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Fate of Damascenone in Wine: The Role of SO_2

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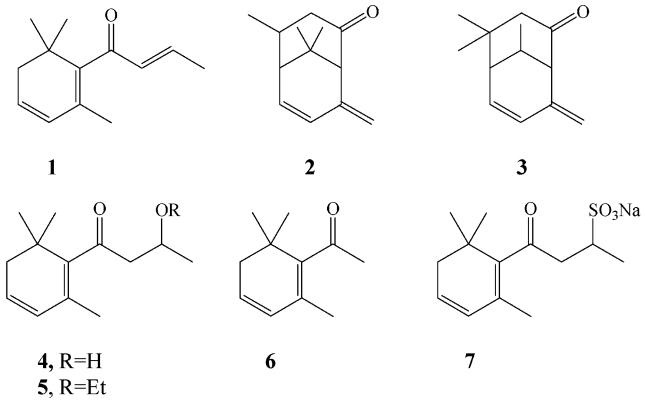
Damascenone has been shown to undergo reaction with common wine components. Following the action of acid and heat alone, two bicyclic compounds, 4,9,9-trimethyl-8-methylenebicyclo[3.3.1]non-6-en-2-one (**2**) and 4,4,9-trimethyl-8-methylenebicyclo[3.3.1] non-6-en-2-one (**3**), were isolated. However, this conversion takes place only very slowly, if at all, under milder conditions (45 °C). When treated with a variety of nucleophiles at pH 3.0 and 5.5, the concentration of damascenone in buffered aqueous ethanol decreased by minor amounts (10−20%) except for cysteine and 2-mercaptoethanol addition at pH 5.5 (∼40 and ∼30%, respectively) and SO_2 (>90% at pH 3.0; 100% at pH 5.5). An adduct from this last combination was prepared and shown to be the C_9 sulfonic acid derivative of damascenone. A detailed investigation into the effect of SO_2 demonstrated that loss of damascenone in model wine was directly related to the concentration of added SO_2 but was essentially unaffected by small changes in pH.

KEYWORDS: Damascenone; sulfur dioxide; sulfonic acid; nucleophilic components; degradation; bicyclodamascenone

INTRODUCTION

β-Damascenone (damascenone) (**1**) is one of the most important aroma compounds known to science. It is thoroughly ubiquitous in nature and is one of the staples of the international perfume industry (*1*). It is characterized by a "stewed apple" or "pear" aroma and has been shown to be a positive contributor to the fruity aroma of red wine varieties (*2*). Its aroma threshold has been measured as 50 ng L^{-1} in model wine (*3*) and as low as 2 ng L^{-1} in water (*4*), making it among the most potent odorants known. Compounds such as damascenone belong to a class of aroma compounds known as the C_{13}-norisoprenoids, which are believed to be degradation products of higher carotenoid species such as neoxanthin (*5*).

Damascenone is formed in wine, initially, in significant amounts (microgram per liter quantities), mainly during fermentation, but the concentration of this compound has been shown to then fall off, by as much as 75% over the first few months of wine maturation (*6*). Wine is a complex medium, containing a myriad of compounds displaying a wide range of chemical reactivities. Damascenone contains two cross-conjugated enone moieties, and so one might imagine that damascenone would be reactive toward nucleophilic species, common to wine, under suitable conditions. In addition, external additives, for example, sulfur dioxide, might contribute to the reduction in concentration of damascenone over time. Demole and Enggist (*7*) have shown that under acidic conditions, damascenone will undergo intramolecular cyclization to produce two bicyclic compounds, assigned on the basis of low-field NMR spectra, as **2** and **3**. However, the conditions they employed (180 °C, *p*-TsOH) are completely atypical for the handling of wine, and it is unclear whether these particular compounds could form under more realistic winelike conditions.



We have conducted a study into the reactivity of damascenone toward mild acid and toward various nucleophilic species in an effort to determine and understand the fate of this important aroma compound in wine.

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MATERIALS AND METHODS

Reagents and Instruments. Chemicals were purchased from Sigma-Aldrich. All solvents used were of pesticide grade from OmniSolv (Darmstadt, Germany). All organic solvent solutions were dried over anhydrous sodium sulfate before being filtered. pH measurements were made with an EcoScan pH 5/6 m (Eutech Instruments, Singapore), which was calibrated before use. Column chromatography was performed using silica gel 60 (230−400 mesh) from Merck. Routine ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini spectrometer at operating frequencies of 300 and 75.5 MHz, respectively. HMBC and CIGAR experiments were run on a Varian Unity Inova 600 MHz spectrometer using Varian vnmr6.1c software. LC-MS spectra were recorded on an API 300 electrospray mass spectrometer, and high-resolution mass spectra were recorded on an Agilent ESI-TOF mass spectrometer with accurate mass capabilities. Model wine buffer solutions in the range pH 2.8−3.4 were prepared by saturating a 10% ethanol solution with potassium hydrogen tartrate and adding 10% tartaric acid solution until the required value was attained. Buffer solutions at pH 5.5 were prepared by saturating a 10% ethanol solution with potassium hydrogen phosphate and adding 10% citric acid solution until the required value was attained.

