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Odor Detection Thresholds and Enantiomeric Distributions of Several 4-Alkyl Substituted γ-Lactones in Australian Red Wine

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The individual enantiomers of γ-octalactone (**1**), γ-nonalactone (**2**), γ-decalactone (**3**) and γ-dodecalactone (**4**) have been synthesized. The (*R*) series of enantiomers was prepared from L-glutamic acid by a strategy involving deamination and reduction to (*S*)-5-oxo-2-tetrahydrofurancarboxaldehyde (*S*)-**7**. The different length side chains were introduced by a series of Wittig reactions, varying in the choice of phosphorane used. Hydrogenation then gave the final γ-lactones **1**−**4**. The (*S*) series of enantiomers was prepared in an analogous fashion beginning with D-glutamic acid. Aroma detection thresholds for all eight enantiomers were determined in a "bag in a box" dry red wine by the application of ASTM method E 679, employing a panel of 25 members. The lowest threshold determined was 8 μg/L for (*R*)-dodecalactone (**4**) while the highest threshold was 285 μg/L for (*R*)-nonalactone (**2**). With the exception of γ-decalactone (**3**) there were statistically significant differences (at the 5% level) in aroma detection thresholds between the two enantiomers of the same lactone. A stable isotope method developed for quantification of the lactones **1**−**4** has been extended for use with chiral phase GC (Rt-βDEXcst capillary column) allowing quantification of the individual enantiomers. The enantiomeric distribution of γ-octalactone (**1**) and γ-nonalactone (**2**) in seven botrytized wines and of **2** in a total of 34 red wines were thus determined; with few exceptions, the (*R*) enantiomer of γ-nonalactone (**2**) was found to be more prevalent than its (*S*) counterpart in the dry red and botrytized white wines analyzed. The same was true for γ-octalactone (**1**) in the botrytized white wines.

KEYWORDS: γ-Lactones; aroma thresholds; Australian wine; enantiomeric distribution; GC-olfaction

INTRODUCTION

γ-Lactones are compounds containing a five-membered cyclic ester, and are common components of many fruits and fruit products, including grapes and wine. Four of the γ-lactones reported as components in grapes and wine, and the focus of this study, are the 4-alkyl substituted lactones shown in **Figure 1**. Kahn et al. first identified γ-nonalactone (**2**) in distilled spirits (*1*). It was later found in wine by Schreier et al. (*2*), who also identified γ-decalactone (**3**) in wine (*3*). The first report of γ-octalactone (**1**) in fortified wines was by Fagan et al. in sherry (*4*), followed later by the earliest report in wine (*5*). The final member of the four, γ-dodecalactone (**4**), was reported by Barbe et al. (*6*).

Mosandl and Günther (*7*) prepared samples of each of the individual enantiomers of, among other compounds, lactones **1**−**4**, and conducted qualitative sensory assessments of each. They were prepared from racemic samples of the γ-lactones via ring opening of the lactones and attachment of a chiral resolving agent, either (*R*)-2-phenylpropionic acid or (1*S*,4*R*)-camphanic acid. Base hydrolysis and relactonization gave the individual enantiomers of the γ-lactones in a high state of optical purity. An informal sensory study was undertaken on the enantiomers; a 1% solution of each compound in propylene glycol was prepared and tested by smelling on strips. The odor descriptors reported are collected in **Table 1**. It was observed that an increase in alkyl chain length led to a decrease in coconut aroma and an increase in fruity-sweet notes. Also, it was suggested that the (*R*)-enantiomers

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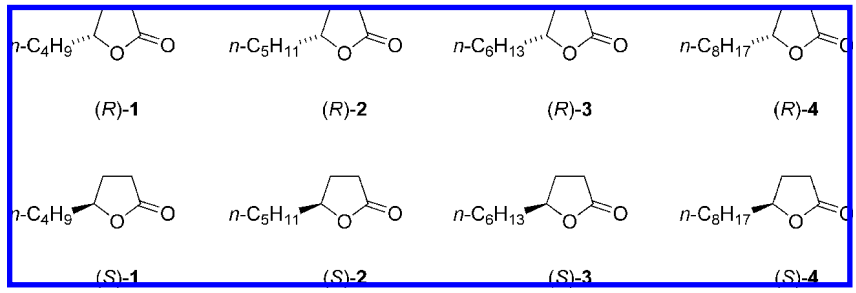

Figure 1. (*R*) and (*S*) enantiomers of the four alkyl substituted γ-lactones chosen for this study.

