DOI:10.1021/jf1038162

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

The Formation of Wine Lactone from Grape-Derived Secondary Metabolites

Joanne Giaccio,[†] Dimitra L. Capone,[‡] Anders E. Håkansson,[‡,§] Heather E. Smyth,[†,‡,∥] Gordon M. Elsey,[†] Mark A. Sefton,[†] and Dennis K. Taylor*,[†]

[†]School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA 5064, Australia, and [‡]The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, Adelaide, 5064, Australia. [§]Current address: H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark. [∥]Current address: Innovative Food Technologies, Department of Employment, Economic Development and Innovation, PO Box 156, Archerfield BC, Qld 4108, Australia

Wine lactone (i.e., 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one, **1a/1b**) was formed hydrolytically at wine pH from both racemic (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**3**) and the corresponding glucose ester **2a** at 45 °C but at room temperature was only formed from the acid **3**. The glucose ester does not appear to be a significant precursor for the formation of wine lactone in wine. The slow formation of wine lactone from the free acid **3** indicates that the acid is not likely to be an important precursor to wine lactone in young wines unless present in high concentration (≫1 mg/L), but could be a significant precursor to wine lactone in wine that is several years old. The wine lactone formed in hydrolysates of the (6*R*)-enantiomer of **3** was partially enriched in the (3*S*,3a*S*,7a*R*)-enantiomer **1a** when the hydrolysis was conducted at pH 3.2 and 100 °C in a closed vessel or under simultaneous distillation−extraction (SDE) conditions, and the enantiomeric excess (ee) varied from 5 to 22%. Hydrolysis of (6*R*)-**3** in sealed ampules at 45 °C and at pH 3.0, 3.2, or 3.4 gave near-racemic wine lactone, but when the hydrolyses were conducted at room temperature, the product was enriched in the (3*S*,3a*S*,7a*R*)-enantiomer **1a** and the ee was greater at higher pH (up to 60% at pH 3.4).

KEYWORDS: Wine lactone; wine; precursor; hydrolysis; chiral analysis; aroma; flavor

INTRODUCTION

The monoterpene lactone, 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one (**1**, **Figure 1**) was first identified as a constituent of koala urine by Southwell in 1975 (*1*). More than twenty years later **1** was found among the volatile constituents of white wine and was implicated as a potentially important contributor to wine aroma (*2*, *3*). Of the eight possible stereoisomers of this so-called "wine lactone" (**1**), only one isomer, (3*S*,3a*S*,7a*R*)-**1a**, was observed in white wines (*4*). The orthonasal detection threshold of **1a** was the lowest of all eight possible stereoisomers of **1**, at 1×10^{-5} ng/L in air (*4*) and 10 ng/L in model wine (*3*), while the retronasal detection threshold for aqueous solutions has been reported as 8 ng/L (*5*). Wine lactone was subsequently described as a constituent of young red wines (*6*), black pepper (*7*), orange juice (*5*) and grapefruit juice (*8*).

Some volatile compounds, formed from the acid-catalyzed transformation of odorless glycoconjugates present in grapes, are known for their important contribution to the aroma of wine (*9*−*12*). In his pioneering study of wine lactone, Guth observed an increase in wine lactone concentration during bottle conservation of a Gewürztraminer wine (*13*), suggesting that this compound might also be formed from grape-derived precursors.

*Corresponding author. Tel: +61-8-8303 7239. Fax: +61-8-8303 7117. E-mail: dennis.taylor@adelaide.edu.au..

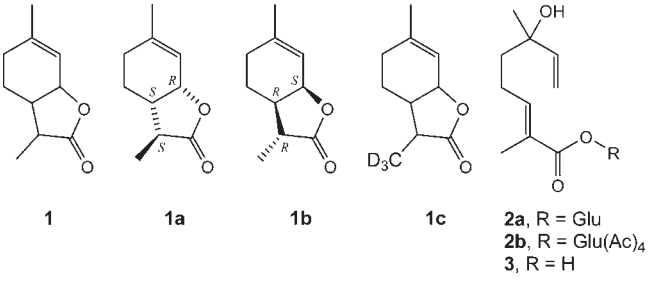

Figure 1. Wine lactone species **1a**−**c** and precursors **2** and **3** used in this study.

