palm wine,[123] and (tentatively) SDE extracts of fermented soybean paste,[124] as a component of both dynamic headspace and SDE extracts of yeast extract pastes[125] and in extracts of mat rush.[126] It was found in various honey samples[8,127−131] including in SDE extracts of goldenrod flower unifloral honey but not in the flowers themselves.[132] The concentration of damascenone in the headspace of tupelo honey was found to be much higher than in that of other types of honey using a semiquantitative analytical method.[133] Other reported sources of damascenone include low-pressure distillates of raw milk from cow, sheep, goat, and water buffalo,[134] pentane/diethyl ether extracts of dill seed,[135] and supercritical CO_2 extracts of cloves[136] (Table 2). It has even been observed in extracts of cultures of *Penicillium* species, which contribute to the flavor of various types of cheese,[137−139] and the headspace of *Staphylococcus pasteuri* during stationary phase growth.[140]

Damascenone in Distilled Alcoholic Beverages and Beer (Table 3). Damascenone is a ubiquitous component of wine made from the grape *Vitis vinifera* (described in the following section) and of many other alcoholic beverages. Some of the earliest reports of the occurrence of damascenone were for distilled alcoholic products and for beer. De Smedt and Liddle[141] observed damascenone in several rum samples "in appreciable quantities", reported as up to 1400 μg/L of alcohol in one sample. Simultaneously, Dubois and Rigaud[142] described finding damascenone in rum as well as a trace of this compound in brandy. Subsequently, Masuda and Nishimura determined a concentration for damascenone in rum of 410 μg/L and of 440 μg/L in sugar cane molasses and concluded that damascenone in rum is derived from the latter.[108] More recently, in an evaluation of the potent odorants of rum and cachaça (produced from distillation of fermented raw sugar cane juice), damascenone was listed as the most potent odorant in both products as determined by CHARM analysis.[143] The presence of damascenone in rum has also been reported by Pino.[144]

Schreier et al.[145] gave the concentration of damascenone in apple brandy as almost 200 μg/L, but did not find this compound in the fermented apple mash from which the brandy was distilled or in the apple mash prior to fermentation. It was considered to be one of the two most potent odorants in an apple cider distillate examined by AEDA[146] and was reported as a component of freshly distilled Calvados (cider distillate).[147]

The concentration of damascenone in a range of grape brandies (24 samples) was first determined by Schreier et al.[148] The mean and range of concentrations, respectively, were 19 and 12−39 μg/L for German grape brandies and 35 and 18−45 μg/L for a set of French grape brandies. Cognac brandies had considerably higher concentrations (102−257 μg/L; mean, 152 μg/L). These higher concentrations were attributed to the distillation method employed for Cognac production. Similar concentrations have been reported in subsequent studies.[108,149] By contrast, in a recent examination of the composition of 11

Table 3. Damascenone in Distilled Alcoholic Beverages and Beer

beverage	concentration (μg/L)	refs
apple brandy	200[a]	145
	nq[b]	146, 147
beer	1.6	8, 169
	1−2	167
	6−25	170
	3	171
	<3	172
	42−157	173−175
	2	152
	nq	108, 164−166,168, 176, 177, 179
grape brandy	12−257	148
	30−110	108
	8	152
	0.9−8.4	149
	180−39360[c]	150
	nq	151, 153−156
plum brandy	nq	162
rum	410	108
	nq	141, 142
		143, 144
tequila	nq	160, 161
whiskey, malt	100−200	108
	nq	159
whiskey, Bourbon	10	108
	9, 11	157
whiskey, grain	tr[d]	108
whiskey, other	16	152

[a] Damascenone observed in the final brandy, but not in the apple mash, either fresh or fermented. [b] nq, not quantified or concentration not reported. [c] Semiquantitative; not detected in one sample. [d] tr, trace.

brandies,[150] the concentration of damascenone was given as ranging from not detected to 39360 μg/L in one sample, but these values were determined with a semiquantitative method only. Damascenone was also listed among the constituents of Cognac by Ferrari et al.[151] Another brandy sample, of undefined origin, was reported to contain 8 μg/L.[152] More recently, damascenone was observed as a constituent of Calvados, Cognac, Armagnac, and Mirabelle brandies.[153] The Armagnac samples appeared to contain less damascenone than the other brandies, although the concentrations of the constituents were not determined and only relative amounts were reported.[153] Damascenone has also been described as a constituent of Chinese brandies[154] and of distillates of fermented grape marc, with concentrations of up to120 μg/L.[155,156]

Masuda and Nishimura reported damascenone concentrations of 100−200 μg/L in two Japanese and two Scotch malt whiskeys.[108] Lower levels were seen in two Bourbon whiskeys (10 μg/L)[108] and in a whiskey of unknown origin (16 μg/L)[152] and only traces in a grain whiskey.[108] Similar concentrations of damascenone in Bourbon whiskey (9 and 11 μg/L) were determined in a study by Poisson and Schieberle,[157] who had earlier shown that damascenone had the highest flavor dilution factor of the odorants investigated.[158] Damascenone has also been detected in three other Scotch whiskey samples[159] and as a constituent of tequila[160,161] and plum brandy.[162]

The presence of damascenone in beer has been widely reported, and increases in damascenone concentration with beer aging are a common observation in many of these studies. The first report of damascenone in beer[163] was for an artificially staled product (beer heated to 50 °C for 3 days), although the only evidence for this assignment was limited mass spectral data. Traces of damascenone in beer were subsequently observed by Masuda and Nishimura in their study of damascenone in a variety of alcoholic beverages.[108] Sen et al.[8] then determined a concentration of 1.6 μg/L in a German lager beer. Subsequently, damascenone was also tentatively identified as a component of both hopped and unhopped beer as well as in samples of hops[164] and reported as a component of aged beer by Narziss et al.[165] It was found in unhopped beers by Kishimoto et al. but was not included in a list of the most potent odorants of hopped beers or hop extracts.[166] The same authors found 1−2 μg/L of damascenone in Japanese beers using SBSE,[167] and others have also detected damascenone in beer with this technique.[168]

Schieberle[169] considered damascenone to be one of the most important odorants of a pale lager beer as determined by flavor dilution experiments. Storage of the fresh beer, spiked with oxygen, for 14 days at 40 °C gave a slight increase in the flavor dilution factor for damascenone, but much larger changes were noted for other odorants.[169] The effect of artificial aging (40 °C for 5 days) for eight beer samples was also described by Chevance et al.[170] In this study, changes in damascenone concentration ranging from no increase to a 25-fold increase as a result of heating were reported. The concentrations given ranged from 6 to 25 μg/L (mean concentration, 12 μg/L) in the unheated beers and from 14 to 210 μg/L (mean concentration, 90 μg/L) in the heated samples. However, these concentrations were determined by GC-FID alone, and there was no verification of peak homogeneity in this study. In a follow-up study, by the same group, in which peak identities were also confirmed by GC-MS, the concentrations of damascenone in unheated and heated beer samples were given as 3 and 9 μg/L, respectively.[171] The amount of damascenone formed in heated, pH-adjusted samples increased with decreasing pH.[171] Somewhat lower concentrations of damascenone (<3 μg/L) in a beer sample stored under various conditions have been measured using HPLC with UV detection.[172] Although the concentration of damascenone in the samples increased during storage, the increases were no more than approximately 50% of the concentration in the fresh beer sample. Consistent with the previous study,[171] the yield of damascenone during storage increased when the pH of the samples was lowered, although the storage temperature and time were not specified for this experiment.[172] These authors also commented that, whereas damascenone concentration might act as a suitable marker for beer aging, this does not mean that damascenone has a direct impact on the organoleptic degradation that beer suffers as a consequence of aging,[172] a view with which we concur. More recently, three papers by Saison et al. also described increases in damascenone concentration as a result of heating.[173−175] These concentrations were cited as from 42 to 157 μg/L in unheated beers to between 230 and 400 μg/L in heated samples. For one beer sample, heating to higher temperatures for a short period gave a greater increase in damascenone concentration than did lower temperatures for longer periods.[175] The increase in damascenone concentration after storage at 28 °C was greater at lower pH, in agreement with the

earlier studies,[171,172] but was unaffected by the presence of oxygen.[175] An increase from 2 to 8 μg/L in damascenone concentration in beer after refluxing for 3 h has also been reported.[152]

Increases in the amount of damascenone in beer samples aged naturally have also been described. The flavor dilution value for damascenone in three beer samples aged at 20 °C for 3 months increased by up to 27-fold,[176] whereas the relative concentration of damascenone increased in another beer sample by 4−5-fold following storage at 18 °C for 25 weeks.[177] Similarly, damascenone was not detected in two freshly brewed hopped beer samples but was observed at a concentration of 2−3 μg/L when the samples were stored for 8 weeks at 28 °C.[178] By contrast, there was no significant difference in the flavor dilution factor for damascenone in a fresh pale lager beer when that beer was aged for 20 °C for a much longer period of 34 months.[179]

Damascenone in Grapes and Wine. Since the first reports of Schreier et al.,[15,180] more than 100 peer-reviewed papers have listed damascenone as a component of grapes and/or wine made from grapes, mostly, but not exclusively, of the species *Vitis vinifera*.[9,16−25,59,108,152,181−272] In the earliest of these, either damascenone was not quantified or relatively high concentrations, based on semiquantitative methods, were reported. More recent papers generally give the concentration of damascenone in wine as between 0.1 and 10 μg/L (Tables 4 and 5).

Among the reports, described in an earlier section, of damascenone being isolated from grape juices under conditions that were sufficiently mild to minimize artifactual formation of this compound,[16−21,23−25] damascenone was quantified in the juices of five cultivars of three *Vitis* species, and the highest concentration (5 μg/kg) was found in a juice of *V. labruscana* cv. Concord.[16] A subsequent study by this group[20] of grapes of this cultivar during ripening showed that the concentration of damascenone in the samples was low (<1 μg/kg) up to veraison and then increased to approximately 2 μg/L after this time. Damascenone was also found in extracts of the leaves of this variety.[20] The effects of ripening have also been described for *V. vinifera* cv. Baga grapes[24] from two vineyards. The damascenone concentration in the grapes from one site appeared to decrease during the ripening period, whereas the converse was the case for the grapes from the second site. A more comprehensive survey of damascenone in grape must was conducted by Camera et al.,[23] who examined 39 must samples from three vintages encompassing four grape cultivars used in Madeira wine production, Boal, Malvasia, Sercial, and Verdelho. The Malvasia musts had the highest mean concentration of damascenone (approximately 8−9 μg/L), followed by Sercial (approximately 5 μg/L) and then Verdelho and Boal (approximately 3 and 2 μg/L, respectively). For the Malvasia musts, there was little difference between the three vintages for damascenone concentration.

Other authors have also described damascenone as a component of grape juice extracts (Table 4). Among these studies, it was reported as a component of Huxelrebe must at a concentration of 1.8 μg/L based on analysis of an SDE extract but was not detected when the same must was analyzed using SBSE.[273] Because the latter technique is highly sensitive for even trace wine volatiles,[215] the damascenone detected was probably entirely an artifact of the SDE process. This interpretation is consistent with the earlier observation of Strauss and colleagues that Riesling musts from a single vineyard contained only traces of damascenone but that the concentration increased to 30−70 μg/L (depending on ripening stage) when the juices were heated at pH 3.0 to 50 °C for 28 days.[21] Similarly, damascenone was observed in SDE extracts of a Muscat of Alexandria must, but not in liquid−liquid or solid phase isolates of the same must.[274] Finally, quantitative data for damascenone in musts have been reported by Camara et al.[230]

Table 4. Damascenone in Grapes

variety	concentration (μg/kg)	refs
Aragonez	nq[a]	265
Arinto	1.3	244
Baga	nq	24
Bical	7[b]	202
	6.7	244
Boal	2[c]	23
Bobal	nq	25
Cabernet franc	1.1[c]	261
	0.021, 0.084	200
Cabernet Gernischt	0.5[c]	261
Cabernet Sauvignon	1.6[c]	261
	0.033, 0.036	200
	nq	18
Campbell-Early	5	108
Catawba	1.5	16
Cayuga white	nq	17
Chardonnay	1.2, 3.8[b]	19
Concord	5	16
	0.3−1.7	20
Delaware	5	108
	0.5	16
Early Sugar	0.09[b]	269
Falangia	2	252
Fernão-Pires/Maria Gomes	94.6[b]	202
	1.6, 95[d]	244
Fiano	tr[e]	253
Ives	0.4	16
Malvasia	8−9[c]	23
Merlot	1.5[c]	261
	3[b]	18
	0.024, 0.029	200
	0.03[b]	269
Mystery	nq	208
Niagara	0.17	16
Petit Verdot	0.63[b]	269
Prime	nq	208
Riesling	nq	17, 229
	tr	21
	tr (<0.3)	266
Sercial	5[c]	23
Seyval blanc	nq	17
Shiraz	2.8−4.4	239
Tempranillo	nq	25
Verdelho	3[c]	23
Vidal blanc	nq	17
unspecified varieties	nq	15

[a] nq, not quantified or concentration data not reported. [b] Semiquantitative data. [c] Mean of several samples. [d] Appears to be the same sample as in ref 202. [e] tr, trace.

