Taste-Guided Isolation of Bitter Lignans from *Quercus petraea* and Their Identification in Wine

Lauriane Sindt,[†,‡] Marine Gammacurta,[†,‡] Pierre Waffo-Teguo,[†,‡] Denis Dubourdieu,[†,‡] and Axel Marchal [*,†,‡]

[†] Université de Bordeaux, ISVV, EA 4577, Unité de recherche OENOLOGIE, 210 chemin de Leysotte, CS 50008, 33882 Villenave d'Ornon, France

[‡] INRA, ISVV, USC 1366 OENOLOGIE, 210 chemin de Leysotte, CS 50008 33882 Villenave d'Ornon, France

ABSTRACT: Quercoresinosides A and B (**1** and **2**), two new lignans, were isolated from a toasted *Quercus petraea* heartwood extract along with a known compound, 3-methoxy-4-hydroxyphenol 1-*O*-*β*-D-(6′-*O*-galloyl)-glucopyranoside (**3**). The purification protocol was based on a taste-guided approach that sought to reveal new bitter compounds released from oak wood into wines and spirits. HRMS and NMR data were used to establish that compounds **1** and **2** are lignan derivatives bearing a glucosyl unit and a galloyl unit at the same positions. Hydrolysis of these compounds showed that they could be distinguished by the absolute configuration of their respective lyoniresinol genin as determined by chiral LC-HRMS in comparison with (+)- and (−)-lyoniresinol standards. Sensory analyses were performed in a non-oaked wine on the pure compounds **1**−**3**. The three molecules exhibited a bitter taste at 2 mg/L that was particularly intense for compounds **2** and **3**. Finally, LC-HRMS demonstrated the occurrence of compounds **1**−**3** in oaked wine and brandy, which supports the hypothesis of their contributions to the increase of bitterness during oak aging.

Flavor has been investigated in recent years both biologically and chemically[1,2] in an attempt to understand the palatability of food and beverages.[3] Many studies have sought to improve the quality of beverages. Instrumental and human methods of detection have demonstrated the molecular mechanisms responsible for the perception of taste and aromas.[4–7] Enological studies have focused on the origins of wine sensory characteristics and have revealed the impact of each winemaking step on the final complexity and typicity of wine.[8] Indeed, wine is a complex matrix for which its chemical diversity is linked directly to a vine variety, cultivated on a given "*terroir*", and mediated by a specific winemaking process. This matrix is composed of many compounds, of which some possess biological properties and are able to stimulate the senses of the tasters.[9] Wine taste is the result of many molecules interacting with each other.[10–12] During the winemaking process, aging transforms the composition of wine. In particular, oak barrel aging improves the color, aroma, structure and taste of wine.[8] Until now, many studies have elucidated the molecular origin of some of the characteristics of the impact of oak on wine such as aging aroma,[13,14] color stabilization,[15] and modulation of somatosensory perceptions.[16,17] Nevertheless, only a few determinants of the gustatory input of oak have been brought to light. Empirical observation of the softening of dry wine during oak aging[18] led to the discovery of new natural oak compounds called quercotriterpenosides.[19,20] Bitterness has also been studied.[21] Concerning ellagitannins described in wine,[16,22,23] a half-tongue test confirmed, in particular, the bitter properties of some compounds of this chemical family.[24] Among the phenolic compounds, lignans have also been shown to exhibit gustatory properties. Indeed, the bitterness of lyoniresinol has been established,[19] as well as its presence in oaked wines and spirits.[25–27] The sensory impact of its two isolated enantiomers has also been studied.[28] Other compounds of the same chemical family have been identified in oak wood but their role in the modification of wine taste during oak aging appears less important than that of lyoniresinol.[29] However,

among the numerous non-volatile compounds identified in oak extracts,[8,30–32] the sensory profiles of only a few have been characterized and many remain unknown.

To investigate the molecular origin of wine bitterness due to oak wood compounds, taste-guided fractionation was undertaken in this study. Pure samples containing a single taste-active compound were thus obtained. The methodology was then applied to an aqueous alcoholic extract of toasted *Quercus petraea* (Matt.) Liebl. (Fagaceae) wood in order to isolate bitter molecules. Then, the purified compounds were identified by HRMS and NMR spectroscopy and their tastes were characterized.

RESULTS AND DISCUSSION

To study wood compounds liable to be found in oaked wine, chips of *Quercus petraea* heartwood were macerated in a H_2O−EtOH solution (50:50; v/v). In a similar manner to bioguided protocols used to discover new bioactive molecules, a taste-guided protocol was set up in order to discover new bitter compounds. A sensory assessment of the bitterness of every fraction obtained was performed after each separation step.

