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Precursors to oak lactone. Part 2: Synthesis, separation and cleavage of several β-D-glucopyranosides of 3-methyl-4-hydroxyoctanoic acid☆

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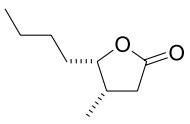
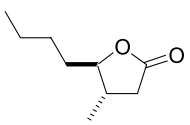
Abstract—The β-D-glucopyranosides of all four stereoisomers of 3-methyl-4-hydroxyoctanoic acid have been prepared. The (3*S*,4*S*) and (3*R*,4*R*) species were prepared from *cis*-5-*n*-butyl-4-methyl-4,5-dihydro-2(3*H*)-furanone (*cis*-oak lactone) by a process involving ring-opening with base and protection of the carboxyl function as its benzyl ester. The glucose unit was introduced by a modified Koenigs–Knorr procedure. A different strategy was necessary for synthesis of the (3*S*,4*R*) and (3*R*,4*S*) compounds. This was based on the reductive ring-opening of *trans*-oak lactone and subsequent protection of the primary alcohol as its *t*-butyldiphenylsilyl ether. Separation of the individual glucosides was effected by preparative thin layer chromatography. Those corresponding to the nature-identical isomers of oak lactone have been shown to produce oak lactone under both acidic hydrolysis and pyrolysis conditions. The galloyl-β-D-glucoside of the *cis*-species, obtained as a natural isolate from the wood of *Platycarya strobilacea*, was also found to produce *cis*-oak lactone upon both acid hydrolysis and pyrolysis. Both the nature-identical (4*S*,5*S*) *cis*-oak lactone and its non-natural (4*R*,5*R*) enantiomer have been prepared from their corresponding glycosides and their aroma thresholds in white wine were determined to be 23 and 82 μg/L (ppb) respectively. The aroma threshold of the nature-identical isomer in a red wine was 46 μg/L.

1. Introduction

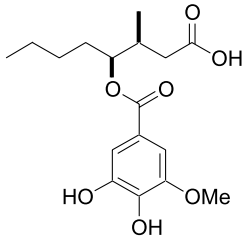
It has long been recognised that the use of oak barrels as vessels for the fermentation and/or maturation of wine can impart favourable sensory characteristics to the beverage. Some 200 oak derived volatile compounds have been identified in wines or spirits that have been so treated,[1] and there are doubtless others awaiting isolation and



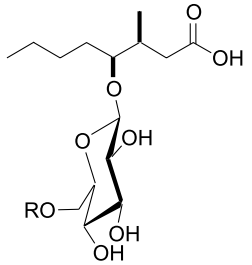
cis-**1**



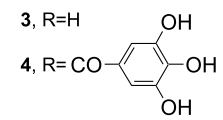
trans-**1**



2



3, R=H



☆ For Part 1, see Ref. 12.

Keywords: Oak lactone; Glycosides; Hydrolysis; Pyrolysis; β-Glucosidase; Aroma thresholds.
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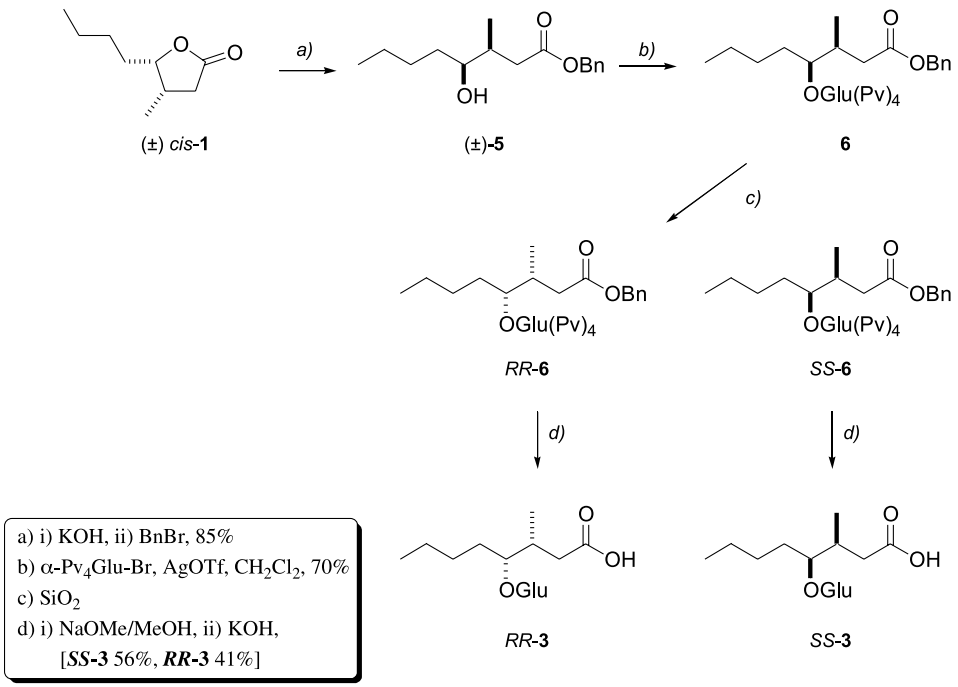
identification. Of these, the most important are considered to be the (4*S*,5*S*) *cis*- and (4*S*,5*R*) *trans*-isomers of 5-*n*-butyl-4-methyl-4,5-dihydro-2(3*H*)-furanone **1**, also known as 'oak lactone' or 'whisky lactone'.[2] These were first identified by Suomalainen and Nykänen[3,4] after an earlier study had incorrectly assigned their structure (tentatively) as a branched δ-nonalactone.[5] Of the two, the *cis*-isomer is considered to be the more important in sensory terms, having a reported aroma threshold for the racemate of 92 μg/L (ppb) in white wine, whereas the aroma threshold for the racemic *trans*-isomer has been reported as 460 ppb in the same medium.[6] Up to now, no thresholds of the naturally occurring isomers in wine have been reported. Aroma descriptors for both isomers include 'coconut', 'citrus' and 'vanilla'.[2,7] A study by a Japanese group has shown a moderate correlation between the presence of oak lactones and the perceived quality of whiskey samples.[8] A recent sensory study showed a positive correlation between the concentration of the *cis*-isomer and the aroma intensity of the 'coconut' descriptor in Chardonnay wine.[9] Similar correlations between this isomer and the aroma intensity of coconut, 'vanilla' and 'berry' were found in Cabernet Sauvignon wines.

Despite their importance to the aroma and flavour of alcoholic beverages, the origin of these compounds remains unclear. *cis*- and *trans*-Oak lactone are already present in green oakwood, but additional quantities of these compounds can be generated in the wood during the drying (seasoning) and coopering processes,[2] in model wine oak extracts heated to 50 °C,[10] and even in the injector block of a gas chromatograph during analysis of oak extracts.[7] Such observations suggest the presence of at least one precursor form of oak lactone in oakwood; to date the literature has provided only three possible candidates. Otsuka et al.[11] isolated, from oakwood powder, a compound to which they assigned the structure **2**. This assignment was based on degradation studies with analysis by tlc, and while the proposed structure was consistent with the observed data, the evidence was not conclusive. Recently, we synthesised an authentic sample of **2** from *cis*-oak lactone and demonstrated that this original structural assignment was erroneous.[12] The second and third potential precursors, **3** and **4**, were isolated from the wood of *P. strobilacea*, a member of the walnut (Juglandaceae) family of trees by Tanaka and Kuono.[13] Compound **4** has subsequently been found in a sample of oakwood.[14] Both **3** and **4** are characterised by the presence of the β-D-glucopyranosyl moiety at C_4 of the open chain form of *cis*-oak lactone, with 4 being further substituted at the 6′ position with the galloyl group. Hydrolysis of both compounds in strong acid produced the expected (4*S*,5*S*) *cis*-oak lactone. To date, no potential precursor to *trans*-oak lactone has been isolated, nor indeed proposed.