Table 5. Damascenone in Wine

variety	concentration (μg/L)	refs
	White Wine	
Agudelo	1.1, 1.9[a]	258
Albariño/Alvarinho	0.9[a,b]	264
	2.1−3.4	254
	nq[c]	245
Blanco Lexitimo	0.8, 1.9[a]	258
Boal	1.3	231
Cayuga white	nq	17
Chardonnay	0.13	16
	66−170	184
	2[b]	248
	1.5, 2.4	262
	137, 190	268
	2.4	272
	nq	189, 217, 219
Clairette	nq	185
Devín	3.1	227
Emir	5−6	194
Falanghina	16−30[a]	220
Fiano	10.4	242
	3	253
	nq	211
Gewürztraminer	0.84	193
	0.84−6.2	195
	0.85	59
Gual	3.35	213
Listán	5.1	213
Loureiro	1.1, 1.3	254
	nq	245
Maccabeo	5	222
	3.5−8.0	240
Malvasia	9.4	213
	1	231
Marmajuelo	5.7	213
Morio-Muscat	nq	22
Muscat de Frontignan	42	183
Muscat of Bornova	10−13	228
Pedro Ximénez	10.2[b]	249
	nq	235
Picpoul	nq	185
Riesling	0.7, 0.8	16
	5−9	266
	10	272
	nq	17, 229
Sauvignon Blanc	3.9	272
	nq	185
Sercial	0.7	231
Seyval blanc	nq	17
Scheurebe	0.98	193
Terret	nq	185
Ugni blanc	nq	185
Verdello	5.75	213

Table 5. Continued

variety	concentration (μg/L)	refs
	0.8	231
Vidal blanc	nq	17, 191
Zelena	0.7[b]	241
not specified/blended	0.39−3.5	216
	3.2[b]	249
	1.3−4.5	152
	3.0	207
	10.1[b]	270
	nq	180, 196, 226, 238, 271
	Red Wine	
Aglianico	4−8	237
Cabernet franc	4	246
	1.7−6.3	200
Cabernet Gernischt	6	246
Cabernet Sauvignon	3	246
	3.3−7.4	200
	nq	185, 199, 201, 203, 215, 232, 267
Carignan	nq	185
Cinsaut	nq	185
Concord	1.6	16
Grenache	3.1	210
	1.2−4	212
	2.8−7.8	200
	2.5	272
	nq	185, 198, 199, 203, 218
Jaen Tinto	nq	263
Merlot	0.23−1.3	197
	1.8−4.5	200
	2.87	9
	5−12	257
	2.0, 2.5	268
	2.4−6.1	275
	nq	199, 201, 203, 223, 232
Negroamaro	2.2, 2.6	268
Pinot noir	4.5−9.4	233
	2.6, 4.1	200
	2.0	272
	nq	267
Primitivo	2.0, 2.4	268
Serradelo	1.6, 5.1[a]	258
Shiraz	3.6	239
	1.1	272
	nq	185, 187
Tannat	3, 3.5	243
Tempranillo	0.7−2.2	262
	nq	203
not specified/blended	0.29−4.7	203
	1.0−6.2	204
	1.36	206
	1.5[b]	214
	0.23−1	243

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Table 5. Continued

variety	concentration (μg/L)	refs
	2.0−4.8	225
	0.5−2.8	152
	0.3−3.4	207
	1.4−4.6	256
	17.5[b]	270
	nq	108, 188, 196, 199, 205, 250, 259
Other Wine Styles		
cava	6.6[b]	249
	nq	235, 260
Champagne	nq	182
Madeira	nq	235
port	1−13	224
	3.3−3.9	152
	2.7	251
Sauternes/botrytized	0.8[b]	249
	nq	234, 235, 247, 255
sherry	2.6[b]	249
	nq	235

[a] Semiquantitative. [b] Mean of several samples. [c] nq, not quantified or concentration not reported.

Most of the earliest reports of damascenone in wine were for white varieties, beginning with the study of Schreier and Drawert.[180] Four years later, Rapp and Knipser[22] listed damascenone among the wine components isolated from a Morio-Muscat wine. They reported, for the first time, the use of Freon 11 extraction as a method for extracting volatile components from alcoholic beverages without the ethanol itself being extracted and with minimal artifact formation due to the low boiling point of the solvent (23.8 °C).

Although many reports of damascenone in white table wines do not include any quantitative data for this compound, some authors have endeavored to determine the concentration of damascenone in this medium (Table 5). Concentrations ranging from 0.13 to 0.85 μg/L were measured by Acree et al.[16] for Riesling and Chardonnay wines. Considerably higher values, 42 μg/L in a Muscat wine[183] and 66−170 μg/L in young Chardonnay wines from six successive vintages,[184] were reported soon after. More than a decade later, damascenone was identified in Scheurebe and Gewürztraminer wines.[193] SIDA was applied, for the first time with wine, to measure the concentration of damascenone in the two samples.[192] The concentrations of damascenone in the Scheurebe and Gewürztraminer wines were cited as 0.98 and 0.84 mg/L, respectively. However, inspection of the tabulated concentration data for all of the components in the two wines clearly shows that the concentration header in the table should have been μg/L but had been misprinted as mg/L.[192]

The concentration of damascenone in five young monovarietal white wines from the Canary Islands can be calculated from the OAVs and threshold concentration data presented by López et al.[213] The highest concentration was found in the Malvasia wine (9.4 μg/L), a value consistent with the concentration found in Malvasia musts by a different group[23] as described above. The concentration in the remaining wines varied from 3 to 6 μg/L.

Cabaraglu et al.[194] found no significant difference in the concentration of damascenone (5−6 μg/L) in an Emir wine made with and without skin contact. The influence of skin contact, prior to vinification, on the composition of Muscat of Bornova wines has also been described.[228] The concentration of damascenone in the resultant wines varied from 10 to 13 μg/L. The damascenone concentration in the wine made with 6 h of skin contact was reported to be significantly higher than that for the control wine (no skin contact) and the wine made with 12 h of skin contact; however, the statistics appear to have been based on analytical rather than treatment replication, the musts were allowed to ferment spontaneously, and the sulfur dioxide addition to the treatments was not identical.

Damascenone concentrations have also been reported for a Gewürztraminer wine,[59] a young white wine,[207] 12 young white wines[216] (variety not specified), another 10 young white wines,[152,249] Macabeo wines,[222,240] a white wine made from Devín grapes,[227] 9 bottles of young Zelena wines,[241] Fiano wine,[242,253] and several Riesling[266,272] and Chardonnay wines.[248,262,272] The individual or mean concentrations cited in these various studies ranged from 0.3 to 10 μg/L. A notable exception to such reports was the recent study of Crupi and colleagues, who reported finding damascenone in two Chardonnay wines at concentrations of 137 and 190 μg/L, contrasting with much lower (<3 μg/L) concentrations in red wines that were also analyzed.[268] The high concentration of damascenone in the Chardonnay wines was also matched by a high concentration of 3-hydroxydamascone in Chardonnay, compared to red varieties.[268] Damascenone was tentatively identified in experimental Falanghina wines produced from musts with various antioxidative treatments, and a concentration range of 16−30 μg/L, based on semiquantitative analysis, was reported.[220] Other reports of damascenone in white wines are listed in Table 5. Among these, it was reported as a component of both grapes and wines of Riesling, Seyval blanc, Vidal blanc, and Cayuga white.[17,266] In contrast, the report of damascenone in an Emir wine specifically noted the absence of this compound in the must from which the wine was made.[194]

Although first identified as a wine component in 1974,[180] reports of the presence of damascenone in red table wine were relatively sparse over the following 20 years. A concentration of 1.6 μg/L in a Concord wine was reported by Acree et al.[16] in 1981, and damascenone was identified by Baumes et al.[185] in five experimental red wines five years later. Although not quantified, damascenone was observed as a component of young Shiraz wines that apparently increased in concentration when the wines were heated to between 42 and 45 °C for 25 days.[187] It was tentatively identified, by GC-O, as a constituent of a wine spoiled by sorbic acid metabolites.[188] From 1998 onward, damascenone began to feature regularly in studies of the composition of red wine (Table 5). Kotseridis et al. reported a concentration range for this compound of 0.2−1.3 μg/L in Merlot noir clone wines[197] and, in separate studies using SIDA,[9,200] a concentration range of 2−8 μg/L in 29 red wines comprising the varieties Cabernet Sauvignon, Cabernet franc, Merlot, Grenache, and Pinot noir. Using the same analytical methodology, they found much lower concentrations (<0.1 μg/L) in six red grape musts.[200] Most subsequent studies of red table wines report similar data. Thus, in a comprehensive investigation of 52 young monovarietal red wines made with Grenache, Tempranillo, Cabernet Sauvignon, and Merlot grapes, the concentration of damascenone ranged from 0.29 to 4.7 μg/L with an average concentration of 1.8 μg/L.

Although the concentration of nearly half of the 47 odorants studied varied significantly between varieties, there was no correlation between damascenone concentration and grape variety.[203] The same group also examined the composition of 57 older red wines from several regions of Spain that had been aged in oak barrels and then in the bottle. The average concentration in the wines was 1.5 μg/L (maximum concentration, 3.4 μg/L).[214] Damascenone was quantified in 10 claret and 20 rosé wines made from Cabernet Sauvignon, Cabernet franc, and Merlot grapes,[204] with similar concentration ranges in each wine type (1.7−6.2 μg/L, mean = 3.4 μg/L, for the rosés; and 1−4 μg/L, mean = 2.7 μg/L, for the clarets). Another study of 10 rosé wines gave a damascenone concentration range of 1.4−4.6 μg/L.[256] The concentration of damascenone in 19 young Grenache wines ranged from 1.2 to 4 μg/L.[212] The mean concentration for the wines made from grapes from very early maturing areas was slightly higher (2.6 μg/L, $n = 7$) than that for the wines made from relatively late or normal maturing areas (1.95 μg/L, $n = 12$), but no statistical analysis was performed on the data. Conversely, a study of the composition of Pinot noir wines from two successive vintages made from grapes of three different ripeness levels gave wines with reported damascenone concentrations between 4 and 10 μg/L.[233] For each vintage, the wine made from the late harvest fruit contained a slightly higher concentration of damascenone, but the treatments do not appear to have been replicated. A study of vine irrigation effects on Merlot wine composition indicated significantly higher concentrations of damascenone in wines made from grapes of partially irrigated (7−12 μg/L) compared to fully irrigated (5−10 μg/L) vines.[257] A follow-up study by this group over three successive vintages confirmed this effect.[275] Other reports of damascenone in red wines are listed in Table 5. In one of these,[215] the use of SBSE enabled tentative identification of the 8-*Z*-isomer of damascenone along with a higher concentration of damascenone itself, which has 8-*E*-configuration. The 8-*Z*-isomer has also been reported as a constituent of SDE extracts of tea leaves.[102]

The presence of damascenone in other wine styles has also been described. It has been identified in the headspace of Champagnes [182] and in samples of Madeira, Pedro Ximénez, sherry, and cava sparkling wines.[231,235,249,260] A concentration range in four 10-year-old Madeira wines of 0.7−1.3 μg/L has been determined,[231] and the same group has compared damascenone concentrations in six of each of Pedro Ximénez, fino, botrytized Sauternes, and cava wines.[249] The mean concentrations in these were 10, 2.6, 0.8, and 6.6 μg/L, respectively. Among the more than 100 wines analyzed by this group, the concentrations measured in the Pedro Ximénez wines, made from sun-dried grapes, were higher (up to 21.7 μg/L) than for any other group of wines. Finally, the concentrations of damascenone in 59 port wines have also been measured.[224] For 14 young port wines (<5 years old), the reported concentration range was 4−13 μg/L, whereas that for the older (10−40 years old) port wines was 1−5 μg/L.[224] Reported concentrations of 2.8 and 3.3 μg/L in single samples of port in two other studies[152,251] are consistent with the above data.