Table 1. Odor Descriptors Reported (*7*) for Lactones **1**−**4** as a 1% Solution of Each Compound in Propylene Glycol

compound	odor descriptors
(*R*)-γ-octalactone (**1**)	coconut notes with almond notes, spicy-green
(*S*)-γ-octalactone (**1**)	coconut notes, fatty
(*R*)-γ-nonalactone (**2**)	soft coconut with fatty-milky aspects, strong, sweet
(*S*)-γ-nonalactone (**2**)	fatty, moldy, weak coconut notes
(*R*)-γ-decalactone (**3**)	caramel, fatty-sweet fruity note, soft coconut, strong
(*S*)-γ-decalactone (**3**)	soft, sweet coconut note with fruity-fatty aspects
(*R*)-γ-dodecalactone (**4**)	bloomy notes with aldehyde and woody aspects, strong, fruity-sweet
(*S*)-γ-dodecalactone (**4**)	fatty-fruity, milky notes

had greater odor strength than the corresponding (*S*)-enantiomers.

In a recent study (*8*) we reported the development of stable isotope dilution methods for the four lactones **1**−**4**, and their application to the quantification of the lactones in Australian wines. We now wish to report the synthesis of all eight enantiomers of lactones **1**−**4** (**Figure 1**). In addition, aroma detection thresholds have been measured for all eight compounds in red wine, the medium in which these compounds are more prevalent, and occur at higher concentrations (*8*). Although we did not detect γ-dodecalactone (**4**) in any of the 178 Australian wines analyzed earlier (*8*), we have included this compound in the aroma threshold detection study as it has been reported as a component of some wines by other authors.

In addition, the quantification method has been extended for use with chiral phase GC and applied to the measurement of the enantiomeric distribution of γ-octalactone (**1**) and γ-nonalactone (**2**) in a selection of wines.

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 (*9*). 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 was performed with Merck silica gel 60 (particle size: 0.040−0.063 mm). Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded as solutions in chloroform-*d* on a Varian Gemini spectrometer operating at frequencies of 300 MHz for proton and 75.5 MHz for carbon nuclei. All compounds gave spectroscopic data that were consistent with the expected structures. Gas chromatography−low resolution mass spectrometry (GC−MS) was performed on an Agilent 6890A gas chromatograph fitted to an Agilent 5973N mass spectrometer, set up as described elsewhere (*8*). Mass spectrometric data are presented as mass to charge ratio (*m*/*z*) and intensity of peak relative to the base peak. Chiral GC−MS was performed with a Rt-βDEXcst capillary column (Restek, Bellefonte, USA), 30 m × 0.25 mm I.D. and 0.25 μm film thickness.

Methods. *(S)-5-Oxo-2-tetrahydrofurancarboxylic Acid (S)*-**6.** To a stirred suspension of L-glutamic acid (**5**) (25.08 g, 168.8 mmol) in H_2O (75 mL) at 0 °C was added simultaneously a solution of $NaNO_2$ (17.72 g, 256.8 mmol) in H_2O (100 mL) and a solution of concentrated HCl (25 mL of a 37% solution, 255.0 mmol) in H_2O (75 mL) over 2 h. The mixture was stirred at room temperature (rt) for 16 h. The water was removed under reduced pressure and the residue extracted into hot ethyl acetate and filtered through celite. The solvent was removed under reduced pressure (coevaporating with toluene). Recrystallization from $CHCl_3$ afforded acid (*S*)-**6** (8.20 g, 37%) as a white crystalline solid.