Winterhalter et al. isolated the tetraacetate of the glucose ester of (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**2b**) from a commercial Riesling wine by multilayer coil countercurrent chromatography (MLCCC) followed by derivatization and flash chromatography (*14*). They suggested that the corresponding acid **3** might be a precursor to wine lactone, by analogy with the conversion of the diol **4** to the ether **5** (**Figure 2a**) (*9*). They hydrolyzed the acid **3** at pH 3.2, 2.5, and 2.0 using simultaneous distillation−extraction (SDE) conditions (*15*) and found wine lactone (**1**) as a major conversion product (**Figure 2b**). Subsequently, the acid **3** was reported as a constituent of Riesling wine (*16*) and has also been tentatively identified in enzyme

 Published on Web 12/28/2010 © 2010 American Chemical Society

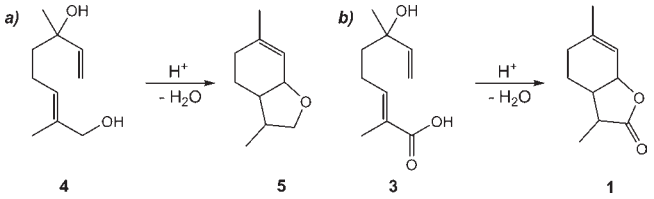

Figure 2. Formation of (**a**) dill ether **5** and (**b**) wine lactone **1** from precursors **4** and **3**, respectively.

hydrolysates of extracts of Semillon, Merlot and Cabernet Sauvignon grapes (*17*, *18*).

Recently, a study of the mechanism for the cyclization of the acid **3** to form wine lactone, involving a proposed 1,3-hydride shift, was reported by Luan et al. (*19*). Cyclization of a deuterium-labeled form of the (6*R*)-enantiomer of **3** under SDE conditions gave a deuterated analogue of the (3*S*,3a*S*,7a*R*)-enantiomer of wine lactone **1a**, together with a smaller concentration of the d_1-analogue of the (3*R*,3a*R*,7a*S*)-enantiomer **1b**. The ratio of d_1-**1a**:**1b** was reported as 4:1 (*19*). This contrasted with an earlier study by Bonnländer (*20*), who obtained wine lactone as a racemate when (6*R*)-**3** was hydrolyzed under the same conditions.

The aim of this study was to investigate the formation of wine lactone from both the glucose ester **2a** and an enantiomerically enriched form of the monoterpenoid acid **3** under wine-conservation conditions, to determine whether these compounds could account for the previously observed increase in wine lactone formation in wine during bottle aging (*13*).

MATERIALS AND METHODS

General. All reagents used were purchased from Aldrich unless otherwise stated. All solvents used were HPLC grade from OmniSolv, with the exception of ethanol, which was fractionally distilled food grade ethanol. The water used was purified by a Milli-Q system. Model wine was 10% ethanol in Milli-Q water saturated with potassium hydrogen tartrate and buffered to desired pH with tartaric acid. Positive ion electron impact (EI) mass spectra were recorded over a scan range of *m*/*z* 35−350 with an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5973N mass spectrometer (MS) with a GERSTEL MPS2Multi Purpose Sampler. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini spectrometer operating at 300 and 75.5 MHz, respectively. Spectra were recorded for deuterated chloroform ($CDCl_3$) solutions.

Synthesis of Substrates and Standards. Racemic **1a**/**1b**, enantiomerically pure **1a** and deuterium (d_3) labeled wine lactone **1c** were all synthesized according to the methods of Guth (*4*). Racemic (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**3**) was prepared as described previously (*21*,*22*). The mass spectrum, ^{1}H NMR and ^{13}C NMR spectra of **3** were essentially identical to those reported previously (*21*) except that we also observed a peak at δ 173.2 (acid carbonyl) in the ^{13}C NMR spectrum. The (6*R*)-enantiomer of **3** was prepared in the same way with the following modification: (6*R*)-(*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienal (0.51 g), prepared from (3*R*)-linalool, was added to silver oxide (1.64 g) in 10% aqueous sodium hydroxide solution (20 mL). The mixture was stirred continuously for 2.5 h at 50 °C then filtered. The aqueous portion was acidified to pH 2 with 10% hydrochloric acid solution, and the product was recovered with diethyl ether to afford 0.29 g of a yellow oil, which was purified by flash chromatography to give the acid **3** as an oil (0.049 g, 11%); $[\alpha]_D$ −21.5 ($CHCl_3$ *c* 6.6), lit. (*23*) −14.6 ($CHCl_3$ *c* 0.52). Portions of both racemic and the (6*R*)-enantiomer of **3** were methylated using diazomethane and the products analyzed by chiral GC−MS; EIMS *m*/*z* (%) 180 (M − 18, 1.5), 166 (3), 165 (4), 151 (6), 148 (10), 139 (15), 138 (20), 128 (10), 125 (15), 121 (35), 120 (17), 117 (37), 112 (20), 97 (47), 95 (30), 93 (20), 80 (15), 71 (100), 55 (35), 43 (60), in good agreement with reported data for the methyl ester (*20*).

