JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Formation of Damascenone under both Commercial and Model Fermentation Conditions

Natoiya D. R. Lloyd,[†,‡,§] Dimitra L. Capone,[§] Maurizio Ugliano,[§] Dennis K. Taylor,[†] George K. Skouroumounis,[†] Mark A. Sefton,[†] and Gordon M. Elsey*[,†,‡]

[†]School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA 5064, Australia

[‡]School of Chemistry, Physics and Earth Sciences, Flinders University, P.O. Box 2100, Adelaide 5001, Australia

[§]The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, Adelaide 5064, Australia

ABSTRACT: The fermentations, at a commercial winery, of six different grape musts encompassing the varieties Riesling, Chardonnay, Sauvignon blanc, Shiraz, Grenache, and Pinot noir were monitored for damascenone concentration. In every case, the concentration of damascenone increased during fermentation from low or undetectable levels to concentrations of several parts per billion. Further increases in damascenone concentration were observed during barrel aging of three of these wines. Two ketones, megastigma-4,6,7-triene-3,9-dione (**4**) and 3-hydroxymegastigma-4,6,7-trien-9-one (**5**), were synthesized and subjected to fermentation conditions using two yeasts, AWRI 796, and AWRI 1537. In the case of the former compound, **4**, synthesis confirmed the original, tentative assignment of the structure and confirmed **4** as a natural product, isolated from honey. Both compounds, under the action of both yeasts, produced appreciable amounts of damascenone (**1**), with ketone **5** and AWRI 796 yeast yielding the highest concentration of **1**.

KEYWORDS: Damascenone, *Saccharomyces cerevisiae*, grasshopper ketone, reductase, norisoprenoids, commercial fermentations, honey

INTRODUCTION

Damascenone (often referred to in the chemical literature as β-damascenone, **1**) is one of the most important natural aroma compounds known and is one of the mainstays of the international perfume industry.[1] Previous research has shown that this compound forms by hydrolysis of certain hydroxylated precursors[2] and their glycoconjugates.[3] The end of the sequence producing **1** is shown in Figure 1; the allenic diol **2** produces damascenone in high yield (>60%) by more than one route, with the dienyne species **3** an intermediate in only one of these.[3]

Guth quantified **1** during production of a Gewurtztraminer wine and observed a significant increase in the concentration of damascenone at the end of alcoholic fermentation.[4] He also found that the concentration of this compound then decreased, by approximately 75% over the first few months of wine maturation.[4] Daniel et al. accounted for this diminution in damascenone content by the action of certain nucleophilic components in wine, especially sulfur dioxide.[5] However, the reason for the rapid formation of damascenone by the end of fermentation remains unclear and is the focus of this paper.

It has long been known that a particular form of *Saccharomyces cerevisiae*, baker's yeast, is able to perform chemical transformations when used as a reagent in chemical synthesis.[6] One mode of action displayed by yeasts concerns the reduction of carbonyl compounds by reductase enzymes.[7] Given that the hydrolysis of diol **2** is likely to account for the formation of damascenone (**1**) itself, the question arises as to whether this precursor compound could be formed under fermentation conditions by reduction of more oxidized species. Two compounds present themselves as possible candidates: the diketone **4** and the hydroxyketone **5** (Figure 2). The former (**4**) has previously been tentatively observed as a component of honey[8,9] as well as being tentatively identified in hydrolysates of extracts of several white wine varieties.[10,11] The latter (**5**) is, as best we can tell, unknown, but its relationship to the known grape component grasshopper ketone (**6**) is obvious. Both Ohloff et al.[12] and Isoe et al.[13] proposed that damascenone could be formed in vivo from **6**, via the allenic triol **7**. Hydrolyses conducted on **7** have shown that it produces damascenone as only a minor component (<1%).[2] However, should dehydration of the C5 alcohol in **6** produce the 9-keto compound **5**, this might then undergo reduction by yeast reductase enzymes to give **2**. Rapid conversion of this compound quantitatively into damascenone (**1**) would then be expected due to the acidic nature of the fermentation environment. In a similar

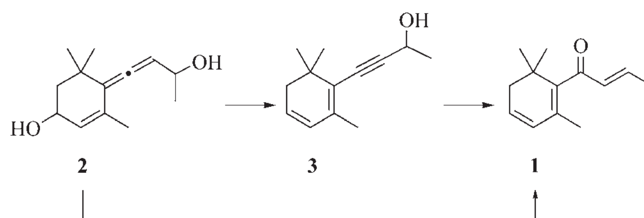

Figure 1. Formation of damascenone (**1**) from allenic diol **2**.