(R)-Octalactone (R)-**1.** (*S*)-**6** (1.48 g, 11.38 mmol) was heated at reflux in oxalyl chloride (2.6 mL, 30.4 mmol) and anhydrous CH_2Cl_2 (2.6 mL) under N_2 for 2.75 h. The solvent and excess oxalyl chloride were removed under reduced pressure to yield a pale yellow oil. This oil, in toluene (5 mL), was added to a suspension of Pd-$BaSO_4$ (0.51 g) in anhydrous toluene (45 mL). H_2 was bubbled through the mixture which was stirred at 60−70 °C for 3 h. The mixture was filtered through celite, rinsed with anhydrous CH_2Cl_2 and concentrated under reduced pressure to yield aldehyde (*S*)-**7**, which was used without further purification. $(CH_3)_3COK$ (1.59 g, 13.5 mmol) was added to a suspension of *n*-propyltriphenylphosphonium iodide (6.23 g, 14.4 mmol) in anhydrous THF (35 mL) and the mixture was stirred under N_2 at 0 °C for 1.5 h. A bright orange solution resulted, indicative of ylide formation. Aldehyde (*S*)-**7** was added in anhydrous THF (5 mL) at 0 °C and stirring was continued under N_2 at rt for 16 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by column chromatography (50% (v/v) ether/X4, R_f = 0.15) to afford alkenes (*S*)-**8** (0.82 g, 52%), a colorless oil, as a 3:1 mixture of *cis*- and *trans*-isomers determined by NMR. This mixture of alkenes (215.9 mg, 1.54 mmol) was stirred in a suspension of Pd-$BaSO_4$ (47.5 mg) with ethyl acetate (5 mL) under an atmosphere of H_2 for 16 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/X4, R_f = 0.39) and purified by column chromatography (50% (v/v) ether/X4, R_f = 0.18) to afford γ-lactone (*R*)-**1** (69.9 mg, 32%) as a colorless oil.

(R)-Nonalactone (R)-**2**, *(R)-Decalactone (R)*-**3** *and (R)-Dodecalactone (R)*-**4.** (*S*)-**6** was converted into the remaining alkenes **9**−**11** *via* procedures analogous to that employed for the synthesis of (**8**), with the difference being the choice of phosphorane used in the Wittig reaction: (*S*)-**9** was prepared using *n*-butyltriphenylphosphonium bromide (43% yield); (*S*)-**10** was prepared using *n*-pentyltriphenylphosphonium iodide (13% yield); and (*S*)-**11** was prepared using *n*-heptyltriphenylphosphonium iodide (35% yield). Conversion of these alkenes into their respective (*R*)-lactones was achieved by hydrogenation over Pd-$BaSO_4$: (*R*)-**2** was prepared over 6 h in 79% yield; (*R*)-**3** was prepared over 24 h in 63% yield; (*R*)-**4** was prepared over 48 h in 53% yield.

(R)-5-Oxo-2-tetrahydrofurancarboxylic acid (R)-**6.** Acid (*R*)-**6** was prepared, as per (*S*)-**6**, from D-glutamic acid (**5**) (15.00 g, 100.9 mmol) in H_2O (50 mL) with sodium nitrite (10.63 g, 154.1 mmol) in H_2O (50 mL) and concentrated HCl (15 mL of a 37% solution, 153. 0 mmol) in H_2O (45 mL). Recrystallization from $CHCl_3$ afforded acid (*R*)-**6** (4.84 g, 37%) as a white crystalline solid.