Procedures. *Preparation of Samples for Analysis of Damascenone (**1**).* For analysis of the damascenone plus miscellaneous nucleophile experiments, an aliquot of the reaction mixture (20 μL) was diluted to 10 mL with model wine in a 15 mL glass screw-cap vial with an aluminum-lined cap (Supelco). To this was added an aliquot (100 μL) of a solution of [$^{2}H_4$]damascenone (*8*) in ethanol (5 μg/mL) as internal standard. Pentane/ethyl acetate (2:1, 3 mL) was added, and the mixture was shaken briefly. A portion of the organic layer was then transferred to a vial for GC-MS analysis. For analysis of the detailed damascenone plus sulfur dioxide experiments, an aliquot (100 μL) of the solution of [$^{2}H_4$]damascenone (*8*) in ethanol (5 μg/mL) was added directly as internal standard to the hydrolysate sample (5 mL). Extraction was performed as described above. For calculating the concentration of the analytes in the wine samples, replicate standards were prepared at the same time as the wine samples, by adding the internal standard solution (100 μL, 5 μg/mL) and a solution of damascenone in ethanol (100 μL, 5 μg/mL) to dichloromethane (1.8 mL) and analyzing this mixture by the GC-MS method (see below) to calculate the relative response factors.

GC-MS Analysis. Samples were analyzed with a Hewlett-Packard (HP) 6890N gas chromatograph fitted with a Gerstel MPS2 autosampler and coupled to an HP 5973N mass spectrometer. The liquid injector was operated in fast liquid injection mode with a 10 μL syringe (SGE, Australia) fitted. The gas chromatograph was fitted with an approximately 30 m × 0.25 mm i.d. J&W fused silica capillary column DB-WAX, 0.25 μm film thickness. The carrier gas was helium (BOC Gases, ultrahigh purity), and the flow rate was 1.2 mL/min. The oven temperature, started at 50 °C, was held at this temperature for 1 min, then increased to 220 °C at 10 °C/min, and held at this temperature for 10 min. The injector was held at 200 °C and the transfer line at 250 °C. The sample volume injected was 2 μL, and the splitter, at 42:1, was opened after 36 s. Fast injection was done in pulsed splitless mode with an inlet pressure of 25.0 psi maintained until splitting. The glass liner (Agilent Technologies) was borosilicate glass with a plug of resilanized glass wool (2−4 mm) at the tapered end to the column. Positive ion electron impact spectra at 70 eV were recorded in the range *m/z* 35−350 for scan runs. For quantification of damascenone, mass spectra were recorded in the selective ion monitoring (SIM) mode. The ions monitored in SIM runs were *m/z* 69, 175, and 190 for damascenone, and 73, 179, and 194 for [$^{2}H_4$]damascenone. Selected fragment ions were monitored for 20 ms each. The underlined ion for each compound was the ion typically used for quantitation, having the best signal-to-noise and the least interference from other components. The other ions were used as qualifiers.

Validation. The method was validated by a series of duplicate standard additions of unlabeled damascenone (1.0−200 μg/L, $n = 9 \times 2$ for compound) to a dry white wine (Australian Chenin Blanc, 11.5% alc/vol, pH 3.40). The standard addition curve obtained was linear throughout the concentration range, with a coefficient of determination (r^2) of 0.996 and the following linear regression equation: $y = 2.59x + 0.122$. To ensure that the accuracy of the analysis was maintained, duplicate control wines, each with and without spiked standard addition of 100 μg of damascenone per liter of wine, were analyzed with every set of samples; the measured damascenone in these samples was always in the range of 95 < [damascenone measured] < 100 μg/L.

Heating of Damascenone under Acidic Conditions. Method A. Damascenone (500 mg, 2.6 mmol) and *p*-toluenesulfonic acid (5 mg) were heated in a sealed tube in a microwave oven (400 W) for 10 min. The two major products were isolated by column chromatography (5% EtOAc/×4) as a mixture (195 mg, 39%). A second chromatography provided pure samples of both **2** and **3** as colorless oils.

Method B. A solution of damascenone (100 mg, mmol) in model wine (10% ethanol, pH 2.8, 50 mL) was heated in a sealed vessel at 100 °C for 11 days. The organic products were extracted with ether and purified by column chromatography (5% EtOAc/×4) to give the same two products as isolated from method A (26 mg, 26%) plus unreacted damascenone.