Received: September 27, 2010
Accepted: December 23, 2010
Revised: December 21, 2010
Published: January 21, 2011

ACS Publications © 2011 American Chemical Society

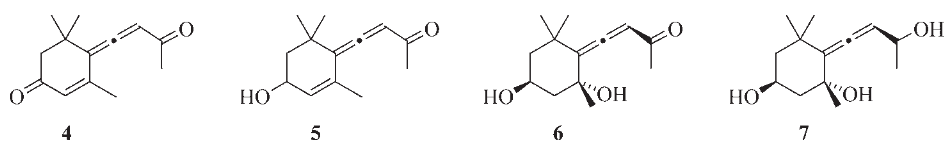

Figure 2. Structural analogues of allene diol **2**.

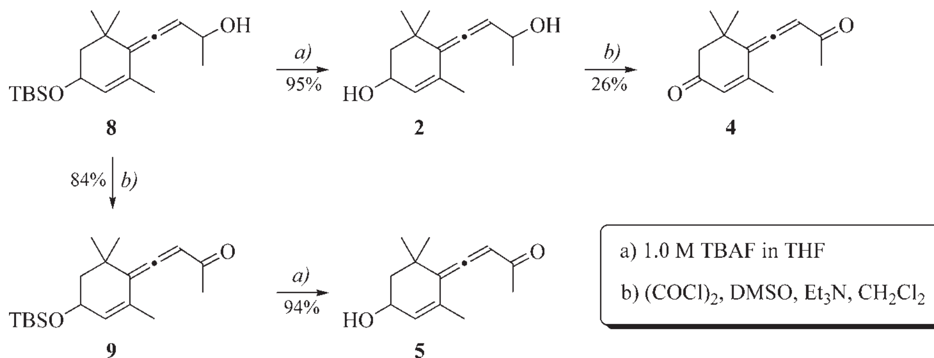

Figure 3. Synthesis of diketone **4** and ketone **5**.

vein, reduction of both carbonyl groups in **4** would also give **2** and, ultimately, damascenone.

This paper demonstrates that the formation of damascenone under fermentation conditions is a general phenomenon and also reports the synthesis of ketones **4** and **5**. In the case of the former compound, it represents the first total synthesis of this compound and confirms it as a natural product. It also demonstrates that both **4** and **5** are able to serve as precursors to damascenone (**1**) under fermentation conditions.

MATERIALS AND METHODS

Materials. Chemicals were purchased from Sigma-Aldrich. All solvents used were of pesticide grade from OmniSolv (Darmstadt, Germany). X4 is a mixed hydrocarbon solvent, with *n*-hexane as the major component. All organic solvent solutions were dried over anhydrous sodium sulfate before being filtered. pH measurements were made with an EcoScan pH 5/6 meter (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 (in $CDCl_3$) with a Bruker Ultrashield Plus spectrometer at operating frequencies of 400 and 100 MHz, respectively. All compounds gave spectroscopic data that were consistent with the expected structures. Mass spectra were recorded on a Hewlett-Packard (HP) 6890 gas chromatograph fitted with a Gerstel MPS2 autosampler and coupled to a HP 5973N mass spectrometer. Commercial wines, sampled at various stages of the vinification and maturation processes, were produced from grapes grown either in the Adelaide Hills (Sauvignon blanc and Pinot noir) or in the Barossa Valley (Riesling, Chardonnay, Grenache, and Shiraz). All of the white wines were fermented to dryness in stainless steel vats, except for the Chardonnay, which was transferred into oak barrels 5 days after yeast inoculation. The red wines were fermented in open fermenters with the Pinot noir and Shiraz wines being transferred, postfermentation, into oak barrels.