(S)-Octalactone (S)-**1**, *(S)-Nonalactone (S)*-**2**, *(S)-Decalactone (S)*-**3** *and (S)-Dodecalactone (S)*-**4.** The (*S*) series of lactones **1**−**4** were prepared from acid (*R*)-**6** via procedures that were entirely analogous to those used for the synthesis of the (*R*) series: (*R*)-**8** in 45% yield; (*R*)-**9** in 45% yield; (*R*)-**10** in 47% yield; and (*R*)-**11** in 43% yield. Conversion of the alkenes into their respective (*S*)-lactones was achieved

by hydrogenation over Pd-$BaSO_4$: (*S*)-**1** over 48 h (41% yield); (*S*)-**2** over 48 h (63% yield); (*S*)-**3** over 72 h (57% yield); and (*S*)-**4** over 6 h (61% yield).

Odor Detection Thresholds. The odor detection thresholds in red wine were determined according to the American Standards for Testings and Materials (ASTM) method E 679 (*10*) which had been previously employed by us for the threshold determination of the four enantiomers of the oak lactones (*11*). The sensory testings took place over approximately one month, with 25 panelists participating in each of the threshold tests. Most panelists had previous experience with difference testing and with sensory evaluation of wine. The judges were Australian Wine Research Institute (AWRI) staff and students of various ethnic origins, aged between 20 and 55, with similar numbers of males and females.

Solutions of the various γ-lactone enantiomers were prepared from stock solutions, and the concentration of each solution was quantified by GC−MS analysis using the corresponding d_7-lactone as internal standard (*8*). The spiked samples were prepared by adding an ethanolic solution (<0.5 mL) of the isomer of interest to 1 L of wine. An equal volume of ethanol was added to the corresponding blank samples. The samples in each individual triangle test were presented in random order and identified only by three-digit random numbers. The sample that was different from the other two was always the spiked sample. Panelists smelled, but did not taste, the samples. Wines were presented as 20 mL portions in randomly coded glasses (Arcoroc Viticole 21.5 cL wine glasses), covered with a watch glass (as part of a triangle test) in ascending order of γ-lactone concentration. Panelists who were successful at detecting all concentrations were retested at lower concentrations, while panelists who were unsuccessful at detecting the highest concentration were retested at higher concentrations. For each judge, an individual best estimate threshold (BET) was calculated, and the group threshold was determined as the geometric mean of the individual BETs.

The red wine used was a "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 was shown to contain no γ-lactones above the limit of analytical detection (LOD) (0.1 μg/L). From the results of the triangle tests, each individual panelist was assigned a best estimate threshold (BET) value. The group BET was then calculated to determine the final threshold value.

The concentrations employed were as follows.

•(*R*)-γ-Octalactone (*R*)-**1**: 3.1, 9.2, 27.5, 82.1, 239 and 726 μg/L, with retest concentrations for the high end of 2292 and 6685 μg/L.
•(*S*)-γ-Octalactone (*S*)-**1**: 9.1, 27.4, 81.3, 244, 739 and 2244 μg/L, with retest concentrations for the high end of 6600 and 19536 μg/L.
•(*R*)-γ-Nonalactone (*R*)-**2**: 9.0, 26.7, 82.5, 246, 727 and 2160 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L, and for the high end of 6678 and 19640 μg/L.
•(*S*)-γ-Nonalactone (*S*)-**2**: 9.0, 27.2, 80.0, 245, 740 and 2200 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L, and for the high end of 6600 and 20000 μg/L.
•(*R*)-γ-Decalactone (*R*)-**3**: 9.0, 26.8, 80.3, 239, 727 and 2199 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L, and for the high end of 6501 and 19120 μg/L.
•(*S*)-γ-Decalactone (*S*)-**3**: 9.1, 27.5, 80.8, 242, 727 and 2222 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L, and for the high end of 6464 and 20200 μg/L.
•(*R*)-γ-dodecalactone (*R*)-**4**: 9.0, 27.4, 82.1, 242, 736 and 2185 μg/L, with retest concentrations for the low end of 0.3, 1.0 and 3.0 μg/L, and for the high end of 6670 and 19780 μg/L.
•(*S*)-γ-Dodecalactone (*S*)-**4**: 3.0, 8.9, 26.9, 79.2, 243 and 733 μg/L, with retest concentrations for the low end of 0.4 and 1.0 μg/L, and for the high end of 2178 and 6534 μg/L.