2. Results and discussion

2.1. Synthesis of glycosides 3

The two diastereomeric *cis*-glucosides were prepared as shown in Scheme 1. Ring opening of racemic *cis*-oak lactone with potassium hydroxide and subsequent trapping of the carboxylate with benzyl bromide gave the benzyl esters **5**, which were successfully converted into their corresponding β-D-glucopyranosides **6** via a modified Koenigs–Knorr procedure.[15] We chose to employ the tetrapivaloylated bromoglucose as the reagent for the introduction of the carbohydrate unit as we have found, in keeping with earlier reports,[16] that this species produces essentially none of the α-anomer. In contrast, the corresponding tetraacetate often yields significant amounts (ca. 10%) of this isomer. After separation of the protected glucosides **6** was effected via preparative tlc,



Scheme 1.

Table 1. Measured and reported specific rotations for all four isomers of oak lactone

Isomer	$[\alpha]_D$,[a] this study	$[\alpha]_D$,[a] lit.[b]
cis (4*S*,5*S*)[c]	−74	−78
cis (4*R*,5*R*)	+79	+76
trans (4*S*,5*R*)[c]	+100	+96
trans (4*R*,5*S*)	−97	−95

[a] Measured as a solution in methanol.
[b] Ref. 23.
[c] Nature-identical isomer.

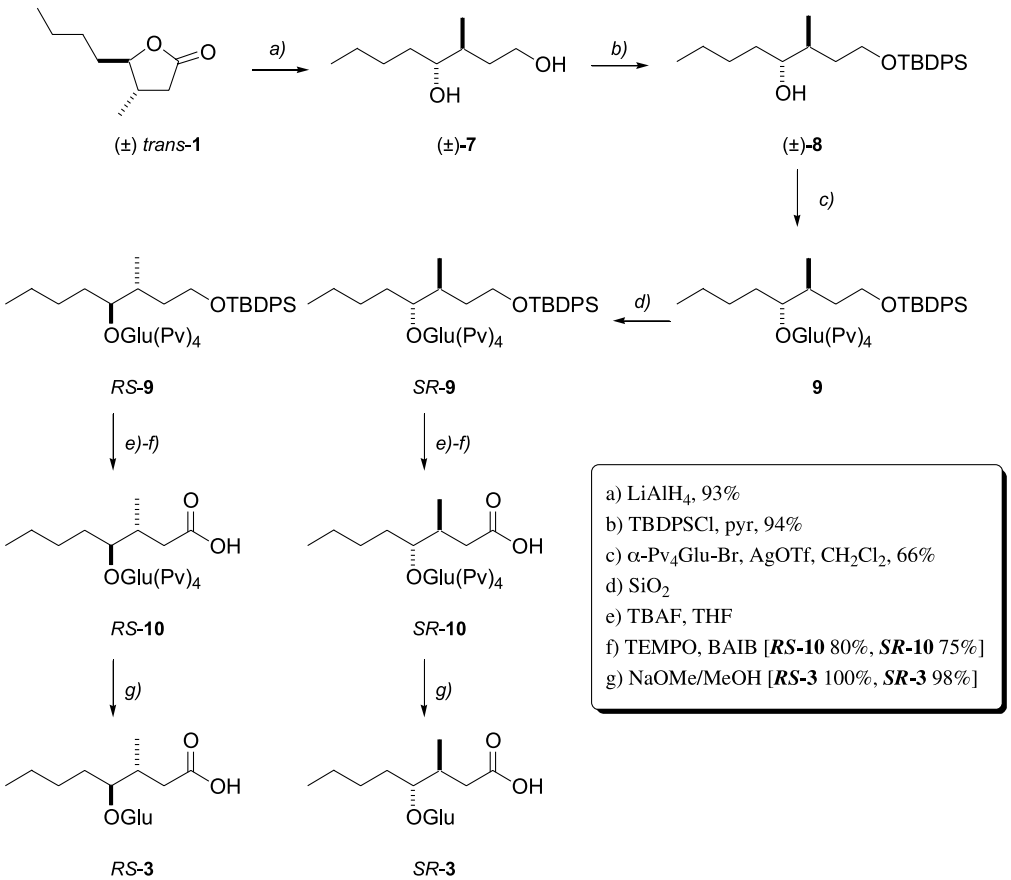
depivaloylation gave the (3*R*,4*R*) and (3*S*,4*S*) isomers of **3**. The stereochemistry of each isomer was assigned based on the results of either of two experiments: strong acid hydrolysis of the glycosylated acid **3** derived from the higher-eluting isomer (on tlc) of **6** produced oak lactone which had a measured specific rotation (in methanol) of $[\alpha]_D=-74$. The other lower-eluting isomer produced oak lactone whose specific rotation was $[\alpha]_D=+79$. Based on the data collected in Table 1, the higher-eluting isomer of **6** is assigned the (3*S*,4*S*) absolute stereochemistry, with the lower-eluting isomer assigned the (3*R*,4*R*) absolute stereochemistry. Quite independently, the stereochemistry of each was also assigned based on the results of chiral GC–MS (Cyclosil B column). A solution of racemic *cis*-oak lactone showed two peaks, with retention times of 14.57 and 14.62 min (Table 2). That the first of these peaks was due to the nature-identical (4*S*,5*S*) isomer was confirmed by analysis of a sample of *cis*-oak lactone obtained from a natural oak extract. Finally, the major synthetic oak lactones produced by hydrolysis of the glucosides **3** also revealed the higher eluting isomer of **6** to have the (3*S*,4*S*) configuration. Further details of the minor oak lactone isomers in the hydrolysates are discussed in the following section.

Table 2. Retention times on chiral GC (Cyclosil B column) of racemic, natural and synthetic isomers of oak lactone

Isomer	Retention times[a] (Cyclosil B)	
Racemic *cis*	14.57	14.62
Natural *cis*	14.57	—
1st *cis* isomer	14.57	—
2nd *cis* isomer	—	14.62
Racemic *trans*	13.93	14.12
Natural *trans*	13.93	—
1st *trans* isomer	—	14.12
2nd *trans* isomer	13.93	—

[a] Minutes.

In the case of the *trans* species, an alternative strategy was required due to problems in both the isolation and handling of the analogous benzyl ester. Although the desired ester could be prepared (as evidenced by NMR), attempts at isolation by chromatography resulted in recovery of only relactonised *trans*-oak lactone. When an attempt was made to glycosylate the crude benzyl ester, the only recovered products were *trans*-oak lactone and the *O*-β-D-glucopyranoside of benzyl alcohol.[16] This rapid lactonisation of the *trans*-isomer, relative to the *cis*-isomer has been found to be a general feature of these two compounds, and a



Scheme 2.

comprehensive kinetic investigation into the chemistry of the aglycone components of both *cis*- and *trans*-**3** has been completed.[17]

The alternative strategy (Scheme 2) employed was based on an earlier synthesis we had reported of the putative oak lactone precursor **2**.[12] Reduction of racemic *trans*-oak lactone with $LiAlH_4$ followed by selective protection of the primary alcohol function gave **8** which, as before, was successfully glycosylated and separated into the two diastereomers of **9**. Removal of the silyl group followed by oxidation and depivaloylation furnished the two *trans*-diastereomers of **3**. As was the case for the two *cis*-glycosides, the stereochemistry of the two *trans*-glycosides was assigned based on both the specific rotations of the two isomers of *trans*-oak lactone produced after cleavage of the sugar moiety in **3**, as well as chiral GC–MS analysis of the derived lactones (Tables 1 and 2, respectively). In this case, the lower-eluting isomer (on tlc) of **9** ultimately provided the nature-identical *trans*-oak lactone, and is therefore assigned (3*S*,4*R*) absolute stereochemistry, while the first-eluting isomer of **9** is assigned (3*R*,4*S*) absolute stereochemistry.