Virtually all studies describing the origin of damascenone in wine point to the grape as the primary source of this compound as a wine component. A single paper by Diaz-Maroto et al.[276] lists damascenone among the components of extracts of shavings of untoasted American, French, Hungarian, and Russian oak woods, but this compound was not detected in extracts of toasted samples. Damascenone has not been reported in a plethora of other studies of the composition of oak used for wine-barrel cooperage despite its distinctive mass spectrum and relative ease of identification at the μg/L level. However, the related compound 3-hydroxydamascone (**14**), which is formed as the major product of hydrolysis of known damascenone precursors (see below), has been identified among a number of other norisoprenoids in oak extracts,[277] and future examination of other oak extracts might well show the presence of damascenone at trace levels. In the study by Diaz-Maroto et al.,[276] it is also possible that the small amounts of damascenone observed were generated artifactually by the SDE conditions used to prepare the extracts for analysis. Guth[195] measured damascenone in a Gewürztraminer stored in either Allier oak barrels or stainless steel tanks for an unspecified time and found a higher concentration in the former (2.8 μg/L compared to 0.84 μg/L), but as the concentration of damascenone in the wine prior to storage was not given; it is not clear whether these data reflect greater damascenone evolution in oak or a greater degree of conversion of damascenone to other products in stainless steel. In a study of changes in red wine composition during barrel aging, Jarauta et al.[225] described an increase and then a decrease in the concentration of damascenone in the wine. After both 6 and 12 months of aging, and in agreement with Guth,[195] the concentration in the barrel-aged wines was slightly higher than in the corresponding stainless steel stored control. On this evidence they proposed that some of the damascenone was oak-derived and included this compound in a list of compounds presumed to be released from oak-derived precursors. However, there was no difference in damascenone concentration in the wine stored in American, compared to French, oak barrels, and it is possible that the slightly higher amount of damascenone in the barrel-aged samples was simply a reflection of small differences between these wines and the stainless steel control in pH and ethanol and sulfur dioxide concentrations, all of which can change during barrel maturation of wine. Bailly et al.[255] also categorize damascenone among the oak-derived components of aged Sauternes but give no explanation for doing so. More recently, Lloyd et al. observed 5-fold increases in damascenone concentration in a commercial Shiraz wine during 300 days of barrel maturation and a doubling of concentration during barrel aging of a Chardonnay and a Pinot noir wine,[272] but this result did not indicate whether the additional damascenone was extracted from oak or formed from grape-derived precursors.

Despite the large number of reports of damascenone as a grape and wine component, there remains no compelling evidence that the concentration of damascenone in wine is linked to grape variety. Comparisons of damascenone concentrations among varieties are confounded by the relatively small number of samples examined in most studies, by the fact that different groups use different analytical methods, many with inadequate details on method validation, and by a whole range of viticultural and wine-making variables. In the one study for which a large range of young monovarietal red wines (52 samples comprising 4 common varieties)[203] were analyzed using the same technique, nearly half the odorants analyzed varied among the varieties, but this did not include damascenone. Rather, this compound seems to be common to most, if not all, grape varieties, and the concentration in wine can depend on many factors, as described in the final sections.

SENSORY IMPACT OF DAMASCENONE

Although there can be little doubt as to the importance of damascenone in the perfume industry,[4,5] surprisingly little is

known about its contribution to the aroma and flavor of most foods and beverages. Buttery et al.[29,30] determined an odor detection threshold for damascenone of 2 ng/L in water and, on this basis, described it as one of the most important odorants in tomato paste. Other authors have also considered damascenone to be an important odorant in various products or commented on its odor intensity.[35,45,74,75,108,116,127,128,143,157,158,169,171,176] The characteristic odor of elderberry juice has been ascribed to damascenone.[75] Such opinions have been based mostly on GC-O assessments or on OAV values, but more detailed sensory assessments such as reconstitution, omission, and addition experiments are usually lacking. Damascenone was listed as having the highest OAV of 15 important odorants in a sample of apple juice. A reconstituted aqueous solution of these odorants in the same concentration as determined in the juice sample was described as giving a typical apple juice-like impression and "in good agreement with the original juice".[47] Similarly, a reconstituted mixture of odorants with OAV > 1 quantified in a reconstituted orange juice sample, and which included damascenone, was said to show "good similarity" to the original juice sample.[60] On the other hand, experiments with a reconstituted mixture of compounds that closely resembled the odor of a whiskey sample showed that the omission of damascenone did not have a significant impact on the aroma of the mixture, even though damascenone had among the highest odor activity values of the constituents.[157]

In contrast to most other foods and beverages, the contribution of damascenone to the odor of wine has been commented on numerous times. As is the case with other products, the majority of such comments have been based only on GC-O assessments or OAV values.[17,59,143,146,158,166,176,188,191,198,199,201,203,206,213,218,232,235,241,247,250,256,258,261] In some cases damascenone has simply been described as an intense odorant, whereas other authors have assumed that, for this reason, it must be an important contributor to wine aroma.

Notwithstanding the limitations of orthonasal and retronasal detection threshold data,[13] these can be useful starting points for assessing whether a food or beverage component might have an impact on the aroma and flavor of that product. Sensory detection thresholds for damascenone, mostly in various aqueous media, have been reported by several groups. In most cases, details on how such thresholds were determined, or even defined, are lacking, making the use of such data problematic.

Following the determination of an orthonasal detection threshold of 2 ng/L for damascenone in water by Buttery et al.,[29,30] using 16−20 panelists, an even lower value of 0.75 ng/L was obtained by Semmelroch et al.,[114] who used "at least five assessors". Ong and Acree[36] reported an odor detection threshold of 10 ng/L in an complex aqueous matrix, determined using a "modified retronasal aroma stimulator" apparatus. Czerny and colleagues obtained detection and recognition thresholds for aqueous damascenone (sample purity checked by GC-O) of 13 and 56 ng/L, respectively, using between 13 and 22 assessors.[14] A retronasal detection threshold of 1 ng/L for solutions in water has also been reported.[98] In his review of the importance of trace odorants in flavors and fragrances,[5] Ohloff described how, when first determining the retronasal detection threshold of damascenone in water, by presenting panelists with decreasing concentrations of damascenone solutions, a threshold of 10000 ng/L was determined. However, when a subsequent determination in which solutions were presented in ascending order of concentration was conducted, a detection threshold some 3 orders of magnitude lower (9 ng/L) was calculated. This was ascribed to a fatiguing effect and high oral persistence of damascenone with the concentrated solutions that were presented first in the former study. Such data illustrate the importance of methodology in determining sensory detection thresholds and the need for caution in accepting published threshold data at face value when not supported by adequate experimental detail.

A more detailed study of both the orthonasal and retronasal detection thresholds for damascenone in both water and in deodorized orange juice ("pumpout") was conducted by Plotto et al. using two different panels of approximately 20 assessors.[278] For the first panel (panel A), comprising assessors who were familiar with difference testing, the group ortho- and retronasal detection thresholds for damascenone in water were 23.7 and 11.4 ng/L, respectively, whereas the corresponding thresholds for the pumpout solutions were 4430 and 1950 ng/L. The second panel (panel B), comprising assessors experienced in sensory assessment tasks, gave lower group ortho- and retronasal detection thresholds for damascenone in water of 14.8 and 6.4 ng/L, respectively, compared to panel A (no data were reported for detection of pumpout by panel B). The authors also observed considerable variation among panelists in their sensitivity to damascenone in both water and pumpout. For the water solutions, individual best-estimate thresholds (BETs) ranged from 0.7 to 1250 ng/L for orthonasal detection, and there was a virtually identical range (1−1250 ng/L) for retronasal detection. An even greater range was observed for the pumpout. In the case of the pumpout solutions for panel A, the distribution of BETs was bimodal, with the respective group ortho- and retronasal thresholds for the more sensitive half of the panel (the "perceivers"), 692 and 387 times lower than those for the less sensitive group, the "nonperceivers". For the water solutions of damascenone, there was no clear bimodal distribution of BETs, and the differences between the perceivers and nonperceivers was less. In water, the group ortho- and retronasal detection thresholds for the perceivers were 8.3 and 2.5 ng/L, respectively, values that are broadly similar to those reported above by others. On this basis, Plotto et al. suggested that these other groups might have employed assessors that were selected for sensitivity to damascenone. For the nonperceivers group, the ortho- and retronasal group threshold values were both 130 ng/L. This more detailed study of sensory detection thresholds for damascenone confirms the need for caution when one attempts to generalize the sensory impact of this or any other compound on the basis of published thresholds that are group averages.

Threshold data for damascenone in various aqueous alcoholic media have also been published. Guth[192] and Pineau et al.[11] both determined orthonasal detection thresholds of 50 ng/L for solutions in 10 and 12% aqueous ethanol, respectively. In the latter case, a panel of some 50 assessors was used. A much higher value of 10000 ng/L was reported for a solution in 40% aqueous ethanol,[108] but because no information on methodology was presented, it is unclear whether this higher value is due solely to the increased alcohol concentration in the medium or might also be due to other factors. SPME has been used to study the effect of ethanol and other major wine components on the concentration of damascenone in the headspace above aqueous solutions.[279] Ethanol had a clear effect, diminishing the amount of adsorption onto the fiber, but it was not clear to what extent this reflected the actual headspace composition and to what extent it resulted from the ethanol modifying the kinetics of partitioning of damascenone between the aqueous and gaseous phase and also competition between the analyte and ethanol for adsorption onto the SPME fiber.

Reported detection threshold data for damascenone in beer and in deodorized or commercial wines are generally much higher than those for solutions in water or aqueous ethanol. Saison and colleagues determined sensory detection thresholds for 26 odorants associated with the aroma of stale beer using a minimum of 18 assessors.[173] Among the compounds studied, they showed a distribution of BETs ranging from 7 to 226 μg/L for 19 assessors for the orthonasal detection of damascenone in beer but then reported a group threshold of 203 μg/L. Clearly, these two sets of data are incompatible. On the basis of the BET data presented,[173] we calculate a group threshold of 39 μg/L. Simpson determined a "flavor" (= orthonasal, R. F. Simpson personal communication) threshold of 50 μg/L for a neutral dry white wine containing no detectable damascenone.[184] A much higher orthonasal detection threshold of 1600 μg/L in a non-aromatic white wine fortified by addition of ethanol to ca. 21% alcohol v/v was reported by Etievant et al.,[183] who concluded, on this basis, that damascenone made no contribution to the aroma of the fortified Muscat wine that was the subject of their study. Lower orthonasal detection values were determined by Pineau and colleagues[11] in their study of the influence of damascenone on wine aroma. These were 140 ng/L in a deodorized white wine (described as having a "neutral" aroma), 850 ng/L in a deodorized red wine (also described as having a "neutral" aroma), 2100 ng/L in a partially deodorized red wine (described as having a caramelized aroma), and 7000 ng/L in a red wine already containing approximately 400 ng/L of damascenone. The authors considered that OAV calculations, usually based on threshold values obtained in water or aqueous ethanol solutions, exaggerated the contribution of damascenone to wine aroma.[11]

Finally, an orthonasal detection threshold of 200 ng/kg for damascenone on cellulose has been reported.[98]

Attempts to reconstitute the aroma of a wine were first reported by Guth.[192] The most important odorants of a Gewürztraminer and a Scheurebe white wine were assigned, on the basis of analysis and calculation of OAVs for the volatile components of the wines. For each wine, the 42 odorants classified as the most important were blended in 10% aqueous ethanolic solution in concentrations equal to those determined in the wines, giving solutions with aromas described as strongly similar to the parent wines. In the case of the mixture reconstituted to match the composition of the Gewürztraminer wine, the aroma was compared to similar mixtures in each of which one of the 42 components was absent. Individual omission of 32 of the components was judged to have little or no effect on the aroma of the mixture. Of the remaining 10 components, removal of damascenone, originally present at a concentration of 0.84 μg/L, was judged to have had a slight effect on the aroma of the mixture.

A similar study was subsequently conducted by Ferreira et al.,[210] who blended the 22 odorants having the highest OAVs in a Grenache rosé wine in 10% aqueous ethanolic solution in concentrations equal to those determined in the wine. The reconstituted mixture was judged to be qualitatively very similar to the original rosé wine, although the two could be distinguished by triangle tests. The individual omission of eight of the odorants from the mixture had no effect on the aroma of the mixture, whereas removal of a further nine had only a slight effect. Among the remaining five odorants, removal of damascenone (3.4 μg/L in the mixture) decreased the odor intensity, but not quality, of the mixture. From this, the authors concluded that the damascenone in the mixture of 22 odorants acted as an aroma enhancer rather than adding a specific qualitative character to the aroma of the wine.