A first step consisted of sequential liquid/liquid partitioning using ethyl acetate (EtOAc) and butan-1-ol (BuOH) to extract compounds from the aqueous solution. This led to three freeze-dried fractions. The BuOH extract was found to express the most intense bitterness while the EtOAc extract exhibited a sweet taste and the aqueous extract was found to be slightly sweet and astringent. These results were in agreement with previous studies on oak-extractible molecules.[19]

Centrifugal partition chromatography (CPC) was then applied to the BuOH extract. A specific solvent system was chosen according to previous tests described in the Experimental Section. Almost 8 g were treated at once within 3 h. CPC tubes were gathered after the performance of LC-HRMS analysis of one out of every five in order to obtain an homogeneous

distribution of the sample mass and the relevant distribution of the eluted molecules between fractions. The freeze-dried CPC fractions provided a total weight representing 89.5% of the initial sample mass.

Gustatometric analysis was performed on the freeze-dried CPC fractions and consisted of a qualitative and quantitative taste evaluation. This technique has already led to the discovery of new taste-active compounds.[19] The tasters were selected according to their level of expertise in detecting tastes and describing gustative perceptions. They used a precise vocabulary concerning fundamental tastes (sweetness, bitterness), tactile sensations (astringency), and somesthetic effects (perception of burning or freshness). Their expertise in wine tasting allowed them to perceive the potential differences between a model solution or wine and the same sample supplemented with wood fractions. The number of tasters was limited due to the small quantities of product available. Sensory test results were recorded in a gustatogram (Table S1, Supporting Information).

Gustatograms are useful for targeting taste fractions in a complex matrix, allowing the purification protocol to be continued appropriately. Four CPC fractions were described as bitter. Gustatograms also have an intensity scale that allowed focusing on the most bitter fraction (VI), which was then used to perform semi-preparative HPLC with UV detection. Ten fractions (A to J) were collected and then submitted to sensory analysis. The gustatogram established for these preparative HPLC fractions presents their taste and bitterness score (Table S2, Supporting Information). Another semi-preparative HPLC separation was then performed to purify compounds **1**−**3**.

Compound **1** showed negative-ion HRESIMS quasi-molecular ion peaks at *m/z* 733.2348 and *m/z* 733.2347, respectively (Figure S2, Supporting Information). Given the isotopic ratio (around 39%), an empirical formula of $C_{35}H_{42}O_{17}$ was attributed. To investigate the nature and the sequence of the functional groups, fragmentation was performed on the pure molecule by

non-resonant activation in the higher collision dissociation (HCD) mode with a 50 arbitrary units collision energy. The presence of ions at *m/z* 581.2239 ($[C_{28}H_{37}O_{13}]^-$) and *m/z* 419.1710 ($[C_{22}H_{27}O_8]^-$), corresponding, in turn, to the neutral loss of $C_7H_4O_4$ and $C_{13}H_{14}O_9$, suggested that compound **1** contains one galloyl group and one hexosyl group (Figure S3, Supporting Information). The fragment ion at *m/z* 313.0566 ($[C_{13}H_{13}O_9]^-$) indicated that these moieties are linked. As a loss of $C_7H_4O_4$ was observed alone (ion at *m/z* 581.2239), it could be proposed that the galloyl group is in the terminal position. Moreover, the empirical formula of the ion at 419.1710 ($[C_{22}H_{27}O_8]^-$) corresponded to lyoniresinol, a lignan identified in oak wood.[25]

The ^{1}H NMR spectrum (Table 1 and Figure S4, Supporting Information) of compound **1** showed two main regions. The first region, between δ_H 7.10 and δ_H 6.40, was characterized by a singlet of a gallate unit at δ_H 7.10 (2H, H-2‴ and H-6‴), a singlet of a 1,3,4,5,6-penta-substituted aromatic ring at δ_H 6.54 (H-2), and another singlet at δ_H 6.41 (2H, H-2′ and H-6′) of a sinapyl unit. The second region, between δ_H 5.0 and δ_H 1.5, was composed of three singlets at δ_H 3.32 (3H, OMe-3), δ_H 3.74 (3H, OMe-5), and δ_H 3.85 (6H, OMe-3′, OMe-5′); two oxymethylene groups at δ_H 3.62 (H-9a) and 3.53 (H-9b), and δ_H 3.88 (H-9′a) and 3.45 (H9′b); three methines at δ_H 1.67 (H-8), 4.36 (H-7′), and 2.09 (H-8′); a methylene at δ_H 2.63 (H-7a) and 2.70 (H-7b) of a benzocyclohexane ring; a doublet at δ_H 4.32 (H-1″) for an anomeric proton of a glucose unit, and six protons of a glucosyl moiety between δ_H 4.50 and 3.0. The anomeric proton signal at δ_H 4.32 (d, J = 7.8 Hz) was indicative of a β-configuration for the glucosyl bond. Therefore, compound **1** could be proposed as a lyoniresinol unit carrying one glucopyranoside moiety and one galloyl moiety, consistent with the HRMS data.

The position of the glucose unit in **1** was determined from the HMBC spectrum, which showed a long-range correlation between the oxymethylene carbon at δ_C 71.2 (C-9′) and H-1″ at δ_H 4.32 (H-1″) of the glucosyl group. The galloyl group was attached to C-6″ at δ_C 62.6 of the glucose moiety via an ester bond. This was confirmed by the shifts of H-6a and H-6b in the

glucose moiety. The relative configuration of the stereogenic carbons of **1** (C-8, C-7′ and C-8′) was established by ROESY NMR spectroscopy. Accordingly, the structure of **1** was assigned as lyoniresinol-9′-*O*-(6″-galloyl)-*β*-glucopyranose.

