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Quantification of Several 4-Alkyl Substituted γ-Lactones in Australian Wines

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Stable isotope dilution assays have been developed for γ-octalactone (**1**), γ-nonalactone (**2**), γ-decalactone (**3**) and γ-dodecalactone (**4**) in both white and red wines for the first time. $^2H_7$-analogues of each lactone were prepared for use as internal standards via a strategy employing ring-opening, esterification and oxidation of the respective starting lactones. The methods were shown to be highly accurate and reproducible ($R^2 \geq 0.999$; SD ≤ 1%). A large selection of Australian wines ($n = 178$) were analyzed for the presence of lactones **1**−**4**. Fifty-eight white wines covering the varieties Chardonnay, Riesling, Sauvignon Blanc, Semillon and Viognier, as well as Botrytis style wines, were analyzed and showed broadly that γ-octalactone (**1**) was the most common lactone, being observed above its limit of detection in 28 of the wines, followed by γ-nonalactone (**2**) in 23 wines. The Botrytis style white wines had the highest concentrations of **1** and **2** (maximum concentrations 8.5 and 59 μg/L respectively). A total of 120 red wines covering the varieties Cabernet Sauvignon, Durif, Merlot, Pinot Noir and Shiraz were also studied and showed γ-octalactone (**1**) and γ-nonalactone (**2**) to be the most common lactones present, in 56 and 57 of the wines, respectively. γ-Decalactone (**3**) was observed in only a small number (13) of red wine samples and not at all in the white varieties. γ-Dodecalactone (**4**) was absent from all 178 samples studied. The highest concentrations of lactones **1**, **2** and **3** in the red wines were 4.2, 39.7 and 4.0 μg/L respectively.

KEYWORDS: γ-Lactones; SIDA; Australian wine; SPE; Botrytis; $^2H_7$-analogues

INTRODUCTION

The structural class of lactones, characterized by the possession of a cyclic ester group, are generally pleasant odorants that contribute a variety of aromas (*1*). They can contain rings of various sizes, but the most common are five- or six-membered rings. The former of these (γ-lactones) are common components of many fruits, including grapes. Four γ-lactones that have been reported in grapes and wine, and which form the focus of this study, are the 4-alkyl substituted lactones shown in **Figure 1**. γ-Nonalactone (**2**) was first identified in distilled beverages by Kahn et al. (*2*) and then later in wine by Schreier et al. (*3*), who also identified γ-decalactone (**3**) in wine (*4*). γ-Octalactone (**1**) was first identified in sherry by Fagan et al. (*5*) with the earliest report in wine following a decade later (*6*). γ-Lactones in wine continue to be described; γ-dodecalactone (**4**) was first reported by Barbe et al. (*7*).

Several methods are described in the literature for the quantification of γ-lactones in wine. Two decades ago, γ-nonalactone was quantified in six white wines and 32 red wines, using a continuous extraction procedure with Freon 11 and employing *iso*-butyl benzoate as the internal standard (*8*). More recently, a study of 52 red wines (comprising the varieties Grenache (17), Tempranillo (11), Cabernet Sauvignon (12) and Merlot (12)) was completed and included the quantification of γ-nonalactone (**2**) using 2-octanol as internal standard and γ-decalactone (**3**) using 4-methyl-2-pentanol (*9*). This study reported average concentrations of 16.2 μg/L and 1 μg/L, for **2** and **3** respectively. In a similar sized study of 57 Spanish red wines (unspecified varieties), γ-nonalactone was quantified using solid-phase extraction as the method of sample cleanup (*10*). Recently, a study of C_8 to C_{12} aliphatic lactones was published by Ferreira et al. and included the γ-lactones **1**−**4** (*11*). The method utilized solid-phase extraction for sample preparation and GC−MS for analysis with 2-octanol again used as the internal standard.

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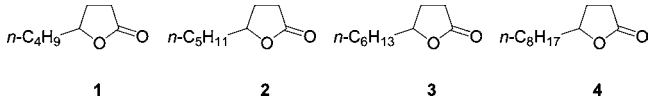

Figure 1. Structures of the four alkyl substituted γ-lactones quantified in wine.