2.2. Chiral GC–MS analysis of oak lactones in hydrolysates

Although NMR spectroscopy indicated that each of the synthetic β-D-glucosides was of high purity, chiral GC–MS analysis gave a more detailed indication of the stereochemical purity of the glycoconjugates, and the oak lactones formed by their hydrolyses. Furthermore, before determining the aroma impact of the individual oak lactone stereoisomers, it was desirable to know the precise composition of the solutions under investigation. Additionally, these data were important in interpreting the results of the hydrolysis and pyrolysis experiments.

Accordingly, solutions of each of the nature-identical oak lactone stereoisomers produced by both strong acid and enzyme hydrolysis of the corresponding β-D-glucosides **3** were examined by chiral GC–MS (Cyclosil B column) and the results are collected in Table 3. That the acid hydrolysis conditions could influence the relative proportion of oak lactone isomers was established by analysis of the acid hydrolysate of the galloyl-β-D-glucoside **4**. This compound was a natural isolate and is therefore expected to be stereochemically pure; this was confirmed by both NMR spectroscopy and, importantly, chiral GC–MS analysis of the enzyme hydrolysate. However, chiral GC–MS analysis of oak lactone from the acid hydrolysate indicated only 92% purity, with 8% of the (4*S*,5*R*) *trans* isomer present. Therefore, we conclude that the newly formed (4*S*,5*R*) *trans*-oak lactone is an artefact of strong acid hydrolysis, indicating the potential for acid-catalysed epimerisation at C_4 under these conditions.

Table 3. Purity (%) of glycosides **3** or **4**, and of oak lactones obtained from hydrolysis of **3** or **4**, as determined by NMR spectrosopy or chiral GC–MS analysis

Sample	Analysis	*SR-trans*	*RS-trans*	*SS-cis*	*RR-cis*
3*S*,4*S*-(**4**)[a]	NMR	—	—	100	—
Enzyme hydrolysate	GC–MS	—	—	100	—
Acid hydrolysate	GC–MS	8	—	92	—
3*S*,4*S*-(**3**)	NMR	—	5	95	—
Enzyme hydrolysate	GC–MS	—	14	86	—
Acid hydrolysate	GC–MS	4	6	90	—
3*S*,4*R*-(**3**)	NMR	94	6	—	—
Enzyme hydrolysate	GC–MS	87	9	2	2
Acid hydrolysate	GC–MS	85	9	3	3

[a] Natural isolate obtained from the wood of *P. strobilacea*.

^{1}H NMR spectroscopy of the (3*S*,4*S*) *cis*-β-D-glucoside **3** indicated it to be 95% pure, with 5% of the (3*R*,4*S*) *trans* isomer present, but neither the (3*S*,4*R*) *trans* nor (3*R*,4*R*) *cis* isomers were present in detectable quantities. This last point was confirmed by enzymatic cleavage of the sugar unit and inspection of the product mixture. The proportion of (4*R*,5*S*) *trans*-oak lactone in the enzyme hydrolysate (14%) is higher than that detected by NMR in the original glucoside, and may reflect some stereoselectivity of the β-glucosidase towards the (3*R*,4*S*) glucoside **3**. Analysis of the strong acid hydrolysate confirmed the absence of the (4*R*,5*R*) *cis* isomer of oak lactone, as well as the presence of 6% of the (4*R*,5*S*) *trans* isomer. However, it also showed the presence of the (4*S*,5*R*) *trans* isomer (4%). As with the hydrolysis of **4**, this isomer of *trans*-oak lactone is presumed to arise from epimerisation at C_4 of the (3*S*,4*S*) glucoside. The other component of this hydrolysate, namely the (4*R*,5*S*) isomer of *trans*-oak lactone appears to be the expected hydrolysis product of the minor component in the original glucoside sample. Given that epimerisation takes place to convert (a small proportion of) *cis*-glucoside into *trans*-oak lactone, one might expect to see the converse taking place. The fact that this hydrolysate contained no detectable *RR cis*-oak lactone may simply reflect the detection limits of the instrument, or alternatively, it may be a manifestation of the thermodynamic preference for formation of the *trans* isomer relative to the *cis*.[17]

Similar considerations of the strong acid and enzyme hydrolysates of the (3*S*,4*R*) *trans*-β-D-glucoside **3** suggested that this diastereomer was of slightly lower purity, approximately 85–90%, with 9% of the (3*R*,4*S*) isomer, as well as smaller amounts (~2–3%) of each of the (3*S*,4*S*) and (3*R*,4*R*) isomers. Although neither the (3*S*,4*S*) nor the (3*R*,4*R*) isomers could be detected by NMR spectroscopy, it is probable that the limited sensitivity of NMR spectroscopy could have impeded their detection at these low concentrations. Their presence in the enzyme hydrolysate is strongly supportive of this.

2.3. Hydrolytic and pyrolytic behaviour of glucosides 3

Although the glucosides **3** present themselves prima facie as candidates for the generation of natural oak lactone, their provenance in oak wood has yet to be established. Their detection in sub-mg/kg amounts by HPLC, unlike that of **4**, is likely to be hampered by the absence of a suitable chromophore.[14] However, it is worth reiterating that (3*S*,4*S*)-**3** has previously been isolated and identified in walnut. Thus, we were keen to investigate the behaviour towards both acidic media (approximating barrel maturation) and pyrolysis (approximating the toasting undergone during cooperage) of the isomers of **3** with absolute

stereochemistry corresponding to the natural isomers of oak lactone. In addition to the glucosides prepared for this study, we also investigated the behaviour of the galloyl-β-D-glucoside **4**.

Table 4 contains quantified amounts of oak lactone produced hydrolytically, and shows that after 48 days at pH 3.0 and 100 °C, the hydrolyses of both the (3*S*,4*S*) and (3*S*,4*R*) isomers of **3** were greater than 90% complete. At the lower temperature (45 °C), no more than trace quantities of oak lactones were produced by either isomer over this time. This is perhaps not surprising as previous studies within our laboratories have shown that hydrolysis of glycosides under mild conditions is extremely slow except where the glycoside is attached at an activated hydroxyl position.[18] Under the same higher temperature conditions, the hydrolysis of the galloyl substituted glycoside **4** was only approximately 20% complete after 35 days. In the case of **4**, the expected *cis*-oak lactone is accompanied by approximately 8% of the *trans*-isomer, produced by epimerisation under the acidic conditions employed, as discussed above. This minor epimerisation is also observed in the hydrolysis of the (3*S*,4*S*) isomer of **3**. Of the 10% *trans* produced, approximately half can be attributed to an impurity in the original glucoside, and the remainder to epimerisation. In contrast, the small proportion of *cis*-oak lactone in the hydrolysate of (3*S*,4*R*)-**3** is likely to arise mostly from impurities in the glucoside, rather than by epimerisation (see Table 3). In this case, lack of epimerisation can be attributed to the thermodynamic stability of the *trans* compared to the *cis* isomer.

Table 4. Quantified amounts of oak lactone produced by the hydrolysis of **3** and **4**, at pH 3.0

Isomer	Temperature (°C)	Time (days)	Total **1**[a]
(3*S*,4*S*)-**3**	45	8	1.1 (0.2)
		48	1.6 (0.3)
(3*S*,4*S*)-**3**	100	8	171.2 (31)
		48	516.2 (93)
		cis:*trans*	90: 10
(3*S*,4*R*)-**3**	45	8	n.d.
		48	n.d.
(3*S*,4*R*)-**3**	100	8	158.0 (28)
		48	521.6 (94)
		cis:*trans*	6: 94
(3*S*,4*S*)-**4**	45	5	n.d.
		35	n.d.
(3*S*,4*S*)-**4**	100	5	17.6 (3)
		35	106.8 (20)
		cis:*trans*	92: 8

[a] μg oak lactone; mean value from two replicates. Values were in agreement to ca. 2%; values in parentheses represent the percentage of oak lactone formed relative to the theoretical maximum.