Methods. *Megastigma-4,6,7-triene-3,9-dione (**4**).* Silylated allenic diol **8** (two diastereoisomers)[14] (0.99 g, 3.07 mmol) in THF (40 mL) was treated with TBAF (1.0 M in THF, 6.1 mL, 6.1 mmol) at room temperature for 16 h (Figure 3). The reaction was quenched by the addition of a saturated solution of sodium bicarbonate, before being diluted with EtOAc (50 mL) and washed with brine (3 × 50 mL). After being dried, the solution was concentrated to give diol **2** as a pale yellow oil (606 mg, 95%) having spectroscopic properties that were identical to those reported earlier.[14]

DMSO (350 mg, 4.45 mmol) in CH_2Cl_2 (5 mL) was added to oxalyl chloride (277 mg, 2.18 mmol) in CH_2Cl_2 (10 mL) at −60 °C over a period of 15 min, and the mixture was allowed to stir at that temperature for a further 30 min. Diol **2** (206 mg, 0.99 mmol) in CH_2Cl_2 (5 mL) was added over 15 min and allowed to stir for a further 45 min. After triethylamine (1.0 g, 9.9 mmol) was added over 15 min, the mixture was allowed to warm to room temperature. Water (5 mL) was added, and after 30 min of stirring, the mixture was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed with, successively, 10% aqueous HCl solution, water, saturated sodium bicarbonate solution, and brine. After drying and concentration, the residue was chromatographed (20% EtOAc/X4) to give the title compound as a pale yellow heavily viscous oil (53 mg, 26%): ^{1}H NMR δ 6.17 (1H, br s, H_4), 5.96 (1H, br s, H_8), 2.42 (2H, s, H_2), 2.24 (3H, s, H_{10}), 2.00 (3H, br s, H_{13}), 1.25, 1.22 (6H, 2s, $H_{11,12}$); ^{13}C NMR δ 214.3 (C_7), 197.5, 197.1 ($C_{3,9}$), 148.5 (C_5), 127.3 (C_4), 117.0 (C_6), 102.5 (C_8), 50.8 (C_2), 37.0 (C_1), 28.7, 28.6 ($C_{10,12}$), 27.2 (C_{11}), 21.5 (C_{13}); MS m/z (%) 204 (M^+, 9), 162 (66), 147 (100), 133 (5), 119 (11), 91 (9), 77 (10), 43 (43).

*3-Hydroxymegastigma-4,6,7-trien-9-one (**5**).* DMSO (1.29 g, 16.58 mmol) in CH_2Cl_2 (20 mL) was added to oxalyl chloride (1.02 g, 8.04 mmol) in CH_2Cl_2 (40 mL) at −60 °C over a period of 15 min (Figure 3). After a further 30 min of stirring, silylated allenic diol **8** (1.19 g, 3.69 mmol) in CH_2Cl_2 (20 mL) was added, and the reaction was allowed to stir for a further 45 min. Triethylamine (3.71 g, 36.7 mmol) was added, and the mixture was allowed to warm to room temperature. Water (90 mL) was added, and after an additional 30 min of stirring, the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed as described above before being dried and concentrated to give a yellow oil (986 mg, 84%).

To this product (460 mg, 1.44 mmol) in THF (40 mL) was added TBAF (2.87 mL of 1 M solution in THF, 2.87 mmol) before being allowed to stir for 16 h at room temperature. The reaction was quenched by the addition of a saturated solution of sodium bicarbonate, before being diluted with EtOAc (25 mL) and washed with brine (3 × 25 mL). After being dried, the solution was concentrated and the residue was chromatographed (20% EtOAc/X4) to give **5** as a pale yellow oil (280 mg, 94%) (†, signals for the two diastereomers overlapping; *, signals for the two diastereomers coincident): ^{1}H NMR δ 6.09, 6.02

(1H, br s, H_8), 5.74* (1H, br s, H_4), 4.62−4.48† (1H, m, H_3), 2.21, 2.20 (3H, s, H_{10}), 2.04−1.92† (1H, m, H_{2a}), 1.79* (3H, br s, H_{13}), 1.54−1.46† (1H, m, H_{2b}), 1.21, 1.18, 1.18, 1.13 (6H, 2s, $H_{11,12}$); ^{13}C NMR δ 213.5* (C_7), 199.0, 198.7 (C_9), 130.3, 130.2, 116.99, 116.96 ($C_{5,6}$), 128.15, 128.10, 102.6, 102.4 ($C_{4,8}$), 65.8, 65.7 (C_3), 45.9, 45.8 (C_2), 35.2, 35.1 (C_1), 29.8, 29.6, 27.9, 27.8, 26.8, 26.7 ($C_{10,11,12}$), 20.9, 20.8 (C_{13}); MS m/z (%) (isomer 1) 206 (M^+, 2), 192 (15), 191 (100), 173 (18), 163 (10), 149 (23), 131 (71), 121 (42), 105 (21), 91 (26), 77 (20), 69 (11), 43 (93); (isomer 2) 206 (M^+, 2), 192 (14), 191 (100), 173 (15), 163 (9), 149 (23), 131 (45), 121 (35), 105 (17), 91 (22), 77 (16), 69 (11), 43 (71).