Although several syntheses of various isotopically labeled analogues of γ-lactones and their application to the analysis of other products have been described (*12*−*16*), no stable isotope dilution method for quantification of these γ-lactones in grapes or wine has so far been reported. SIDA is the most accurate method for the determination of volatile flavor compounds in food and beverages, including wine, and employs isotopically labeled analogues, typically deuterated compounds, as internal standards. We wish to report a simple synthesis of d_7-labeled analogues of each of the γ-lactones **1**−**4** as well as the development of SIDA methods for their quantification. Finally, we have analyzed a large sample ($n = 178$) of Australian red and white wines, comprising ten varieties, for their lactone content in order to determine the range of concentrations that might be expected in these varieties.

MATERIALS AND METHODS

Materials. Wines were purchased commercially from several wine retail outlets in Adelaide, South Australia. Chemicals were purchased from Sigma-Aldrich and either used as supplied or dried and distilled using standard procedures (*17*). In the cases of the commercial samples of the γ-lactones, the molar amounts reported in the synthetic procedures have been corrected to reflect the actual purity (97%) of the samples supplied. X4 is a mixed hydrocarbon solvent, with *n*-hexane as the major component. Reactions employing moisture sensitive reagents were handled under N_2 and performed in flame dried glassware. TLC was performed with Merck silica gel 60 F_{254} (20 × 20 cm) aluminum sheets, and column chromatography utilized Merck silica gel 60 (particle size: 0.040−0.063 mm). Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Varian Gemini 200 or 300 spectrometer operating at frequencies of 300 MHz for proton and 75.5 MHz for carbon nuclei, respectively. All compounds gave spectroscopic data that were consistent with the expected structures. Gas chromatography−low resolution mass spectrometry (GC−MS) was performed with an Agilent 6890A gas chromatograph fitted to an Agilent 5973N mass spectrometer. High resolution mass spectrometry was performed with a Bruker BioApex 47e FTMS fitted with an Analytica electrospray source (ESI).

Methods. *(±)-$^2H_7$-γ-Octalactone (d_7-**1**).* To a stirred solution of (±)-γ-octalactone (**1**) (4.99 g, 34.04 mmol) in MeOH (65 mL) was added KOH (2.23 g of 85% grade, 33.78 mmol), and the mixture was then stirred at room temperature (RT) for 3 days. The solvent was removed under reduced pressure to afford the potassium carboxylate as a white solid (6.24 g). This salt (5.21 g, 26.27 mmol) was dissolved in DMSO (100 mL) with heating; to this cooled solution was added isopropyl bromide (12.2 mL, 123.43 mmol), and the mixture was stirred at RT for 20 h. The solution was diluted with H_2O (100 mL) and extracted with Et_2O (75 mL × 3). The combined organic extracts were washed with H_2O (100 mL × 3), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The resulting oil was purified by column chromatography (CH_2Cl_2, then 5% (v/v) Et_2O/CH_2Cl_2, $R_f = 0.14$ in the latter solvent) to afford alcohol **5** as a colorless oil (4.24 g, 85%).

To a stirred solution of DMSO (4.6 mL, 64.11 mmol) in anhydrous CH_2Cl_2 (90 mL) at −78 °C under N_2 was added oxalyl chloride (16.2 mL of a 2 M solution in CH_2Cl_2, 32.38 mmol) over 2 min. After the mixture was stirred at −78 °C for 30 min, a solution of alcohol **5** (4.37 g, 21.60 mmol) in anhydrous CH_2Cl_2 (15 mL) was added and the resulting mixture was stirred at −78 °C for 45 min. Et_3N (20 mL, 142.77 mmol) was added slowly, and the cloudy solution was stirred at −78 °C for 30 min, at 0 °C for 30 min and finally at RT for 30 min. The reaction was poured into a rapidly stirred solution of $NaHSO_4$ (1.0 M, 125 mL), the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (75 mL × 3). The combined organic extracts were concentrated under reduced pressure, and the residue was taken up in Et_2O (100 mL) and then washed with a 1 M solution of $NaHSO_4$ (40 mL × 3), H_2O (40 mL), saturated $NaHCO_3$ (40 mL) and brine (40 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure to yield keto ester **9** as a yellow oil (4.37 g, quantitative), which was used without further purification.

