How stereochemistry influences the taste of wine: Isolation, characterization and sensory evaluation of lyoniresinol stereoisomers

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Abstract:

Wine expresses its beauty by sending a sensory message to the taster through molecules coming from grapes, yeast metabolism or oak wood. Among the compounds released during barrel aging, lyoniresinol has been recently reported as a relevant contributor to wine bitterness. As this lignan contains three stereogenic carbons, this work aimed at investigating the influence of stereochemistry on wine taste by combining analytical and sensorial techniques. First, an oak wood extract was screened by Liquid Chromatography–High Resolution Mass Spectrometry to target isomers separable in a symmetric environment and a diastereoisomer called *epi*-lyoniresinol was isolated for the first time. Then, an original racemic resolution based on natural xylose-derivatives was carried out to obtain lyoniresinol enantiomers. Chiroptical spectroscopic measurements associated with theoretical calculations allowed the unambiguous determination of their absolute configuration. The taste properties of all these stereoisomers revealed that only one lyoniresinol enantiomer is strongly bitter whereas the other one is tasteless and the diastereoisomer is slightly sweet. The presence of these three compounds was established in an oaked Bordeaux wine by chiral and non-chiral chromatography, suggesting the significant influence of stereochemistry on wine taste.

Keywords: Chiral Liquid Chromatography–High Resolution Mass Spectrometry, Vibrational circular dichroism, Counter Current Chromatography, Lignan, Oak wood, Bitterness

Highlights

Targeted screening by LC–HRMS was used to search for stereosiomers of lyoniresinol.

Epi-lyoniresinol was isolated and identified for the first time in *Quercus* genus.

Two lyoniresinol enantiomers were separated.

Vibrational circular dichroism was used to determine their absolute configuration.

Among lyoniresinol isomers in wine, only (+)-lyoniresinol exhibits bitterness.

1. Introduction

Among the aesthetic pleasures in life, wine tasting plays a special role in that, unlike music, poetry or paintings, wine is physically absorbed by the taster. Therefore, even though cognitive processing is primordial [1], [2], the emotion caused by wine tasting is above all due to direct contact between wine stimuli and the taster's sensory receptors. These stimuli are volatile and non-volatile compounds that are responsible for the odors and tastes of wine, respectively. The identification of such taste-active molecules has been a subject of intense research for decades, demonstrating that they can be released from grapes, synthesized by micro-organisms during fermentation or chemically modified during bottle storage [3]. Oak wood is another source of active molecules; during barrel aging, both volatile and non-volatile compounds are released from wood to wine, whose organoleptic properties are thus highly modified [4]. Recently, a lignan from oak wood called lyoniresinol was shown to exhibit bitterness [5]. Another study showed that lyoniresinol is present in wines aged in new oak barrels at concentrations higher than its perception threshold (1.5 mg/L in white wine), establishing its key contribution to the increase in bitterness observed during oak aging [6].

Since the presence of functional groups can modulate the sensory attributes of wine molecules [7], natural derivatives of lyoniresinol have also been sought in oak wood extract. Galloyl, glucosyl and xylosyl derivatives were thus discovered, some of them exhibiting bitterness but with a lower intensity than lyoniresinol [6].

Beyond functional groups, stereochemistry also plays an important role on organoleptic properties. This importance is well known for numerous volatile compounds whose absolute configuration influences both the intensity and the nature of the aroma (*S*- and *R*-enantiomers of limonene smell of lemon and orange, respectively [8]). Similarly, stereochemistry also influences taste attributes. For the first time in 1886, Piutti isolated d-asparagine, the enantiomer of L-asparagine, demonstrating that these compounds are sweet and tasteless, respectively [9]. After this discovery, other compounds were shown to have different taste characteristics according to their stereochemistry, such as naringin diastereoisomers with their distinct bitterness [10], and alapyridaine enantiomers whose dextrorotary form has a sweet taste whereas the levorotatory form has no taste at all [11].