*4,9,9-Trimethyl-8-methylenebicyclo[3.3.1]non-6-en-2-one (**2**):* δ_H ($CDCl_3$) 6.34 (1H, d $J = 9.8$ Hz, H_7), 5.93 (1H, m, H_6), 5.00 (1H, s, H_{11a}), 4.99 (1H, s, H_{11b}), 2.77 (1H, br s, H_1), 2.45 (1H, m, H_4), 2.13−2.02 (2H, m, H_3), 1.99 (1H, m, H_5), 0.97 (3H, d $J = 6.8$ Hz, H_{10}), 0.97, 0.95 (6H, 2 × s, $H_{12,13}$); δ_C ($CDCl_3$) 210.8 (C_2), 139.9 (C_8), 129.7 (C_6), 129.4 (C_7), 116.8 (C_{11}), 64.7 (C_1), 45.9 (C_5), 41.9 (C_3), 36.3 (C_9), 33.2 (C_4), 26.5 (C_{12}), 26.0 (C_{13}), 19.3 (C_{10}); MS, *m/z* (%) 190 (44), 175 (15), 148 (54), 146 (47), 133 (28), 120 (38), 119 (76), 105 (100), 91 (33), 79 (19), 77 (27), 69 (54), 41 (19).

*4,4,9-Trimethyl-8-methylenebicyclo[3.3.1]non-6-en-2-one (**3**):* δ_H ($CDCl_3$) 6.31 (1H, d $J = 9.9$ Hz, H_7), 5.96 (1H, m, H_6), 5.03 (1H, s, H_{12a}), 4.99 (1H, s, H_{12b}), 2.97 (1H, br s, H_1), 2.40−2.28 (2H, m, $H_{3a,9}$), 1.91−1.80 (2H, m, $H_{3b,5}$), 1.06, 1.04 (6H, 2 × s, $H_{10,11}$), 0.92 (3H, d $J = 6.7$ Hz, H_{13}); δ_C ($CDCl_3$) 210.4 (C_2), 137.8 (C_8), 130.1 (C_6), 128.8 (C_7), 117.5 (C_{12}), 59.7 (C_1), 47.9 (C_3), 46.8 (C_5), 40.8 (C_4), 31.2 (C_9), 28.5 (C_{10}), 27.7 (C_{11}), 17.5 (C_{13}); MS, *m/z* (%) 190 (11), 175 (12), 134 (7), 106 (85), 105 (24), 91 (100), 83 (15), 77 (11), 41 (11).

*Conversion of **2** and **3** into Their Respective 2,4-Dinitrophenylhydrazones.* Ketone **2** (14 mg, 0.07 mmol) in ethanol (0.6 mL) was treated with 0.5 mL of a solution of 2,4-dinitrophenylhydrazine [prepared by dissolving 2,4-dinitrophenylhydrazine (200 mg) in concentrated H_2SO_4 (1 mL) and diluted with water (1.4 mL) and 95% EtOH (5 mL)] and left to stand for 2 days at 5 °C. The resultant precipitate was filtered and washed successively with ethanol, saturated sodium bicarbonate solution, and water before being recrystallized from 95% EtOH to give orange needles (12 mg, 44%): mp 136−140 °C; δ_H ($CDCl_3$) 11.10 (1H, s, NH), 9.05 (1H, d $J = 2.5$ Hz, H_{16}), 8.22 (1H, dd $J = 9.8$ and 2.5 Hz, H_{18}), 7.91 (1H, d $J = 9.8$ Hz, H_{19}), 6.28 (1H, d $J = 10.0$ Hz, H_7), 5.80 (1H, dd $J = 10.0$ and 6.6 Hz, H_6), 4.94 (2H, br s, H_{11}), 2.92 (1H, br s, H_1), 2.47 (1H, dd $J = 15.7$ and 5.2 Hz, H_{3a}), 2.27 (1H, m, H_4), 1.94 (1H, m, H_5), 1.74 (1H, dd $J = 15.7$ and 11.7 Hz, H_{3b}), 0.98 (3H, d $J = 6.7$, H_{10}), 0.94 (6H, 2 × s, $H_{12,13}$); δ_C ($CDCl_3$) 162.6 (C_2), 142.2 (C_8), 145.4, 137.5, 130.0, 130.0, 123.5, 116.5 (ArC), 129.9 (C_7), 129.1 (C_6), 116.2 (C_{11}), 57.3 (C_1), 46.3 (C_5), 35.5 (C_9), 31.4 (C_4), 27.9 (C_3), 26.7 (C_{12}), 26.1 (C_{13}), 19.6 (C_{10}).

