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Identification of Natural Oak Lactone Precursors in Extracts of American and French Oak Woods by Liquid Chromatography–Tandem Mass Spectrometry

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A method for the screening of potential natural oak lactone precursors in oak wood extracts using LC–MS/MS combined with information-dependent acquisition was developed. The method was applied to extracts of American and French oak woods. As a result, *cis*-3-methyl-4-galloyloxyoctanoic acid (ring-opened *cis*-oak lactone gallate), (3*S*,4*S*)- and (3*S*,4*R*)-3-methyl-4-*O*-*β*-D-glucopyranosyloctanoic acid (ring-opened *cis*- and *trans*-oak lactone glucoside), and (3*S*,4*S*)-3-methyl-4-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranosyloctanoic acid (ring-opened *cis*-oak lactone galloylglucoside) were identified as natural oak lactone precursors in the extracts by comparison with the respective synthetic reference compounds. In addition, the ring-opened oak lactone rutinoside was tentatively identified in the extracts. Three apparent isomers of the ring-opened *cis*-oak lactone galloylglucoside were also observed.

KEYWORDS: Oak; oak lactone; oak lactone precursor; LC–MS/MS

INTRODUCTION

The (4*S*,5*S*) *cis*-isomer (**1a**) and the (4*S*,5*R*) *trans*-isomer (**1b**) of 5-butyl-4-methyl-4,5-dihydro-2(3*H*)-furanone (**Figure 1**), also known as the "oak" or "whiskey" lactones, are extracted from oak wood into wine during fermentation and/or maturation. The concentration of the *cis*-oak lactone isomer in oak wood was found to be 4–13 times higher than that of the *trans*-oak lactone depending upon geographical oak origin (*1*). Recent sensory studies have shown that the odor detection threshold for the natural *trans*-isomer was approximately 7 times higher than for the natural *cis*-isomer in both white and red wine matrices (*2*). Hence, of the two, the *cis*-isomer is considered to be the more important volatile compound responsible for an oak aroma character such as "coconut", "vanilla", or "dark chocolate" added to wine (*3*).

Lactonization of ring-opened oak lactone (3-methyl-4-hydroxyoctanoic acid) goes to completion at wine pH, and the half-lives of the formation of the *trans*- and *cis*-oak lactone isomers were calculated to be 6.6 and 86.6 h, respectively, in model wine at pH 3.3 (*4*). This process might explain why oak aroma in wine was reported to continue to intensify for some time after the removal of oak chips (*5*). A similar observation was also reported by Pollnitz (*6*), who observed a small increase in the concentration of *cis*-oak lactone (but not the *trans*-isomer) in model wine extracts of oak chips following the removal of the chips.

Tanaka and Kouno (*7*) isolated two derivatives of the ring-opened oak lactone from the wood of *Platycarya strobilacea* (Juglandaceae family). These derivatives were identified as (3*S*,4*S*)-3-methyl-4-*O*-*β*-D-glucopyranosyloctanoic acid (**3a**, ring-opened *cis*-oak lactone glucoside) and (3*S*,4*S*)-3-methyl-4-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranosyloctanoic acid (**4**, ring-opened *cis*-oak lactone galloylglucoside). Subsequently, the ring-opened *cis*-oak lactone galloylglucoside was also identified in extracts of oak wood, and the release of oak lactone from this compound was confirmed by hydrolysis with sulfuric acid (*8, 9*).

Although the existence of the ring-opened *cis*-oak lactone glucoside in oak wood has not yet been confirmed, it was also considered to be a potential oak lactone precursor by Wilkinson et al. (*10*). Consequently, the ring-opened *cis*- and *trans*-oak lactone glucosides (**3a** and **3b**, respectively) were synthesized, and the release of the respective oak lactones from the glucosides as well as from the galloylglucoside (**4**) was confirmed under pyrolysis conditions similar to those expected during the toasting of oak wood. Otsuka et al. (*11*) previously described several potential oak lactone precursors isolated from oak wood powder, which included a compound thought to be 3-methyl-4-(3′-*O*-methylgaloyloxy)octanoic acid. This assignment, however, was