(*E*)-*2,6-Dimethyl-6-hydroxyocta-2,7-dienoic acid glucose ester* (**2a**). 2,3,4,6-Tetraacetyl-α-D-glucopyranosyl bromide (2.42 g) and then silver carbonate (1.80 g) were added to a solution of racemic **3** (0.96 g) in dry pyridine/toluene (1:1, 50 mL). The reaction mixture was heated for 5 h at 60 °C, after which it was cooled and filtered through Celite 521, which was then washed with dichloromethane. The combined organic layer was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (0− 2% MeOH in dichloromethane), giving the tetraacetate **2b** (2.52 g) as a white solid. The ^{1}H NMR data ($CDCl_3$) were virtually identical to those previously reported by Winterhalter et al. (*14*). ^{13}C NMR ($CDCl_3$) δ: 169.6, 169.1, 168.4, 168.3, 164.7, 144.7, 143.4, 125.3, 111.3, 91.0, 72.0, 71.7, 71.6, 69.2, 66.9, 60.5, 39.3, 27.2, 27.1, 22.7, 19.7, 19.6, 19.5, 11.1, in reasonable agreement with data reported for a C_6D_6 solution (*21*). A saturated solution of ammonia in methanol (85 mL) was added to a solution of the tetraacetate **2b** (2.52 g) in methanol (85 mL) at 0 °C. The mixture was kept at this temperature for 3.5 h and the solvent then removed by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (5−10% MeOH in dichloromethane) to afford the glucose ester **2a** (400 mg) as a white solid. ^{1}H NMR ($(CD_3)_2CO$) δ: 6.89 (1H, tq, *J* 7.6, 1.5 Hz, H_3); 5.95 (1H, dd, *J* 17.3, 10.7 Hz, H_7); 5.55 (1H, d, *J* 7.8 Hz, $H_{1'}$); 5.26 (1H, dd, *J* 17.3, 1.9 Hz, H_{8a}); 5.01 (1H, dd, *J* 10.7, 1.8 Hz, H_{8b}); 4.56−4.22 (3H, m, $H_{2',3',4'}$); 3.86−3.36 (8H, m, $H_{5',6',OH(x5)}$); 2.38−2.18 (2H, m, H_4); 1.82 (3H, d, *J* 1.5 Hz, C_{2Me}); 1.65−1.59 (2H, m, H_5); 1.27 (3H, s, C_{6Me}). ^{13}C NMR ($(CD_3)_2CO$) δ: 166.8, 146.5, 145.0, 127.7, 111.8, 95.5, 78.4, 78.0, 73.9, 72.7, 71.2, 62.5, 41.6, 28.5, 24.3, 12.3.

Preparation of Samples for Hydrolysis. Model wine (50 mL) at pH 3.0 or 3.4 was spiked with a stock solution (in ethanol) of either the racemic acid **3** or the glucose ester **2a** to give final concentrations of 252 μg/L and 495 μg/L, respectively. To avoid dissolved oxygen in the solutions, an anaerobic hood was used to prepare the solutions and transfer them into 50 mL ampules. The ampules were sealed under nitrogen, and stored in darkness at 45 °C or at room temperature in a temperature controlled laboratory (21 ± 1 °C). The hydrolyses of the (6*R*)-enantiomer of the acid **3** were conducted in a similar manner except that the substrate concentration was 5 mg/L and the model wine pH was 3.0, 3.2, or 3.4. Formation of wine lactone from the (6*R*)-enantiomer of **3** using simultaneous distillation extraction was undertaken as described previously (*20*) using an aqueous phosphate/citrate buffer, pH 3.2, and pentane:diethyl ether 1:1.