Ketone **3** (20 mg, 0.1 mmol) in ethanol (0.8 mL) was treated exactly as above, with 0.7 mL of a solution of 2,4-dinitrophenylhydrazine. The product was collected and recrystallized from 95% EtOH to give orange needles (25 mg, 64%): mp 174−177 °C; δ_H ($CDCl_3$) 11.10 (1H, s, NH), 9.06 (1H, d $J = 2.5$ Hz, H_{16}), 8.22 (1H, dd $J = 9.6$ and 2.5 Hz, H_{18}), 7.92 (1H, d $J = 9.6$ Hz, H_{19}), 6.27 (1H, d $J = 9.9$ Hz, H_7), 5.86 (1H, dd $J = 9.9$ and 6.6 Hz, H_6), 5.00 (1H, br s, H_{12a}), 4.96 (1H, br s, H_{12b}), 3.14 (1H, br s, H_1), 2.26 (1H, m, H_9), 2.18 (1H, d $J = 14.6$ Hz, H_{3a}), 2.02 (1H, d $J = 14.6$ Hz, H_{3b}), 1.85 (1H, m, H_5), 1.05, 0.97 (6H, 2 × s, $H_{10,11}$), 0.89 (3H, d $J = 6.7$ Hz, H_{13}); δ_C ($CDCl_3$) 162.3 (C_2), 140.4 (C_8), 145.5, 137.5, 129.9, 129.1, 123.6, 116.4 (ArC), 129.9 (C_6), 129.5 (C_7), 117.2 (C_{12}), 52.6 (C_1), 47.4 (C_5), 39.4 (C_4), 34.4 (C_3), 31.9 (C_9), 28.7 (C_{10}), 27.5 (C_{11}), 17.7 (C_{13}).

General Procedure for Heating of Damascenone with Various Nucleophiles. Damascenone (0.1 g/L) was added to buffered aqueous ethanol at either pH 3.0 or pH 5.5. The required nucleophile was added to give a concentration of 0.1 g/L, and aliquots (5 mL) were sealed in

glass ampules and stored at 45 °C for 1, 2, or 3 months. After the required time, the ampules were removed and stored at −20 °C prior to analysis. The ampules were opened, and the damascenone was quantified as detailed above. Nucleophilic species added in this experiment were cysteine, sodium acetate, mercaptoethanol, ethylamine, and SO_2 (added as sodium metabisulfite).

Heating of Damascenone with SO_2. Damascenone (1 mg/L) was added to buffered aqueous ethanol at either pH 3.2 or pH 3.4. Sodium metabisulfite was added to each to give sulfur dioxide concentrations of either 80 ppm (120 mg/L $Na_2S_2O_5$) or 200 ppm (300 mg/L). It was noted that addition of these quantities of sodium metabisulfite had only a negligible effect (~0.01 pH unit for 300 mg added) on the pH of the solutions. Aliquots of each were sealed in glass ampules and stored at either room temperature (for 2, 4, 6, and 8 days and 2, 3,w and 4 weeks) or 45 °C (for 12 h and 1, 2, 3, 4, 5, 6, 7, 10, and 14 days). After the required time had elapsed, the ampules were opened, and the damascenone was quantified as described above.

*9-Hydroxymegastigma-3,5-dien-7-one (**4**).* To a solution of 2,5,5-trimethyl-1-acetylcylcohexa-1,3-diene (**6**) (*9*) (50 mg, 0.31 mmol) in dry THF (2 mL) at −78 °C was added dropwise LiHMDS (0.34 mL, 1 M in THF, 0.34 mmol). After 1 h of stirring at this temperature, acetaldehyde (0.017 mL, 0.31 mmol) was added and the reaction allowed to stir for 90 min, during which time the solution was allowed to warm to room temperature. The reaction was quenched with NH_4Cl solution, the solvent removed, and the product extracted with ether. The ether extracts were washed with water and brine and dried (Na_2SO_4), and the solvent was evaporated. The alcohol was purified by column chromatography (30% EtOAc/×4) to yield **4** (34 mg, 54%) as a colorless oil: δ_H ($CDCl_3$) 5.89−5.75 (2H, m, $H_{3,4}$), 4.29 (1H, m, H_9), 2.77 (1H, dd J = 18.3 and 2.7 Hz, H_{8a}), 2.63 (1H, dd J = 18.3 and 8.9 Hz, H_{8b}), 2.09 (2H, dd J = 4.1 and 1.5 Hz, H_2), 1.72 (3H, s, H_{13}), 1.22 (3H, d J = 6.5 Hz, H_{10}), 1.08, 1.07 (6H, 2 × s, $H_{11,12}$); δ_C ($CDCl_3$) 211.8, 141.3, 128.0, 127.9, 127.8, 63.8, 53.2, 39.7, 33.9, 26.2, 26.1, 22.3, 19.1; MS, *m*/*z* (%) 208 (4), 149 (39), 133 (31), 122 (31), 121 (100), 107 (20), 105 (42), 91 (25), 77 (15), 43 (38).