As well as their determination of the retronasal detection thresholds of damascenone in wine and various wine-like media, Pineau et al.[11] also studied the orthonasal detection threshold of ethyl cinnamate, ethyl caproate, and 2-isobutyl-3-methoxypyrazine in aqueous ethanol solution in 12% aqueous ethanol with and without 50 ng/L of damascenone added to both the test samples and controls. Although no statistics were presented to show whether differences were significant, the addition of damascenone appeared to result in lower detection thresholds for ethyl cinnamate and ethyl caproate and a slightly higher threshold for 2-isobutyl-3-methoxypyrazine. In agreement with Ferreira et al.,[210] the authors concluded that damascenone might act indirectly rather than directly on wine aroma, increasing fruitiness and decreasing methoxypyrazine odor. Some related experiments on the perception of esters were reported by Escudero et al.[243] Triangle tests showed that an aqueous ethanolic solution of esters could be distinguished from the same solution to which damascenone (850 ng/L) and β-ionone (140 ng/L) had been added, and the latter solution was described as having a "sweeter" aroma. This difference was not, however, observed when the solutions were made up in a dearomatized wine, a result that could be considered consistent with the higher detection thresholds for damascenone, reported by Pineau et al. for wine-like compared to hydroalcoholic solutions.[11] Higher concentrations of damascenone (3500 ng/L) and β-ionone (230 ng/L) conferred more raisin- and plum-like aromas to the solution of esters in aqueous ethanol.[243] The effects of these higher concentrations in dearomatized wine were not reported.

A further reconstitution/omission study has been reported by Escudero et al.,[222] using a Maccabeo wine that was stripped of its volatile components with XAD resin and then spiked with a number of volatile compounds at the concentration at which they occurred in the original wine. Removal of damascenone from the reconstituted mixture made a significant difference to the aroma of the mixture, but when the concentration of damascenone in the white wine was doubled (from 4750 to 9500 ng/L), there was no significant change in the aroma of the wine. This was ascribed to the relationship between damascenone concentration and perceived aroma intensity, which was shown in an earlier study, using GC-O,[280] to be weak. In that study, panelists could discern differences in odor intensity only when the differences in damascenone concentration were >10-fold.

Other evaluations of the impact of damascenone on wine aroma have relied on correlations between aroma descriptor intensities and compound concentrations. A comprehensive study[214] of the aroma and composition of 57 barrel-aged Spanish red wines, in which the concentration of damascenone had mean and maximum values of 1.5 and 3.4 μg/L, respectively, showed that what were considered to be the more pleasant aromas were positively correlated with the concentration of a group of components including damascenone, whereas the converse was the case for the 'animal-leather-phenolic' descriptor. The descriptor 'total fruits' depended primarily on damascenone concentration. The same group also conducted a chemometric study relating aroma compound concentration with sensory descriptors of six Merlot wines.[223] Again, damascenone was positively correlated with the 'fruity' descriptor. Addition studies were also conducted with these wines in which individual compounds were added to the wine in which they were found at lowest concentration to bring the concentration to an amount equivalent to the

highest measured. Addition of damascenone (tripling the concentration), or the single addition of the four other compounds that were positively correlated with "quality" (and in contrast to the addition of compounds negatively associated with "quality"), had little or no effect on the aroma of the wine. However, when these five compounds were added together as a group, the aroma of the wine was modified, with enhanced 'toasted' and 'fruity' notes and suppression of 'pyrazine' and 'eucalyptus' aromas. It is not clear, however, whether the combination of all five compounds was necessary for this effect, or only some of them.

In contrast to the above observations, another study[204] of 20 rosé and 10 claret wines found that 'fruity' aroma was correlated with, and enhanced by the addition of, 3-mercaptohexanol, its *O*-acetate, and ethyl 2-phenylacetate, but there was no such correlation with damascenone, which had a 4-fold concentration range in each group of wines. Similarly, a study of the composition and sensory properties of 35 Albariño wines determined the concentration of damascenone in the samples but did not include damascenone in a list of compounds correlated with specific odor descriptors.[264]

With the exception of several detection threshold determinations, little is known of the contribution of damascenone to flavor (retronasal) perception. Buettner[219] studied the oral persistence of odorants from two Chardonnay wines using a modified SBSE device to sample odorants present in the oral cavity. Damascenone was detected analytically (by GC-MS) after, but not prior to, consumption of wine and was among the group of odorants with the highest oral persistence. This observation is consistent with a similar, but informal, observation reported by Ohloff.[5] A related study, using palm wine, showed the presence of damascenone among the odorants exhaled immediately after swallowing, but damascenone was not among the odorants exhaled 20 s later.[123]

Despite the detailed sensory studies outlined above, the contribution of damascenone to wine aroma remains less than clear-cut. We concur with Pineau et al.[11] that GC-O intensities and OAV calculations based on detection thresholds in water or aqueous ethanol solution are a poor guide to the sensory impact of damascenone in more complex matrices such as wine. Although there seems little doubt that the presence or absence of damascenone can affect the aroma of experimentally concocted mixtures of wine volatiles at wine-like concentrations, addition studies with real wines have failed to provide convincing evidence that the same is the case for such wines. Some, but not all, correlation studies indicate a link between damascenone concentration and wine aroma. If damascenone does have a sensory impact on wine, then the evidence to date indicates that it probably acts as an enhancer of aroma intensity, particularly of fruity-type aromas.

Of course, failure to demonstrate that two solutions with different concentrations of damascenone also have statistically different aroma values does not mean that the aromas are identical. Differences can sometimes be blurred by a high level of "noise" in sensory data and confounded by great variability in the capacity of individuals to detect the aromas of specific compounds.

Apart from the oral persistence study of Buettner[219] and several sensory threshold determinations, virtually nothing is known about the contribution of damascenone to the flavor of wine as perceived retronasally. When both ortho- and retronasal detection thresholds have been determined in the same study,[278] the latter was lower than the former by a factor of 2, so it is possible that damascenone is more important to the flavor than to the aroma of wine. Finally, nothing is known about the contribution of damascenone to the flavor of grapes other than that its concentration in grape must is usually greater than its sensory detection threshold in water. Given the importance attached by wine producers to grape tasting as a means of determining when wine grapes are suitable for harvest, this might also prove to be a useful area for further investigation.

FORMATION AND FURTHER TRANSFORMATION OF DAMASCENONE DURING PLANT PRODUCT PROCESSING AND STORAGE

The observation that damascenone could be isolated from the essential oil of rose petals but not from the petals themselves quickly led to the hypothesis that the damascenone was formed chemically from some form of precursor or precursors as a result of the application of heat.[5] This supposition is supported by numerous other examples, discussed above, in which damascenone either appeared or increased in concentration in various products following heating. The observation of damascenone as an apparent natural product in a number of cases is not inconsistent with such a hypothesis, as chemical formation of damascenone could also take place during plant growth, providing that precursors are sufficiently reactive at room temperature for such transformations to take place to at least some extent. Several reports[152,171,172] of increases in damascenone concentration in beer following accelerated aging showed that such increases were greater when the pH was lowered and support the view that, in some cases at least, the formation of damascenone is acid catalyzed.

In 1982, Williams and colleagues used a C18 reverse phase (RP) absorbent to separate grape secondary metabolites from major grape juice components such as sugar and organic acids.[281] Hydrolysis of the C18 RP isolate at 100 °C and at pH 1 or 3 gave a wide variety of volatile products, including damascenone. This study and subsequent research have shown that such isolates are rich in glycoconjugates and yield aglycones on treatment with glycosidase enzymes or with acid. In subsequent studies in the same laboratory,[19,282,283] C18 RP isolates of Chardonnay and Semillon grape juices heated to 50 °C for 28 days at pH 3.2 also gave significant quantities of damascenone. This compound, however, was not generated when the isolates were treated with a glycosidase enzyme preparation. The same result was also observed in a subsequent and analogous study of Riesling wine[284,285] and of Cabernet Sauvignon and Merlot juices.[18] In the studies of grape extracts, smaller concentrations of damascenone were also found in freon extracts of the juices prior to C18 RP extraction. Buttery and co-workers[40] used XAD-2 resin to isolate a pool of secondary metabolites from fresh tomatoes. In agreement with the results above, damascenone was not included in a list of aglycones released by treatment of this fraction with a β-glucosidase but was formed in significant quantities when the extract was subjected to SDE at 100 °C over 3 h, conditions considered appropriate in view of the fact that tomato processing often involves similar temperatures. Consistent with the pH effects observed in heated beer samples,[152,171,172] greater amounts of damascenone were isolated when the SDE process was conducted at a pH of 3, compared to 4.1 or 5.[40]

Many other studies have also shown the generation of damascenone by heating reverse phase isolates of various

products under a variety of acid conditions and temperature.[20,178,200,239,240,265,266,282,286−299] In some cases, generation of damascenone by acid hydrolysis of such precursor fractions was conducted following enzyme treatment.[290,299] In addition to the examples given above, some authors have also reported that enzyme treatment alone did not generate damascenone.[295,299] Other reports, however, indicate that damascenone might sometimes also be generated by enzyme activity. It has been listed as among the components liberated from tobacco extracts by Pectinol C,[105] from extracts of linden flowers by Pectinex C,[300] and from extracts of kiwifruit by a β-glucosidase enzyme.[301] Humpf et al.[302] described the liberation of damascenone in small quantities by treatment of an XAD 2 isolate of blackberry fruit with a Rohapect D5L enzyme preparation and in substantial quantities (200 μg/kg) when extracts of blackberry leaves were treated with the same enzyme. However, the products of the latter treatment also included a high concentration of vitispirane, a spiroether that is also known to be formed under acid hydrolysis conditions,[19,282,285,303−305] so it is possible that the damascenone and vitispirane observed were artifacts formed during the diethyl ether concentration step (conducted at 45 °C) prior to analysis by GC-MS or else were formed in the GC injector port. Damascenone was formed by enzyme hydrolysis of C18 isolates of fresh blackberries, but this was conducted at a relatively acidic pH of 3.1.[306] It was formed when grape musts were subjected to heat treatment at 100 °C for 15 min and then treated with glycosidase enzyme,[202,244] but it is not possible to determine from the papers during which step the damascenone was formed. Loscos et al.[298] observed small amounts of damascenone in enzyme hydrolysates of extracts of seven grape varieties but considered this to be most likely an artifact of sample handling. Finally, the effect of moisture content on damascenone formation in raw coffee beans during storage has been described and ascribed to possible enzyme activity.[113]

A number of studies have shown that more than one component of RP isolates of foodstuffs or beverages can be responsible for the generation of damascenone by acid hydrolysis under laboratory conditions. Some of these hydrolytic conditions comprised low pH and high temperature, so the observation of multiple "precursors" in these isolates does not mean that all such compounds will contribute to the formation of damascenone during food or beverage production, much less to damascenone as a natural product. The specific structures of the compounds identified in some of these studies are described in the following section. Winterhalter et al.[285] used droplet countercurrent chromatography to fractionate a C18 RP isolate of a Riesling wine and monitored the fractions by SDE at a wine-like pH (3.2). There were at least three separate compounds in the fractions that gave damascenone under these conditions. Roberts and colleagues[286] fractionated an XAD-2 isolate of an apple juice using HPLC. They monitored the fractions for damascenone precursors by heating at pH 2.0 to 80 °C for 20 min and showed the presence of at least eight damascenone precursors among the various fractions. These results were said to indicate a complex biogenesis of damascenone, although the hydrolytic conditions were hardly biomimetic. Size exclusion chromatography suggested that these precursors included mono-, di-, and trisaccharides. Multiple precursors were also indicated in a similar study of extracts of cell cultures of Concord grapes[287] in which fractions were heated to 90 °C for 20 min at pH 2.0. Countercurrent chromatography was used to fractionate rose oil by Winterhalter et al.,[288] who showed that the damascenone precursor fraction, monitored by SDE at pH 2.5, eluted with the glycoconjugate fraction. Subsequently, further fractionation of the acetylated products of a rose oil extract using solid phase chromatography showed that at least four components of the oil could give damascenone when heated to pH 2 for 20 min at 90 °C.