Analysis of Samples for Wine Lactone. Each hydrolysate of racemic acid **3** and glucose ester **2a** (50 mL) was spiked with a solution (100 μL) of d_3-wine lactone **1c** as internal standard (0.25 μg/mL in ethanol). After mixing, the sample was added to sodium hydrogen carbonate (3 g) so that any residual organic acids that might be converted to wine lactone in the GC injector block would remain in the aqueous phase. Pentane/ethyl acetate (2:1, 5 mL) was added, and the mixture was shaken thoroughly. After settling, the organic layer was removed and concentrated with a stream of nitrogen to approximately 0.4 mL, prior to analysis. Hydrolysates of (6*R*)-**3** were worked up in the same way but without the addition of internal standard.

Method Validation. A calibration curve for wine lactone was obtained with spiked standard additions to model wine (pH 3.3). Wine lactone was added to give concentrations of 0, 100, 250, 500, 1000, 2000, 4000, and 8000 ng/L. All spiked samples were prepared, extracted and analyzed in duplicate as described above. Six replicates of the 2000 and 500 ng/L spiked samples were prepared, extracted and analyzed to test the repeatability of the method. The calibration curve was linear throughout the concentration range with a correlation coefficient (r^2) of 0.9996 and with standard deviations of 5.8% and 0.6% at 2000 and 500 ng/L, respectively. The limits of quantification and detection were 30 ng/L and 10 ng/L respectively.

Instrumental Analysis. Samples were analyzed by GC−MS. The GC was fitted with a DB-WAX fused silica capillary column (J&W, 122−7032, 30 m × 0.25 mm, 0.25 μm film thickness) for quantification and a CycloSil-B fused silica chiral capillary column (Agilent 113-6632, 30 m × 0.32 mm × 0.25 μm film thickness) for chiral analysis. The carrier gas was helium (Air Liquide or BOC gases, ultrahigh purity), linear velocity 39 cm/s, flow rate 1.2 mL/min, vacuum compensated at the mass spectrometer interface. For quantification, the oven temperature was started at 50 °C, held at this temperature for 1 min, increased to 240 at 10 °C/min, and held at this temperature for 10 min. For chiral analysis, the oven temperature was started at 60 °C, held at this temperature for 1 min, increased to 150 at 10 °C/min, then increased to 230 at 3 °C/min and held at this temperature for 5 min. The injector, in pulsed splitless mode, was held at 220 °C (200 °C for