The pyrolysis of both **3** and **4** was investigated by adsorbing the desired compound onto oakwood powder, and then subjecting the whole to conditions which were expected to closely mimic barrel toasting temperatures.[19] The oakwood for this experiment was chosen for its intrinsically low levels of oak lactone, even after toasting. In contrast to their hydrolytic behaviour, the pyrolysis of all three glycosides resulted in reasonably rapid formation of significant amounts of the oak lactones, (20–30% conversion, Table 5) again with epimerisation of *cis* to *trans* being more important than the converse.

Table 5. Quantified amounts of oak lactone produced in the pyrolysis of **3** and **4**

Isomer	*cis*-**1**[a]	*trans*-**1**[a]	Yield[b]
Control untoasted	n.d.	n.d.	—
Control toasted	0.2	0.1	–
(3*S*,4*S*)-**3** toasted	60.0	7.1	29
cis:*trans*	89	11	
(3*S*,4*R*)-**3** toasted	4.0	55.4	26
cis:*trans*	6	94	
(3*S*,4*S*)-**4** toasted	36.9	2.0	15
cis:*trans*	5	95	

[a] (μg/g wood); mean value from three replicates. Values were in agreement to ca. 2%.
[b] Yield defined as the percentage of total oak lactone produced relative to the theoretical maximum.

2.4. Sensory evaluation of (4*S*,5*S*)-*cis*-1 and (4*R*,5*R*)-*cis*-1

In order to satisfactorily assess the aroma impact of the individual stereoisomers of **1**, it is necessary to know the precise composition of the solution under investigation. Accordingly, solutions of each of the four isomers of **1** produced were examined by chiral GC–MS (Cyclosil B column) with the results collected in Table 6. While no one of the solutions is 'pure' in the literal meaning of the word, they are each strongly enriched in their respective isomer. Given that the reported threshold for racemic *cis*-oak lactone (92 ppb in white wine) is much lower than the reported threshold for racemic *trans*-oak lactone (460 ppb) in the same medium, it is apparent that small amounts of *trans*-isomer in the solution of predominantly *cis*-oak lactone would be expected to have little impact on the sensory properties of the solution. Conversely, however, small amounts of the more potent *cis*-isomer in the predominantly *trans*-solution would be problematic. Consequently, sensory analysis was limited to the *cis*-enriched solutions.

Table 6. Chiral GC–MS analysis of the composition of oak lactones produced by strong acid hydrolysis of **3**

Sample	*SR-trans*	*RS-trans*	*SS-cis*	*RR-cis*
SS-cis	4	6	90	—
RR-cis	5	7	4	84
SR-trans	85	9	3	3
RS-trans	2	96	1	1

A duo–trio test[20] was conducted to establish whether or not there was a perceptible difference in aroma impact between the two *cis*-enriched solutions; a young (<12 months old) neutral, dry white wine (2002 South Australian Chenin Blanc) was spiked with either (4*S*,5*S*)-*cis*-**1** or (4*R*,5*R*)-*cis*-**1** (161.7 μg/L) and presented to a panel of 25 judges, 20 of whom correctly identified the sample which differed from the reference. These data show that the two *cis*-isomers are significantly different (at the 99% confidence level). Furthermore, they bring into question the wisdom of conducting sensory impact studies using racemic *cis*-**1**.

The aroma detection thresholds[21] of solutions of each of the *cis* isomers of **1** were determined in the same neutral white wine. The aroma detection threshold for the solution enriched in (4*S*,5*S*)-*cis*-**1** (90% pure) was calculated to be 23 μg/L. The best estimate threshold for each panellist was the geometric mean of the highest concentration missed and the next higher concentration tested. The group threshold was calculated as the geometric mean of the individual best estimate thresholds. The distributions of best-estimate thresholds for individual panellists are shown in Figure 1. Informal descriptors used by the panel to describe this isomer included: 'fruity', 'coconut', 'woody', 'caramel', 'buttery', and 'vanilla'. The aroma detection threshold for the solution rich in the non-natural isomer, (4*R*,5*R*)-*cis*-**1** (84% pure) was similarly calculated to be 82 μg/L, with informal descriptors including 'lime', citrus, 'honey', 'coconut', 'vanilla', 'burnt apple' and 'cinnamon'. Finally, the aroma detection threshold of the solution rich in (4*S*,5*S*)-*cis*-**1** was determined in red wine to be 46 μg/L, with similar informal descriptors to those from the white wine study.

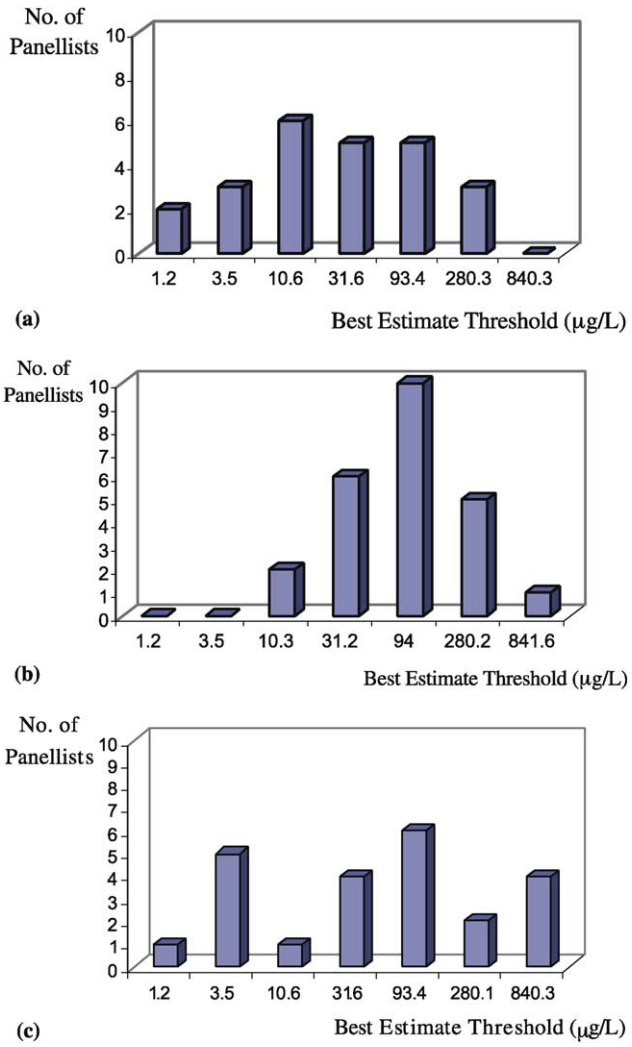


Figure 1. Histograms showing best estimate threshold distributions for (a) (4*S*,5*S*)-*cis*-**1** in white wine, (b) (4*R*,5*R*)-*cis*-**1** in white wine, and (c) (4*S*,5*S*)-*cis*-**1** in red wine.

3. Conclusions

Hydrolytic studies at wine pH have shown that the simple or substituted glucosides **3** and **4** are not likely to be significant sources of oak lactones during barrel maturation of wines unless microorganisms with β-glucosidase activity are present. Such derivatives cannot explain the observation[10] that oak lactone concentration in pH 3 oak extracts can increase after the oak is removed from soaking. In contrast, such compounds are likely to be important sources of additional oak lactone formed during barrel manufacture and toasting.