*9-Ethoxymegastigma-3,5-dien-7-one (**5**).* To metallic sodium (50 mg, 2.2 mmol) was added dry ethanol (1 mL). After the sodium had completely dissolved, a portion of the sodium ethoxide solution (0.065 mL, 0.14 mmol) was added to a stirred solution of damascenone (27 mg, 0.14 mmol) in dry EtOH (2 mL). The reaction was stirred for 90 min before being quenched with NH_4Cl solution and concentrated under reduced pressure. The product was extracted with ether, washed (H_2O, brine), and dried (Na_2SO_4) and the solvent evaporated to give a 3:2 mixture of product plus damascenone. The product was purified by column chromatography (CH_2Cl_2) to yield **5** (14 mg, 42%) as a pale yellow oil: δ_H ($CDCl_3$) 5.87−5.75 (2H, m, $H_{3,4}$), 4.02 (1H, m, H_9), 3.58 (1H, dq J = 9.1 and 7.0 Hz, H_{14a}), 3.44 (1H, dq J = 9.1 and 7.0 Hz, H_{14b}), 2.95 (1H, dd J = 17.6 and 6.9 Hz, H_{8a}), 2.50 (1H, dd J = 17.6 and 5.5 Hz, H_{8b}), 2.08 (2H, dd J = 3.8 and 1.2 Hz, H_2), 1.74 (3H, s, H_{13}), 1.20 (3H, d J = 6.2 Hz, H_{10}), 1.15 (3H, t J = 7.0, H_{15}), 1.09, 1.07 (6H, 2 × s, $H_{11,12}$); δ_C ($CDCl_3$) 208.4, 142.0, 128.3, 127.6, 127.6, 70.9, 64.1, 52.7, 39.8, 33.9, 26.2, 26.1, 20.0, 19.0, 15.5; MS, *m*/*z* (%) 236 (5), 221 (7), 192 (3), 175 (6), 149 (73), 133 (49), 122 (100), 121 (85), 107 (60), 105 (54), 91 (40), 73 (76), 45 (72). HRMS (ESI): found 237.1857 ($M + H^+$), $C_{15}H_{25}O_2$ requires 237.1855; found 259.1673 ($M + Na^+$), $C_{15}H_{24}O_2Na$ requires 259.1674.

*Sulfonic Acid Derivative (**7**).* A mixture of damascenone (95 mg, 0.5 mmol) and $Na_2S_2O_5$ (150 mg) in DMF (5 mL) was heated at 45 °C for 3 days. The DMF was removed, and the product was dissolved in H_2O, washed with EtOAc, and extracted with acetonitrile/EtOAc (5:1) to yield, after drying and concentration, a yellow solid (51 mg, 35%): δ_H (D_2O) 6.03−5.87 (2H, m, $H_{3,4}$), 3.47 (1H, ddq J = 9.3, 3.0, and 6.7 Hz, H_9), 3.35 (1H, dd J = 19.1 and 3.0 Hz, H_{8a}), 2.94 (1H, dd J = 19.1 and 9.3 Hz, H_{8b}), 2.14 (2H, dd J = 4.1 and 1.5 Hz, H_2), 1.72 (3H, s, H_{13}), 1.37 (3H, d J = 6.7 Hz, H_{10}), 1.09 (6H, 2 × s, $H_{11,12}$); δ_C (D_2O) 212.2 (C_7), 140.8 (C_6), 129.3 (C_3), 129.0 (C_5), 127.7 (C_4), 50.7 (C_9), 47.3 (C_8), 39.3 (C_2), 33.3 (C_1), 25.6 (C_{11}), 25.6 (C_{12}), 18.4 (C_{13}), 15.2 (C_{10}); LC-MS (negative ion mode), *m*/*z* 271 (M − Na).

*Formation of the Sulfonic Acid Derivative (**7**) under Wine Conditions.* Damascenone (100 mg, 0.5 mmol) and sodium metabisulfite (100 mg, 0.5 mmol, equivalent to 1.0 mmol SO_2) were added to model wine (500 mL, pH 3.2) and stirred at room temperature for 4 weeks. The mixture was extracted first with ethyl acetate to remove unreacted damascenone, and then the products were extracted with acetonitrile and ethyl acetate (5:2), and the solvent was removed. NMR analysis of the crude mixture showed the sulfonic acid **7** to be the major product.

RESULTS AND DISCUSSION

Loss of Damascenone under Acidic Conditions. Demole and Enggist (*7*) have reported that damascenone undergoes acid-catalyzed intramolecular cyclization to give two products, which they assigned as **2** and **3** on the basis of low-field NMR and mass spectral data. We repeated the original experiment under modified conditions (400 W microwave, with added *p*-TsOH) and obtained two compounds that were isolated by silica chromatography. For both compounds, NMR and mass spectral data obtained matched those reported for **2** and **3**. In an attempt to obtain crystals for X-ray crystallographic analysis, each of the compounds was converted into the corresponding 2,4-dinitrophenylhydrazone derivative. Unfortunately, the crystals produced proved unsuitable for such analysis. However, long-range coupling experiments (HMBC, CIGAR) on the hydrazone derivatives were in complete agreement with the reported structures.

In contrast to the initial experiment, which was conducted in the absence of solvent, the reaction was repeated in 10% aqueous ethanol solution (pH 2.8). After being heated at 100 °C for 11 days, the same two major products were observed in the product mixture.