In general, and as described above, the concentration of damascenone in grapes can be lower, often much lower, than the concentration in the corresponding finished wine. The ability to measure specific quality parameters in wine grapes is a sought-after goal of wine producers and enologists alike. To be able to measure potential wine damascenone concentration by examining the grapes from which the wine is to be made might provide one such quality parameter. Kotseridis et al.[200] used acid hydrolysis (pH 2.2, 100 °C, 60 min) of C18 RP isolates to measure hydrolytically releasable damascenone in red wine grapes in which the concentration of free damascenone was negligible. They then compared the grape hydrolytically releasable damascenone with the concentration of free and remaining hydrolytically releasable damascenone in the corresponding 1-year-old wines made from the same grapes. All damascenone concentrations were conducted using a properly validated GC-MS method using d_4-damascenone as internal standard. The concentration of damascenone in the finished wines varied from 34 to 67% of the hydrolytically releasable damascenone in the grapes, with a mean value of approximately 50%. A similar result was obtained by Loscos et al.,[298] who found a statistically significant correlation between the concentration of damascenone released by harsh acid hydrolysis (pH 2.5, 100 °C, 1 h) and that released by fermentation of RP isolates of several grape varieties. In this latter study, the concentration of damascenone in the fermented wines varied from 15 to 38% of the hydrolytically releasable damascenone in the grapes, with a mean value of approximately 28%. These lower proportions presumably reflect the slightly higher pH used by Loscos et al.[298] compared to that used earlier.[200] Kotseridis et al.[200] described their assay as being able to "predict approximately the levels of free and hydrolytically liberated damascenone in the wines". However, in both of these studies, the results were from several samples only and the wines were produced by microvinification, using the same yeast in the latter study[298] and presumably also in the former.[200] Loscos et al.[240,307] have subsequently shown that fermentation with different yeast strains can have a significant impact on the concentration of damascenone in wines made from the same must. A much broader study of a greater number of samples, varieties, and fermentation conditions is still required to determine whether predictive assays such as the one described above can be of use in winemaking and viticulture. The complex nature of the relationship between damascenone in wine and that measured in hydrolysates of grape-derived precursors is illustrated by the data of Kotseridis et al.,[200] which showed that the free plus hydrolytically liberated damascenone in the finished wines was sometimes higher (up to 145%) and sometimes lower (down to 60%) of the free plus hydrolytically liberated damascenone in the corresponding grapes.

A recent study of Riesling grapes and wines described a negative, rather than positive, correlation between free plus hydrolytically released damascenone in grapes and free damascenone in wine made from those grapes.[266] In this study, the concentration of damascenone in heated wines (pH 2, 100 °C, 1 h) was slightly less than in the unheated wines, leading the authors to conclude that the conversion of grape damascenone precursors to damascenone during vinification was quantitative, a

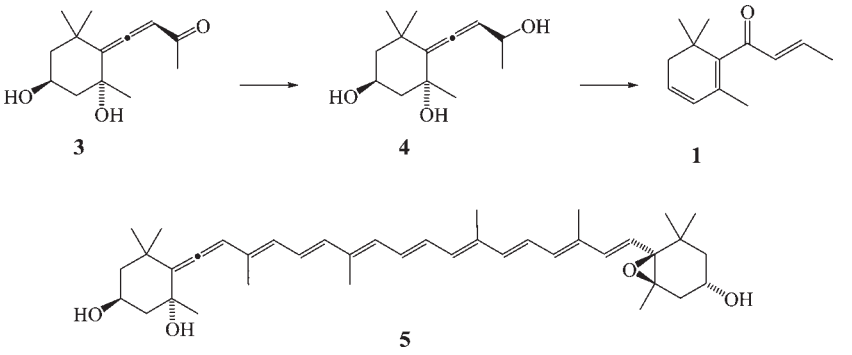

Figure 2. Formation of damascenone from neoxanthin as proposed by Ohloff et al. and Isoe et al.[314,315]

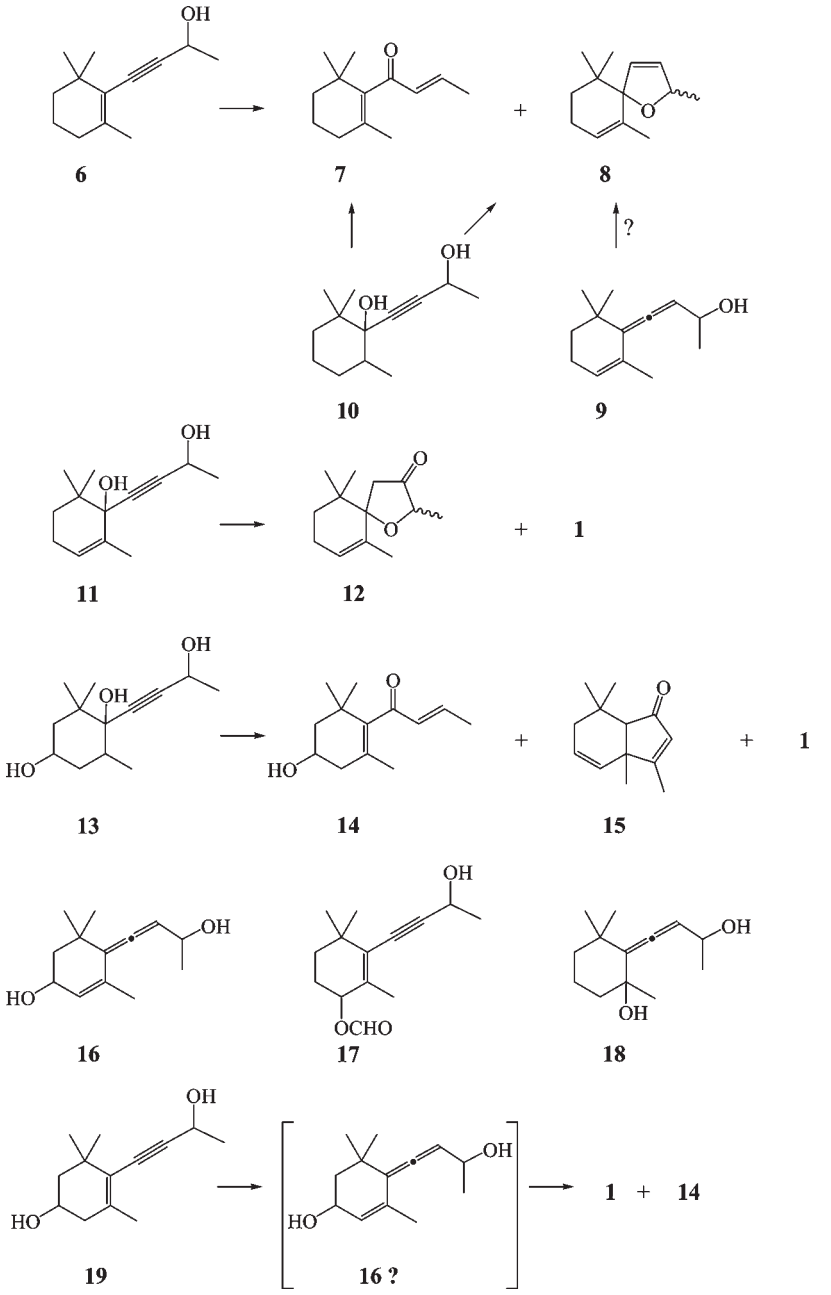

Figure 3. Compounds discussed by Ohloff et al. and Isoe et al.[314,315] in their studies of model compounds related to damascenone formation.

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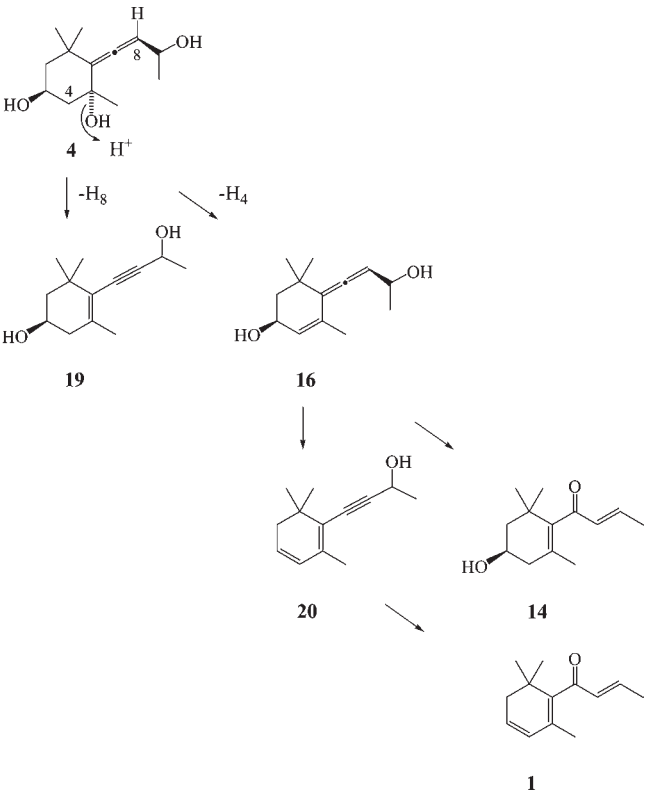
conclusion that contrasts with the observations described above and with the isolation of damascenone precursors from other Riesling wines (see below). However, damascenone is known to react with a range of wine components as well to rearrange to bicyclic products under harsh acid hydrolysis conditions,[308] and the amount of damascenone found in these heated wines[266] is presumably that resulting from both formation and degradation processes.

Other authors have also used hydrolytically liberated or "bound" damascenone as an indicator of changes in flavor potential of grape samples. Shure and Acree[20] used hydrolysis of C18 RP isolates at pH 2 and 90 °C to measure changes in bound damascenone during Concord berry development. They found that both free and bound damascenone increased in concentration toward the end of the ripening period. Girard et al.[209] used steam distillation at pH 2 to determine the "relative concentration" of bound damascenone in a study of Gewürztraminer grape development. Changes, as a result of various viticultural practices, in the concentration of damascenone released by strong acid hydrolysis from grape isolates have also been reported by others.[239,266,292,293]

Identification of Damascenone Precursors and Chemical Mechanisms for Damascenone Formation during Plant Product Processing and Storage. Structurally, damascenone is based on the megastigmane carbon skeleton **2**[309](Figure 1). Such compounds, commonly classified as C_{13} norisoprenoids, are assumed to be apocarotenoids, that is, formed from the degradation of carotenoids by the action of carotenoid cleavage dioxygenases which have been isolated from various plants, including *V. vinifera* (see, e.g., refs 310−313). Much of the evidence for this assumption is the similarity between most megastigmanes and the terminal component of plant carotenoids. Because such compounds are generally not oxygenated at C_7 but commonly oxygenated at C_9, presumably as a result of the oxidative cleavage of the acyclic portion of carotenoids, the key step in damascenone formation has been thought to be transposition of oxygen from C_9 to C_7.[282] Soon after the structural elucidation of damascenone, both Ohloff et al.[314] and Isoe et al.[315] suggested that such a transposition could take place with allenic intermediates. Ohloff et al.[314] hypothesized that damascenone (**1**) could be formed from an allenic triol **4** derived from the known ketone **3** ("grasshopper ketone"), which could in turn be derived directly by enzymatic cleavage of neoxanthin (**5**) (Figure 2). Because of potential difficulties in synthesizing the triol **4**, they chose to study the behavior of acetylenic analogues under acidic conditions (Figure 3). Thus, warming the acetylenic alcohol **6** to 45 °C in aqueous formic acid gave a mixture of β-damascone (**7**) and dehydrotheaspirane (**8**) in a ratio of 3:1. They suggested that the spiro compound **8** was formed via the allene **9**. Similarly, the hydrate **10** gave the same products, **7** and **8**, in a ratio of 55:45 in aqueous sulfuric acid at 70 °C. Hydrolysis of the unsaturated analogue of **10**, that is, **11**, under conditions similar to those employed for **6** gave damascenone (**1**) and the spiroketone **12** in good yield but in a ratio of 1:9. Finally, treatment of the acetylenic triol **13** in aqueous sulfuric acid at room temperature gave damascenone (**1**) along with 3-hydroxydamascone (**14**) and the ketone **15** in a ratio of 4:15:1. The authors suggested that damascenone might have been formed from triol **13** via an intermediate such as the diol **16**.[314]