Keto ester **9** prepared above (0.50 g, 2.50 mmol) was heated under reflux in 35% (w/v) DCl/D_2O solution (1.5 mL) and D_2O (8.5 mL) under N_2 for 6 days. Any loss in volume of the DCl/D_2O solution was replaced as required. The reaction mixture was allowed to cool and was then extracted with Et_2O (10 mL × 3). The organic extracts were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. To the residue was added $NaBD_4$ (0.48 g, 11.26 mmol) and D_2O (10 mL) and the mixture was stirred under N_2 at RT for 24 h. The reaction was quenched by the careful addition of 35% (w/v) DCl/D_2O solution (pH 2) and stirred at RT under N_2 for 24 h. The product was extracted with Et_2O (25 mL × 3), and the organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by column chromatography (50% (v/v) Et_2O/hexanes, $R_f = 0.18$) to afford γ-lactone (d_7-**1**) as a colorless oil (0.28 g, 75%).

Labeled nonalactone, decalactone and dodecalactone were prepared as described for labeled octalactone, using the following quantities.

*(±)-$^2H_7$-γ-Nonalactone (d_7-**2**).* (±)-γ-Nonalactone (**2**) (4.97 g, 30.86 mmol) and KOH (2.24 g of 85% grade, 33.93 mmol) in MeOH (50 mL) generated the potassium carboxylate as a white solid (6.79 g). This salt (5.28 g, 24.87 mmol) was converted into ester **6** in DMSO (130 mL) by treatment with isopropyl bromide (12.0 mL, 121.43 mmol). Purification by column chromatography (5% (v/v) Et_2O/CH_2Cl_2, $R_f = 0.26$) afforded a colorless oil (4.62 g, 86%).

Ester **6** (4.17 g, 19.28 mmol) was oxidized under Swern conditions of DMSO (4.3 mL, 59.93 mmol) in anhydrous CH_2Cl_2 (85 mL) with oxalyl chloride (15 mL of a 2 M solution in CH_2Cl_2, 30.00 mmol) and Et_3N (17 mL, 121.34 mmol). The product **10** was obtained as a yellow oil (4.14 g, quantitative) and used without further purification.

Keto ester **10** (0.49 g, 2.30 mmol) in D_2O (8 mL) was heated at reflux in 35% (w/v) DCl/D_2O solution (2 mL) for 15 days, followed by reduction with $NaBD_4$ (0.21 g, 4.89 mmol) in D_2O (10 mL). Acidification with 35% (w/v) DCl/D_2O solution (pH 2) and stirring for 24 h. afforded, after purification by column chromatography (50% (v/v) Et_2O/hexanes, $R_f = 0.23$), (d_7-**2**) as a colorless oil (49.2 mg, 13%).

*(±)-$^2H_7$-γ-Decalactone (d_7-**3**).* (±)-γ-Decalactone (**3**) (5.00 g, 29.08 mmol) and KOH (2.13 g of 85% grade, 32.27 mmol) in MeOH (50 mL) generated the potassium carboxylate as a white solid (6.77 g). Treatment of this salt (5.68 g, 25.09 mmol) in DMSO (150 mL) with isopropyl bromide (12.0 mL, 121.43 mmol) afforded alcohol **7** (5.17 g, 89%) as a colorless oil after purification by column chromatography (50% (v/v) Et_2O/CH_2Cl_2, $R_f = 0.27$).

Oxidation of ester **7** (4.53 g, 19.67 mmol) using DMSO (4.4 mL, 61.33 mmol) in anhydrous CH_2Cl_2 (85 mL) with oxalyl chloride (15 mL of a 2 M solution in CH_2Cl_2, 30.00 mmol) and Et_3N (17 mL, 121.34 mmol) gave the keto ester **11** as yellow oil (4.61 g, quantitative), which was used without further purification. Labeled γ-decalactone (d_7-**3**) was synthesized from keto ester **11** (0.51 g, 2.25 mmol) in D_2O (8.5 mL) via a three step sequence of 35% (w/v) DCl/D_2O solution (1.5 mL) for 29 days, $NaBD_4$ (0.50 g, 11.65 mmol) in D_2O (10 mL) and 35% (w/v) DCl/D_2O solution (pH 2). Purification by column chromatography (50% (v/v) Et_2O/hexanes, $R_f = 0.20$) afforded (d_7-**3**) as a colorless oil (0.16 g, 40%).