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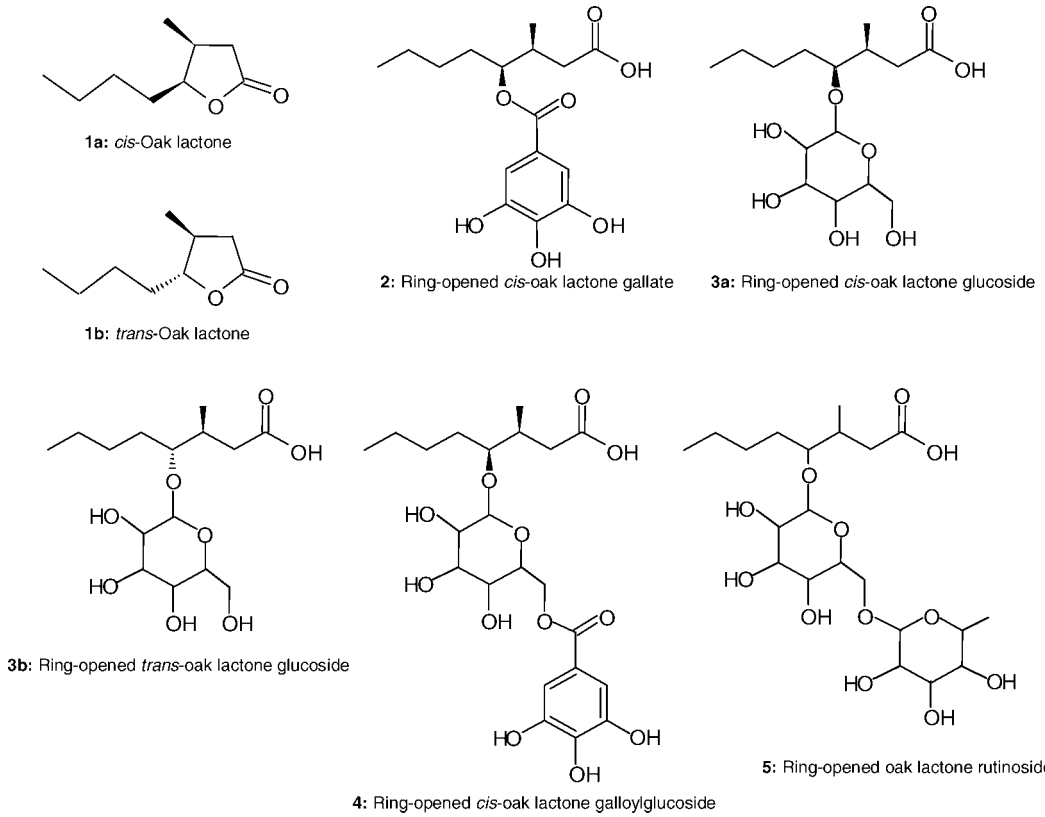

Figure 1. Structures of oak lactones and their precursors.

Table 1. Mass Spectra and LC Retention Times of Reference Compounds and Potential Natural Oak Lactone Precursors Screened in American and French Oak Extracts by LC–MS/MS

Reference Compounds										
reference compound	t_R (min)	precursor ion (*m*/*z*)	enhanced product ions (*m*/*z*)					product ions of *m*/*z* 173 (*m*/*z*)		
glucoside[a]	13.9	335.2	173.0	155.2	143.2	127.2	113.0	155.2	137.4	127.1
gallate[b]	23.5	325.2	173.2	169.1	124.9			155.0	136.8	127.3

Compounds Screened by LC–MS/MS											
peak number	t_R (min)	precursor ion (*m*/*z*)	enhanced product ions (*m*/*z*)							product ions of *m*/*z* 173 (*m*/*z*)	
1	12.9	481.0	335.6	205.7	173.8	163.4	143.4	131.3	115.8	155.0	137.0	127.0
2	13.9	335.0	173.5	155.4	143.4	127.6	113.4			155.2	137.0	127.0
3, 4, 5, 6	16.0–19.0	487.7	469.7	335.4	271.2	211.3	173.3	169.7	125.0	155.2	137.0	127.2
7	23.5	325.1	173.4	169.3	125.6					155.2	137.2	127.1
8	33.7	343.2	325.3	281.3	227.2	187.3	173.2	125.2		155.3		127.3
9	39.0	359.2	253.1	217.2	203.2	173.2						
10	41.2	373.2	337.2	313.0	291.4	217.2	173.2	153.2		155.0		127.0

[a] Ring-opened *cis*-oak lactone glucoside. [b] Ring-opened *cis*-oak lactone gallate.

shown to be incorrect by Raunkjær et al. (*12*), who synthesized both this compound and the corresponding gallate (**2**).

These various studies indicated that the formation of oak lactone from oak lactone precursors could occur in two steps: liberation of the ring-opened oak lactone from oak lactone precursor by either pyrolysis (toasting) or enzymic activity, followed by ring closure (lactonisation) to yield oak lactone (*4, 10*). However, so far only the ring-opened *cis*-oak lactone galloylglucoside has been confirmed to be present in oak wood. The purpose of this study was therefore to screen for potential natural oak lactone precursors in American and French oak extracts using liquid chromatography–tandem mass spectrometry (LC–MS/MS) combined with information-dependent acquisition (IDA) and subsequently to characterize and identify the screened compounds using various LC–MS/MS techniques.