Two other (very minor) components of this reaction mixture were the two C_9 adducts of damascenone—the hydrate **4** and the ethanol adduct **5**. Both have been observed previously as components in the acid hydrolysis of suspected damascenone precursors (*10*), although their structures were only tentatively assigned. Authentic samples of each were therefore prepared, and the assignment confirmed. The hydrate **4** was prepared by addition of acetaldehyde across the enolate of 2,5,5-trimethyl-1-acetylcylcohexa-1,3-diene (**6**) (*9*). The ethanol adduct **5** was prepared by treating damascenone with sodium ethoxide in ethanol. From analysis of the NMR spectra, it was clear that the reaction had proceeded as expected. In both compounds the two protons on C_8 presented as the AB portion of an ABX system, with the X portion (the lone hydrogen on C_9) further coupled to the protons on C_{10}. Also, in both cases, the signals for H_3 and H_4 were essentially unchanged from those of damascenone itself, confirming the regiochemistry of the products.

Loss of Damascenone Due to Nucleophilic Attack. There are many species present in wine that could conceivably undergo reaction with damascenone and account for its loss in aging wines. These include various carboxylic acids, amines, and thiols. An initial experiment was set up in which damascenone was sealed with one of the added nucleophilic species, indicated in **Figure 1**, in buffered aqueous ethanol solution (pH 3.0). As all of the solutions (including the control) were prepared from aqueous ethanol buffered with tartrate/tartaric acid, they all contained these species as potential nucleophiles, with the capacity to form adducts such as **4** and **5** and conjugates with tartrate, as well as with the added nucleophiles listed in **Figure 1**.

The solutions were heated at 45 °C for 60 days, and the remaining damascenone was then quantified. **Figure 1** shows the damascenone remaining after 60 days. In most cases, the falloff in damascenone content was minor (10−20%). Therefore, under these conditions, the rate of formation of compounds **2**−**5**,

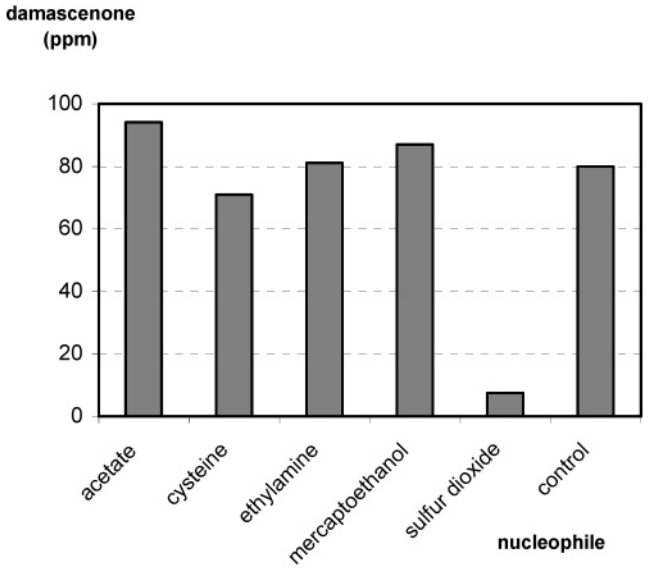

Figure 1. Damascenone remaining after 60 days at 45 °C, pH 3.0.

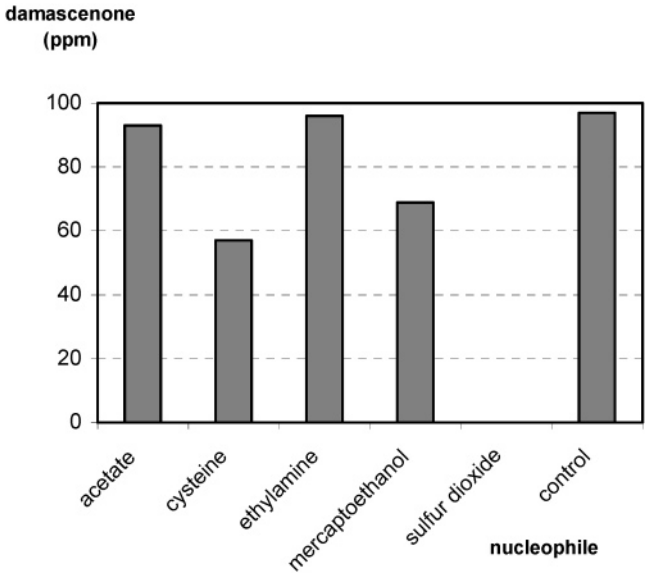

Figure 2. Damascenone remaining after 90 days at 45 °C, pH 5.5.

or of adducts with most of the nucleophiles present, is insufficient to account for the decrease in damascenone during bottle storage. However, when SO_2 was added, the concentration of damascenone decreased to <10% of the original value. It was anticipated that increasing the pH to higher values might result in an increase in concentration of the conjugate bases of the added nucleophiles and thus an increase in the nucleophilicity of the medium. A further experiment was conducted at pH 5.5, and the results are collected in **Figure 2**. Other than reaction with sulfur dioxide, the higher pH (and longer reaction time) had little effect except for the levels of damascenone with cysteine and mercaptoethanol as the added nucleophiles, for which the concentrations of damascenone fell to approximately 60 and 70% of its original value, respectively. Although these results indicate a propensity for damascenone to react with thiols under certain conditions, it is clear that the reductions observed in the damascenone content are still insufficient to account for the reported losses (*6*) of damascenone (up to 75% over 3 months at cellar temperature).