Yeast Strains and Fermentations. The two yeasts used in the laboratory fermentations, *S. cerevisiae* strains AWRI 796 and AWRI 1537, were maintained on yeast−malt extract (YM) medium (Amyl Media, Dandenong, Australia) supplemented with 1.5% agar and stored at 4 °C. All strains were obtained from The Australian Wine Research Institute Culture Collection (Adelaide, Australia). Fermentations were carried out in a chemically defined grape juice-like (CDGJ) medium as described by Ugliano et al.[15] The medium was sterilized by filtration through a 0.2 μm sterile membrane and, after spiking with either megastigma-4,6,7-triene-3,9-dione (**4**) or 3-hydroxymegastigma-4,6,7-trien-9-one (**5**) at concentrations of 1000 μg/L, was divided into 200 mL aliquots. The inoculation procedure was as follows. A loopful of yeast cells was incubated in 10 mL of YM medium with shaking for 24−48 h at 28 °C. Preadaptation of the cells to the fermentation medium was carried out by inoculating 100 μL of the cultures into 20 mL of 50% (v/v) CDGJ medium. These subcultures were grown at 28 °C until a biomass of (1−2) × 10^8 cells/mL, determined microscopically using a hemocytometer, was reached, after which they were inoculated in the CDGJ medium at a final concentration of 1 × 10^6 cells/mL. Fermentations were carried out at 18 °C in 250 mL Erlenmeyer flasks sealed with fermentation water locks and shaken at 180 oscillations per minute. Flasks were kept in a thermostatically controlled water bath with recirculating water acting as coolant, to ensure optimal dispersion of the heat generated during fermentation. Samples (1 mL) for monitoring the progress of alcoholic fermentation were removed daily under sterile conditions with a needle and syringe via a sample port closed with a rubber Suba seal and were analyzed enzymatically for total reducing sugar concentration (Roche Molecular Biochemicals) using an automated Cobas FARA centrifugal analyzer. For each yeast strain, triplicate fermentations of CDGJ medium with and without spiked compounds were performed. Noninoculated samples of CDGJ medium containing the precursor compounds was used to evaluate acid-catalyzed hydrolysis of ketones **4** and **5**. Upon completion of fermentation (residual sugars < 1 g/L), samples were cold-settled for 5 days at 5 °C, with sterile nitrogen supplied at low pressure to prevent the ingress of air into the flasks. The wines were then racked off gross yeast lees and submitted to extraction and analysis of damascenone.

Preparation of Samples for Damascenone Analysis. Buffer solutions were prepared by saturating a 10% ethanol solution with potassium hydrogen tartrate and adding 10% tartaric acid solution until the required value was reached. Damascenone was quantified by the SIDA method reported in Daniel et al.,[5] which utilized d_4-damascenone as internal standard.

Extraction of Commercial Honey Samples. Two commercially purchased honeys, Golden Nectar Organic Real Leatherwood Honey (Tasmania) and Manuka Blend Honey (New Zealand), were extracted according to procedures based on those previously used by Rowland et al.[16] and Alissandrakis et al.,[17] respectively. The honeys were diluted with water, when necessary, and extracted, under sonication, with pentane/dichloromethane (2:1) for the manuka honey sample and with pentane/dichloromethane (2:1) followed by ethyl acetate for the leatherwood honey sample. In each case the extracts were concentrated by careful distillation and then sealed and kept refrigerated until analysis.

*GC-MS Conditions for Honey Extract Analysis and Co-injection with Authentic Diketone **4**.* 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 60 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 1.5 mL/min. The oven temperature, started at 50 °C, was held at this temperature for 1 min, then increased to 200 °C at 15 °C/min, further increased to 245 °C at 5 °C/min, and held at this temperature for 20 min. The injector was held at 220 °C and the transfer line at 245 °C. The sample volume injected was 2 μL and was introduced in pulsed splitless mode with an inlet pressure of 45.0 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.