MATERIALS AND METHODS

Reference Compounds. (3*RS*,4*RS*)-3-Methyl-4-galloyloxyoctanoic acid (**2**) (**Figure 1**) was synthesized in-house as previously reported (*12*). (3*S*,4*S*)-3-Methyl-4-*O*-*β*-D-glucopyranosyloctanoic acid (**3a**) and (3*S*,4*R*)-3-methyl-4-*O*-*β*-D-glucopyranosyloctanoic acid (**3b**) were also synthesized in-house (*10*). (3*S*,4*S*)-3-Methyl-4-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranosyloctanoic acid (**4**) was provided by T. Tanaka of Nagasaki University (Nagasaki, Japan) (*7*).

Preparation of Oak Extracts from Oak Wood. Fine shavings (1 mm thickness) were taken from three oak wood samples, two staves of American oak (A1, seasoned for 1 year; A2, seasoned for 2 years) and one stave of French oak (F2, seasoned for 2 years), provided by A. P. John Coopers (Tanunda, South Australia). The oak shavings (10 g) were soaked in 200 mL of methanol for 1 week at room temperature, after which time the shavings were removed by filtration. The filtrate

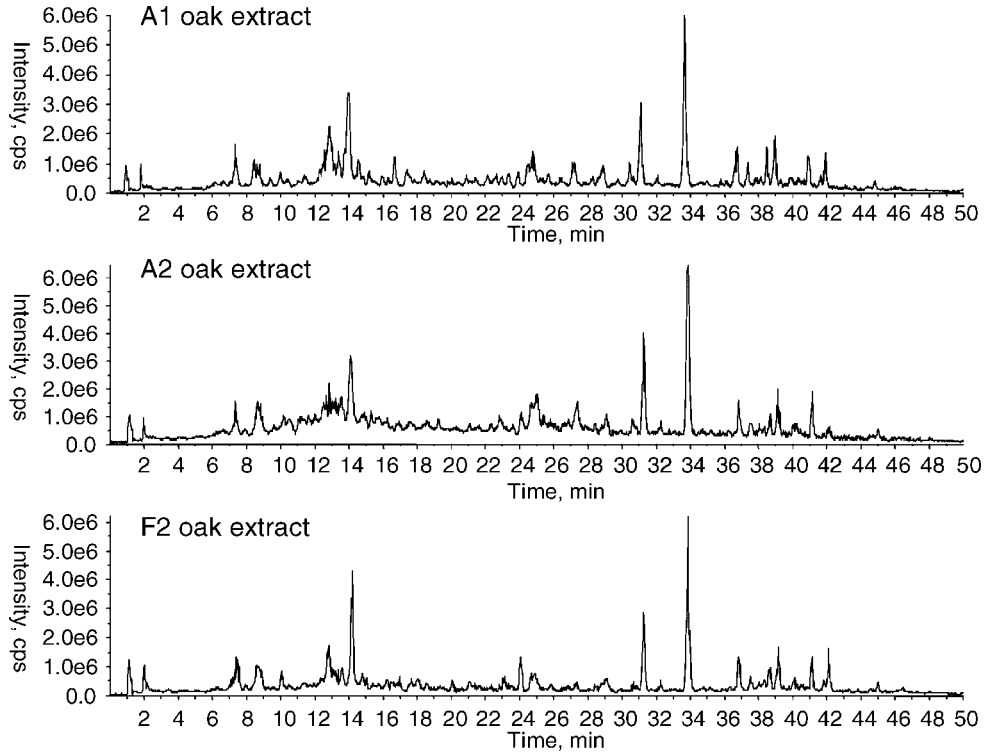

Figure 2. Total ion chromatograms of the survey scan (precursor ion scan for *m*/*z* 173) obtained from the analysis of the A1, A2, and F2 oak extracts by LC–MS/MS.