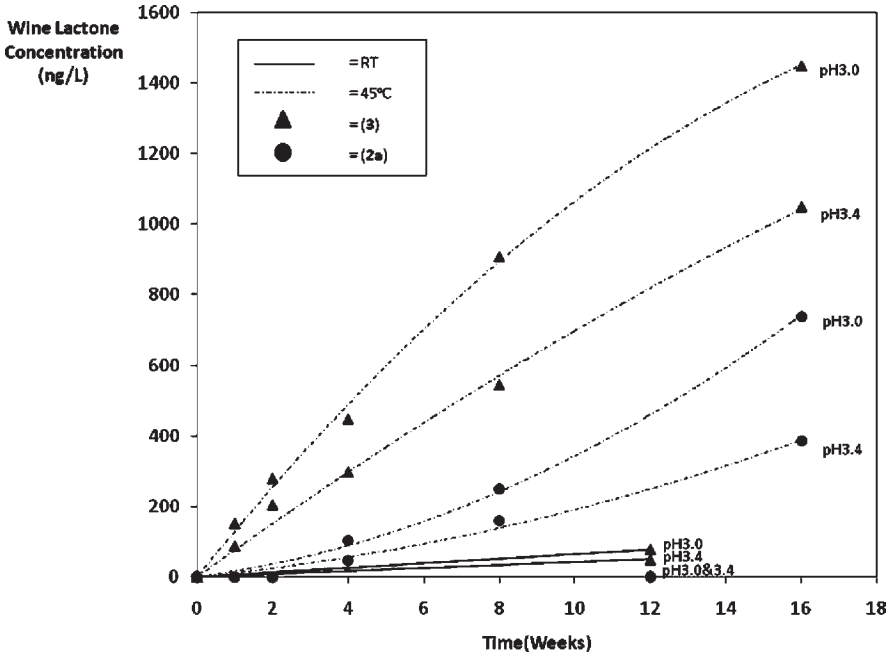

Figure 3. Hydrolytic formation of wine lactone **1** from glucose ester **2a** and acid **3**. All points mean data for duplicate samples, which did not vary by more than 5% from the mean except for **2a**, pH 3.4, 45 °C, week 4 (±11%) and **3**, pH 3.0, 45 °C, week 1 (±8%). Ester **2a** at RT did not produce detectable levels of **1**.

Table 1. Ratio of Enantiomers of Wine Lactone (**1a**:**1b**) Formed from Hydrolysis of (6*R*)-**3** under Various Reaction Conditions

		temperature			other	
pH		RT[a]	45 °C[a]	100 °C[b]	SDE 1[b,c]	SDE 2[b,d]
3.0	ratio[e] A[f]	65.5:34.5 ± 0.4	51.0:49.0 ± 0.1			
	ratio B[g]	69.0:31.0 ± 0.5	51.5:48.5 ± 0.2			
3.2	ratio A	72.5:27.5 ± 1.8	51.5:48.5 ± 0.5	53.5:46.5 ± 0.4	58.5:41.5 ± 0.2	52.0:48.0 ± 1.1
	ratio B	78.0:22.0 ± 2.2	52.0:48.0 ± 0.7	54.5:45.5 ± 0.5	61.0:39.0 ± 0.2	52.5:47.5 ± 1.4
3.4	ratio A	74.0:26.0 ± 0.7	52.5:47.5 ± 0.2			
	ratio B	80.0:20.0 ± 0.9	53.0:47.0 ± 0.3			

[a] Average of duplicate measurements of triplicate experiments. [b] Average of duplicate measurements of single experiments. [c] Aqueous phase heated slowly to boiling point. [d] Aqueous phase heated rapidly to boiling point. [e] In all cases ratio refers to **1a**:**1b**. [f] Uncorrected ratio. [g] Ratio corrected for initial stereochemical purity of **3** by the formulas

$$\text{corrected value (1a)} = (\text{uncorrected value (1a)} - 9.5) \times (100/81)$$

$$\text{corrected value (1b)} = 100 - \text{corrected (1a)}$$

chiral analysis) and the transfer line at 240 °C (230 °C for chiral analysis). The splitter, at 44:1, was opened after 36 s. The sample injection volume was 2 μL. The liner used was resilanized borosilicate glass, tapered, with a plug (2−4 mm) of resilanized glass wool near the column interface. The residence time for the needle in the injector block was 100 ms. Positive ion electron impact spectra at 70 eV were recorded in the range m/z 35−350 for scan runs. For quantification and chiral analysis of wine lactone, mass spectra were recorded in selected ion monitoring (SIM) mode. The ions monitored were m/z 154, 146, and 126 (**1c**) and 151, 138, and 123 (**1**) for quantification (**1a**/**1b**) and 166, 151, 138, and 123 for chiral analysis (**1a** and **1b**). The second eluting peak during chiral chromatography of the racemic wine lactone had an identical retention time to the synthetic (3*S*,3a*S*,7a*R*)-enantiomer of wine lactone.

RESULTS

The evolution of wine lactone from the acid **3** and from the glucose ester **2a** in aqueous ethanol, at room temperature and at 45 °C, and at pH 3.0 and 3.4 is shown in **Figure 3**. Only the isomers **1a** and **1b** (indistinguishable by achiral analysis) were observed in the hydrolysates. Wine lactone was formed from both the acid **3** and the glucose ester **2a** at 45 °C, but at room temperature was only formed in detectable quantities from the acid (LOD = 10 ng/L). As expected for an acid-catalyzed transformation, wine lactone was formed faster at the lower pH.

To study the stereoselectivity of wine lactone formation from the acid **3**, the (6*R*)-enantiomer was also prepared from commercially available (6*R*)-linalool. The enantiomeric purity of the product **3** was determined by performing chiral GC analysis on the corresponding methyl ester. This showed that the enantiomers were present in a ratio of 6*R*:6*S* = 90.5:9.5. Chiral analysis of the (6R)-linalool used as starting material showed the presence of no more than 5% of the (6*S*)-isomer, indicating that there had been a minor amount of racemization during the synthesis of **3**.