Method Development for Chiral Analysis. The method used for quantification of the individual enantiomers of octalactone (**1**) and nonalactone (**2**) was based precisely on that used for quantification of the racemic lactones (*8*), with the following modifications: a chiral (Rt-βDEXcst capillary column, 30 m × 0.25 mm i.d. and 0.25 μm film thickness) column was employed. The carrier gas was helium at 1.1 mL/min. The initial column temperature was 50 °C, held for 1 min, then increased to 220 at 4 °C/min and held for 10 min with the transfer line at 220 °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 25.0 psi was applied. The same target and qualifier ions were used as in the earlier report (*8*).

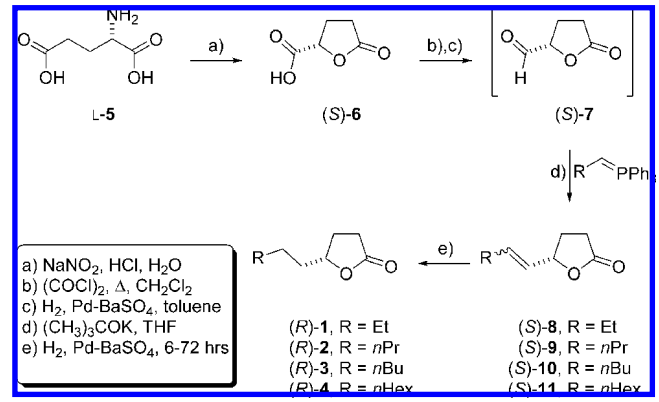

Figure 2. Synthetic scheme for the preparation of the (*R*) lactones from L-glutamic acid.

GC-O Purity Check on Lactones 1−4. The individual enantiomers of **1**−**4** were analyzed by GC-O as described in an earlier report (*19*) with the following modifications: The Gerstel MPS2 autosampler was fitted with a liquid injector and 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 2.0 mL/min. The oven temperature started at 50 °C and was held at this temperature for 1 min, then increased to 240 at 10 °C/min and held at this temperature for 10 min. The injector was held at 200 °C and the transfer line at 240 °C. The sample volume injected was 2 μL, and the splitter, at 45:1, was opened after 30 s. Fast injection was done in pulse 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.

RESULTS

Synthesis of the Enantiomerically Pure γ-Lactones 1−4. By adapting a procedure used previously for the synthesis of the sex pheromone of the Japanese beetle, (5*R*,*Z*)-5-(1-decenyl)dihydro-2(3*H*)-furanone, the individual enantiomers of **1**−**4** were prepared (*12*). This sequence began with either L- or D-glutamic acid and utilized the stereospecificity of the deamination reaction (*13*). **Figure 2** shows the conversion of L-glutamic acid into the (*R*) series of enantiomers. L-Glutamic acid (**5**) was treated with nitrous acid (generated in situ from hydrochloric acid and sodium nitrite) to produce acid (*S*)-**6**. The reaction is known to proceed with complete retention of configuration (*13*). This acid was obtained in low yield (37%), consistent with the findings reported in the literature (*14*). Acid (*S*)-**6** was converted into its corresponding acid chloride, which was then immediately converted into the aldehyde (*S*)-**7** by treatment with hydrogen gas, at 60−70 °C, in toluene with palladium on barium sulfate as catalyst. Attempts to purify this aldehyde were unsuccessful, and it was therefore used immediately upon preparation. The next step in this pathway was the Wittig reaction that enabled the addition of the alkenyl side chain. By altering the phosphorane used, the length of the side chain was easily manipulated to produce the alkenyl analogues (**8**−**11**) of the desired γ-lactones. The final γ-lactone products were obtained after hydrogenation of the corresponding alkenyl precursors. A range of catalysts were tested including palladium on carbon, palladium on barium sulfate, palladium hydroxide and palladium on calcium carbonate. As reported in the