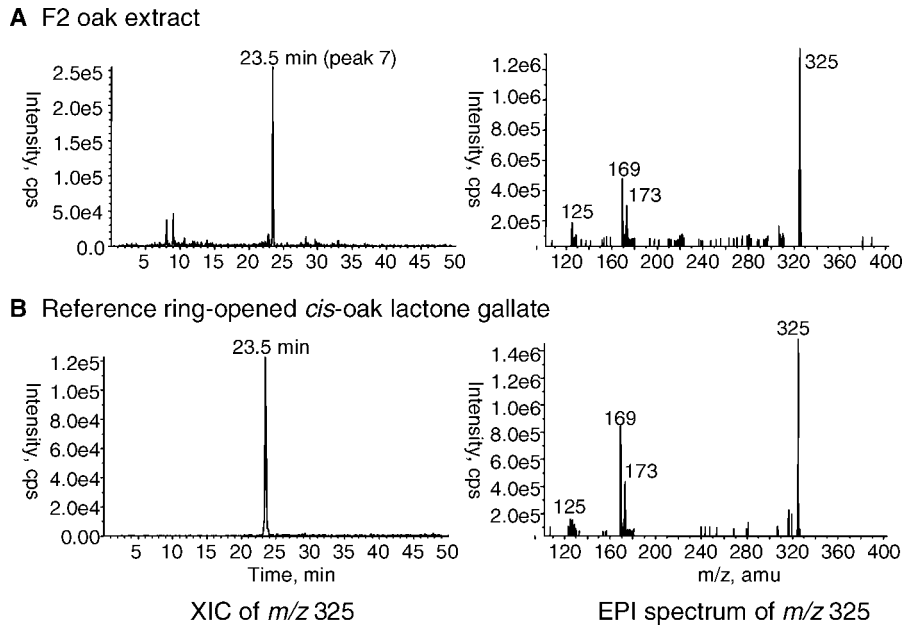

Figure 3. Extracted ion chromatograms (XIC) and enhanced product ion (EPI) spectra of *m*/*z* 325 (peak 7) obtained from the analysis of (**A**) the F2 oak extract and (**B**) reference ring-opened *cis*-oak lactone gallate by LC–MS/MS.

was concentrated in vacuo at 30 °C and then reconstituted with 10 mL of distilled water. The aqueous filtrate was loaded onto a C18 Sep-Pak cartridge (Waters) using a vacuum manifold (Alltech Associates Inc.). After the cartridge was washed with water (3 × 10 mL), the retained compounds were eluted with 5 mL of methanol.

A 1 mL aliquot of the methanolic extract from A1, A2, or F2 was concentrated to semidryness by evaporation of the solvent with nitrogen gas. The residue was reconstituted with 200 μL of a methanol/water mixture (1:1, v/v), followed by analysis using LC–MS/MS.

Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS). All LC–MS/MS experiments were carried out with a 4000 Q TRAP hybrid tandem mass spectrometer equipped with a turbo ionspray source (Applied Biosystems/MDS Sciex, Concord, ON) combined with an Agilent 1200 HPLC system equipped with a binary pump, a degasser, an autosampler, and a column oven (Agilent, Santa Clara, CA). Data acquisition and processing were performed using Analyst, version 1.4.2 (Applied Biosystems/MDS Sciex).

LC Conditions. A 5 μL aliquot of the oak extract was injected and chromatographed on a 150 mm × 2 mm (inside diameter), 4 μm Synergi Hydro-RP 80A column (Phenomenex, Torrance, CA). The column temperature was maintained at 25 °C during the LC run. A binary gradient with mobile phases consisting of 0.1% acetic acid in water (v/v, solvent A) and acetonitrile (v/v, solvent B) was used. The elution conditions were as follows: flow rate of 300 μL/min, linear gradient from 10 to 50% solvent B over 30 min, from 50 to 90% over 15 min,

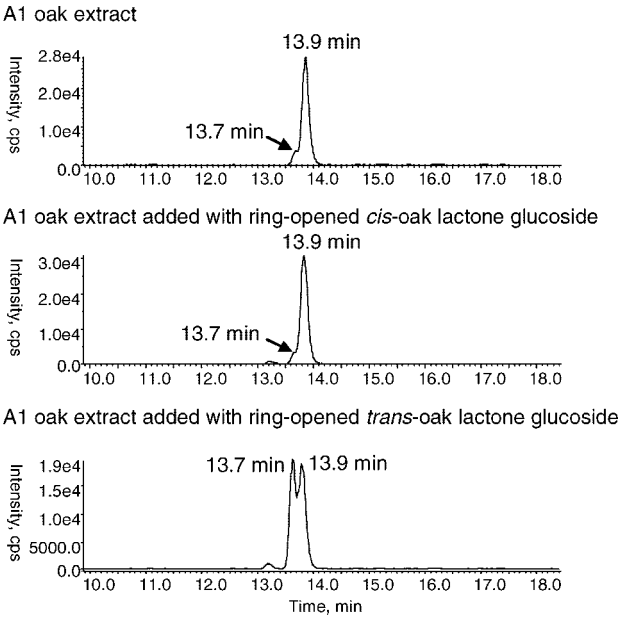

Figure 4. Chromatograms obtained from the analysis of (**A**) A1 extract alone, (**B**) A1 extract added with the reference ring-opened *cis*-oak lactone glucoside, and (**C**) A1 extract added with the reference ring-opened *trans*-oak lactone glucoside by LC–MS/MS in selected reaction mode.

and 90% for 5 min (50 min run). The column was then re-equilibrated for 5 min before the next injection. The effluent from the column was passed directly to the turbo ionspray interface.