The (6*R*)-enantiomer of **3** was hydrolyzed at a variety of temperatures and pH values, and the wine lactone formed was analyzed by chiral GC−MS. In each case, the actual stereoselectivity of the conversion, taking into account the initial enantiomeric purity of **3**, was calculated and is displayed in **Table 1**.

At room temperature, and after a reaction time of nine months, there was a clear excess of the natural enantiomer of wine lactone **1a**. The ratio of **1a**:**1b** increased with increasing pH, reaching a maximum value of 4:1 at pH 3.4. Such a degree of enantioselectivity was not evident at the higher temperature of 45 °C, and the

wine lactone formed was near-racemic with just a slight enantiomeric excess (ee) of 2% of the natural form **1a** at pH 3.0 and 3.2 and 5% at pH 3.4. The ratios of **1a**:**1b** were highly consistent between triplicate hydrolysates for each set of experimental conditions at these lower temperatures, and the variation between treatment replicates (< 2%) was no greater than the variation between replicate analyses of the same sample. Two hydrolyses were also carried out at 100 °C and an intermediate pH of 3.2 using SDE conditions described previously (*19*, *20*). In this case, the ratio of **1a**:**1b** was more variable, with the ee being 5% and 22% in the two experiments. A third hydrolysis at this temperature and pH, but conducted in a closed vessel, gave an ee of 9% **1a**.

DISCUSSION

Wine lactone was formed hydrolytically from both the monoterpene acid **3** and glucose ester **2a**. Not surprisingly, wine lactone was formed more readily from the former. The rates of formation of wine lactone from the acid at 45 °C were broadly consistent with those reported in an earlier study by Winterhalter and Bonnländer (*16*) at 40 °C. After 3 months at room temperature, wine lactone was just detectable in the hydrolysates of the acid **3** at both pH values but not in the hydrolysates of the glucose ester **2a**. Of course, detectable levels of wine lactone might also be formed after longer periods of time from the glucose ester **2a**. Nevertheless, considering the initial concentration of the glucose ester in this study (495 μg/L), the glucose ester does not appear to be a direct major precursor for the formation of wine lactone in wine.

The low levels of wine lactone formed at room temperature from the monoterpene acid **3** indicate that it is unlikely to be an important precursor for wine lactone in young wines unless present in high concentration (≫1 mg/L), but could be an important precursor for wine lactone in older wine.

Guth (*4*) observed only one enantiomer, **1a**, during chiral analysis of unspecified white wine samples. Although subsequent data on the distribution of wine lactone isomers in other wines is lacking, this observation by Guth indicates that, should naturally occurring wine lactone be formed by acid-catalyzed transformation of the acid **3**, then hydrolysis of a single enantiomer of **3** would be expected to be highly enantioselective. Bonnländer (*20*) and Luan et al. (*19*) have reported conflicting data on the stereochemical outcome of converting the (6*R*)-enantiomer of the acid **3** to wine lactone by SDE, although both used similar, if not identical, reaction conditions. In the former case, racemic wine lactone was formed. Luan et al. (*19*), on the other hand, reported the formation the isomers **1a** and **1b** in a ratio of 4:1. This latter result was obtained using a deuterium-labeled substrate, and it was not clear whether the discrepancy between the two sets of results was a result of an isotope effect in the latter study. The use of deuterium-labeled substrates showed that, at 100 °C, the natural isomer **1a** was formed from (6*R*)-**3** *via* a proposed mechanism utilizing a 1,3-hydride shift, but whether this process is limited to experiments conducted at elevated temperatures, or also takes place at lower temperatures, was not investigated; nor does this mechanism alone explain the formation of the enantiomer **1b**. Lastly there was no assessment of the optical purity, as opposed to the chemical purity, of their variously substituted analogues of **3**.