Loss of Damascenone Due to SO_2. The most striking entries in **Figures 1** and **2** are those corresponding to SO_2 as the added

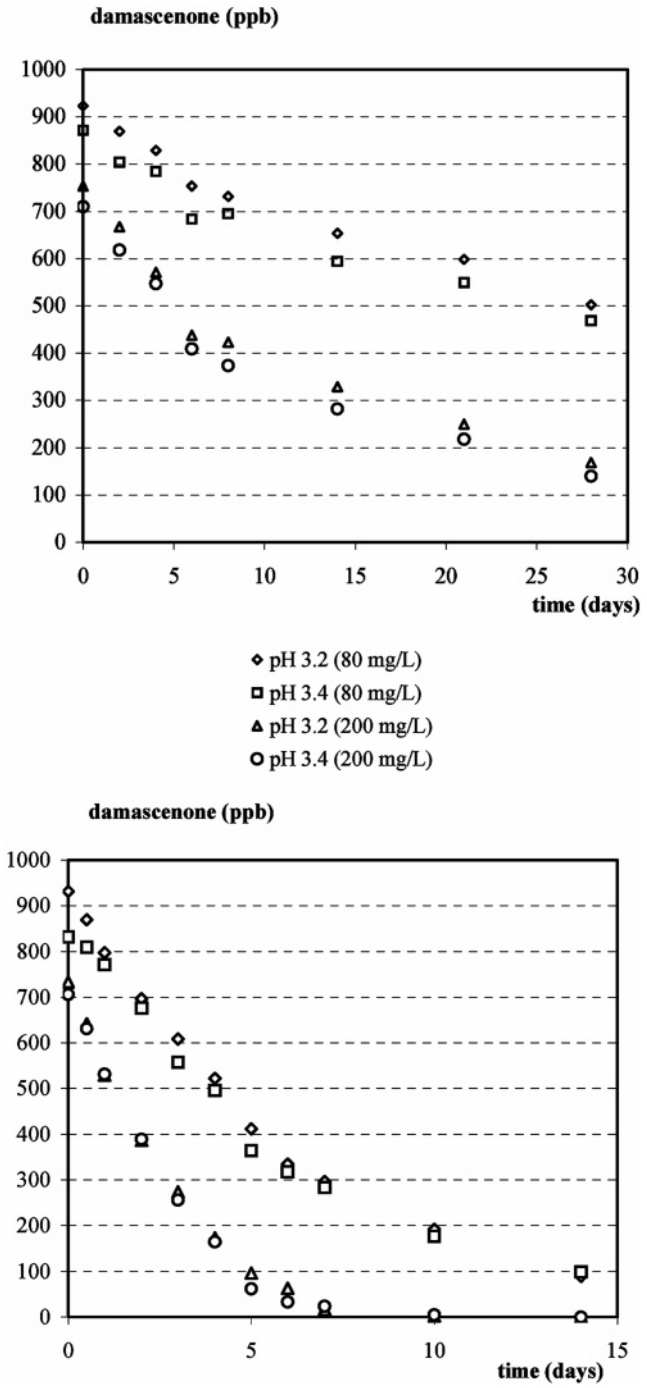

Figure 3. Effect of pH and added SO_2 on the level of damascenone over time, at 25 °C (upper) and 45 °C (lower).

nucleophile (introduced as sodium metabisulfite). After 60 days at pH 3.0 and 45 °C, the level of damascenone remaining was <10% of the original value. After 90 days at pH 5.5 and 45 °C, there was no detectable damascenone (LOD ~ 0.5−1% of starting concentration); that is, it had been completely consumed. Sulfur dioxide (or its aqueous equivalent, bisulfite) is known to react with carbonyl compounds to produce bisulfite adducts. Accordingly, sodium metabisulfate was reacted with damascenone in DMF and the product extracted with acetonitrile/ethyl acetate. Spectral analysis revealed that the product isolated was in fact the sulfonic acid derivative **7**, where the sulfonate functionality had been formed by 1,4-addition to the α,β-unsaturated enone, rather than 1,2-addition across the ketone. The regiochemistry of addition was established from the proton

NMR spectrum where the two protons on C_8 and the proton on C_9 form an ABX system (J = 19.1, 9.3, and 3.0 Hz). The spectrum is in close agreement with those for **4** and **5**. Examples from the literature reporting the observation of this unexpected regiochemistry are extremely uncommon. Pfoertner (*11*) observed conjugate addition when he treated various substituted chalcones with sodium sulfite and sodium pyrosulfite in aqueous methanol. Bisulfite has also been observed to add across a double bond in certain heteroaromatic systems; Pitman and Sternson (*12*) have measured equilibrium constants for such processes.