MS/MS Conditions. All mass spectrometric data were obtained in negative ion mode. Nitrogen gas was used for the curtain, nebulizer, turbo, and collision gases. The turbo ion source parameters were set at −4300 V for the ion-spray voltage, −70 V for the declustering potential, −10 V for the entrance potential, 50 psi for gas 1 (nebulizer) and gas 2 (turbo), 14 psi for the curtain gas, and 500 °C for the turbo gas temperature. The collision potential was optimized in a range of 30–40 V as appropriate, and the collision gas pressure was set to be high.

The mass spectrometer was a hybrid instrument combining triple quadrupole (QQQ; Q1 for the first mass analyzer, Q2 for the collision cell, and Q3 for the second mass analyzer) and linear ion trap (LIT) technologies (*13, 14*). The second mass analyzer (Q3) can be switched from Q to LIT or vice versa. Accordingly, this instrument can be used as a QQQ and QQLIT tandem mass spectrometer.

For precursor ion scan mode, the instrument was operated as a QQQ device. The resolution of Q1 and Q3 was set to low, and Q1 was scanned from *m*/*z* 250 to 800 with a step size of 0.4 amu and a scan time of 2.0 s; Q3 was held at a set *m*/*z* value. For product ion scan mode, the instrument was operated as a QQQ device. The resolution of Q1 and Q3 was set to unity, and Q3 was scanned from *m*/*z* 50 to 520 with a step size of 0.2 amu and a scan time of 1.0 s; Q1 was held at a set *m*/*z* value. For enhanced product ion scan mode, the instrument was operated as a QQLIT device. The LIT parameters were as follows: scan rate, 4000 amu/s; Q0 trapping, yes; LIT full time, 20 ms; Q3 entry barrier, 8 V; step size, 0.12 amu; and scan range, *m*/*z* 100–800. The resolution of Q1 was set to low. For MS/MS/MS (MS3) scan mode, the instrument was operated as a QQLIT device. The LIT parameters were as follows: scan rate, 4000 amu/s; Q0 trapping, yes; LIT full time, 50 ms; Q3 entry barrier, 8 V; scan range, *m*/*z* 50–200; excitation time, 100 ms; and AF2, 20–30. The resolution of Q1 was set to low. For selected reaction monitoring mode (SRM), the instrument was operated as a QQQ device. The resolution of Q1 and Q3 was set to unit resolution. The ion transition was monitored with a dwell time of 50 ms.

RESULTS AND DISCUSSION

Development of a Screening Method for Potential Oak Lactone Precursors. The reference ring-opened *cis*-oak lactone glucoside (**3a**) and *cis*-oak lactone gallate (**2**) were used for the development of a screening method for potential natural oak lactone precursors. The enhanced product ion spectra of these reference compounds (1–10 μg/mL) were obtained by LC–MS/MS (**Table 1**). The major fragment ions were found to be *m*/*z* 173, 155, 143, 127, and 113 from the glucoside (*m*/*z* 335) and *m*/*z* 173, 169, and 125 from the gallate (*m*/*z* 325). The fragment ion at *m*/*z* 173 was common to both the reference compounds (**2** and **3a**) and appeared to represent a deprotonated ion of the ring-opened oak lactone moiety (3-methyl-4-hydroxyoctanoic acid). The further characterization of the ion at *m*/*z* 173 by MS3 was performed as follows. The first precursor ion of either *m*/*z* 335 or 325 was fragmented in the collision cell (Q2), and the resulting fragment ions, including *m*/*z* 173, were trapped in LIT (Q3). The second-generation precursor ion (*m*/*z* 173) was isolated and fragmented in LIT to obtain the MS3 spectrum of *m*/*z* 173 (**Table 1**). The MS3 spectra of the reference compounds exhibited the same major ions at *m*/*z* 173, 155, 127, and 137 in order of abundance, where *m*/*z* 155 appeared to be a lactonized fragment ion (oak lactone) resulting from a loss of 18 amu from *m*/*z* 173, followed by losses of H_2O (*m*/*z* 137) or a CO group (*m*/*z* 127). These fragment ions supported the ion at *m*/*z* 173 representing the ring-opened oak lactone.

On the basis of the MS/MS characterization of these reference compounds, a screening method was established using LC–MS/MS combined with information-dependent acquisition (IDA) (*14, 15*) as follows. First, the precursor ion scan for *m*/*z* 173 was performed as a survey scan to search for the potential oak lactone precursors; then the enhanced product ion scan of these surveyed precursor ions was automatically performed as a dependent scan for confirmation, and subsequently, the second-generation precursor ion at *m*/*z* 173 was further fragmented by MS3 scan to confirm its identity.