We first repeated the hydrolysis of (6*R*)-**3** under the SDE conditions reported previously (*19*, *20*). Two experiments gave different degrees of selectivity. After the amount of each enantiomer of wine lactone formed from the racemic component of the acid **3** was subtracted from the measured total, the ratios of **1a**:**1b** formed from the excess (6*R*)-enantiomer in the starting acid were calculated (**Table 1**). In one case, this ratio of **1a**:**1b** was close to racemic (ee **1a** = 5%), while on the second occasion, a greater proportion of **1a** was formed (ee 22%). A third hydrolysis was conducted at the same temperature (100 °C) and pH (3.2) but in a closed vessel, and gave an ee of **1a** of 9%.

While SDE is a rapid and convenient method for generating and isolating hydrolysis products, the conditions of SDE do not always reliably mimic processes in which products are heated but without distillation. Not only are volatile products removed from the reaction mixture once distillation commences but volatile reaction intermediates can also be removed and trapped in the organic phase before they can react further, thus potentially distorting the outcome of the hydrolytic process (*24*). The degree to which this can happen would then depend on the time that elapses before the temperature of the aqueous phase reaches the boiling point, how quickly the aqueous phase boils, and how effective extraction into the organic phase is during solvent mixing. Such variables might have been responsible for the different results obtained by Luan et al. (*19*) and by Bonnländer (*20*). We confirmed that no racemization of **1a** takes place in aqueous solution at 100 °C and that there is no conversion of **3** to wine lactone in the boiling organic phase, thus eliminating the possibility that variation in these processes also contributed to the discrepancy in the published results.

In contrast to the experiments under SDE conditions, the stereochemical outcome of wine lactone formation from (6*R*)-**3** at room temperature was highly reproducible and weighted toward formation of the **1a** enantiomer, with the proportion of **1a** increasing with increasing pH. At 45 °C, however, the wine lactone formed was near-racemic with a maximum ee of **1a** of 6% at pH 3.4. This result, along with others (*25*), further highlights the need for caution in extrapolating from data obtained from even mildly accelerated conditions to processes that occur at room temperature. Indeed, it is not even certain whether the mechanism or mechanisms of wine lactone formation are the same at these different temperatures or to what extent racemization of the starting substrate **3** competes with wine lactone formation under the different sets of conditions.

The predominant formation of the (3*S*,3a*S*,7a*R*)-enantiomer of wine lactone (**1a**) from (6*R*)- 2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**3**) at room temperature is consistent with the finding, in wine, of this isomer only by Guth (*4*). Over time, sensorially significant quantities of **1a** could be formed, along with the relatively odorless enantiomer **1b** from the acid **3**, and possibly also indirectly from the glucose ester **2a**, should **3** be formed from **2a** by esterase action of wine microorganisms, including fermentation yeasts. Confirmation of these possibilities requires quantification and chiral analysis of both **1a**, **1b** and **3** in a range of young and older wines.

ABBREVIATIONS USED

Ee, enantiomeric excess; SDE, simultaneous distillation extraction.

ACKNOWLEDGMENT

We wish to thank our colleagues, especially George Skouroumounis and Kevin Pardon for assistance with the synthetic aspects, as well as Katryna van Leeuwen for assistance with the hydrolytic studies.

LITERATURE CITED

(1) Southwell, I. A. Essential oil metabolism in the Koala. III. Novel urinary monoterpenoid lactones. *Tetrahedron Lett*. **1975**, *24*, 1885−1888.

(2) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem*. **1997**, *45*, 3022−3026.