Compound **2** was obtained as a minor constituent, and its molecular formula of $C_{35}H_{42}O_{17}$ was established by negative HRESIMS (*m/z* $[M-H]^-$, 733.2347), again corresponding to a lyoniresinol unit with a glucopyranoside moiety and a galloyl moiety. The ^1H and ^{13}C NMR data (Table 1) of **2** were closely comparable to those of **1**. Some differences were observed between the ^1H NMR signals of (H-7′), associated respectively to δ_H 4.36 and δ_H 4.19 for compounds **1** and **2**. Interestingly, the same observations were made for two structurally similar lignan diastereoisomers, tarennanosides A and B.[34] The HMBC and ROESY spectra of **1** and **2** (Figure S6 and S8, Supporting Information) showed the same correlations, indicating that they should have the same relative configuration of their stereogenic carbons. This suggested that compound **2** is a diastereoisomer of **1**. The position of the glucose unit in **2** was determined by a HMBC experiment, which showed a long-range correlation between the oxymethylene carbon at δ_C 71.1 (C-9′) and H-1″ at δ_H 4.18 (H-1″) of the glucosyl group. The galloyl group was attached to C-6″ at δ_C 64.3 (C-6″) of the glucose moiety via an ester bond. This was confirmed by the shifts of H-6a and H6b in the glucose moiety. Thus, the structure of **2** was concluded to be a lyoniresinol-9′-*O*-(6″-galloyl)-*β*-glucopyranose isomer. The relative configuration of the chiral centers (C-8, C-7′ and C-8′) was deduced in the same manner as for **1**.

As compounds **1** and **2** carry the same functional groups at the same positions, show the same relative configuration, and exhibit different specific optical rotations (+10 and −26, respectively), they may be differentiated by the absolute configuration of their genin. Indeed, lyoniresinol is naturally found as a mixture of both enantiomers.[35] In previous work, these enantiomers were isolated and their absolute configuration determined by use of vibrational circular dichroism (VCD) associated with theoretical calculations. The small quantities

available of compounds **1** and **2** did not allow chiroptic measurements. Thus, the hydrolysis of these molecules followed by the comparison of chiral chromatography data to those previously obtained for lyoniresinol enantiomers[28] was implemented to determine the absolute configuration of the genins corresponding to **1** and **2**.

Compounds **1** and **2** were heated in acidic conditions under an inert atmosphere in order to hydrolyze the glucose-gallate moiety in each case. After 12 h, LC-HRMS analysis on a C_{18} column showed that the reaction was not complete but a significant amount of lyoniresinol was already present. Since degradation products appeared, the hydrolysis was stopped and the crude reaction mixtures were evaporated in vacuo and analyzed by LC-HRMS equipped with a chiral column. Extracted ion chromatograms corresponding to the lyoniresinol deprotonated molecule [$C_{22}H_{27}O_8^-$] showed one peak for each sample at 5.83 min and 7.03 min for the hydrolysis mixture obtained from compounds **1** and **2**, respectively (Figure 1). A comparison with pure standards of lyoniresinol enantiomers obtained as described by Cretin et al.[28] demonstrated that the genin of compound **1** is (8*R*,8′*R*,7′*S*)-lyoniresinol, while that of compound **2** is (8*S*,8′*S*,7′*R*)-lyoniresinol.

Therefore, compounds **1** and **2** were assigned as (+)-(8*R*,8′*R*,7′*S*)-lyoniresinol-9′-*O*-(6″-galloyl)-*β*-D-glucopyranose, and (-)-(8*S*,8′*S*,7′*R*)-lyoniresinol-9′-*O*-(6″-galloyl)-*β*-D-glucopyranose, respectively, and have been named quercoresinosides A and B.

The negative-ion HRESIMS of compound **3** showed a deprotonated-molecular ion $[M - H]^-$ at *m/z* 453.1033. Considering the mass accuracy specifications of the spectrometer and the isotopic ratio observed (23%), the empirical formula $C_{20}H_{22}O_{12}$ was assigned to compound **3**. The NMR data (Table 1) and specific optical rotation (−24) were compared to literature data, thus revealing this compound to be 3-methoxy-4-hydroxyphenol 1-*O*-*β*-D-(6′-*O*-galloyl)-glucopyranoside. This phenol has been identified in the acorns of *Quercus mongolica* and the bark of *Quercus acutissima* previously,[33] but its taste has not been described before.

The taste-guided purification protocol led to two preparative HPLC bitter fractions, B and F. Compound **3** represented 11.5% w/w of fraction B while compounds **1** and **2** isolated from fraction F represented respectively 5.6% w/w and 2.2% w/w. As major compounds of the bitter fractions, these molecules were expected to exhibit a bitter taste.