Simultaneously with the work of Ohloff et al.,[314] Isoe et al.[315] also examined the hydrolysis of **11** at 100 °C in aqueous formic acid, obtaining the same products (**1** and **12**). When the reaction was conducted at room temperature, however, the rearranged formate ester **17** was obtained, along with an oxidized analogue. They also hydrolyzed the allenic diol **18** in acetic acid at room temperature, obtaining β-damascone (**7**) in 35% yield. An even greater yield of **7** (78%) was obtained when the tetrahydropyranyl ether of the tertiary alcohol in **18** was hydrolyzed. Isoe et al. proposed a sequence for damascenone formation that was essentially the same as that of Ohloff et al., except that they suggested that 3-hydroxydamascone (**14**) was the intermediate between triol **4** and damascenone. However, Ohloff et al.[314] showed that 3-hydroxydamascone (**14**) was not converted to damascenone in 30% sulfuric acid but instead reacted slowly to form a bicyclic product, a result that was subsequently confirmed by us [316] at milder pH values (3 and 1). The corresponding glucoside of **14** similarly failed to give any damascenone under the same conditions.[316] Despite these results and without any supporting data, a few other authors have also proposed 3-hydroxydamascone as a precursor to damascenone.[56,170,178,317] Curiously, in a subsequent review,[5] Ohloff presumed 3-hydroxydamascone to be a direct precursor of damascenone despite the evidence to the contrary in his earlier paper. In this review,[5] Ohloff also suggested that damascenone might be formed, along with 3-hydroxydamascone (**14**) from the acetylenic diol **19**, which had recently been isolated from Burley tobacco. He also proposed the allenic diol **16** as an intermediate in this conversion, with damascenone formed via the C_3 cation and 3-hydroxydamascone directly via side-chain rearrangement. These products (**1** and **14**) were said to be formed when **19** was treated with 30% sulfuric acid.[5]

Scheme 1. Formation of Damascenone and Other Products from Allene Triol 4 As Proposed by Skouroumounis and Sefton[316]



Since these early proposals, we have conducted an extensive investigation into the hydrolytic chemistry of these and other possible damascenone precursors as well as some analogues. Hydrolytic studies at pH 1 and 3 and a variety of temperatures ranging from 20 to 100 °C were conducted on the enyne diol **19**, proposed by Ohloff[5] as a possible precursor to damascenone.[282] The higher pH (3) is still at the low end of the range for most foodstuffs and beverages, whereas the former was employed for comparative purposes. In agreement with that earlier study,[5] **1** and **14** were formed, with the latter as the major product.[282] However, at room temperature and pH 3, the conversion was extremely slow. After 3 months, <10% of the enyne diol **19** was converted to products, and only a very low yield of damascenone was obtained (<0.2%). At 100 °C, conditions approximating the processing of many foodstuffs, approximately a fourth of the diol **19**, was converted to **1** and **14** after 4 h at pH 3. The proportion of damascenone in the product mix also increased with the more forcing conditions. Nearly complete conversion and trace amounts of other products were evident at pH 1 and high temperature. Subsequent hydrolytic studies with the corresponding C_9 glucoside of **19**[316,318] showed that this reacted even more slowly than the aglycone (by a factor of 8), although the ratio of **1** to **14** was somewhat higher for hydrolysis of the glucoside. In all of these studies, the ratio of damascenone (**1**) to 3-hydroxydamascone (**14**) at any given pH and temperature remained constant over time, confirming that the former was not formed from the latter.

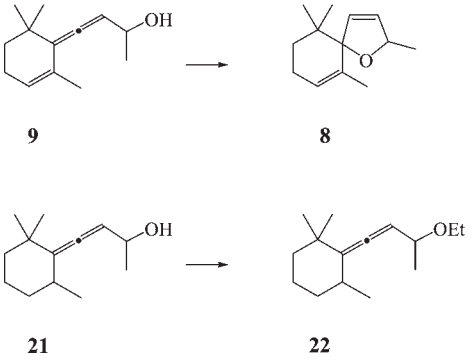
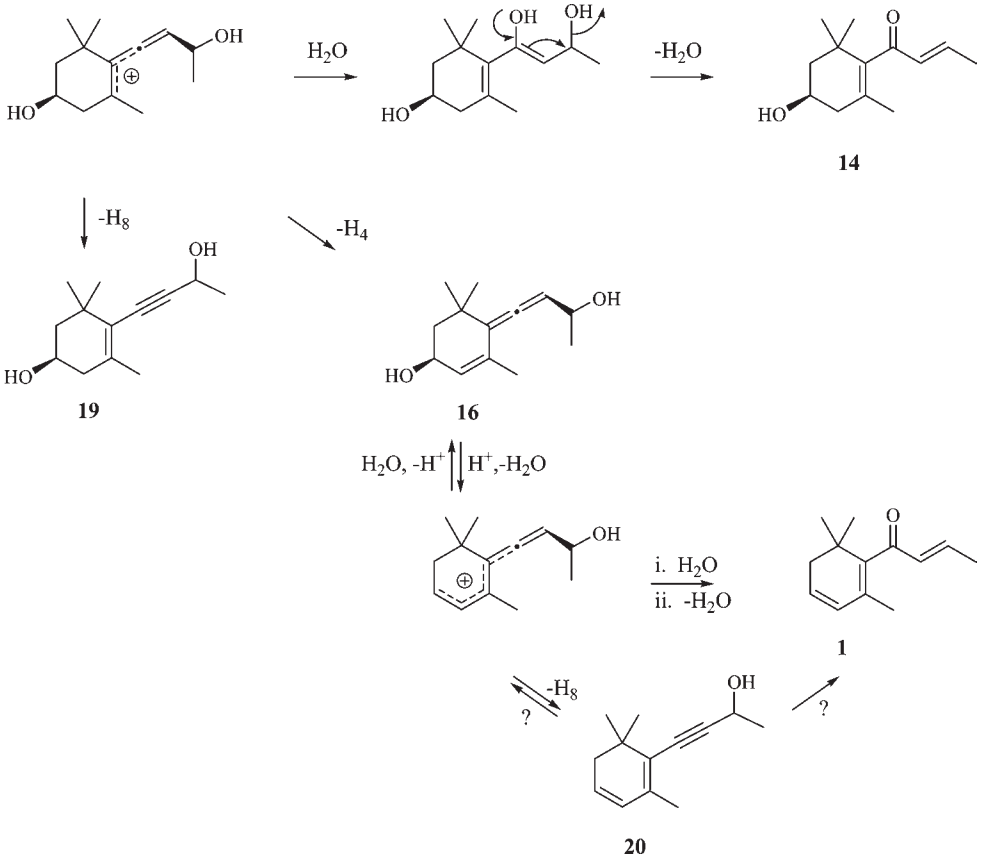


Figure 4. Desoxy analogues of allenic damascenone precursors and their hydrolysis products.[323]

The results show that whereas the diol **19** (or its glycoconjugates) might be able to act as a damascenone precursor during processing of acidic foodstuffs at high temperature, it is not a significant precursor to damascenone in unprocessed foods or beverages or those processed under milder conditions or which have significantly higher pH values. Even in an acidic product such as wine, which can be aged over a considerable period of time, the diol **19** will form damascenone only very slowly. The results also illustrate the pitfalls in relying on hydrolyses under forcing conditions to monitor damascenone precursors in products that are never subject to such conditions.

To test the hypothesis[314,315] that the triol **4** might also be involved in the formation of damascenone, this compound was synthesized and hydrolyzed at a pH of 3.0.[318] In contrast to the enyne diol **19**, the triol **4** reacted rapidly. At 80 °C, it was

Scheme 2. Revised Scheme for the Formation of Damascenone and Other Products from a C_5-Carbocation Derived from Triol 4 under Acid Conditions[323]



completely converted to three products after 1 h: damascenone (7% yield) plus 3-hydroxydamascone **14** (72%) and the diol **19** (21%). The same products were formed at lower temperatures but more slowly and with a smaller proportion of damascenone in the products. Even at room temperature, more than half of the triol **4** was consumed after 24 h. Two apparent intermediates were also observed in this study and tentatively assigned the structures **16** and **20**.[318] These hydrolytic studies showed that hydrolysis of the triol **4**, but not the diol **19**, was fast enough to account for the presence of damascenone in grapes and other fruits as well as young wines. In the case of grapes, this hypothesis has been supported by the observation of the three main products of the hydrolysis of triol **4** as grape constituents in free or glycoconjugated form and in proportions similar to those observed in the hydrolytic studies.[19,319] The presumed progenitor of the triol **4**, the so-called grasshopper ketone,[3] has also been observed as a grape component in glycoconjugated form.[18,19,283,320] Furthermore, recent research on carotenoid cleavage dioxygenase enzymes has shown that these can generate grasshopper ketone directly from neoxanthin.[347,348]

Following these observations, a revised proposal was put forward to explain the sequence of reactions involved in the formation of the products of allene triol **4** hydrolysis (Scheme 1).[316] In this scheme, loss of the reactive tertiary C-5 hydroxyl would be accompanied by either loss of the C-8 proton leading to enyne diol **19**, which could only react further under forcing conditions, or loss of the C-4 proton to give the diol **16**. This, in turn, could give either damascenone via loss of the C-3 hydroxyl and formation of the alcohol **20** or 3-hydroxydamascone via loss of the C-9 hydroxyl as suggested by Ohloff et al.[5] Subsequently, the assignments of the proposed intermediates **16** and **20** were confirmed by synthesis.[321,322] Hydrolysis of **20** at 25 °C indeed gave damascenone as the sole product. The time for half of the alcohol **20** to be converted to damascenone was 40 h at pH 3.0 and 65 h at pH 3.2.[323] Hydrolysis of the various synthetic isomers of the diol **16** under the same conditions, however, also gave damascenone as the only major product, accompanied by trace amounts of C-9 adducts. The alcohol **20** was observed as an intermediate in the conversion, and no trace of 3-hydroxydamascone (**14**) was formed.[322] The conversion of diol **16** to damascenone was slightly faster than the conversion of alcohol **20**, with half-lives of the former of 32 and 48 h at pH 3.0 and 3.2, respectively.[323] This meant that, although some of the damascenone generated from **16** was apparently being formed via **20**, the latter was not an obligatory intermediate in the conversion, which proceeded via at least two pathways. Chiral analysis of the products of short-term hydrolysates of pure enantiomers of the diol **16** showed that the hydroxyl at C-3 was completely epimerized in recovered starting material, whereas the stereochemistry at C-9 remained intact.[322] The reactivity at C-9 was further examined in a similar study of model allenic alcohols **9** and **21** (Figure 4), which are desoxy analogues of the diol **16** and triol **4**, respectively (Figure 4).[323] Compared to the triol **4** and diol **16**, hydrolysis of **9** in 10% aqueous ethanol proceeded relatively slowly. At both 25 and 45 °C and pH 3, the enantiomerically pure forms of **9** gave isomeric dehydrotheaspiranes **8** as the only products, and chiral analysis of these products showed that there had been no epimerization at C-9 prior to cyclization. Hydrolysis of the alcohol **21** proceeded even more slowly under these conditions, giving the corresponding ethyl ether **22** as the sole product.[323] No transposition of oxygen from C-9 to C-7 took place for either substrate, indicating that such transpositions are unlikely to account for damascenone formation in nature.

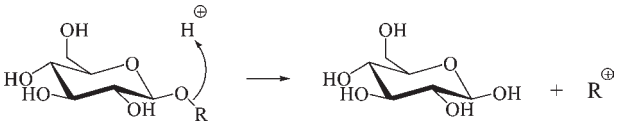


Figure 5. Hydrolysis of glycosides of activated alcohols.