Potential Natural Oak Lactone Precursors in Oak Woods. The oak extracts from A1, A2, or F2 were analyzed by the screening method in searching for natural oak lactone precursors. Total ion chromatograms of the precursor ion scan of all the oak extracts similarly exhibited numerous peaks which were detected throughout the LC run (**Figure 2**). Approximately 50 different precursor ions for *m*/*z* 173 were detected from each of the oak extracts. These surveyed precursor ions were automatically characterized by enhanced product ion and MS3 scans. These ions were subsequently sorted on the basis of their enhanced product and MS3 spectra as well as the LC elution profiles. This indicated that most of the surveyed precursor ions were excluded as potential candidates for molecular ions $[M - H]^-$ of oak lactone precursors, on the basis of the following considerations. A precursor ion was an odd-numbered $[M - H]^-$ ion, assuming a nitrogen atom was unlikely to be present in oak lactone precursors; was fragmented to *m*/*z* 173 by the dependent scan (the survey scan was performed in low resolution so that a precursor ion for an ion 1 or 2 amu smaller or larger than *m*/*z* 173 was also observed); did not have fragmentation patterns which were frequently found, e.g., sequential losses of 18 or 14–16 amu from a precursor ion; and was not repeatedly detected over a wide range of retention times. Subsequently, 10 peaks with seven different precursor ions were investigated more closely (**Table 1**).

Precursor Ion *m*/*z* 325 (peak 7). Peak 7 with precursor ion *m*/*z* 325 was found in the A1, A2, and F2 oak extracts (**Table 1**). The ion was consistent with the molecular ion $[M - H]^-$ of the reference ring-opened *cis*-oak lactone gallate (**2**). The enhanced product ion spectrum and retention time of peak 7 found in the F2 oak extract (**Figure 3A**) were virtually identical

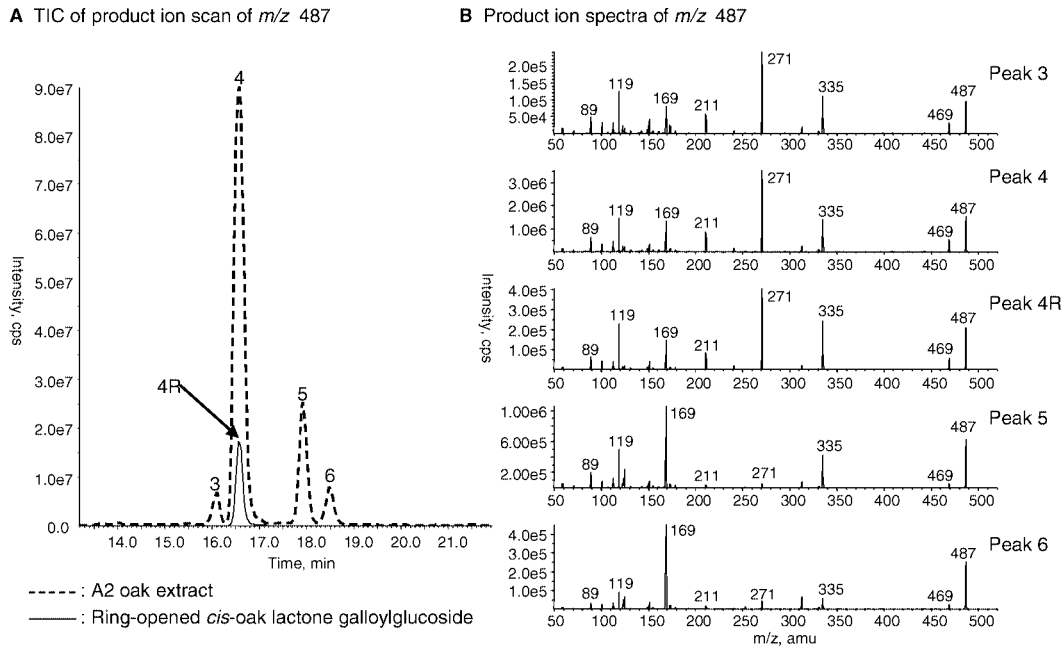

Figure 5. (**A**) Total ion chromatograms of the product ion scan of *m*/*z* 487 and (**B**) product ion spectra of *m*/*z* 487 obtained from the analysis of the F2 oak extract (peaks 3, 4, 5, and 6) and reference ring-opened *cis*-oak lactone galloylglucoside (peak 4R) by LC–MS/MS.

to those of the reference gallate (**Figure 3B**). In addition, the MS3 spectrum of *m*/*z* 173 agreed with that of the reference compound. Therefore, the ring-opened *cis*-oak lactone gallate was confirmed as a compound of the extracts of American and French oak woods.