Compounds **1**−**3** were dissolved in non-oaked white wine (Bordeaux 2011) at 2 mg/L and the taste of each solution was characterized in comparison to the same wine as a reference. (±)-Lyoniresinol, for which the sensory properties have been characterized already with a perception threshold in white wine of 1.5 mg/L,[29] was used as a bitter standard. The three compounds (**1**−**3**) exhibited a bitter taste. On a 0-5 scale representing bitterness intensity assessed as a consensus between the panelists, compound **3** scored 3/5, compound **1** 1/5, and compound **2** was intensively perceived (5/5). The same conditions (2 mg/L in non-oaked white Bordeaux) were applied to lyoniresinol. Its bitterness was assessed at 2/5 for this compound, which demonstrates the interest in **2** and **3**, for which the tastes were perceived as more potent on the scale used. The results suggested that compounds **2** and **3** could have perception thresholds lower than 1.5 mg/L.

Oak aging mastery is one of the main concerns of winemakers since it contributes to revealing the sensory qualities of great wines. To determine the relevance of taste-active oak compounds in an enological study on wine and brandy taste, it was considered important to inquire into their presence in a commercial wine and a commercial cognac aged in oak barrels.

LC-HRMS mass measurement accuracy allowed screening samples by targeting characteristic *m/z* ions of specific empirical formulas. Thus, extracted ion chromatograms (XIC) of *m/z* ratios specific to compounds **1** to **3** were compared for an oak wood extract, an oaked wine and a brandy (cognac) matured in oak barrels (Figure 2). Similar signals were detected in all samples. The specificity of mass measurement (<5 ppm) and similarity in retention time (<0.04 min) demonstrated that compounds **1**-**3** were present in oaked wine and cognac. In

addition, the analysis of these samples in the HCD fragmentation mode revealed fragments with *m/z* 419.1710 at the retention time of compounds **1** and **2** (data not shown), which supported the observations made. Moreover, the XIC corresponding to *m/z* 733.2349 also exhibited additional minor peaks suggesting the occurence of quercoresinoside isomers in oak wood, wine and cognac. A previous study revealed the presence of **3** in oaked whiskey[36] but it has not been identified before in wine or in cognac. This finding highlights the presence of these two new compounds in a commercial oaked wine and a commercial brandy. It furthers knowledge on wine lignans including compounds arising from grapes[37–39] and oak wood.[29,31]

The tastes of these three compounds were also described and the bitterness of quercoresinosides A (**1**) and B (**2**) and 3-methoxy-4-hydroxyphenol 1-*O*-*β*-D-(6′-*O*-galloyl)-glucopyranoside (**3**) was noticeable. The further establishment of a perception threshold and quantification studies would determine their real impact on wine taste. The influence of cooperage parameters and aging conditions on their concentrations in wine will also need to be investigated.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2000 polarimeter. The sodium emission wavelength was set at 589 nm and the temperature at 293K. The samples were solubilized in methanol and the final value resulted from a mean of ten measures of 10 seconds each. NMR experiments were conducted on a Bruker Avance 600 NMR spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz) equipped with a 5 mm TXI probe. All 1D (proton) and 2D (COSY, ROESY, HMBC, and HSQC) spectra were acquired at 300 K in methanol-d_4, which gave as reference the solvent signal (^{1}H δ 3.31; ^{13}C δ 49.00).[40] Data analysis was performed with Bruker Topspin version 3.2. The LC-HRESIMS platform consisted of an HTC PAL® autosampler (CTC Analytics AG, Zwingen, Switzerland), an Accela U-HPLC system with quaternary pumps, and an Exactive Orbitrap mass spectrometer equipped

with a heated electrospray ionization (H-ESI) probe (both from Thermo Fisher Scientific, Bremen, Germany). CPC was performed on a Spotprep II LC paired up with a SCPC-100 + 1000 (Armen Instrument, Saint-Avé, France), both controlled by Armen Glider Prep V5.0 software. A Waters Prep 150 LC including a 2545 Quaternary Gradient Module and a 2489 UV/visible detector was used for the last steps of purification. A Sunfire Prep C_{18} OBD column (250 × 19 mm, 5 µm particle size, Waters, Guyancourt, France) equipped with a SunFire Prep C_{18} guard column (10 × 19 mm, Waters) was chosen after LC-HRMS tests on various analytical columns.

Sample preparation, extraction, centrifugal partition chromatography and high performance liquid chromatography were performed with ultrapure water (Milli-Q purification system, Millipore, France) and HPLC grade solvents (acetonitrile, ethanol, ethyl acetate, methanol, butan-1-ol and propan-2-ol, VWR International, Pessac, France). LC-HRMS chromatographic separations were performed with LC-MS grade acetonitrile and deionized ultrapure water (Optima, Fisher Chemical, Illkirch, France). Two commercial wines were used in this study: a white non-oaked Bordeaux 2011 (100% Sauvignon Blanc, 12.5% v/v) for sensory analysis and a red Pessac-Léognan 2014 (60% Cabernet Sauvignon, 40% Merlot, 13.5% v/v) aged in new oak barrels for chemical analysis. A commercial brandy (Cognac XO) aged in oak barrels was also analyzed.