(±)-$^2H_7$-γ-Dodecalactone (d_7-4). (±)-γ-Dodecalactone (**4**) (6.04 g, 29.55 mmol) and KOH (2.26 g of 85% grade, 34.24 mmol) in MeOH (50 mL) generated the potassium carboxylate as a white solid (7.90 g). This salt (6.41 g, 25.19 mmol), after treatment with isopropyl bromide (12.0 mL, 121.43 mmol) in DMSO (150 mL), afforded ring-opened alcohol **8** (5.18 g, 80%) as a colorless oil upon purification by column chromatography (50% (v/v) Et_2O/CH_2Cl_2, $R_f = 0.27$).

Swern oxidation of alcohol **8** (4.76 g, 18.42 mmol) using DMSO (4.3 mL, 59.93 mmol) in anhydrous CH_2Cl_2 (85 mL) with oxalyl

chloride (15 mL of a 2 M solution in CH_2Cl_2, 30.00 mmol) and Et_3N (17 mL, 121.34 mmol) afforded **12** as a yellow oil (4.87 g, quantitative) which was used without further purification. A three step sequence of 35% (w/v) DCl/D_2O solution (2 mL) for 17 days, $NaBD_4$ (0.24 g, 5.60 mmol) in D_2O (10 mL) and 35% (w/v) DCl/D_2O solution (pH 2) was used for the conversion of **12** (0.51 g, 1.98 mmol) into labeled γ-dodecalactone (d_7-**4**). Purification by column chromatography (50% (v/v) Et_2O/hexanes, $R_f = 0.23$) afforded γ-lactone (d_7-**4**) as a colorless oil (69.2 mg, 17%).

Method Development for Solid-Phase Extraction (SPE) Analysis with the d_7-Internal Standards. *Sample Preparation (11).* Varian Bond Elut-ENV 200 mg prepacked 3 mL cartridges (Varian, Australia) were placed in an extraction manifold system and conditioned by rinsing with MeOH (2 mL) and H_2O (4 mL). Wine (50 mL) spiked with the d_7 standards (50 μL of a 10 μg/mL solution; equivalent to 10 μg of lactone per L of wine for each γ-lactone) was passed through, and the cartridges were rinsed with H_2O (5 mL) and then 40% (v/v) MeOH/H_2O with 1% (w/v) $NaHCO_3$ (20 mL). The cartridges were allowed to dry by passing air through for 30 min. The analytes were extracted with CH_2Cl_2 (2 mL), dried through a glass pipet with $MgSO_4$ and concentrated under a gentle stream of N_2 at RT to fit into a vial insert (100 μL) for analysis by GC−MS.

Calibration Functions and Validation of Method. The method was validated by a series of duplicate standard additions of the analytes (0 μg/L, 0.5 μg/L, 1 μg/L, 2 μg/L, 5 μg/L, 10 μg/L, 25 μg/L, 50 μg/L and 100 μg/L) to a red wine ("bag in a box" dry red wine, pH 3.50, 12.8% EtOH, SO_2 levels 117 mg/L total and 21 mg/L free) and to a white wine ("bag in a box" fresh dry white wine, pH 3.23, 9.2% EtOH, SO_2 levels 155 mg/L total and 16 mg/L free) containing the d_7 standards (50 μL of a 10,000 μg/L solution; spike at 10 μg/L for each γ-lactone). The blank wines were analyzed and shown to contain no γ-lactones (LOD < 0.1 μg/L, determined as the point at which the quantifying target ion and one qualifier ion were observable). The reproducibility of the analyses was determined at two concentrations, 5 μg/L and 25 μg/L, by spiking seven samples of the same wine at each of these concentrations with the analytes.