■ RESULTS AND DISCUSSION

Formation of Damascenone during Commercial Scale Wine Production. We first sought to determine whether the observation, described by Guth,[4] of a significant increase in damascenone concentration following fermentation of a grape must was an isolated or common phenomenon. Figure 4 shows the evolution of damascenone over the course of fermentation in six grape varieties, three each of white and red wines, in a commercial winery. In every case, it is evident that the concentration of **1** increased during fermentation. Three of the wines, Riesling, Sauvignon blanc, and Grenache, were bottled or blended soon after vinification and were therefore not sampled further. The remaining three wines were further aged in oak barrels for 9−10 months prior to sale. The concentration of damascenone in these three barrel-aged wines increased between 2- and approximately 5-fold (data not shown) during the period of barrel maturation. In a study of changes in red wine composition during barrel-aging, Jarauta et al.[18] reported changes in the concentration of damascenone. After both 6 and 12 months of aging, and in agreement with Guth,[4] the concentration in the barrel-aged wines was slightly higher than in the corresponding stainless steel-stored control.

The data in Figure 4 show the importance of damascenone formation during primary fermentation. Formation was also significant during barrel aging. Such formation might be due solely to the well-documented generation of damascenone by acid-catalyzed transformation of grape-derived precursors,[3] but the rapid evolution during alcoholic fermentation in particular suggests that biological mechanisms could contribute to damascenone in wine during both of these stages of wine production. One way in which such a process could take place is if grape-derived norisoprenoids (apocarotenoids) are transformed by biological reduction to acid-labile damascenone precursors. To test this hypothesis, we prepared ketones **4** and **5** and subjected them to fermentation conditions.

Synthesis of Ketones 4 and 5. The synthesis of ketones **4** and **5** is shown in Figure 3 and began with silylated allene **8**, previously prepared as an intermediate in the synthesis of **2**.[14] Oxidation of the 9-hydroxy function using Swern conditions[19] gave the corresponding 9-keto compound in excellent yield. Deprotection with TBAF in THF afforded the 9-keto target compound **5** in nearly quantitative yield. The other target compound, diketone **4**, was prepared from **8** by deprotection followed by a double Swern oxidation. The compound thus produced showed NMR properties almost identical to those