Plant Material. Oak wood used in this study was supplied by the cooperage company Seguin-Moreau. It was sampled in January 2014 from a batch of staves designed to provide barrels for wine aging. The botanical species was assigned to *Quercus petraea* according to the method described by Marchal et al.[41] The staves were air-dried for two years and toasted according to the cooperage process. They were then reduced to chips. A voucher specimen is deposited in the Institute of Vine and Wine Sciences with the reference BCTR-14-01.

Extraction and Isolation. The oak wood material (600 g) was macerated in 5.5 L of H_2O−EtOH solution (50:50; v/v) for two weeks. Filtration (0.45 µm) was used to remove wood chips and particles. The solution containing soluble wood compounds was concentrated in vacuo by evaporation of ethanol and, partly, water. The concentrated aqueous solution (500 mL) obtained after ethanol removal from the oak chip maceration was extracted five times with ethyl acetate (500 mL). The remaining aqueous phase was extracted four times with butan-1-ol (400 mL). The organic layers of each extraction step were combined, evaporated in vacuo and re-suspended in water before freeze-drying. The EtOAc extract (10.20 g), the BuOH extract (7.94 g), and the H_2O extract (27.79 g) were stored in air and under light protective conditions.

Biphasic systems of solvents were prepared in small quantities (5−10 mL) in tubes and 600 µL of each phase was added to 1 mg of extract sample. After shaking and dissolution of the sample, an aliquot (100 µL) of each phase was evaporated to dryness, resolubilized with 1 mL of H_2O−MeOH (95:5; v/v), and applied to LC-HRMS. The partition coefficient, Kd, was calculated as the ratio of the solute area in each phase. The ternary biphasic system H_2O−propan-2-ol−EtOAc (10:1:10; v/v/v) was chosen for exhibiting a homogeneous separation of the molecules between the two phases. The solvents were pumped by a four-way quaternary high pressure gradient pump, and an automatic high pressure 30 mL injection valve was used to introduce the sample in the system. The separation was performed at room temperature. The sample (7.94 g) was dissolved in 30 mL of biphasic system and filtered. The separation was conducted in the 1 L rotor at 1200 rpm in ascending mode. The flow rate was set at 25 mL/min during elution (120 min) and 40 mL/min during extrusion (60 min). Collection was automatically performed in 25 mL tubes by a Spot prep fraction collector set to one tube/min during elution and two tubes/min during extrusion. A 10 µL aliquot was removed every five tubes for LC-HRMS analysis. The CPC fractions were constituted according to the

elution profile of the major compounds. The tubes of each fraction were gathered, evaporated and freeze-dried.

The CPC fraction VI was bitter, still abundant (441 mg), and chemically complex. A first semi-preparative HPLC method aimed at obtaining smaller fractions suitable for a further fractionation step that would lead to pure compounds. The chromatogram was subdivided according to the presence of the main peaks. The mobile phase was composed of water containing 0.05% of trifluoroacetic acid (A) and acetonitrile (B). Elution was conducted at 20 mL/min and collection was performed manually in 25 mL tubes. The gradients were specifically adapted to the fractions by LC-HRMS optimization. The CPC fraction VI was eluted with the following gradient: 0−2 min, 15% B; 20 min, 50% B; 22−30 min, 100% B. Fractions B and F from semi-preparative HPLC were bitter and were respectively subfractioned with a 0−8 min, 11% B; 18 min, 14% B; 20−30 min, 100% B gradient and in isocratic mode with 14% B. Before each injection, a 10 min equilibration phase was applied manually. The samples were dissolved at 5−50 mg in 200 µL of methanol-H_2O (25:75; v/v) and 0.45 µm filtered. Elutions were monitored by UV detection at 280 nm. The fractions were evaporated in vacuo to remove organic solvent before re-solubilization in water and freeze-drying. A pure molecule was isolated from fraction B (compound **3**, 3.1 mg) and two from fraction F (compounds **1**, 2.5 mg and **2**, 1.0 mg, (Figure S1, Supporting Information)).

*(+)-Quercoresinoside A (**1**):* white amorphous powder; $[\alpha]^{20}_{D}$ +10 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD, 600 MHz), and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; (−)-HRMS *m/z* 733.2348 (calcd for $C_{35}H_{41}O_{17}^{-}$, 733.2349).

*(−)-Quercoresinoside B (**2**):* white amorphous powder; $[\alpha]^{20}_{D}$ -26 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD, 600 MHz), and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; (−)-HRMS *m/z* 733.2347 (calcd for $C_{35}H_{41}O_{17}^{-}$, 733.2349).