(3) Guth, H. Quantification and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027−3032.
(4) Guth, H. Determination of the configuration of wine lactone. *Helv. Chim. Acta* **1996**, *79*, 1559−1571.
(5) Buettner, A.; Schieberle, P. Evaluation of aroma differences between hand-squeezed juices from valencia late and navel oranges by quantification of key odorants and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 2387−2394.
(6) Lopez, R.; Ferreira, V.; Hernandez, P.; Cacho, J. F. Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: A comparative study. *J. Sci. Food Agric.* **1999**, *79*, 1461−1467.
(7) Jagella, T.; Grosch, W. Flavour and off-flavour compounds of black and white pepper (*Piper nigrum* L.) 1. Evaluation of potent odorants of black pepper by dilution and concentration techniques. *Eur. Food Res. Technol.* **1999**, *209*, 16−21.
(8) Buettner, A.; Schieberle, P. Characterization of the most odor-active volatiles in fresh hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). *J. Agric. Food Chem.* **1999**, *47*, 5189−5193.
(9) Strauss, C. R.; Wilson, B.; Williams, P. J. Novel monoterpene diols and diol glycosides in Vitis vinifera grapes. *J. Agric. Food Chem.* **1988**, *36*, 569−573.
(10) Humpf, H.-U.; Winterhalter, P.; Schreier, P. 3,4-Dihydroxy-7,8-dihydro-β-ionone β-D-glucopyranoside: natural precursor of 2,2,-6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0^{1,6}]undec-4-ene (Riesling acetal) and 1,1,6-trimethyl-1,2-dihydronaphthalene in red currant (*Ribes rubrum* l.) leaves. *J. Agric. Food Chem.* **1991**, *39*, 1833−1835.
(11) Cox, A.; Skouroumounis, G. K.; Elsey, G. M.; Perkins, M. V.; Sefton, M. A. Generation of (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene from C_{13}-norisoprenoid precursors. *J. Agric. Food Chem.* **2005**, *53*, 6777−6783.
(12) Winterhalter, P.; Schreier, P. C13-Norisoprenoid glycosides in plant tissues: an overview on their occurrence, composition and role as flavour precursors. *Flavour Fragrance J.* **1994**, *9*, 281−287.
(13) Guth, H. Comparison of different white wine varieties in odor profiles by instrumental analysis and sensory studies. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 714; American Chemical Society: Washington, DC, 1998; pp 39−54.
(14) Winterhalter, P.; Messerer, M.; Bonnländer, B. Isolation of the glucose ester of (E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid from Riesling wine. *Vitis* **1997**, *36*, 55−56.
(15) Winterhalter, P.; Baderschneider, B.; Bonnländer, B., Analysis, structure, and reactivity of labile terpenoid aroma precursors in riesling wine. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 714; American Chemical Society: Washington, DC, 1998; pp 1−12.
(16) Winterhalter, P.; Bonnländer, B. Aroma-active benzofuran derivatives: analysis, sensory properties, and pathways of formation. In *Aroma Active Compounds in Foods*; Takeoka, G. R., Güntert, M., Engel, K.-H., Eds.; ACS Symposium Series 794; American Chemical Society, Washington, DC, 2001; pp 21−32.
(17) 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.
(18) Sefton, M. A. Hydrolytically-released volatile secondary metabolites from a juice sample of Vitis vinifera grape cvs Merlot and Cabernet Sauvignon. *Aust. J. Grape Wine Res.* **1998**, *4*, 30−38.
(19) Luan, F.; Degenhardt, A.; Mosandl, A.; Wüst, M. Mechanism of wine lactone formation: demonstration of stereoselective cyclization and 1,3-hydride shift. *J. Agric. Food Chem.* **2006**, *54*, 10245−10252.
(20) Bonnländer, B. Isolierung, Charakterisierung und Synthese von Aromavorläufern aus Wein, Dillkraut und Lindenblüten. Ph.D. Thesis, Technische Universität Braunschweig, Braunschweig, Germany 2003.
(21) Bonnländer, B.; Baderschneider, B.; Messerer, M.; Winterhalter, P. Isolation of two novel terpenoid glucose esters from Riesling wine. *J. Agric. Food Chem.* **1998**, *46*, 1474−1478.
(22) Dalcanale, E.; Montanari, F. Selective oxidation of aldehydes to carboxylic acids with sodium chlorite-hydrogen peroxide. *J. Org. Chem.* **1986**, *51*, 567−569.
(23) Zou, K.; Tong, W.; Liang, H.; Cui, J.; To, G.; Zhoa, Y.; Khang, R. Diastereomeric saponins from *Albizia julibrissin*. *Carbohydr. Res.* **2005**, *340*, 1329−1334.
(24) Daniel, M. A.; Capone, D. L.; Sefton, M. A.; Elsey, G. M. Riesling acetal is a precursor to 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) in wine. *Aust. J. Grape Wine Res.* **2009**, *15*, 93−96.
(25) Cox, A.; Capone, D. L.; Elsey, G. M.; Perkins, M. V.; Sefton, M. A. Quantitative analysis, occurrence and stability of (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) in wine. *J. Agric. Food Chem.* **2005**, *53*, 3584−3591.

Received for review September 30, 2010. Revised manuscript received December 7, 2010. Accepted December 8, 2010. 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).