literature, extensive ring opening occurred to produce substantial (~50%) quantities of the alkyl carboxylic acids (*12*). The most effective means of hydrogenation was through the use of palladium on barium sulfate, although the presence of the ring opened acid was detected in every case. Purification by column chromatography with an aluminum oxide plug followed by silica gel proved to be the best method for sample cleanup, with the final products **1**−**4** having (*R*)-configuration obtained in low to excellent yields. The entire sequence was repeated beginning with D-glutamic acid (*15*) to give the (*S*) series of compounds. In all eight cases, chiral GC−MS revealed that the synthetic samples all contained >99% of the desired enantiomer, i.e. enantiomeric excess (ee) >98%.

Aroma Thresholds for the Enantiomerically Pure γ-Lactones 1−4. Although there are limited threshold data for γ-lactones in wine, we are not aware of any reported threshold data for the individual stereoisomers of **1**−**4** in any medium. This information was considered necessary to better assess whether or not these γ-lactones might contribute to the overall aroma of wine at the concentrations measured and to evaluate any sensory differences between the enantiomers. Previous studies concerning the sensory properties of the γ-lactones have produced reported thresholds for racemic γ-nonalactone (**2**) of 30 μg/L in a dry white wine (*16*) and 460 μg/L in a sweet white wine (*17*). Racemic γ-decalactone (**3**) has a reported threshold of 10 μg/L in a model wine solution (*18*); however model wine thresholds are not always reliable indicators of threshold values in wine (*19*). With the γ-lactones consistently detected at significantly higher levels in red wines (*8*), in agreement with other literature reports (*20*), it was felt that they were more likely to be important to the aroma of red wine than to that of white wine. Thus, red wine was chosen as the medium for this sensory threshold study.

Recently, Czerny et al. conducted a re-evaluation of the odor thresholds of some 84 compounds which had previously been characterized as important food odorants (*20*). They correctly pointed out that in order to be confident about the veracity of the determined threshold, all compounds should undergo an initial purity check by GC-O to confirm that samples are not contaminated by any trace odor-active compounds. We concur, and accordingly, solutions of each of the eight compounds under investigation here were individually assessed several times at different concentrations and found to contain no odors other than those of the specific target compounds themselves.

In this study, the (*R*) and (*S*) isomers of γ-octalactone (**1**) had odor thresholds of 238 μg/L and 135 μg/L, respectively. (*R*)-γ-Nonalactone (**2**) was determined to have the highest odor threshold value of all the compounds tested, at 285 μg/L, while its enantiomeric (*S*) counterpart had a threshold approximately one-third that value, at 91 μg/L. (*R*)- and (*S*)-γ-decalactone (**3**) were the only pair of enantiomers with similar thresholds, at 34 μg/L and 47 μg/L, respectively. The (*R*) isomer of γ-dodecalactone (**4**) was found to be the most potent compound in this study, with an odor detection threshold of 8 μg/L. Its (*S*) enantiomer had an odor threshold value of 39 μg/L, almost five times as large: the largest difference (by ratio) found among the pairs of enantiomers for this series of γ-lactones.

Enantiomeric Distribution of the γ-Lactones 1 and 2 in Red Wines and Botrytized White Wines. The analytical method described previously was extended for use with a chiral gas chromatography column, using d_7-analogues as internal standards (*8*). Since these labeled standards were prepared as racemic mixtures, when applied with a chiral (Rt-βDEXcst capillary) GC column, the method allowed quantification of the individual (*R*)- and (*S*)-enantiomers of the lactones. The calibration functions were developed from the previously prepared wine extracts purified through the SPE procedure in both white and red wine. Precision and accuracy of the method were verified at two different concentrations, 2.5 μg/L and 12.5 μg/L, with the difference between the spiked level and the average measured level falling within the acceptable range of error (≤3.6%). The limit of quantification was 2 μg/L for each enantiomer, and better than baseline resolution was achieved for each enantiomeric pair.