Precursor Ion *m*/*z* 335 (peak 2). Precursor ion *m*/*z* 335 was observed at a retention time of 13.9 min (peak 2) in all the oak extracts which was consistent with the molecular ions $[M - H]^-$ of the ring-opened oak lactone glucoside (**Table 1**). The enhanced product ion, MS3 spectra, and retention time of peak 2 agreed with those of the reference ring-opened *cis*-oak lactone glucoside (**3a**) (**Table 1**). Therefore, the ring-opened *cis*-oak lactone glucoside appeared also to be a constituent of the extracts. However, we considered the possibility that peak 2 might have been the *trans*-isomer (**3b**) due to the close retention times and product ion spectra of the reference compounds (**3a** and **3b**) (**Figure 4**). Therefore, for further confirmation of peak 2, a LC co-injection experiment of the oak extract and reference compound was carried out. The A1 oak extract alone or with the addition of the same volume of the reference *cis*- or *trans*-isomer of the glucoside in 50% methanol (10–50 μg/mL) was analyzed by LC–MS/MS in SRM, for which the ion transition of *m*/*z* 335 → 173 was specifically monitored for the presence of the glucosides. The A1 oak extract alone exhibited two unresolved peaks at 13.7 and 13.9 min, and the latter peak was dominant (**Figure 4A**). The addition of the reference *cis*-isomer enhanced the latter peak at 13.9 min (**Figure 4B**), while the reference *trans*-isomer enhanced the former peak at 13.7 min (**Figure 4C**). As a result, the *cis*- and *trans*-isomers of the glucoside were both confirmed as compounds in the extract. The level of the *cis*-isomer in the oak extract was found to be considerably higher than that of the *trans*-isomer, in general agreement with the relative abundance of the lactones themselves in oak-aged wines. The ring-opened *trans*-oak lactone glucoside was also detected in the A2 and F2 extracts.

Precursor Ion *m*/*z* 487 (peaks 3, 4, 5, and 6). The survey scan chromatogram of all the oak extracts exhibited at least four peaks derived from precursor ion *m*/*z* 487, which eluted at close retention times ranging from 16 to 19 min (**Table 1**). Ion *m*/*z* 487 was consistent with the molecular ions $[M - H]^-$ of the ring-opened *cis*-oak lactone galloylglucoside. To confirm this assignment, the F2 oak extract and the reference *cis*-oak lactone galloylglucoside (**4**) were separately analyzed by LC–MS/MS in the product ion scan of *m*/*z* 487. The total ion chromatogram (TIC) of the product ion scan clearly exhibited four peaks at 16.1, 16.6, 17.9, and 18.5 min (peaks 3, 4, 5, and 6, respectively) (**Figure 5A**). The product ion spectrum and retention time of peak 4 (16.6 min) were essentially identical to those of the reference compound (peak 4R) (**Figure 5A,B**), confirming the presence of the ring-opened *cis*-oak lactone galloylglucoside in the oak extract. In addition, the other three peaks (peaks 3, 5, and 6) gave the same product ions as peak 4, which included *m*/*z* 469, 335, 271, 211, 169, 119, and 89 (**Figure 5B**). The presence of two isomers (*cis* and probably *trans*) of the galloylglucoside has been reported by Masson et al. (*8*). Peak 3 was considered to be the ring-opened *trans*-oak lactone galloylglucoside since it eluted slightly earlier than the *cis*-isomer (peak 4) and gave a product ion spectrum virtually identical to that of the *cis*-isomer [similar chromatographic and mass spectrometric aspects were observed in the simple ring-opened *cis*- and *trans*-oak lactone glucosides (**3a** and **3b**, respectively)]. Fragment ion *m*/*z* 169 was presumably derived from the gallic acid moiety esterified with a hydroxyl of the glucose (*8*). The relative intensity of this fragment ion for peaks 5 and 6 was significantly greater than that for peaks 3 and 4, suggesting that peaks 5 and 6 might represent the glucose esterified with gallic acid at a secondary alcohol group (C2′–4′) rather than at the primary alcohol group (C-6′, **4**).