Instrumental Analysis. An Agilent 6890A gas chromatograph fitted to an Agilent 5973N mass spectrometer was used with a 60 m × 0.25 mm i.d. 0.25 μm Innowax capillary column (Agilent, USA). The carrier gas was helium at 1.5 mL/min. The initial column temperature was 50 °C, held for 1 min, then increased to 190 at 40 °C/min. and finally to 250 at 5 °C/min and held for 20 min with the transfer line at 250 °C. The injection was carried out with a Gerstel MPS2 auto sampler using a 10 μL syringe in pulse splitless mode. During splitless mode, a pressure pulse of 45.0 psi was applied. The injected volume was 2 μL. The mass spectrometer detector was initially used in scan mode with recording in the range of *m*/*z* 35−350. For method development, validation and analysis, the mass spectrometer detector was used in selected ion monitoring (SIM) mode with a solvent delay of 5 min. The ions monitored for the analytes were *m*/*z* 85, 86, 100, 128 and the ions monitored for the d_7 standards were *m*/*z* 90, 91, 107, 135. The underlined ions were the target ions used for quantification, and the remaining ions were the qualifying ions used for verification of compound identity and peak homogeneity.

RESULTS AND DISCUSSION

Synthesis of the d_7-Labeled Analogues of the γ-Lactones. As each of the γ-lactones included in this study was commercially available, our syntheses of the labeled analogues began with these purchased samples, and were based on that previously reported by Pollnitz et al. (*18*) for the preparation of labeled analogues of the oak lactones; the strategy employed is displayed in **Figure 2**. Ring opening of each of the lactones was effected by the use of potassium hydroxide in DMF, with the resulting carboxylate species trapped as their respective isopropyl esters. Swern oxidation (*19*) provided the 4-keto substituted esters **9**−**12**, in which the α-hydrogens are activated toward exchange. Under the acidic conditions employed, the four hydrogens α to the ketone plus the two hydrogens α to the carboxyl function

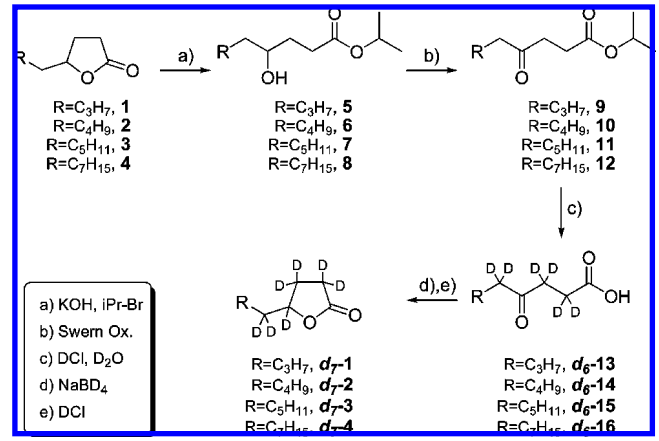

Figure 2. Synthetic scheme for the preparation of the d_7-labeled γ-lactones **1**−**4**.

all underwent exchange to give the d_6-labeled keto-acids **13**−**16**. The seventh deuterium atom was introduced by reduction of the 4-keto function with $NaBD_4$ in D_2O. Acidification and concomitant lactonization provided the desired targets d_7-**1**−d_7-**4**. The position of the deuterium atoms on the lactone ring as well as on the side chain allowed each mass spectrum fragment for the analytes to be distinguished from the corresponding fragment in the labeled analogues, as well as providing greater choice in the selection of target and qualifier ions needed for the SIDA method.

Development of the Analytical Method. *Target and Qualifier Ions.* Solutions of the γ-lactones and the deuterated analogues were analyzed by GC−MS in scan mode to enable selection of suitable target and qualifier ions. With the facile cleavage of the alkyl side chain, the spectra were dominated by a large base peak (*m*/*z* 85 for unlabeled; *m*/*z* 90 for labeled) with smaller peaks for the other fragment ions. With the molecular ion of low abundance, the ions selected were *m*/*z* 85, 86, 100 and 128 for lactones **2**−**4**, and *m*/*z* 90, 91, 107 and 135 for the respective labeled standards. In the case of lactone **1**, the ions at *m*/*z* 128 (unlabeled) and 135 (labeled) were not observed. The base peaks were used for quantification by peak area and the remaining ions used as qualifiers to ensure correct compound identification and peak homogeneity. For the quantification of the γ-lactones, the mass spectra were recorded in selected ion monitoring (SIM) mode.