Interestingly, lyoniresinol has three stereogenic centers, suggesting the potential existence of eight stereoisomers. Among the several plant species from which lyoniresinol has been isolated, it has been observed as a mixture of both 8*R*,8′*R*,7′*S*- and 8*S*,8′*S*,7′*R*-enantiomers

with variable relative abundance [12]. In *Quercus* genus oak wood, specific optical rotation measurement indicated that the two enantiomers are present at similar concentrations [13]. Nevertheless, their individual gustatory properties have never been assessed. Furthermore, some lyoniresinol diastereoisomers have been identified in various plants [14], [15], [16], [17] but never in the *Quercus* genus. Nonier et al. only evoked the presence of one lyoniresinol isomer in oak wood by comparing GC–MS spectra but no further investigations were carried out to verify this hypothesis [18], [19].

Knowledge is therefore lacking about the existence of lyoniresinol isomers in oak wood and their organoleptic properties. Considering the strong bitterness developed by lyoniresinol in racemic mixture, the isolation, characterization and sensory study of such stereoisomers might be of particular relevance.

For this reason, we first sought lyoniresinol diastereoisomers in oak wood extract by targeted screening and Counter Current Chromatography (CCC) isolation guided by liquid chromatography coupled with high resolution mass spectrometry (LC–HRMS). Then, an original enantiomeric resolution was implemented by hydrolysis of natural xyloside derivatives present in oak wood. Both enantiomers were isolated and their absolute configuration was determined unambiguously for the first time by means of chiroptical spectroscopic measurements. The gustatory properties of all the purified compounds were assessed and their presence in wine aged in oak barrels was studied to investigate whether the stereochemistry of lyoniresinol might influence the taste of wine and more generally the pleasure of the consumer.

2. Materials and methods

2.1. Chemicals and materials

Oak wood used in this study was heartwood staves of *Quercus petraea* from various French forests. The staves were 2-year air-dried and then reduced to chips by the cooperage industry (Seguin Moreau, Merpins, France). Two wines were used in this study: a non-oaked white Bordeaux 2013 (100% Sauvignon blanc, 13% vol. alc.) for sensory tests and a red Margaux 2012 (90% Cabernet Sauvignon and 10% Merlot, 13.5% vol. alc.) aged in new French oak barrels for 15 months for chemical analysis.

All solvents were HPLC grade (VWR International, Pessac, France) except acetonitrile used for HRMS analysis (Optima® LCMS grade, Fisher Scientific, Fair Lawn, USA) and deionized water (MilliQ, Millipore, Bedford, USA). Lyoniresinol, lyoniside ((+)-lyoniresinol

9′-O-β-xylopyranoside) and nudiposide ((−)-lyoniresinol 9′-O-β-xylopyranoside) were isolated as described previously by Marchal et al., 2014 [6].

2.2. Preparation of pre-purified oak wood extract

Oak wood extract was obtained from a hydro-alcoholic solution (50:50; EtOH/H2O) of wood chips (250 g/L) for two weeks at room temperature. After a 0.45 µm filtration, the hydro-alcoholic solution was concentrated in vacuo to remove ethanol. The aqueous solution was extracted three times with 200 mL of ethyl acetate (EtOAc). The combined organic layers were evaporated to dryness, suspended in water and freeze-dried to obtain a brownish free-flowing powder of EtOAc prepurified extract (1.9 g).

2.3. LC–HRMS analysis

The LC–HRMS apparatus 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 HESI I probe (both from Thermo Fisher Scientific, Les Ulis, France). Liquid chromatography separation was carried out on a C18 column (Hypersil Gold 2.1 mm × 100 mm, 1.9 µm particle size, Thermo Fisher Scientific) with water (Eluent A) and acetonitrile (Eluent B) as mobile phases. The flow rate was set at 600 µL/min and the injection volume was 5 µL. Eluent B varied as follows: 0 min, 14%; 0.5 min, 14%; 1.5 min, 19%; 2 min, 19%; 4.5 min, 38%; 4.6 min, 98%; 6.9 min, 98%; 7 min, 14%; 8.6 min, 14%. Chiral chromatography analysis were performed on a Chiralpak® IB-3 column (2.1 mm × 150 mm, 3 µm particle size) with a flow rate set at 300 µL/min and an isocratic elution mode (water/acetonitrile; 80:20; v/v).