Taken together, these various studies give a much clearer picture of the way in which various end products can be formed from the allenic triol **4**. Under mild acid conditions, the formation of all such products is presumably initiated by loss of the tertiary C-5 hydroxyl to give the corresponding carbocation, which can then react in several ways (Scheme 2). Loss of the C-8 proton would give the enynediol **19**, whereas hydration at C-7 and keto−enol tautomerism accompanied by loss of the C-9 hydroxyl presumably accounts for 3-hydroxydamascone formation. A minor pathway is initiated by loss of the C-4 hydrogen to give the diol **16**. Formation of the C-3 carbocation from **16** leads to damascenone, either directly via hydration at C-7 (analogous to formation of 3-hydroxydamascone from **4**) or via the acetylenic alcohol **20** formed by loss of the C-8 proton (analogous to formation of **19**). Under mild conditions, transposition of oxygen in simple secondary allenic alcohols to form α,β-unsaturated ketones does not take place and occurs only with more highly conjugated secondary allenic alcohols such as **16**, with tertiary allenic alcohols such as **4** (giving 3-hydroxydamascone), or with highly conjugated acetylenic alcohols such as **20** (giving damascenone).[323]

Detailed studies of the mechanism of damascenone formation from precursor forms have been conducted primarily with aglycones, because of the relative ease of synthesis of desired substrates and ease of analysis of products and intermediates by gas chromatography. Nevertheless, plants generally accumulate such compounds as glycoconjugates,[324,325] and therefore a complete understanding of damascenone formation in nature as well as in processed foods and beverages requires an understanding of the chemical behavior of such conjugates. Kotseredis et al. measured the amount of damascenone generated by heating red wine samples to 45 °C and showed that the increases in damascenone concentration were significantly less if the wines were first treated with an enzyme preparation with glycosidase activity.[200]

Several studies of the hydrolytic behavior of glycosides and their corresponding aglycones have shown that, in general, the former are converted to products significantly more slowly than are the latter.[316,323,326−328] In aqueous ethanol at pH 3.0 and 80 °C, geraniol glucoside was converted to linalool and other transformation products approximately 10 times more slowly than was geraniol itself.[316] This result was in accordance with a similar study in which an 8-fold difference between geraniol and its glucoside in this transformation at 100 °C was reported.[327] At 50 °C, the difference in reaction rates was even greater.[316] A similar trend in reaction rate difference was observed for other model aglycones and glycosides.[316] No geraniol was formed from its glucoside under the reaction conditions, showing that solvolysis of geraniol glucoside took place entirely via cleavage of the ether linkage (Figure 5), rather than of the glycosidic bond. It appears that the former process takes place under mild acid conditions only when a stabilized carbocation (in this case allylic)

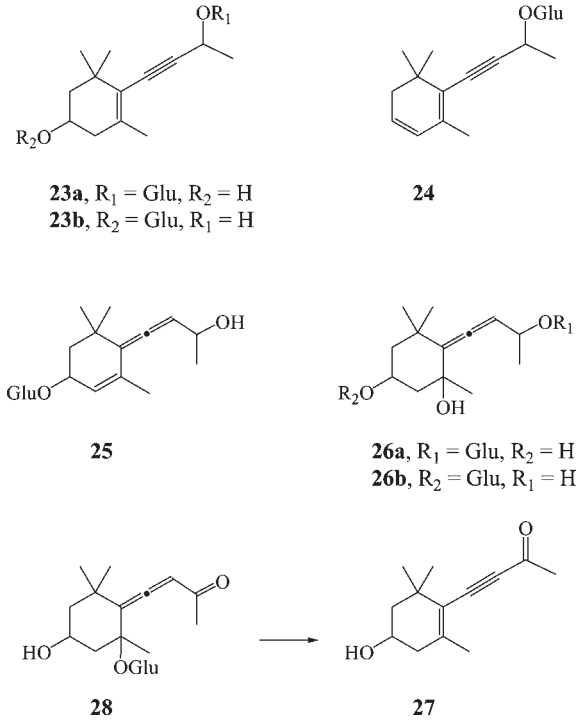


Figure 6. Glycoconjugates related to damascenone formation.

can be formed[18] and that hydrolysis of unactivated glycosides requires lower pH and higher temperatures.[329]

The influence of glycoconjugation on the hydrolysis of several precursors to damascenone has also been reported (Figure 6). As discussed above, the hydrolysis of the C-9 glucoside **23a** proceeded some 8 times more slowly than that of the aglycone **19**.[316] The same two main products, damascenone and 3-hydroxydamascone, were formed in the hydrolysates, but the yield of damascenone from the glucoside **23a** was greater. Presumably, by slowing the acid-catalyzed cleavage of the carbon−oxygen bond at C-9, the presence of the glucopyranosyl moiety has promoted alternative transformations to take place.

Glycoconjugation also slowed the rate of damascenone formation from the glucosides (**24** and **25**), but only by approximately 2-fold.[323] In the latter case, this was despite breakage of the C−O bond at C-3 not being rate determining, suggesting that the effect of glycoconjugation on this step was probably much greater than indicated by a comparison of the rates of product formation. The rate-determining step for hydrolysis of **24** to damascenone is not known.

Two recent studies have been conducted by the same group on the solvolysis of glucosides of the allenic triol **4**.[326,328] In the first of these studies,[326] a synthetic sample of the 3*S*,9*S*-isomer of the allene triol 9-glucoside **26a** was subjected to hydrolysis at pH 2 and 4 at three temperatures: 40, 60, and 90 °C. They reported that no damascenone was formed at pH 4 at any temperature, and even at pH 2, damascenone was formed only at 90 °C. At this temperature, damascenone and 3-hydroxydamascone were formed after 30 min in 5 and 8% yields, respectively (determined by a validated GC-MS method), accompanied by a 13% yield of the enyne diol glucoside **23a** (details of quantification not given). Several other nonvolatile compounds were also observed in the hydrolysates but could not be identified. When the reaction time was increased to 4 h, no additional damascenone was formed (no information on the other products was given). This suggests that the triol glucoside **26a** was considerably less reactive (no apparent reaction at pH 2 and 60 °C) than the corresponding aglycone, which could be converted to the final products at room temperature and pH 3.[318] The ratio of damascenone to 3-hydroxydamascone in the glucoside hydrolysate was also very different from that observed for the aglycone,[318] perhaps due to steric or inductive effects on the competing pathways leading from the C-5 cation shown in Scheme 2.[323]

In the second report of the hydrolysis of glycosylated precursors by this group,[328] their study was widened to both C-3 and C-9 glucosides of both the allene triol **4** and the enyne diol **19**, but comparisons of all four glucosides in model solutions were confined to relatively harsh conditions, not generally encountered in food or beverage production. At a pH of 5.4 and 120 °C, none of the four glucoconjugates were converted to damascenone. In contrast to this, however, when a green tea infusion was used as the medium under otherwise identical conditions, increases in damascenone concentration (equivalent to up to a 1% conversion of the added glucoside) in the medium appeared to take place with all four substrates. There was no obvious explanation for the different behaviors of the two media, and the authors speculated that as yet unidentified interactions with other metabolites might have been responsible for the observations. The experiments were apparently not replicated, so it is unclear which increases in damascenone were statistically significant.

At pH 3, hydrolyses were reported only for the C-9 glucosides at 90 °C.[328] The allene triol glucoside **26a** was converted to damascenone in an apparently non-time-dependent manner, whereas no damascenone was observed in the hydrolysates of the enyne diol glucoside **23a**. The latter result is consistent with the earlier study of this compound,[316] which showed that, at this pH and 100 °C, the rate of conversion of the enyne diol glucoside to damascenone was slow and yields were low, even after several hours of heating. At a pH of 2.0 and 90 °C, all four glucosides were converted to damascenone. Both the C-3 and C-9 glucosides **26a** and **26b** gave similar yields of damascenone, that is, 6−8% (the paper consistently discusses transformation "rates", although it is clear from the context that "yields" were meant), and the formation of damascenone was largely complete after 10 min. Lower yields of damascenone were obtained from the enyne diol glucosides **23a** and **23b** in a time-dependent manner (over 30 min). For reasons that are unclear, the yield of damascenone was greater for the C-3 glucoside **23b**. The authors also reported that the percentage conversion of the C-3 glucoside **26b** to **23b** was nearly 3 times greater than for the corresponding transformation of the triol 9-glucoside **26a**. This provides a further example of the influence of glycoconjugation on product distribution in addition to those discussed above.

A recent paper[268] reported ratios of damascenone to 3-hydroxydamascone in two Chardonnay wines as well as two each of three red varietal wines. The concentration of these compounds was 1−2 orders of magnitude higher in the Chardonnay wines, and the ratio of damascenone to 3-hydroxydamascone in the Chardonnay was around 1:3 compared to 1:10 for the remaining wines. This was attributed to differences in the position of glycosylation on the allene triol **4** determining the breakdown of glycoconjugated forms of the intermediate diol **16** into either damascenone or 3-hydroxydamascone (**14**), despite earlier evidence that **14** is not formed at all from **16**.[322] The authors

analyzed only free forms of 3-hydroxydamascone in the wines, and therefore the observed ratios might indicate nothing more than different proportions of 3-hydroxydamascone in free compared to glycoconjugated forms in the different wines. Additionally, as the red and white wines were made in different ways, these concentrations could also result from yeast or other influences on damascenone during winemaking.

The evidence to date indicates that, when present in plants or plant products, the triol **4**, in aglycone form, is sufficiently reactive to form damascenone at room temperature and mildly acidic pH. Similarly, the triol **4**, the diol **19**, and their glucosides are capable of generating damascenone under mildly acidic conditions and elevated temperature such as encountered in the preparation of processed foods. There is not yet, however, sufficient evidence to support the hypothesis that apocarotenoid glycoconjugates are able to form damascenone at room temperature or to account for damascenone formation during steam distillation of less acidic products such as leaves or flower petals. Further studies that better mimic the conditions under which damascenone can be formed in such products are still required.

Several papers indicate the possibility that glycosidase enzymes (which in some cases, at least, operate via protonation of the glycosidic moiety)[330] are capable of initiating solvolytic processes that might lead, inter alia, to damascenone formation. As discussed above, damascenone has been reported as a component of enzyme hydrolysates of reverse phase isolates of plant extracts,[105,300−302] although other such studies have noted the presence of this compound in acid, but not enzyme, hydrolysates. Chevance et al.[170] reported a substantial increase in the concentration of damascenone in a dark ale following treatment with a glycosidase enzyme but, as described above, did not verify GC peak homogeneity in their study. Näf et al.[331] described the direct formation of damascenone plus 3-hydroxydamascone when the pentaacetate of the 9-glucoside **26a** was treated with sodium methoxide followed by a β-glucosidase enzyme. As another example of apparent acid-catalyzed rearrangement accompanying enzyme hydrolysis, Osorio et al.[332] reported the formation of the acetylenic ketone **27** from grasshopper ketone 5-glucoside **28** following treatment with almond emulsin. Nevertheless, the triol **4** is extremely labile and has been identified in enzyme hydrolysates by some authors,[18,41] and the possibility that some of these observations result from artifactual degradation of enzyme hydrolysis products cannot yet be discounted.

Of the various synthetic apocarotenoids that have been shown to generate damascenone by mild acid hydrolysis, three (the enyne diol **19**, the allene triol **4**, and the acetylenic alcohol **20**) have been reported as being isolated from natural sources, in either aglycone or glycoconjugated form. In some cases, these compounds were obtained from fractions monitored specifically for their damascenone-generating potential. The enyne diol **19** was first isolated, from Burley tobacco, in 1976,[333] subsequently observed as a component of grape juices,[19,282] and tentatively identified as a component of black tea infusions.[97] It was also observed in glycosidase hydrolysates of reverse phase isolates of grape juices[18,19,282,285] and of purple passionfruit.[334] The amount observed in the enzyme hydrolysates of Chardonnay grape isolates was sufficient to account for the damascenone formed by mild acid treatment of the same extracts.[19] A study of the glycoconjugate fraction obtained from a Riesling[285] wine showed that, when this isolate was further fractionated, the diol **19** was observed in the enzyme hydrolysates of some, but not all, fractions that also yielded damascenone on acid hydrolysis. This

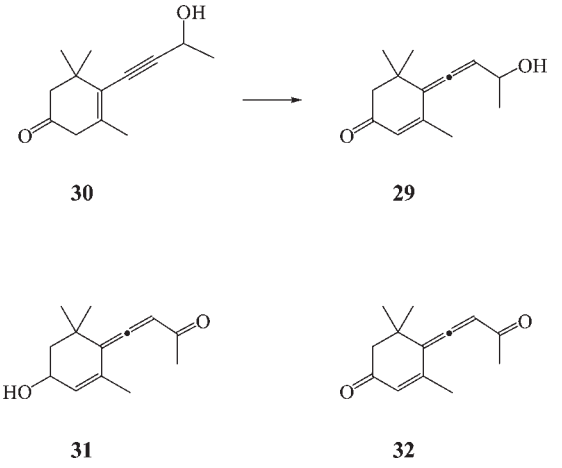


Figure 7. Some oxidized analogues of damascenone precursors.

study was, however, confounded by the possibility of further oxidation by glycosidase enzyme preparations of homoallylic cyclohexenols (such as **19**) to the corresponding cyclohexenones (such as the allenic ketone **29**), presumed to be formed by rearrangement of **30** (Figure 7), a problem that was not recognized at the time.[319] The isolation of the oxidized form of **19**, that is, the 9-ketone **27**, led Winterhalter and colleagues[334] to speculate that **19** might be formed from **27**, which in turn could be formed by oxidative cleavage of a corresponding carotenoid, thus constituting an alternative biogenesis of damascenone to that proposed by Ohloff et al.[314] and Isoe et al.[315] from neoxanthin **5**. The ketone **27**, however, could just as easily be formed in vivo by oxidation of **19** or dehydration and rearrangement of grasshopper ketone **3**.[332]

Both the C-3 and C-9 glucosides of the diol **19**, that is, **23a** and **23b**, were first isolated as natural products by Winterhalter and colleagues from a Riesling wine and from rose flowers.[288,289,335,336] They were subsequently isolated from green tea infusions, and the stereochemistry of both was assigned as 3*R*,9*R* on the basis of their identity with synthetic samples.[328,337] Roberts et al.[286] have tentatively identified an arabinosyl glucoside of **19** as a constituent of apple juice.