4. Experimental

4.1. General

Chemicals were purchased from Sigma-Aldrich. Commercial oak lactone (50:50) was fractionated by spinning band column distillation to give pure fractions of both the *cis*- and *trans*-isomers. All solvents used were HPLC grade from OmniSolv and HiPerSolv. Column chromatography was performed using silica gel 60 (230–400 mesh) from Merck. Preparative thin layer chromatography was performed using glass-backed silica gel 60 plates (20×20 cm) from Merck. All organic solvent solutions were dried over anhydrous sodium sulfate before being filtered. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini spectrometer at operating frequencies of 300 and 75.5 MHz, respectively. Mass spectra were recorded on a Hewlett–Packard (HP) 6890 gas chromatograph fitted with liquid HP 6890 series injector and coupled to a HP 5973 mass spectrometer. Optical rotations were measured with a PolAAr 21 polarimeter. Microanalyses were performed at Microanalytical Services, University of Otago, New Zealand. Oak lactone was quantified by the SIDA method reported previously.[7]

4.1.1. (3*R*,4*R*) and (3*S*,4*S*) Benzyl 3-methyl-4-hydroxyoctanoate (±5). To a solution of *cis*-oak lactone (1.10 g, 7.05 mmol) in methanol (30 mL) was added potassium hydroxide (400 mg, 7.05 mmol) and the reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated and the residue dissolved in DMF (30 mL). To this solution was added benzyl bromide (840 μL, 7.07 mmol) and the reaction mixture stirred at room temperature for 16 h. The solution was diluted with water (10 mL) and extracted with ether (3×20 mL). The combined organics were washed with water (2×20 mL), dried and concentrated. The resulting oil was purified by column chromatography (20% ethyl acetate in hexane) to give a colourless oil (1.59 g, 85%). [Found: C, 72.7; H 9.2. $C_{16}H_{24}O_3$ requires C, 72.69; H 9.15%]; δ_H ($CDCl_3$) 7.40–7.25 (5H, m, Ar*H*), 5.12 (2H, s, CH_2Ar), 3.55 (1H, m, H_4), 2.53 (1H, dd, J=15.2, 6.6 Hz, H_{2a}), 2.29 (1H, dd, J=15.2, 7.6 Hz, H_{2b}), 2.28 (1H, m, H_3), 1.44–1.24 (6H, m, $H_{5,6,7}$), 0.92 (3H, d, J=6.9 Hz, H_9), 0.89 (3H, t, J=7.2 Hz, H_8); δ_C ($CDCl_3$) 173.4, 135.9, 128.5, 128.2, 126.9, 74.2, 66.2, 38.4, 35.4, 33.8, 28.4, 22.7, 14.0 13.6; ESI-MS (80% MeOH) 287.2 ($M+Na^+$).

4.1.2. (3*R*,4*R*) and (3*S*,4*S*) Benzyl 3-methyl-4-*O*-(2′,3′,4′,6′-tetrapivaloyl-β-D-glucopyranosyl) octanoate (6).

2,3,4,5-Tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide, prepared according to Kunz et al.[16] (3.5 g, 6.04 mmol) was added to a solution of (±**5**) (1.6 g, 6.01 mmol) in dichloromethane (25 mL) containing silver triflate (1.6 g, 6.23 mmol) and 2,6-lutidine (700 μL, 6.04 mmol). The reaction mixture was stirred at room temperature in the dark for 16 h before being quenched with saturated sodium bicarbonate solution and extracted with dichloromethane (2×15 mL). The combined organic extracts were washed with brine (25 mL), dried and concentrated. The crude product was purified by column chromatography eluting with 10% ethyl acetate in hexane to give a colourless resin (3.2 g, 70%). [Found: C, 66.3; H 9.0. $C_{42}H_{66}O_{12}$ requires C, 66.12; H 8.72%]; A solution of this diastereomeric mixture in dichloromethane was loaded onto preparative tlc plates (20 cm×20 cm, Silica 60) and eluted two or three times (as required) with dichloromethane. The plates were visualised under UV light, the fractions of interest removed and extracted with 80% dichloromethane in methanol (2×50 mL) and methanol (2×50 mL).

Compound (3*S*,4*S*)-**6**. δ_H ($CDCl_3$) 7.45–7.29 (5H, m, Ar*H*), 5.25 (1H, dd, *J*=9.3, 9.3 Hz, $H_{3'}$), 5.15 (1H, d, *J*=12.0 Hz, CH_2Ar), 5.08 (1H, dd, *J*=9.3, 9.3 Hz, $H_{4'}$), 5.06 (1H, d, *J*=12.0 Hz, CH_2Ar), 5.01 (1H, dd, *J*=9.3, 7.8 Hz, $H_{2'}$), 4.52 (1H, d, *J*=7.8 Hz, $H_{1'}$), 4.18 (1H, dd, *J*=12.3, 1.8 Hz, $H_{6a'}$), 3.92 (1H, dd, *J*=12.3, 5.4 Hz, $H_{6b'}$), 3.56–3.34 (2H, m, $H_{4,5'}$), 2.52–2.14 (3H, m, $H_{2,3}$), 1.54–1.20 (6H, m, $H_{5,6,7}$), 1.21, 1.15, 1.11, 1.10 (36H, 4s, C*Me*$_3$), 0.90–0.80 (6H, m, $H_{8,9}$); δ_C ($CDCl_3$) 178.0, 177.2, 176.5, 176.4, 172.7, 135.9, 128.6, 128.4, 128.3, 99.8, 80.7, 72.8, 71.9, 71.8, 68.2, 66.1, 61.9, 38.8, 38.7, 38.7, 38.6, 37.2, 33.0, 31.2, 27.8, 27.3, 27.2, 27.1, 27.1, 22.6, 14.4, 13.9; ESI-MS (80% MeOH) 785.6 ($M+Na^+$).

Compound (3*R*,4*R*)-**6**. δ_H ($CDCl_3$) 7.42–7.28 (5H, m, Ar*H*), 5.29 (1H, dd, *J*=9.2, 9.5 Hz, $H_{3'}$), 5.16 (1H, d, *J*=12.3 Hz, CH_2Ar), 5.11 (1H, dd, *J*=9.5, 10.0 Hz, $H_{4'}$), 5.02 (1H, d, *J*=12.3 Hz, CH_2Ar), 4.99 (1H, dd, *J*=9.2, 7.9 Hz, $H_{2'}$), 4.55 (1H, d, *J*=7.9 Hz, $H_{1'}$), 4.18 (1H, dd, *J*=12.1, 1.8 Hz, $H_{6a'}$), 3.94 (1H, dd, *J*=12.1, 4.9 Hz, $H_{6b'}$), 3.68–3.56 (2H, m, $H_{4,5'}$), 2.57 (1H, dd, *J*=15.3, 4.8 Hz, H_{2a}), 2.22 (1H, dd, *J*=15.3, 8.4 Hz, H_{2b}), 2.16 (1H, m, H_3), 1.54–1.20 (6H, m, $H_{5,6,7}$), 1.17, 1.13, 1.13, 1.09 (36H, 4s, C*Me*$_3$), 0.88 (3H, t, *J*=6.8 Hz, H_8), 0.82 (3H, d, *J*=6.6 Hz, H_9); δ_C ($CDCl_3$) 177.9, 177.1, 176.3, 176.3, 173.2, 136.1, 128.4, 128.1, 128.0, 98.9, 80.3, 72.4, 71.9, 71.5, 68.0, 65.9, 61.7, 38.8, 38.7, 38.7, 38.7, 38.0, 32.7, 31.2, 28.1, 27.1, 27.1, 27.0, 27.0, 22.8, 13.9, 13.1.; ESI-MS (80% MeOH) 785.6 ($M+Na^+$).

4.2. General procedure for depivaloylation of *cis*-glycosides (6)

Sodium metal (130 mg, 5.6 mmol) was dissolved in methanol (5 mL) and the resulting solution was added to a solution of (3*R*,4*R* and 3*S*,4*S*) **6** (337.5 mg, 0.44 mmol) in methanol (10 mL). The reaction mixture was stirred at room temperature for 16 h. The mixture was then stirred for a further 30 min in the presence of acidified Amberlite IRC-50 (H) ion exchange resin. The reaction mixture was filtered, concentrated in vacuo to remove methyl pivalate, and the residue obtained dissolved in water (10 mL). Potassium hydroxide (194.6 mg, 3.48 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was acidified to pH 3 with 10% hydrochloric acid solution and extracted with ether (10 mL). The crude aqueous glycoside solution was then purified by chromatography on XAD-2 resin to give a colourless resin (106.1 mg, 71%).