To investigate whether **7** could form under more typical wine conditions, damascenone was treated with sodium metabisulfite in a model wine (pH 3.2, room temperature, 4 weeks). The product was extracted with acetonitrile/ethyl acetate and analyzed by NMR, which showed that the major component was indeed **7**.

A detailed quantitative assessment of the effect of SO_2 on the concentration of damascenone over time was conducted. Two concentrations of SO_2 (80 and 200 mg/L), two pH values (3.2 and 3.4), and two temperatures (25 and 45 °C) were chosen, with the final results displayed in **Figure 3**. As expected, temperature played a key role in the rate of consumption of damascenone, but this rate appeared to be effectively independent of pH, within the narrow range employed in this experiment. The time zero points show reduced levels of damascenone, relative to the spiked amount (1000 ppb). The discrepancies appear to closely parallel the amount of added sulfur dioxide; when added at 80 mg/L the discrepancies are ~10%, but when added at 200 mg/L, the discrepancies are ~25%. They are most likely to be an artifact of the storage process. The individual time zero samples were stored at −20 °C for ~1 month prior to analysis, and under these conditions some slow but significant reaction could take place. All of the samples were analyzed at the end of the experiment. However, the discrepancies in the time zero points are substantially less than the changes in damascenone concentration over the course of the experiment and do not alter in any way the conclusions drawn.

Decreases in damascenone content during wine maturation can be attributed to several factors, but it is the interaction with sulfur dioxide that is likely to be the most important process accounting for such losses. These results also explain recent observations (*13*) in our laboratories that the loss of damascenone in white wine over time is less when such wines are stored under an air headspace (with resultant consumption of SO_2) rather than in an anaerobic environment.

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LITERATURE CITED

(1) Pickenhagen, W. In *Flavor Chemistry—Thirty Years of Progress*; Teranishi, E. L. W., Hornstein, I., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; pp75−87.

(2) Aznar, M.; López, R.; Cacho, J.; Ferreira, V. Prediction of aged red wine aroma properties from aroma chemical composition. Partial least squares regression models. *J. Agric. Food Chem.* **2003**, *51*, 2700−2707.

(3) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027−3032.

(4) Buttery, R. G.; Teranishi, R.; Ling, L. Identification of damascenone in tomato volatiles. *Chem. Ind.* **1988**, 238.

(5) Winterhalter, P.; Rouseff, R. Carotenoid-derived aroma compounds: an introduction. *Carotenoid-Derived Aroma Compounds*; Winterhalter, P., Rouseff, R., Eds.; ACS Symposium Series 802; American Chemical Society: Washington, DC, 2002; pp 1−17.

(6) Guth, H. Comparison of different white wine varieties in odor profiles by instrumental analysis and sensory studies. In *The Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 714; American Chemical Society: Washington, DC, 1999; pp 39−54.

(7) Demole, E.; Enggist, P. A chemical study of Virginia tobacco flavor (*Nicotiana tabacum* L.). I. Isolation and synthesis of two bicyclodamascenones. *Helv. Chim. Acta* **1976**, *59*, 1938−1943.

(8) Kotseridis, K.; Baumes, R.; Skouroumounis, G. K. Synthesis of labelled [$^2H_4$] β-damascenone, [$^2H_2$] 2-methoxy-3-isobutylpyrazine, [$^2H_3$] α-ionone, and [$^2H_3$] β-ionone for quantification in grapes, juices and wines. *J. Chromatogr. A* **1998**, *824*, 71−78.

(9) Phaff, R.; Bischofberger, N.; Mathies, P.; Petter, W.; Frei, B.; Jeger, O. Carbonyl vs. epoxyketone photochemistry: Photolysis of 1,2;3,4-diepoxy-2,6,6-trimethyl-1-cyclohexyl methyl ketone. *Helv. Chim. Acta* **1985**, *68*, 1204−1216.

(10) Skouroumounis, G. K.; Sefton, M. A. Acid-catalyzed hydrolysis of alcohols and their β-D-glucopyranosides. *J. Agric. Food Chem.* **2000**, *48*, 2033−2039.

(11) Pfoertner, v. K.-H. Substituted alkyl sulfonates by addition of sodium hydrogen sulfide to chalcones. *Helv. Chim. Acta* **1980**, *63*, 664−667.

(12) Pitman, I. H.; Sternson, L. A. Method for estimating the equilibrium constants for covalent addition of nucleophilic reagents to heteroaromatic molecules. *J. Am. Chem. Soc.* **1976**, *98*, 5234−5238.

(13) Capone, D. L.; Simpson, R. F.; Cox, A.; Duncan, B.; Skouroumounis, G. K.; Sefton M. A. New insights into wine bottle closure performance—flavour 'scalping' and cork taint. In *Proceedings of the 12th Australian Wine Industry Technical Conference*; Blair, R. J., Pretorius, I. S., Eds.; Australian Wine Industry Technical Conference: Adelaide, SA, 2004; in press.

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