*(−)-3-Methoxy-4-hydroxyphenol 1-O-β-D-(6'-O-galloyl)-glucopyranoside (**3**):* white amorphous powder; [α]$^{20}_{D}$ -24 (*c* 0.09, CD_3OD); ^{1}H NMR (CD_3OD, 600 MHz), see Table 1; ^{13}C NMR (MeOD, 150 MHz), and (−)-HRMS *m/z* 453.1038 (calcd for $C_{20}H_{21}O_{12}^{-}$, 453.1039).

Acid Hydrolysis of Compounds 1 and 2. The acidic hydrolysis of **1** and **2** was performed under a CO_2 atmosphere. Compounds **1** and **2** were solubilized separately in an aqueous solution of trifluoroacetic acid (6 mol/L). The solution was heated under reflux at 80 °C for 12 h in an inert atmosphere (CO_2). The hydrolysis reaction was monitored by LC-HRMS. The crude reaction mixtures were then evaporated in vacuo to remove acid and freeze-dried before chiral analysis.

A C_{18} column was used as the stationary phase (Hypersil Gold 2.1 mm × 100 mm, 1.9 µm particle size, Thermo Fisher Scientific). The mobile phases were water (A) and acetonitrile (B). The flow rate was 600 µL/min, and eluent B varied as follows: 0 min, 8%; 0.3 min, 8%; 1.5 min, 13%; 2 min, 19%; 4.5 min, 30%; 4.6 min, 98%; 6.9 min, 98%; 7 min, 8%; 8 min, 8%. The injection volume was 5 µL. HRESIMS acquisitions were carried out in the negative ionization mode at 3 kV. The vaporizer temperature of the source and the capillary temperature were set, respectively, at 320 °C and 350 °C, the nitrogen sheath gas at 75, the auxiliary gas at 18 and the sweep gas at 0 (arbitrary units). The capillary voltage, the tube lens voltage offset and the skimmer voltage were set at −60 V, −135 V and −26 V, respectively. A mass range of 200−800 Th was acquired for 6 min in the full-scan MS mode with a mass resolution of 25 000 (m/Δm, fwhm at *m/z* 200). In addition, purified molecules were also submitted to non-resonant activation at 50 arbitrary units in a higher collisional energy (HCD) cell situated at the far end of the C-trap region. The mass analyzer was calibrated each week using Pierce® ESI Negative Ion Calibration solution (Thermo Fisher Scientific). For chiral analysis, a Chiralpak® IB-3 column (2.1 mm × 150 mm, 3 µm particle size, Chiral Technologies, Illkirch-Graffenstaden, France) was used in the isocratic elution mode (H_2O−CH_3CN; 80:20; v/v) at 300 µL/min.

Sensory Analysis. Gustatory analyses were performed in a specific room equipped with individual booths and 20 °C air-conditioning. Each freeze-dried fraction was tasted at a specific concentration related to its mass percentage of the stock sample. The pre-purified extracts were dissolved in a 12% vol. H_2O−EtOH solution made of mineral water (eau de source de Montagne, Laqueuille, France) and distilled ethanol, and tasted in normalized glasses. The purified compounds were also tasted in non-oaked white wine at the concentration of 2 mg/L. Five wine-tasting experts (three females, two males, age range 25–64 years) were asked to describe the sensory perception by comparing the solutions supplemented with the fraction/molecule to a blank solution. These panelists were researchers in enology and professionals in wine tasting. They were informed of the nature and risks of the present study and were asked to give their consent to participate in the sensory analyses. Taste was described and bitterness was evaluated on an intensity scale from 0 (not detectable) to 5 (strongly detectable). Even if the fractions and compounds were purified from commercial oak wood used for cooperage and observed in oaked wines, the panelists were advised not to swallow but to spit out the samples after tasting.

ASSOCIATED CONTENT

Supporting Information

The following data are available as supplementary material:

Full-scan LC-HRESIMS chromatogram of fraction F. One-dimensional (^{1}H) and two-dimensional (COSY, ROESY, HSQC and HMBC) NMR spectra of **1**−**2**. HRMS spectra of compounds **1** and **2** in the full-scan and HCD mode.

AUTHOR INFORMATION

Corresponding Author

E-mail: axel.marchal@u-bordeaux.fr. Tel +33557575867

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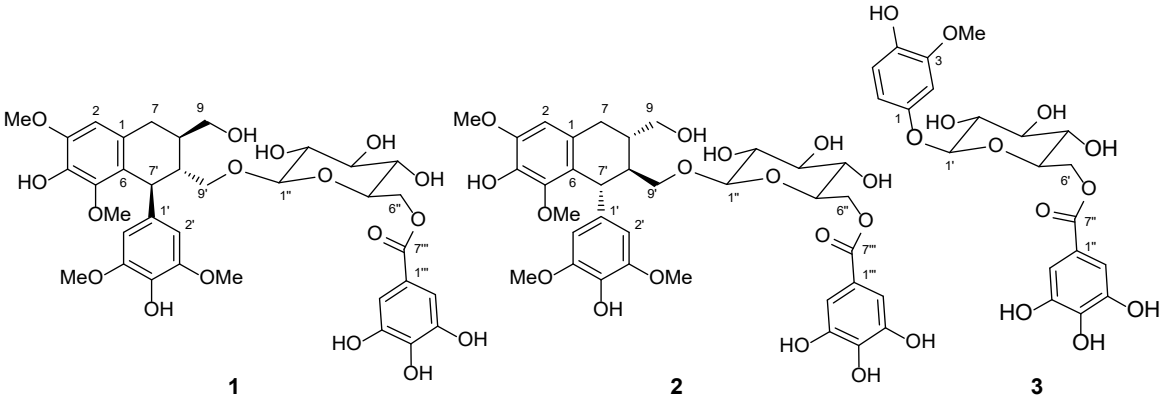
Table 1. NMR Spectroscopic Data for Compounds 1−3 (600 MHz, CD_3OD)