4.2.1. (3*S*,4*S*) 3-Methyl-4-*O*-β-D-glucopyranosyloctanoic acid (3). The free glycoside (33.5 mg, 56%) was obtained from (3*S*,4*S*)-**6** (135.5 mg, 0.18 mmol) as described above, $[\alpha]_D$=−29.8 (*c* 0.67, CH_3OH), lit.[13] $[\alpha]_D$=−23; δ_H (CD_3OD) 4.31 (1H, d, *J*=7.8 Hz, $H_{1'}$), 3.82 (1H, dd, *J*=11.8, 2.2 Hz, $H_{6a'}$), 3.67 (1H, dd, *J*=11.8, 5.2 Hz, $H_{6b'}$), 3.63 (1H, m, H_4), 3.40–3.18 (3H, m, $H_{3',4',5'}$), 3.16 (1H, dd, *J*=9.0, 7.8 Hz, $H_{2'}$), 2.62 (1H, dd, *J*=14.6, 4.8 Hz, H_{2a}), 2.27 (1H, m, H_3), 2.15 (1H, dd, *J*=14.6, 8.6 Hz, H_{2b}), 1.62–1.24 (6H, m, $H_{5,6,7}$), 0.95 (3H, d, *J*=6.6 Hz, H_9), 0.91 (3H, t, *J*=7.1 Hz, H_8); δ_C (CD_3OD) 178.5, 104.9, 84.3, 79.0, 78.5, 76.3, 72.6, 63.7, 38.9, 35.1, 32.9, 30.0, 24.6, 16.0, 15.3; ESI-MS (80% MeOH) 359.4 ($M+Na^+$).

4.2.2. (3*R*,4*R*) 3-Methyl-4-*O*-β-D-glucopyranosyloctanoic acid (3). The free glycoside (22.7 mg, 41%) was obtained from (3*R*,4*R*)-**6** (154.4 mg, 0.20 mmol) as described above, $[\alpha]_D$=−16.4 (*c* 0.55, CH_3OH); δ_H (CD_3OD) 4.26 (1H, d, *J*=7.7 Hz, $H_{1'}$), 3.85 (1H, dd, *J*=12.6, 1.9 Hz, $H_{6a'}$), 3.72–3.58 (2H, m, $H_{6b',4}$), 3.40–3.20 (3H, m, $H_{3',4',5'}$), 3.15 (1H, dd, *J*=9.1, 7.7 Hz, $H_{2'}$), 2.66 (1H, dd, *J*=15.1, 6.8 Hz, H_{2a}), 2.26 (1H, m, H_3), 2.12 (1H, dd, *J*=15.1, 7.6 Hz, H_{2b}), 1.62–1.22 (6H, m, $H_{5,6,7}$), 0.95–0.89 (6H, m, $H_{8,9}$); δ_C (CD_3OD) 178.8, 104.9, 83.9, 79.0, 78.6, 76.2, 72.6, 63.9, 39.5, 35.5, 33.0, 30.1, 24.7, 15.4, 15.3; ESI-MS (80% MeOH) 359.4 ($M+Na^+$).

4.2.3. (3*R*,4*S*) and (3*S*,4*R*) 3-Methyloctan-1,4-diol (±7). LAH (540 mg, 14.2 mmol) was added to a cooled solution of *trans*-oak-lactone (2.0 g, 12.82 mmol) in anhydrous THF (40 mL). The reaction mixture was then heated at reflux overnight before being quenched by addition of acetone (5.0 mL) followed by addition of a solution of sodium hydroxide (1.0 M, 4.0 mL) and saturated sodium sulfate. The resulting solids were removed by filtration and the filtrate concentrated and coevaporated with CH_3CN (3×10 mL) to give the product (1.91 g, 93%) as a colourless oil. δ_H ($CDCl_3$) 3.80–3.58 (2H, m, H_1), 3.43 (1H, m, H_4), 1.75–1.25 (9H, m, $H_{2,3,5,6,7}$), 0.94 (3H, d, *J*=6.6 Hz, H_9), 0.90 (3H, d, *J*=7.2 Hz, H_8); δ_C ($CDCl_3$) 75.9, 60.5, 36.3, 35.2, 34.1, 28.0, 22.8, 16.5, 14.0.

4.2.4. (3*R*,4*S*) and (3*S*,4*R*) 1-*t*-Butyldiphenylsilyloxy-3-methyloctan-4-ol (±8). TBDPSCl (4.22 g, 15 mmol) and (±**7**) (2.2 g, 13.75 mmol) in pyridine (40 mL) were stirred at room temperature for 72 h, after which time the solvent was removed. The residue was diluted with dichloromethane (100 mL) and washed with saturated copper sulfate solution (100 mL), saturated sodium bicarbonate solution (100 mL) and water (100 mL). The organic phase was then dried and concentrated to give a pale yellow oil, which was purified by column chromatography (100% dichloromethane) to give a colourless oil (5.17 g, 94%). [Found: C, 75.6; H, 9.6. $C_{25}H_{38}O_2Si$ requires C, 75.32; H 9.61%]; δ_H ($CDCl_3$) 7.75–7.65 (4H, m, Ar*H*), 7.47–7.36 (6H, m, Ar*H*),

3.80–3.62 (2H, m, H_1), 3.45 (1H, m, H_4), 1.80–1.25 (9H, m, $H_{2,3,5,6,7}$), 1.09 (9H, s, *t*Bu), 0.93 (3H, t, J=6.0 Hz, H_8), 0.89 (3H, d, J=6.6 Hz, H_9); δ_C ($CDCl_3$) 135.6, 133.5, 129.6, 127.6, 75.7, 62.0, 35.9, 34.5, 33.8, 28.2, 26.8, 22.8, 19.1, 16.2, 14.1.

4.2.5. (3*R*,4*S*) and (3*S*,4*R*) 1-*t*-Butyldiphenylsilyloxy-3-methyl-4-*O*-(2′,3′,4′,6′-tetrapivaloyl-β-D-glucopyranosyl)octane (9). The alcohol (±**8**) (2.83 g, 7.1 mmol) was glycosylated in an identical manner to that described above for the *cis*-species, using 2,3,4,5-tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide (4.11 g, 7.1 mmol), silver triflate (1.824 g, 7.1 mmol) and 2,6-lutidine (820 μL, 7.1 mmol). The crude product was purified by column chromatography (10% ethyl acetate in hexane) to give a colourless resin (4.2 g, 66%). [Found: C, 68.5; H 9.2. $C_{51}H_{80}O_{11}Si$ requires C, 68.27; H 8.99%]; The individual glycoside diastereomers were separated in an identical manner to that described for **6**.

Compound (3*R*,4*S*)-**9**. δ_H ($CDCl_3$) 7.68–7.62 (4H, m, Ar*H*), 7.48–7.35 (6H, m, Ar*H*), 5.26 (1H, dd, J=9.3, 9.3 Hz, $H_{3'}$), 5.10 (1H, dd, J=9.3, 9.3 Hz, $H_{4'}$), 5.01 (1H, dd, J=9.3, 7.8 Hz, $H_{2'}$), 4.50 (1H, d, J=7.8 Hz, $H_{1'}$), 4.18 (1H, dd, J=12.0, 1.8 Hz, $H_{6a'}$), 3.93 (1H, dd, J=12.0, 5.1 Hz, $H_{6b'}$), 3.72–3.44 (4H, m, $H_{1,4,5'}$), 1.58–1.48 (1H, m, H_3), 1.48–1.20 (8H, m, $H_{2,5,6,7}$), 1.20, 1.14, 1.14, 1.11 (36H, 4s, COCMe_3), 1.06 (9H, s, SiCMe_3), 0.84 (3H, t, J=6.9 Hz, H_8), 0.79 (3H, d, J=6.9 Hz, H_9). δ_C ($CDCl_3$) 178.0, 177.2, 176.4, 176.3, 135.5, 134.0, 129.7, 127.7, 99.4, 82.1, 72.9, 71.8, 71.6, 68.1, 61.8, 61.8, 38.8, 38.7, 38.7, 35.2, 32.0, 29.2, 27.9, 27.3, 27.2, 27.0, 27.0, 26.9, 22.6, 14.5, 14.0; ESI-MS (80% MeOH) 919.8 ($M+Na^+$).