The allene triol **4** has been isolated as a natural product from fresh leaves of *Helianthus annuus*[338] and observed as an aglycone from enzyme treatment of reverse phase isolates of starfruit[41] and Merlot grapes.[18] Earlier, the 9-glucoside **26a** was isolated, as the pentaacetate derivative, from the leaves of *Lycium halimifolium*, the essential oil of which contains damascenone as the main product.[331] The same compound was subsequently identified as a component of leaves of *Premna subscandens*.[339] More recently, Suzuki et al.[326] reported identifying the 3*S*,9*R*-isomer of the 9-glucoside **26a** in extracts of *R. damascena* flowers by LC-MS comparison with a synthetic sample. They also reported identification of the 3*S*,9*S*-analogue, but as the ion intensity ratios of the synthetic sample do not match those of the corresponding peak in the isolate, this latter assignment is, at best, tentative. In a subsequent paper by the same group, the C-9 glucoside **26a**, with 3*S*,9*R*-stereochemistry, was isolated from a green tea infusion and the structure confirmed by comparison with a synthetic sample.[328]

The acetylenic alcohol **20** has been reported as a constituent of rum,[340] a product containing high concentrations of damascenone (see above). However, no experimental evidence was presented in

support of this identification and, as far as we are aware, this compound has not been cited elsewhere as a natural product or as a constituent of foods or beverages.

A direct formation of damascenone via thermal degradation of carotenoid precursors has been proposed to explain the substantial increase in damascenone, from <0.3 to 255 μg/kg, in coffee beans when heated to medium roast.[115] Free radical chemical oxidation of neoxanthin has been shown to generate grasshopper ketone **3**, but no damascenone was observed in the reaction.[341] By contrast, Bezman and colleagues[342] were able to form damascenone by treatment of 9′-*cis*-neoxanthin with peroxyacetic acid at high temperatures (90−130 °C). Ferreira et al.[251] described an experiment in which a port wine was saturated with oxygen, supplemented with lutein, and then heated to 60 °C for up to 87 h. The concentration of damascenone increased and then decreased in the supplemented but not in the nonsupplemented (control) sample. However, as the sample of lutein (which is structurally unrelated to damascenone) was impure, the amount added was not specified and the initial concentration of damascenone in the supplemented wine at the beginning of the experiment was only half that in the control wine, the reasons for the changes in damascenone concentration remain unclear.

The role of fermentation in determining the concentration of damascenone in alcoholic beverages (mainly wine) has been described by several groups. In a study of beer production, Chevance et al.[170] analyzed a sample of wort and reported a damascenone concentration of 450 μg/L, which was then undetected after fermentation. When the wort was supplemented with either 500 or 1000 μg/L damascenone, the concentration of this compound was measured as half the calculated initial plus added material at the beginning of fermentation, and this value was reduced to 10−16% by the end of fermentation. There is no obvious reason why damascenone should be totally removed by fermentation from unsupplemented wort but only partially diminished in the supplemented ferments and, as discussed above, these concentrations were determined by GC-FID alone and there was no verification of peak homogeneity in this study. However, the results for the supplemented ferment indicate that yeast might play a role in at least partially removing damascenone during the fermentation of beer. In contrast to this paper,[170] Kishimoto described an increase in damascenone concentration after fermentation of wort and measured much smaller concentrations of damascenone (<1 μg/L) in their samples.[167] Saison et al. subjected a 1-year-old beer to refermentation with brewing yeast and found substantial decreases in the concentration of various aldehydes but no change in the concentration of damascenone.[174]

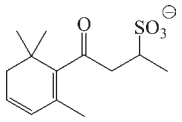
Some reports of damascenone in grapes and wine indicate that the concentration of damascenone is generally higher in the latter. Chisholm et al.[17] described an increase in the odor intensity of damascenone following fermentation of grape musts. Subsequently, Guth[195] used SIDA to quantify damascenone during the production of a Gewürztraminer wine and showed that the concentration increased from only a trace in the must to 6.2 μg/L at the end of fermentation. Similarly, increases in damascenone concentration in six commercial ferments, encompassing six grape varieties, have been described by Lloyd et al.[272] These concentrations all rose from sub-μg/L levels in the must to between 1 and 10 μg/L at the end of fermentation.[272] Some papers indicate the importance of yeast strain or fermentation conditions to the concentration of damascenone in finished wine,[240,307,343] but whether this is due to biochemical effects on damascenone generation on further degradation of damascenone once formed, or a combination of both processes, is unresolved. Apart from biological processes, acid hydrolysis of precursor forms during fermentation might contribute to the increase in damascenone concentration during fermentation. Ugliano and Moio[253] measured an increase in damascenone concentration of 3 μg/L during fermentation of a Fiano must but also found an increase to 2 μg/L when a sterile sample of the must was simply held at the same temperature for the same time. On the other hand, a study of wine production during fermentation[240] described concentrations of damascenone in finished wine that could not be accounted for in terms of the amount of damascenone generated by acid hydrolysis of reverse phase isolates of the corresponding must. Of course, this observation might simply indicate that solid phase extraction is not efficient in isolating all of the damascenone precursor material, a possibility which is consistent with other observations of this group that spiking ferments with solid phase extracts of grapes gives relatively small increases in damascenone concentration compared to unspiked ferments.[294,307]

Biochemical contributions to damascenone formation during fermentation could include processes whereby secondary metabolites are converted to reactive damascenone precursors that then generate damascenone through acid hydrolysis. An increase in damascenone concentration during malolactic fermentation has been reported by some authors,[237,344,345] whereas others[195] observed no such increase. Ugliano and Moio associated this increase with the glucosidase activity of the bacteria that were the subject of their study.[237] As described above, various authors have reported damascenone formation following treatment of various extracts with glycosidase enzymes.[105,300−302] Izquierdo Cañas et al.[344] suggested that an increase observed by them during secondary fermentation might just be due to acid hydrolysis alone over the time period of secondary fermentation. Lloyd et al.[272] demonstrated that adding oxidized forms of potential damascenone precursors such as the ketones **31** and **32** (Figure 7) to ferments resulted in significant generation of damascenone, but whether such oxidized precursors are present in raw materials used to make fermented beverages remains to be demonstrated. Stingl et al.[303] generated damascenone by SDE treatment of apple leaf extracts but reported no increase in damascenone concentration when the extracts were first treated with baker's yeast.

Changes to Damascenone Concentration during Wine Storage. The observed formation of damascenone during food processing, beer aging, etc., has led to the considerable body of research into the generation of damascenone from precursor forms, as detailed above, but less is known of the processes that might diminish the concentration of damascenone in foods and beverages over time. Given the acidic nature of wine and the demonstration of the presence of acid-labile damascenone precursors, it might be expected that the concentration of damascenone in wine would increase during bottle aging, but this is not always the case. Rapp and Güntert[346] reported a decrease in the concentration of damascenone in white wine aged for 7 years. Similarly, Guth measured a rapid increase in the concentration of damascenone during fermentation of a Gewürztraminer must, but the concentration of damascenone in the resultant wine then diminished by more than two-thirds during storage in stainless steel tanks over 4 months.[195] Decreases in damascenone concentration during aging are also implied by the data of

Silva Ferreira and dePinho, who, in a survey of white and port wines of different vintages, found a trend to lower concentrations of damascenone in older wines.[216,224] Other authors, on the other hand, have noted or suggested increases in damascenone concentration during early periods of wine conservation. Lopez et al.[199] found a greater amount of damascenone in a blended barrel-aged red wine compared to three younger wines and suggested that the presence of damascenone was somehow linked to wood maturation, although the wines being compared were not made from the same musts. The same group[225] noted an increase in the concentration of damascenone in a red wine during the first 6 months of storage in stainless steel. They also reported an increase and then a decrease in damascenone concentration during storage of the same wine in oak barrels over a longer period (up to 24 months), but the data are confounded by the concentrations having been determined for wines taken from a different set of barrels at each sampling time. Lloyd et al. also noted increases in damascenone concentration during barrel aging of Chardonnay, Shiraz, and Pinot noir wines.[272] Both increases and decreases in damascenone concentration following micro-oxygenation have been noted.[267] Finally, an investigation of the evolution and degradation of volatile wine components during heating, to 50 °C, of experimental wines produced by fermentation of model media supplemented with RP isolates from eight different grape varieties showed that, in every sample, the concentration of damascenone reached a maximum after heating for 1 week and then diminished during further heating for up to 9 weeks.[297]

A detailed study of the composition of wine stored on yeast lees using three yeast strains has been described recently.[307] Some wine samples were supplemented with solid phase grape isolates, either before or after fermentation. During storage over the first 3 months, both increases and decreases in damascenone concentration were observed, depending on the yeast strain, but after 9 months on lees, the concentration of damascenone decreased in every case. These data indicate competing processes of damascenone generation and degradation. It is not clear to what extent it was the yeast lees contact that contributed to loss of damascenone as no control experiments in which wines were aged without lees were reported. However, one yeast strain, the one giving the lowest concentration of damascenone at the end of fermentation, also gave a consistently lower percentage decrease of this compound during maturation, resulting in the 9-month-old wines all having similar damascenone concentrations. These data indicate that the yeast lees might be contributing to damascenone loss, although it must be borne in mind that wines were fermented with a particular yeast strain and then stored on the lees of that same strain. As the yeast strain had clearly affected the postfermentation wine composition, it is not clear to what extent the different yeast storage results were a consequence of different prematuration wine compositions. Other evidence for a direct influence of lees on loss of damascenone was that when strong acid hydrolysates of solid phase grape isolates, containing damascenone, were added to model wines with or without lees from one of the yeast strains, the damascenone concentration diminished some 8 times more quickly in the presence of the lees.[307] However, as the rate of damascenone loss in even the control experiment (model wine, no lees) was much greater than encountered with the same lees strain in real wine, it is debatable as to what extent such model experiments give an insight into processes taking place with real wines. A recent paper has described the partitioning of damascenone between sparkling wine and yeast lees.[271]



33

Figure 8. Adduct formed from damascenone and sulfur dioxide.

Chemical Mechanisms for Degradation of Damascenone in Wine. Apart from the possibility of biological processes decreasing the amount of damascenone in foods and beverages, chemical transformations could also account for such decreases. Such reactions in wine-like media have been reported.[308] Damascenone was shown to be degraded by acid alone, but the rate of the reaction was slow and unlikely to account for observed losses of damascenone during wine maturation. Damascenone also reacted with various nucleophilic wine components, but most of them reacted relatively slowly. The reaction of damascenone with sulfur dioxide, however, was rapid at wine pH, with 50% loss at room temperature in the presence of 80 mg/L sulfur dioxide after 30 days, and total loss of damascenone under more forcing conditions. The product of the reaction of damascenone with sulfur dioxide was shown to be the adduct **33** (Figure 8), formed by conjugate addition to the damascenone side chain.[308]

The control model wine solution used by Loscos et al.[307] in their study of yeast lees effects on wine components described above also contained sulfur dioxide, and the reported rate of damascenone loss in this solution was consistent with the kinetic data reported for this reaction.[308] The presence of sulfur dioxide has been shown to suppress the formation of damascenone in port samples heated to 45 or 60 °C.[224]

Reaction with sulfur dioxide could also explain why loss of damascenone during wine maturation has been more commonly reported with white wines (see above), which usually contain higher concentrations of free SO_2, and is consistent with a report of greater increases in damascenone concentration during heating of red, compared to white, wine samples.[152] Reports of greater concentrations of damascenone in wine samples matured in oak, compared to stainless steel,[195,225] might also simply reflect the more rapid binding or oxidation of SO_2 in oak storage.

CONCLUSION

Not only is damascenone an important component of perfumes, it is found in a wide variety of food products and beverages and is among the most heavily researched of all aroma compounds. A variety of detailed chemical studies have shed light on how damascenone can be formed chemically from polyhydroxylated apocarotenoids and their glycoconjugates, but these do not fully explain which of the various potential precursors can account for damascenone formation during steam distillation of weakly acidic material and during fermentation. Although sensory studies have gone some way toward determining the impact of damascenone on wine aroma (but not flavor), virtually nothing is known of the contribution of this compound to the aroma and flavor of other products. These questions will undoubtedly form the basis for many future studies on this ubiquitous and fragrant substance.

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