	1		2			3	
position	δ_c, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	position	δ_C, type	δ_H (*J* in Hz)
1	128.5, C		130.8, C	-	1	153.5, C	
2	105.7, CH	6.54 s	107.6, CH	6.55 s	2	104.6, CH	6.70 d (2.6)
3	146.0, C		145.9, C	-	3	150.0, C	
OMe-3	58.6, CH_3	3.32 s	59.8, CH_3	3.28 s	4	143.9, C	
4	137.3, C		137.3, C		5	116.9, CH	6.62 d (8.5)
5	147.3, C		146.9, C		6	111.0, CH	6.57 dd (8.6, 2.6)
OMe-5	55.1, CH_3	3.74 s	56.4, CH_3	3.84 s	7	56.8, CH_3	3.70 s
6	124.8, CH		124.5, CH				
7	31.9, CH_2	2.63 dd (13.0, 3.2) 2.70 dd (15.2, 4.5)	32.2, CH_2	2.69 brd 2.69 brd			
8	41.2, CH	1.67 m	39.3, CH	1.73 m			
9	65.9, CH_2	3.53 dd (10.5, 8.9) 3.62 brd	65.8, CH_2	3.53 dd (10.6, 6.6) 3.61 dd (10.9, 4.1)			
1'	132.7, C		134.8, C				
2', 6'	104.8, CH	6.41 s	107.0, CH	6.38 s			
3', 5'	147.2, C		147.2, C				
OMe-3', 5'	54.8, CH_3	3.85 s	56.44, CH_3	3.68 s			
4'	150.9, C		138.9, C				
7'	40.5, CH	4.36 d (6.1)	43.2, CH	4.19 d (7.2)			
8'	44.6, CH	2.09 m	45.1, CH	2.07 m			
9'	71.2, CH_2	3.45 dd (9.7, 4.2) 3.88 dd (9.8, 5.9)	71.1, CH_2	3.57 dd (10.0, 4.7) 3.88 dd (10.1, 4.7)			
1"	102.7, CH	4.32 d (7.8)	104.3, CH	4.18 d (7.7)	1'	104.7, CH	4.72 d (7.6)
2"	73.7, CH	3.30 m	75.1, CH	3.24 dd (7.7, 9.4)	2'	72.3, CH	3.44 m
3"	76.1, CH	3.43 m	77.9, CH	3.37 m	3'	78.5, CH	3.47 m
4"	71.6, CH	3.42 m	70.6, CH	3.48 m	4'	75.6, CH	3.44 m
5"	75.6, CH	3.52 m	75.1, CH	3.44 m	5'	76.2, CH	3.70 m
6"	62.6, CH_2	4.35dd (11.8, 5.6) 4.49dd (11.8, 1.9)	64.3, CH_2	4.41 dd (12.0, 4.2) 4.47 dd (11.9, 2.0)	6'	65.4, CH	4.42 dd (11.8, 6.7) 4.59 dd (11.8, 1.9)
1"'	119.5, C		122.4, C		1"	122.3, C	
2"', 6"'	108.3, CH	7.10 s	110.1, CH	7.10 s	2", 6"	111.0, CH	7.10 s
3"', 5"'	144.6, C		145.0, C		3", 5"	147.4, C	
4"'	138.1, C		138.3, C		4"	140.8, C	
7"'	166.6, C		166.7, C		7"	169.5, C	