Optimization of the Extraction Procedure. Initially solid-phase microextraction (SPME) was investigated as the extraction technique. However, the method developed with this technique proved problematic; when applied to the quantification of the lactones in real wine samples, some samples suffered from interference of coeluting compounds that gave several of the selected ions. Although the method was satisfactory for many of the wines analyzed, it was not sufficiently robust to allow quantification of all four lactones in all of the wines chosen (*20*). In particular, the use of SPME, for some wines, gave chromatograms in which a compound with a retention time almost identical to that of **4**, and a major peak in the mass spectrum at *m*/*z* 85 was present. We initially assigned this peak to lactone **4**. However, closer analysis of the mass spectrum revealed that the qualifier ions, required to confirm its identity, were absent. It was therefore decided to utilize solid-phase extraction (SPE) as a means of cleaning up the samples. SPE is an analytical technique used widely for either sample extraction or sample cleanup. A recently published method for the determination of aliphatic lactones in wine featuring SPE (*11*) has been extended here to incorporate the d_7 analogues of

Table 1. Minimum and Maximum Concentrations of γ-Lactones **1** and **2** in Commercial White Wines

	minimum[a]	maximum[a]	$n^{b,c}$
	Chardonnay ($N = 12$)		
1	<0.1	1.2	3
2	<0.1	0.8	2
	Riesling ($N = 12$)		
1	0.28	1.7	12
2	<0.1	3.3	3
	Sauvignon Blanc ($N = 10$)		
1	<0.1	<0.1	0
2	<0.1	0.2	1
	Semillon ($N = 10$)		
1	<0.1	3.5	7
2	<0.1	0.5	2
	Viognier ($N = 7$)		
1	<0.1	<0.1	0
2	<0.1	4.7	6

[a] All values in μg/L. [b] Number of wines in which the lactone exceeded LOD (0.1 μg/L). [c] Concentrations of γ-decalactone (**3**) and γ-dodecalactone (**4**) were, in all cases, below the LOD.

the γ-lactones as internal standards. We found that by using this alternative form of sample preparation, interfering compounds including that initially misidentified as **4** were removed. The calibration functions obtained for standard additions of each γ-lactone to both white ("bag in box" fresh dry white) and red ("bag in box" dry red) wine were linear throughout the concentration range (0−100 μg/L), with excellent coefficients of determination ($R^2 \geq 0.999$). The method was verified by the analysis of seven replicates at two different concentrations (5 μg/L and 25 μg/L) and found to be within an acceptable range for accuracy and precision (SD ≤ 1%).

Analysis of Australian Wines. A large wine survey of 178 commercial single variety wines (58 white wines and 120 red wines) was conducted to determine the robustness of the method and to quantify the four target γ-lactones (Tables A and B, respectively, in the Supporting Information) using the SIDA method developed with the d_7 internal standards. The quantified values listed in the tables were the result of single determinations.

White Wines. For the dry white wines, the varieties chosen were Chardonnay (12 wines), Riesling (12 wines), Sauvignon Blanc (10 wines), Semillon (10 wines) and Viognier (7 wines) (**Table 1**). The wines represented a wide cross-section of quality, including wine from both the high end and the low end of the market. The wines were chosen from the different wine-making regions within Australia. The ages of the wines ranged from <12 months old (vintage 2006) back to the 1994 vintage.

The concentration of the γ-lactones in the dry white wines ranged from below the limit of detection (0.1 μg/L) to 4.7 μg/L for γ-nonalactone (**2**) in a Viognier. Neither γ-decalactone (**3**) nor γ-dodecalactone (**4**) was detected in any of the white wines studied. In general, more white wines contained **1** than **2**, particularly for the Riesling and Semillon varieties, while γ-lactones were absent from all but one of the Sauvignon Blanc wines.

In contrast to Ferreira et al. (*11*), who did not find γ-octalactone (**1**) in dry white wine, we found this compound in nearly half of the dry white wines analyzed, albeit at low concentrations (**Table 1**). Conversely, the concentrations of γ-nonalactone (**2**) in the present study were significantly lower than those reported by others (*8*, *11*, *21*, *22*).

Table 2. Concentrations of γ-Lactones **1** and **2** in Seven Commercial Botrytized Wines

vintage	**1**[a]	**2**[a]
2005	5.9	59.3
2005	3.7	33.1
2004	4.3	30.6
2004	7.1	35.8
2002	5.1	24.8
2001	8.5	49.7
1999	<0.1	8.9

[a] All values in μg/L.