Compound (3*S*,4*R*)-**9**. δ_H ($CDCl_3$) 7.68–7.62 (4H, m, Ar*H*), 7.44–7.33 (6H, m, Ar*H*), 5.29 (1H, dd, J=9.6, 9.3 Hz, $H_{3'}$), 5.07 (1H, dd, J=9.9, 9.6 Hz, $H_{4'}$), 4.99 (1H, dd, J=9.3, 7.8 Hz, $H_{2'}$), 4.56 (1H, d, J=7.8 Hz, $H_{1'}$), 4.21 (1H, dd, J=12.3, 1.8 Hz, $H_{6a'}$), 3.92 (1H, dd, J=12.3, 6.0 Hz, $H_{6b'}$), 3.76–3.58 (3H, m, $H_{1,5'}$), 3.48 (1H, m, H_4), 1.90–1.20 (9H, m, $H_{2,3,5,6,7}$), 1.18, 1.14, 1.14, 1.11 (36H, 4s, COCMe_3), 1.03 (9H, s, SiCMe_3), 0.88 (3H, t, J=6.6 Hz, H_8), 0.82 (3H, d, J=6.6 Hz, H_9); δ_C ($CDCl_3$) 178.0, 177.2, 176.5, 176.3, 135.5, 134.0, 129.5, 127.6, 99.0, 82.6, 72.6, 71.9, 71.6, 68.3, 62.2, 62.0, 38.8, 38.7, 38.7, 33.7, 32.3, 30.4, 28.2, 27.2, 27.2, 27.1, 27.0, 26.9, 22.9, 15.8, 14.0; ESI-MS (80% MeOH) 919.8 ($M+Na^+$).

4.3. General procedure for deprotection and oxidation of 9

TBAF (230 mg, 0.72 mmol) was added to a solution of (3*R*,4*S*) and (3*S*,4*R*)-**9** (540 mg, 0.6 mmol) in THF (20 mL) and stirred at room temperature for 16 h during which time the solution became yellow in colour. The solvent was evaporated and the residue dissolved in ethyl acetate (30 mL) and washed with saturated sodium bicarbonate solution (10 mL), 5% citric acid solution (10 mL) and water (2×10 mL). The organic phases were dried and concentrated and the crude product was purified by column chromatography (10% ethyl acetate in hexane) to give (3*R*,4*S*) and (3*S*,4*R*) 3-methyl-4-*O*-(2′,3′,4′,6′-tetrapivaloyl-β-D-glucopyranosyl)octan-1-ol as a colourless oil (230 mg, 87%). [Found: C 63.9; H 9.3. $C_{35}H_{62}O_{11}$ requires C, 63.80; H 9.48%]. To this mixture (159 mg, 0.241 mmol) was added TEMPO (50 mg, 0.32 mmol) and BAIB (200 mg, 0.6 mmol) in acetonitrile (2 mL) and water (3 mL).[22] After stirring at room temperature for 48 h, the reaction mixture was extracted with ethyl acetate (2×30 mL) and the extracts washed with 5% citric acid solution (15 mL) and water (15 mL), before being dried and concentrated to give an orange oil. The crude product was purified by column chromatography (2% methanol in dichloromethane) to give (3*R*,4*S*) and (3*S*,4*R*) 3-methyl-4-*O*-(2′,3′,4′,6′-tetrapivaloyl-β-D-glucopyranosyl)octanoic acid (**10**) as a pale yellow resin (132 mg, 81%).

4.3.1. (3*R*,4*S*) 3-Methyl-4-*O*-(2′,3′,4′,6′-tetrapivaloyl-β-D-glucopyranosyl)octanoic acid (10). Conversion of (3*R*,4*S*)-**9** (144.0 mg, 0.16 mmol) into the acid (3*R*,4*S*)-**10** was accomplished as outlined above to give a colourless oil (87.0 mg, 80%); δ_H ($CDCl_3$) 5.31 (1H, dd, J=9.4, 9.0 Hz, $H_{3'}$), 5.11 (1H, dd, J=9.8, 9.4 Hz, $H_{4'}$), 5.03 (1H, dd, J=9.4, 7.8 Hz, $H_{2'}$), 4.61 (1H, d, J=7.8 Hz, $H_{1'}$), 4.22 (1H, br d, J=12.2 Hz, $H_{6a'}$), 3.97 (1H, dd, J=12.2, 5.3 Hz, $H_{6b'}$), 3.66 (1H, m, $H_{5'}$), 3.50 (1H, m, H_4), 2.6–2.46 (1H, m, H_{2a}), 2.18–1.96 (2H, m, $H_{2b,3}$), 1.50–1.10 (6H, m, $H_{5,6,7}$) 1.20, 1.15, 1.14, 1.10 (36H, 4s, CMe_3), 0.91 (3H, d, J=6.3 Hz, H_9), 0.85 (3H, t, J=7.1 Hz, H_8); ESI-MS (80% MeOH) 695.4 ($M+Na^+$).

4.3.2. (3*S*,4*R*) 3-Methyl-4-*O*-(2′,3′,4′,6′-tetrapivaloyl-β-D-glucopyranosyl)octanoic acid (10). (3*S*,4*R*)-**9** (202.2 mg, 0.23 mmol) was treated as outlined above to give the acid (3*S*,4*R*)-**10** as a colourless oil (113.9 mg, 75%); δ_H ($CDCl_3$): 5.29 (1H, dd, J=9.5, 9.5 Hz, $H_{3'}$), 5.09 (1H, dd, J=9.5, 9.3 Hz, $H_{4'}$), 4.98 (1H, dd, J=9.5, 7.9 Hz, $H_{2'}$), 4.57 (1H, d, J=7.9 Hz, $H_{1'}$), 4.23 (1H, br d, J=12.0, $H_{6a'}$), 3.94 (1H, dd, J=12.0, 5.5 Hz, $H_{6b'}$), 3.66 (1H, m, $H_{5'}$), 3.47 (1H, m, H_4), 2.42–2.32 (1H, m, H_{2a}), 2.24–1.95 (2H, m, $H_{2b,3}$), 1.50–1.10 (6H, m, $H_{5,6,7}$), 1.19, 1.12, 1.12, 1.08 (36H, 4s, CMe_3), 0.94 (3H, d, J=6.5 Hz, H_9), 0.87 (3H, t, J=6.7 Hz, H_8); ESI-MS (80% MeOH) 695.4 ($M+Na^+$).

4.3.3. (3*R*,4*S*) 3-Methyl-4-*O*-β-D-glucopyranosyloctanoic acid (3). Protected glycoside (3*R*,4*S*)-**10** (87.0 mg, 0.13 mmol) was converted into the free glycoside according to the procedure outlined above, but omitting the second KOH step, to give (3*R*,4*S*)-**3** (43.5 mg, 100%); δ_H (CD_3OD): 4.33 (1H, d, J=7.7 Hz, $H_{1'}$), 3.85 (1H, dd, J=11.7, 2.4 Hz, $H_{6a'}$), 3.69 (1H, dd, J=11.7, 5.2 Hz, $H_{6b'}$) 3.57 (1H, app. q, H_4), 3.39–3.16 (4H, m, $H_{2',3',4',5'}$), 2.54 (1H, dd, J=14.6, 4.6 Hz, H_{2a}), 2.26 (1H, m, H_3), 2.14 (1H, dd, J=14.6, 8.8 Hz, H_{2b}), 1.62–1.24 (6H, m, $H_{5,6,7}$), 0.99 (3H, d, J=6.7 Hz, H_9), 0.92 (3H, t, J=7.2 Hz, H_8); δ_C (CD_3OD): 178.4, 105.0, 85.0, 79.0, 78.5, 76.2, 72.6, 63.7, 39.3, 35.4, 32.9, 29.1, 24.6, 16.8, 15.3; ESI-MS (80% MeOH) 359.4 ($M+Na^+$); $[\alpha]_D$=−15.5 (*c* 0.52, CH_3OH).