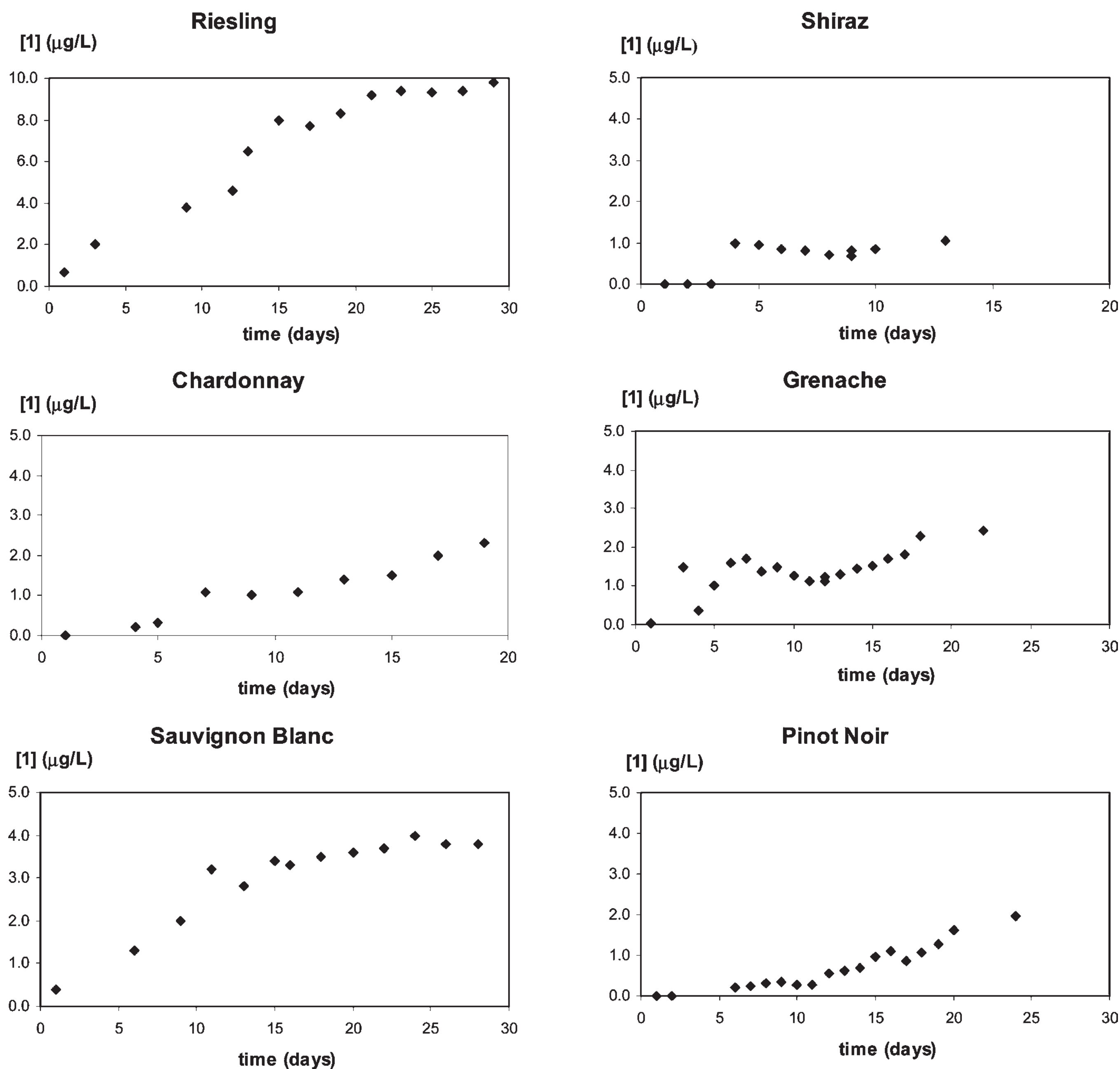

Figure 4. Evolution of damascenone during fermentation of six wine varieties under commercial production conditions. Time zero points correspond to yeast inoculation, and the final points indicate when the ferments were considered to be dry. Data points are from single samplings.

reported for a honey component, tentatively identified as this structure earlier.[8]

With the synthetic sample of diketone **4** in hand, we confirmed this compound as a constituent of two honey samples. The peaks assigned as **4** in the gas chromatograms of the extracts were symmetrically enhanced on admixture with the authentic sample, and the mass spectra of these peaks and of **4** were identical. To the best of our knowledge, this is the first confirmation of **4** as a natural product. The diketone **4** had also been tentatively identified in grape samples that had been treated with a glycosidase enzyme extract,[10,11] although the identity of the grape component that yielded this aglycone is unclear.[20]

Fermentations Using Ketones 4 and 5. The two yeasts used in this study were *S. cerevisiae* strains AWRI 796 and AWRI 1537. Model ferments were individually spiked with the substrates **4** and **5** at concentrations of 1000 μg/L (ppb). Control ferments containing each of the ketones, but no yeast, as well as controls with neither yeast nor ketone were also conducted and demonstrated that, in the absence of yeast, no damascenone was formed directly from either compound. Final levels of damascenone (**1**) were measured as reported previously using d_4-damascenone as internal standard.[5] The results of the fermentations are collected in Table 1.

Quantification of damascenone in the final samples indicated that, when yeast was present, addition of the ketones to the fermentation medium resulted in the formation of damascenone. No damascenone was detected in nonspiked reference fermentations. Ketone **5** produced, in all cases, higher levels of

Table 1. Formation of Damascenone (1) from Diketone 4 and Ketone 5 after Fermentation with Various Yeasts

compound[a]	yeast[b]	[**1**][c]	SD[d]
4	A	12.7	3.5
	B	1.1	0.3
5	A	103.0	6.1
	B	54.0	4.3
control[e]	A	nd	
	B	nd	
control (**4**)[f]		nd	
control (**5**)[f]		nd	
control[g]		nd	

[a] All spiked fermentations contained compounds at starting concentrations of 1 mg/L. [b] A, AWRI 796 yeast; B, AWRI 1537 yeast. [c] Final concentrations of damascenone expressed in μg/L (ppb). [d] Standard deviation (ppb) from triplicate fermentations. [e] Conducted with yeast, but no spike. [f] Conducted with spike, but no yeast. [g] Conducted with neither spike nor yeast.