Precursor Ion *m*/*z* 481 (peak 1). Peak 1 with precursor ion *m*/*z* 481 was found in all the oak extracts. The MS3 spectrum was similar to those of the previously discussed reference compounds (**2** and **3a**), demonstrating that peak 1 might also be a candidate for an oak lactone precursor (**Table 1**). Peak 1 eluted at 12.9 min which was earlier than the ring-opened oak lactone glucosides (14 min), galloylglucoside (16–19 min), or gallate (24 min), suggesting that the compound had a more hydrophilic nature than the monoglucoside and galloyl derivatives. Ion *m*/*z* 481 appeared to be consistent with the molecular ion $[M - H]^-$ of the ring-opened oak lactone rutinoside (**5**) which was supported by the enhanced product ion spectrum

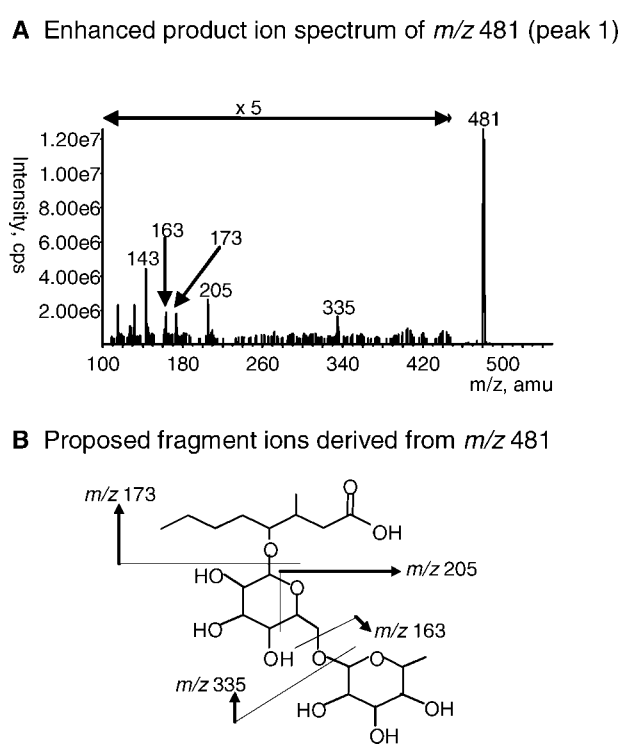

Figure 6. (**A**) Enhanced product ion (EPI) spectrum of *m*/*z* 481 (peak 1) and (**B**) proposed assignment of fragment ions.

(**Figure 6A,B**). The sequential losses of 146 (*m*/*z* 335) and 162 (*m*/*z* 173) from the precursor ion are usually observed in the fragmentation of rutinoside resulting from the sequential cleavages of glycosidic bonds (*16*, *17*).

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Precursor Ions *m*/*z* 343, 359, and 373 (peaks 8, 9, and 10, respectively). The MS3 spectra of peaks 8 and 10 were similar, in part, to those of the reference compounds, but peak 9 did not yield any fragment ions (**Table 1**). The characteristic fragment ion of glycosides was an aglycone ion resulting from the elimination of a dehydrated sugar moiety, e.g., the elimination mass of 162 amu (*m*/*z* 335 → 173) for the ring-opened oak lactone glucoside. In the enhanced product ion spectrum, the elimination masses from the precursor ions of peaks 8, 9, and 10 were found to be 170 amu (*m*/*z* 343 → 173), 186 amu (*m*/*z* 359 → 173), and 200 amu (*m*/*z* 373 → 173), respectively, which are unlikely to represent the loss of any common dehydrated sugar or sugar derivative moiety (**Table 1**) (*16–19*). On the other hand, ions *m*/*z* 187, 203, and 217 were derived from precursor ions *m*/*z* 343, 359, and 373, respectively, resulting from a neutral loss of 156 amu which was the same as the mass of oak lactone. This neutral loss was also observed in the ring-opened oak lactone gallate (*m*/*z* 325 → 169). Ions *m*/*z* 343, 359, and 373 were 18, 34, and 48 amu greater than the molecular ion $[M - H]^-$ of the gallate (*m*/*z* 325), respectively. These differences in mass from the gallate were consistent with the addition of water (18 amu) and/or the substitution of a hydrogen with a hydroxyl group (16 amu), which would usually make a compound more hydrophilic. However, these peaks eluted approximately 10 min later than the gallate. We therefore concluded that these ions were from compounds unlikely to represent molecular ions of gallate-related compounds. Taking these observations into consideration, we could not confirm that peaks 8, 9, and 10 are potential oak lactone precursors.

In summary, using the LC–MS/MS screening method, the ring-opened *cis*-oak lactone gallate (**2**), ring-opened *cis*- and *trans*-oak lactone glucosides (**3a** and **3b**, respectively), and ring-opened *cis*-oak lactone galloylglucoside (**4**) together with three other isomers of this compound were observed as components of American and French oak wood extracts. In addition, the ring-opened oak lactone rutinoside (**5**) was tentatively identified. Although a synthetic sample (*12*) of the methylated gallate proposed by Otsuka et al. (*11*) as an oak lactone precursor could be easily detected by the screening method, the compound was not observed in any of the extracts (data not shown). This last observation is in keeping with our earlier suggestion (*12*) that it was a misidentification.

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