Table 2. Group Best Estimate Thresholds (BET, μg/L), Standard Errors (SE) and Upper and Lower 95% Confidence Intervals (CI) for Lactones **1**−**4** in Red Wine

			95% CI	
compound	BET	SE	lower	upper
(*R*)-**1**	238	25	188	287
(*S*)-**1**	135	17	101	169
(*R*)-**2**	284	51	185	384
(*S*)-**2**	91	15	61	121
(*R*)-**3**	33	5	23	42
(*S*)-**3**	47	7	33	61
(*R*)-**4**	8	1	6	10
(*S*)-**4**	41	5	32	50

Table 3. Enantiomeric Distribution of γ-Nonalactone (**2**) in Several Red Wine Varieties

	R (%)	*S* (%)
Cabernet Sauvignon (15)[a]		
high	63	54
low	46	37
average	62	38
Durif (2)[a]		
high	62	39
low	61	38
average	62	38
Merlot (4)[a]		
high	68	52
low	48	32
average	59	41
Pinot Noir (4)[a]		
high	63	40
low	60	37
average	61	39
Shiraz (9)[a]		
high	61	52
low	48	39
average	56	44

[a] Number in parentheses indicates total number of wines studied.

From the large selection (120) of red wines reported previously (*8*), those which contained γ-nonalactone (**2**) at levels greater than 4 μg/L (a total of 34 wines) were analyzed using the chiral method (**Table 3**). No other γ-lactones were present in high enough concentrations to warrant chiral analysis. However, should these be detected in other varieties at higher concentrations, as reported by Ferreira et al. (*21*), then the method for the other γ-lactones can be applied. In the wines analyzed using the chiral method, the (*R*)-enantiomer for γ-nonalactone was present, on average, in greater amounts than the (*S*)-enantiomer, across the five different red wine varieties (**Table 3**). There was, however, no overwhelming predominance of any one isomer, with the highest average of 62% for the (*R*)-enantiomer in Durif followed closely by 61% in Pinot Noir. The average for the (*R*)-enantiomer across all red wines analyzed

Table 4. Enantiomeric Distribution of γ-Octalactone (**1**) and γ-Nonalactone (**2**) in Seven Botrytis Style Wines

	γ-octalactone (**1**)		γ-nonalactone (**2**)	
	R (%)	*S* (%)	*R* (%)	*S* (%)
high	71	50	80	38
low	50	29	62	20
average	62	38	75	25

by the chiral method was calculated to be 59%, or an 18% enantiomeric excess in favor of the (*R*)-isomer.

The only style of white wines in which any lactones were found at levels high enough to allow analysis by the chiral method were botrytized wines (*8*). In fact, both γ-octalactone (**1**) and γ-nonalactone (**2**) were found at such levels in six out of seven, and seven out of seven wines, respectively. The enantiomeric distribution of **1** and **2** in these wines is shown in **Table 4**. As with the red wines, the (*R*) enantiomer was found to be the more prevalent of the two, with average levels for this enantiomer of 62% and 75% for lactones **1** and **2**, respectively.

DISCUSSION

A general trend for a decrease in odor threshold value was observed with an increase in alkyl chain length (**Table 2**), a trend which had been observed previously for thresholds determined in water on racemic samples of the lactones **1**−**4** (*22*). This earlier study, which concerned the aroma of apricot, found that the lactones **3** and **4** were approximately an order of magnitude more potent than the lactones **1** and **2**, which is broadly similar to the results reported in the present study. In the present study, there was a decrease in threshold value for the (*R*)-enantiomers from γ-octalactone (**1**) (238 μg/L) to γ-dodecalactone (**4**) (8 μg/L) of nearly 30-fold; the only exception to this trend was γ-nonalactone (**2**). The corresponding decrease in threshold value for the (*S*)-enantiomers from γ-octalactone (**86b**) (135 μg/L) to γ-dodecalactone (**89b**) (39 μg/L) was only by a factor of approximately three and a half.