Table 3. Minimum and Maximum Concentrations of γ-Lactones **1**−**3** in Commercial Red Wines

	minimum[a]	maximum[a]	$n^{b,c}$
	Cabernet Sauvignon ($N = 30$)		
1	<0.1	1.3	8
2	<0.1	33.9	21
3	<0.1	4.0	3
	Durif ($N = 6$)		
1	0.25	4.2	6
2	<0.1	39.7	4
3	<0.1	0.8	1
	Merlot ($N = 25$)		
1	<0.1	2.1	9
2	<0.1	19.1	8
3	<0.1	1.1	2
	Pinot Noir ($N = 17$)		
1	<0.1	1.1	3
2	<0.1	34.8	9
3	<0.1	<0.1	0
	Shiraz ($N = 42$)		
1	<0.1	2.2	30
2	<0.1	21.4	15
3	<0.1	0.9	7

[a] All values in μg/L. [b] Number of wines in which the lactone exceeded LOD (0.1 μg/L). [c] Concentrations of γ-dodecalactone (**4**) were, in all cases, below the LOD.

Neither γ-decalactone (**3**) nor γ-dodecalactone (**4**) was detected in any of the white wines, in agreement with previously reported values close to zero (*11*, *21*, *22*).

The botrytized wines studied showed much higher values of **1** and **2** than any of the dry white wines (**Table 2**), although, again, neither **3** nor **4** was observed. With the single exception of **1** in one sample, the lowest concentration observed for these two lactones in botrytized wine was greater than the highest concentration observed in the dry white wines.

A large increase in concentration of **2** in botrytized wine has been reported before by Genovese et al. (*23*). The average concentration of **2** in unaffected wines of the Fiano variety was measured by those authors at 6.3 μg/L, while those which had been infected by *Botrytis cinerea* produced average concentrations of 43.2 μg/L. They also reported increases in the concentration of **3** from 3.7 μg/L (unaffected) up to 15.5 μg/L. γ-Lactones were previously reported as characteristic aroma compounds of Tokaji Aszú wines (*24*).

Red Wines. As was the case with the white wines, the red wines were chosen from across the different Australian wine-making regions, and also covered a wide range of quality. They comprised Cabernet Sauvignon (30 wines), Durif (6 wines), Merlot (25 wines), Pinot Noir (17 wines) and Shiraz (42 wines) (**Table 3**). The ages of the red wines ranged from <12 months old (vintage 2006) back to the 1991 vintage.

Compared with the dry white wines, the red wines analyzed were found to contain significantly higher levels of the γ-lac-

tones, ranging from below the limit of detection (0.1 μg/L) to 39.7 μg/L for **2** in a Durif. The highest level of **1** was also found in a Durif, at 4.2 μg/L.

In general most of the red wines contained substantially higher concentrations of **2** than of **1**, and the former compound was present at a concentration above 10 μg/L in nearly a third of the Cabernet Sauvignon wines. A small number of the red wines (13) contained γ-decalactone (**3**), albeit at concentrations below 1 μg/L in ten of these. The highest concentration of **3** was 4.0 μg/L in a Cabernet Sauvignon wine. Once again, γ-dodecalactone (**4**) was not measured above the limit of detection in any of the red wines.

In these Australian red wines, the concentrations measured for lactones **1**−**3** were broadly similar to those reported previously (*11*), except that we found lower concentrations of **2** in some wines. The two studies differ however, in the concentration of γ-dodecalactone (**4**) measured. The earlier study found this compound in Spanish red wines, particularly the aged reds, whereas we did not detect this compound above 0.1 μg/L in the 120 Australian red wines analyzed.

In summary, the method described here is sensitive, accurate, precise and can be easily applied to large numbers of wines. Accurate quantification of γ-lactones, each of which has attractive aroma properties (*1*), will enable a better understanding of the contribution of these compounds to the sensory attributes of wines made from different varieties, under different climatic conditions.

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Supporting Information Available: ^{1}H NMR,^{13}C NMR, and HRMS data for all the compounds studied. Individual quantification data results for lactones **1**−**4**, from all 178 wines included in the survey. This material is available free of charge via the Internet at http://pubs.acs.org.

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