damascenone than did diketone **4**. The particular yeast strain used also showed an effect, with higher final concentrations of damascenone generally associated with the use of AWRI 796. This type of yeast strain effect on damascenone concentration in wine has been previously reported,[21] although, to date, no evidence has been proposed for the mechanisms operating. The results reported here demonstrate that a mechanism involving reduction of the ketone functional group by the yeast, followed by an acid-catalyzed rearrangement of the resulting compound **2**, leading to formation of significant quantities of damascenone during fermentation is feasible. This supports the view that the occurrence of key aroma compounds in fermented products can occur not only via acid-catalyzed reactions but also via a sequential two-step process requiring both yeast activity and acidic conditions, as previously demonstrated for rose oxide[22] and proposed for hotrienol and linalool oxides.[15]

Studies on the removal of wort aldehydes by yeast enzymes have indicated that different enzymes can be involved in the biological reduction of carbonyls during fermentation, including alcohol dehydrogenase, aldose reductase, and NADPH reductase.[23] Yeast alcohol dehydrogenases have also been found responsible for the reduction of monoterpenoid ketones.[24,25] Differences between yeast strains and species with regard to their ability to reduce carbonyls have also been reported, in agreement with the results observed here. Moreover, in our study, fermentations with AWRI 796 took 8−10 days longer to reach dryness, resulting therefore in a longer time of contact between yeast enzymes and the substrate. This might have contributed to the higher damascenone concentrations observed.

In the case of 3-hydroxymegastigma-4,6,7-trien-9-one (**5**), the yield of damascenone was approximately 11% with the AWRI 796 yeast and approximately 6% with the AWRI 1537 yeast. Previous studies indicated that damascenone can be generated via acid-catalyzed reactions from different precursors present in grapes, yielding **1** at different rates depending on their chemical structure.[3,14] The observations presented herein indicate that the formation of damascenone during fermentation is also dependent on the chemical structure of different precursor compounds acting as substrates for yeast activity. Although it is possible that the lower yield observed for ketone **5** is a result of the yeast having to effect two reductions, other factors might be involved. Furthermore, the extent of conversion of **4** and **5** to damascenone might be even greater than indicated by the data in Table 1 should damascenone itself also be susceptible to biological transformation, once formed.

AUTHOR INFORMATION

Corresponding Author

*Phone: +61 8 8313 0096. Fax: +61 8 8303 7116. E-mail: Gordon.Elsey@adelaide.edu.au.

Funding Sources

This project was supported by the School of Agriculture, Food and Wine, the University of Adelaide, as well as by Australia's grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian government. The University of Adelaide and AWRI are part of the Wine Innovation Cluster (www.wineinnovationcluster.com).

ACKNOWLEDGMENT

We thank Prue and Stephen Henschke for the commercial wine samples, Katryna van Leeuwen for assistance in preparation of commercial wine samples for analysis, and other colleagues for helpful discussions and feedback.

REFERENCES

(1) Pickenhagen, W. In *Flavor Chemistry − Thirty Years of Progress*; Teranishi, R., Wick, E. L., Hornstein, I., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999: pp 75−87.

(2) Skouroumounis, G. K.; Massy-Westropp, R. A.; Sefton, M. A.; Williams, P. J. Precursors of damascenone in fruit juices. *Tetrahedron Lett.* **1992**, *33*, 3533–3536.

(3) Daniel, M. A.; Puglisi, C. J.; Capone, D. L.; Sefton, M. A.; Elsey, G. M. Rationalising the formation of damascenone: Synthesis and hydrolysis of damascenone precursors and their analogues, in both aglycone and glycoconjugate form. *J. Agric. Food Chem.* **2008**, *56*, 9183–9189.

(4) 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.

(5) Daniel, M. A.; Elsey, G. M.; Capone, D. L.; Perkins, M. V.; Sefton, M. A. Fate of damascenone in wine: the role of SO_2. *J. Agric. Food Chem.* **2004**, *52*, 8127–8131.

(6) Csuk, R.; Glänzer, B. I. Baker's yeast mediated transformations in organic chemistry. *Chem. Rev.* **1991**, *91*, 49–97.

(7) Ema, T.; Sugiyama, Y.; Fukumoto, M.; Moriya, H.; Cui, J.-N.; Sakai, T.; Utaka, M. Highly enantioselective reduction of carbonyl compounds using a reductase purified from baker's yeast. *J. Org. Chem.* **1998**, *63*, 4996–5000.

(8) Tan, S. T.; Wilkins, A. L.; Holland, P. T.; McGhie, T. K. Extractives from New Zealand unifloral honeys. 2. Degraded carotenoids and other substances from heather honey. *J. Agric. Food Chem.* **1989**, *37*, 1217–1221.