1 **2** **3**

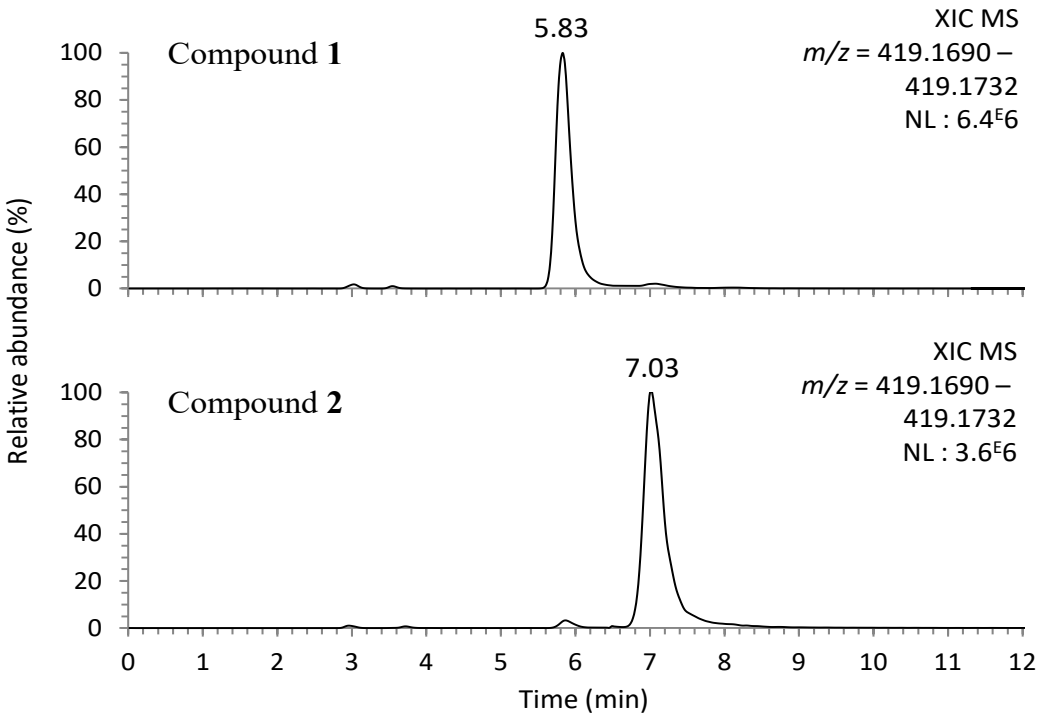


Figure 1. Negative LC-HRESIMS extracted ion chromatograms (XICs), *m/z* 733.2349, of hydrolysis products of compounds **1** (top) and **2** (bottom) on a chiral column.

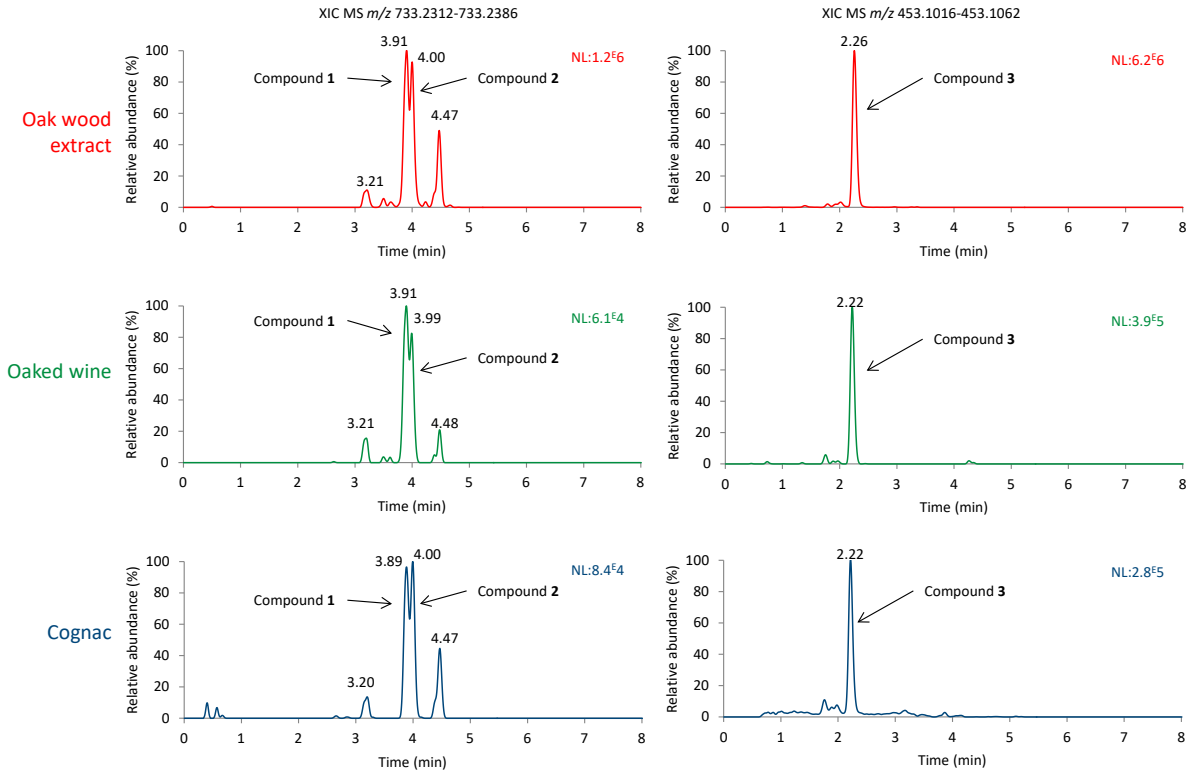


Figure 2. Negative LC-HRESIMS extracted ion chromatograms of an oak wood extract, an oaked wine, and a cognac (top to bottom) corresponding to $[M - H]^-$ ions of compounds **1** and **2** (right) and **3** (left).

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