A statistical analysis of the data was conducted in order to determine their significance. Standard errors (**Table 2**) of the geometric mean of the individual best estimate thresholds were determined according to the method of Norris (*23*). The mean threshold values were considered to be significantly different ($p < 0.05$) if the confidence intervals surrounding the means (±1.96 SE's) did not overlap. This analysis indicated that in the case of three of the four lactones (**1**, **2** and **4**), the detection threshold of the (*R*) enantiomer was significantly higher than that of the corresponding (*S*) enantiomer ($p < 0.05$), i.e. the (*S*) enantiomers were sensorially more potent than the (*R*) enantiomers. Furthermore, the threshold values of the corresponding enantiomers of lactones **1** and **2** were not significantly different from one another, but both were significantly higher than those of the other lactones. Neither isomer of lactone **3** was significantly different from the (*S*) enantiomer of lactone **4**, but all three were different from the (*R*) enantiomer of **4**.

When the threshold values are related to the concentration of γ-lactones quantified in the red wines (*8*), it is evident that none of the γ-lactones were measured above their individual odor detection thresholds in any of the Australian wines investigated. It might therefore be tempting to conclude that this series of γ-lactones does not have any impact on red wine aroma. However, these odor threshold values illustrate merely the concentration at which 50% of the sampled population could detect a difference between a spiked sample, of one single compound, compared with the control. Sensory studies by Jarauta et al. have been conducted on a mixture of lactones, including **1**−**4**, based on the average concentration at which they were measured in wine (*24*). Their results suggested that lactones might be significant contributors to wine aroma as a cluster, rather than as individual odorants, and highlighted the synergistic or additive effect a group of aroma compounds can potentially have on perceived odor.

When considering the odor properties of a collection of compounds with similar odor properties, it is necessary to consider the compounds both as a whole and as individual odorants. With a larger number of compounds presented together, there is a greater probability that members on a sensory panel could detect at least one of the odorants under investigation even when each compound is below its group odor detection threshold concentration for a group of panelists. This is a direct result of the variation in sensitivity of panel members to individual compounds. For an understanding of the possible effects of combinations of odorants, whether they be synergistic or hypoadditive (*25*, *26*), odor threshold values would need to be determined for each panelist on the individual compounds as well as different combinations of the compounds, a complex and time-consuming task.

With few exceptions, the (*R*) enantiomer of γ-nonalactone (**2**) was found to be more prevalent than its (*S*) counterpart in all of the dry red and botrytized white wines analyzed. The same was true for γ-octalactone (**1**) in the botrytized white wines. Further evaluation of the isomeric distribution data, in terms of vintages analyzed, revealed that the average ratio (*R*):(*S*) for lactone **2** was slightly higher (62:38) in older (vintage 1995−2000) red wines than in younger (2004−2006) red wines (56:44). Wines which fall in the middle of these age ranges (2001−2003) also fell into the middle in terms of ratios (59:41). However, it should be reiterated that only wines in which the level of **2** was higher than 4 μg/L were included in the detailed distribution study.

In summary the individual enantiomers of the lactones **1**−**4** have been synthesized in high enantiomeric excess and their odor detection thresholds determined (in red wine) for the first time. The results indicate that the lactones are unlikely to contribute individually to the aroma of red wine, but does not exclude the possibility that they might be more important when considered as a group.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, MS and optical rotation data for the (*R*) and (*S*) enantiomers of lactones **1**−**4** plus NMR data for intermediates in the syntheses. Calibration functions and accuracy and precision data for the chiral quantification method. This material is available free of charge via the Internet at http://pubs.acs.org.

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