(9) D'Arcy, B. R.; Rintoul, G. B.; Rowland, C. Y.; Blackman, A. J. Composition of Australian honey extractives. 1. Norisoprenoids, monoterpenes, and other natural volatiles from blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys. *J. Agric. Food Chem.* **1997**, *45*, 1834–1843.

(10) Sefton, M. A.; Francis, I. L.; Williams, P. J. Free and bound volatile secondary metabolites of *Vitis vinifera* grape cv. Sauvignon Blanc. *J. Food Sci.* **1994**, *59*, 142–147.

(11) Sefton, M. A.; Francis, I. L.; Williams, P. J. The free and bound volatile secondary metabolites of *Vitis vinifera* grape cv. Semillon. *Aust. J. Grape Wine Res.* **1996**, *2*, 179–183.

(12) Ohloff, G.; Rautenstrauch, V.; Schulte-Elte, K. H. Model reactions for the biosynthesis of damascone compounds and their preparative application. *Helv. Chim. Acta* **1973**, *56*, 1503–1513.

(13) Isoe, S.; Katsumura, S.; Sakan, T. The synthesis of damascenone and β-damascone and the possible mechanism of their formation from carotenoids. *Helv. Chim. Acta* **1973**, *56*, 1514–1516.

(14) Puglisi, C. J.; Daniel, M. A.; Capone, D. L.; Elsey, G. M.; Prager, R. H.; Sefton, M. A. Precursors to damascenone: synthesis and hydrolysis of four isomeric 9-dihydroxymegastigma-4,6,7-trienes. *J. Agric. Food Chem.* **2005**, *53*, 4895–4900.

(15) Ugliano, M.; Bartowsky, E.; McCarthy, J.; Moio, L.; Henschke, P. A. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *J. Agric. Food Chem.* **2006**, *54*, 6322–6331.

(16) Rowland, C. Y.; Blackman, A. J.; D'Arcy, B. R.; Rintoul, G. B. Comparison of organic extractives found in leatherwood (*Eucryphia lucida*) honey and leatherwood flowers and leaves. *J. Agric. Food Chem.* **1995**, *43*, 753–763.

(17) Alissandrakis, E.; Daferera, D.; Tarantilis, P. A.; Polissiou, M.; Harizanis, P. C. Ultrasound-assisted extraction of volatile compounds from citrus floweres and citrus honey. *Food Chem.* **2003**, *82*, 575–582.

(18) Jarauta, I.; Cacho, J.; Ferreira, V. Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: an analytical study. *J. Agric. Food Chem.* **2005**, *53*, 4166–4177.

(19) Omura, K.; Swern, D. Oxidation of alcohols by "activated" dimethyl sulfoxide. A preparative steric and mechanistic study. *Tetrahedron* **1978**, *34*, 1651–1660.

(20) Sefton, M. A.; Williams, P. J. Generation of oxidation artifacts during the hydrolysis of norisoprenoid glycosides by fungal enzyme preparations. *J. Agric. Food Chem.* **1991**, *39*, 1994–1997.

(21) Loscos, N.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *J. Agric. Food Chem.* **2007**, *55*, 6674–6684.

(22) Koslitz, S.; Renaud, L.; Kohler, M.; Wüst, M. Stereoselective formation of the varietal aroma compound rose oxide during alcoholic fermentation. *J. Agric. Food Chem.* **2008**, *56*, 1371–1375.

(23) Vesely, P.; Duncombe, D.; Lusk, L.; Basarova, G.; Seabrooks, J.; Ryder, D. The impact of fermentation temperature on yeast reductase activity. *Master Brew. Assoc. Am. Tech. Q.* **2004**, *41*, 282–292.

(24) van Dyk, M. S.; van Rensburg, E.; Rensburg, I. P. B.; Moleleki, N. Biotransformation of monoterpenoid ketones by yeasts and yeast-like fungi. *J. Mol. Catal. B* **1998**, *5*, 149–154.

(25) Carballeira, J. D.; Valmaseda, M.; Alvarez, E.; Sinisterra Gago, J. V. *Gongronella butleri*, *Schizosaccharomyces octosporus* and *Diplogelasinospora grovesii*: novel microorganisms useful for the stereoselective reduction of ketones. *Enzyme Microb. Technol.* **2004**, *34*, 611–623.