4.3.4. (3*S*,4*R*) 3-Methyl-4-*O*-β-D-glucopyranosyloctanoic acid (3). Protected glycoside (3*S*,4*R*)-**10** (113.9 mg, 0.17 mmol) was converted into the free glycoside according to the procedure outlined above, to give (3*S*,4*R*)-**3** (55.9 mg, 98%); δ_H (CD_3OD): 4.30 (1H, d, J=7.7 Hz, $H_{1'}$), 3.86 (1H, dd, J=11.8, 2.2 Hz, $H_{6a'}$), 3.68 (1H, dd, J=11.8, 5.3 Hz, $H_{6b'}$) 3.58 (1H, app. q, H_4), 3.40–3.14 (4H, m, $H_{2',3',4',5'}$), 2.59 (1H, dd, J=15.3, 5.0 Hz, H_{2a}), 2.24 (1H, m, H_3), 2.10

(1H, dd, J=15.3, 8.5 Hz, H_{2b}), 1.60–1.24 (6H, m, $H_{5,6,7}$), 0.97 (3H, d, J=6.8 Hz, H_9), 0.94 (3H, t, J=7.2 Hz, H_8); δ_C (CD_3OD): 178.8, 104.3, 83.8, 78.9, 78.6, 76.1, 72.6, 63.8, 39.7, 35.9, 32.3, 28.8, 24.9, 17.9, 15.3; ESI-MS (80% MeOH) 359.4 ($M+Na^+$); $[\alpha]_D$=−21.3 (c 0.7, CH_3OH).

4.4. General procedure for strong acid hydrolysis of glycosides 3 to oak lactone (1)

(3*R*,4*S*)-**3** (36.3 mg, 0.11 mmol) was dissolved in water (9 mL), concentrated sulfuric acid (1 mL) and dioxane (1 mL) and refluxed for 16 h. The cooled mixture was extracted with ether (2×10 mL), washed with water (10 mL), dried and concentrated. The resulting oil was purified by column chromatography (20% ether in pentane) to give (4*R*,5*S*)-*trans*-**1** as a colourless oil (8.5 mg, 62%): $[\alpha]_D$=−97 (c 0.34, CH_3OH), lit.[23] $[\alpha]_D$=−95.

(4*S*,5*R*)-*trans*-**1** was prepared as above (17.1 mg, 78%) $[\alpha]_D$=+100 (c 0.48, CH_3OH), lit.[23] $[\alpha]_D$=+96.

(4*S*,5*S*)-*cis*-**1** was prepared as above (10.5 mg, 60%) $[\alpha]_D$=−74 (c 0.42, CH_3OH), lit.[23] $[\alpha]_D$=−78.

(4*R*,5*R*)-*cis*-**1** was prepared as above (12.1 mg, 63%) $[\alpha]_D$=+79 (c 0.50, CH_3OH), lit.[23] $[\alpha]_D$=+76.

4.5. Aroma detection thresholds of (4*S*,5*S*)-*cis*-1 and (4*R*,5*R*)-*cis*-1

The aroma threshold of (4*S*,5*S*)-*cis*-**1** in a young (<12 months old) neutral dry white wine (2002 South Australian Chenin blanc) was determined according to the American Society for Testing and Materials (ASTM) method E 679, using 24 judges. The judges were of European origin, aged between 20 and 50, with similar numbers of males and females. The white wine had a free sulfur dioxide content of 24 mg/L. Wines were presented (as part of a triangle test) in ascending order of (4*S*,5*S*)-*cis*-**1** concentration, at 2.0, 6.1, 18.5, 53.9, 161.7 and 485.1 μg/L. Panellists smelt, but did not taste the samples. Those who could detect the spiked wines at all of these concentrations were then tested at lower concentrations; conversely, those who could not detect the spike at any of the concentrations were tested at higher concentrations. The aroma threshold of (4*R*,5*R*)-*cis*-oak lactone was determined in the above manner. The aroma threshold of (4*S*,5*S*)-*cis*-**1** was also determined in a young (2002 South Australian Shiraz) red wine, as described above.

4.6. Mild acid hydrolysis of glycosides (3*S*,4*S*)-3, (3*S*,4*R*)-3 and (3*S*,4*S*)-4

A solution of the required glycoside was prepared by dissolving either **3** (1.2 mg) or **4**[13] (1.7 mg) in water (1000 mL containing 10% EtOH) buffered to pH 3.0 by saturating a solution with potassium hydrogen tartarate, and adjusting the pH with 10% aqueous tartaric acid solution. Portions (7 mL) of the solutions were sealed in glass ampoules and heated at the temperatures and times indicated in Table 2. The ampoules were then opened and analysed for oak lactone content as described by Pollnitz et al.[7]

4.7. Enzyme hydrolysis of glycosides (3*S*,4*S*)-3, (3*S*,4*R*)-3 and (3*S*,4*S*)-4

To a solution of the required glycoside (~20 mg) in pH 5.0 buffer solution (9 mL) was added either AR2000 enzyme (~30 mg) or almond emulsion β-glucosidase enzyme (~30 mg), and warmed at 30 °C for 48 h. The reaction mixture was acidified to pH 1.0 with 10% hydrochloric acid, allowed to stand overnight, and then extracted with ether (2×25 mL). The combined ether extracts were dried and concentrated. A small portion was dissolved in dichloromethane (approx. 1 ppm) and analysed by chiral GC–MS.

4.7.1. Pyrolysis of glycosides (3*S*,4*S*)-3, (3*S*,4*R*)-3 and (3*S*,4*S*)-4. To a sample of powdered oakwood (1 g) in a large glass ampoule (50 mL) was added a solution of either glycoside **3** (500 μg in EtOH, 100 μL) or glycoside 4[13] (800 μg in EtOH, 100 μL). The ampoules were sealed and heated at 235 °C for 30 min (equivalent to a heavy toasting). The ampoules were then opened and extracted with model wine (20 mL) for 72 h, prior to analysis for oak lactone content.[7]

4.7.2. GC–MS chiral analysis. Samples of oak lactone produced by strong acid hydrolysis of the various glycosides, **3** or **4**, dissolved in dichloromethane were analysed with a Hewlett–Packard (HP) 6890 gas chromatograph fitted with liquid HP 6890 series injector and coupled to a HP 5973 mass spectrometer. The liquid injector was operated in fast liquid injection mode with a 10 μL syringe (SGE, Australia) fitted. The gas chromatograph was fitted with an approx. 30 m×0.25 mm J and W fused silica capillary column Cyclosil-B, 0.25 μm film thickness. The carrier gas was helium (BOC gases, Ultra High Purity), flow rate 1.2 mL/min. The oven temperature was started at 50 °C, held at this temperature for 1 min, then increased to 220 °C at 10 °C/min and held at this temperature for 10 min. The injector was held at 220 °C and the transfer line at 240 °C. The sample volume injected was 2 μL and the splitter, at 42:1, was opened after 36 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 resilanised glass wool (2–4 mm) at the tapered end to the column. Positive ion electron impact spectra at 70 eV were recorded in the range m/z 35–350 for scan runs. All solvents were Mallinckrodt nanopure grade, and verified for purity by GC–MS prior to use.

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