Mass acquisitions were performed in negative Fourier transform mass spectrometry (FTMS) ionization mode. The chromatographic conditions as well as the ionization and spectrometric parameters are described in Supplementary Data.

2.4. Centrifugal partition chromatography fractionation

The detailed description of the Centrifugal partition chromatography (CPC) apparatus, its preparation and the procedure implemented for choosing the system are presented in Supplementary Data. The quaternary system (*n*-heptane/ethyl acetate/acetonitrile/water 1:4:1.29:4 v/v) was used to fractionate the EtOAc prepurified extract in a 100 mL rotor. For each injection, the extract (0.95 g) was dissolved in 10 mL of a mixture consisting of upper and lower phases (4 mL and 6 mL, respectively) of the system, 0.45 µm filtered and injected. The

experiment was performed in ascending mode at 2500 rpm with a 10 mL/min flow rate for 45 min. The Spot prep fraction collector was set at 1 tube/min. Every five CPC tubes, an aliquot (10 µL) was taken, evaporated, dissolved in 1 mL of water/methanol 95:5 and analyzed by LC–HRMS to constitute a fraction enriched in compound **2**. To obtain such a fraction, CPC tubes were pooled, evaporated in vacuo, suspended in water and freeze-dried.

2.5. Preparative High Performance Liquid Chromatography purification

The details of the apparatus and procedures used for High Performance Liquid Chromatography (HPLC) purification are presented in Supplementary Data. For each injection, aliquots (25–40 mg) of samples to be purified were introduced manually into the system. Chromatographic peaks were collected manually just downstream the UV detector. Samples obtained after successive injections were pooled, evaporated in vacuo to remove acetonitrile and freeze-dried twice.

2.6. Racemic resolution of lyoniresinol enantiomers

Lyoniside (57.4 mg) and nudiposide (58.6 mg) were separately solubilized in a 4 mol L^{-1} TFA solution and placed under reflux at 80 °C. Experiments were performed in parallel under a nitrogen atmosphere for 14 h. After reaction, the solutions were evaporated in vacuo to remove TFA traces, suspended in water and freeze-dried twice. The crude reaction mixtures were purified using preparative HPLC.

2.7. NMR experiments

All 1D and 2D NMR experiments were performed 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 NMR spectra were acquired at 300 K in methanol-d_4. ^{1}H and ^{13}C chemical shifts were referenced to solvent signals. Data were processed using TOPSPIN software (Bruker). Molecule assignments were obtained by two-dimensional ^{1}H–^{1}H COSY, ^{1}H–^{1}H ROESY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC experiments.

2.8. Polarimetry

A JASCO P-2000 polarimeter with a sodium emission wavelength (λ = 589 nm) was used to determine the specific optical rotations of lyoniresinol stereoisomers in methanol at 293 K.

2.9. Vibrational circular dichroism measurements

Infrared (IR) and Vibrational Circular Dichroism (VCD) spectra were recorded with a ThermoNicolet Nexus 670 FTIR spectrometer equipped with a VCD optical bench [20]. In this optical bench, the light beam was focused by a BaF_2 lens (191 mm focal length) onto the sample, passing an optical filter, a BaF_2 wire grid polarizer (Specac) and a ZnSe photoelastic modulator (Hinds Instruments, Type II/ZS50). The light was focused by a ZnSe lens (38.1 mm focal length) onto a 1 × 1 mm^2 HgCdTe (ThermoNicolet, MCTA* E6032) detector. IR and VCD spectra were recorded at a resolution of 4 cm^{-1} by co-adding 50 scans and 36000 scans (12 h acquisition time), respectively. The sample was held in a fixed path length (100 μm) cell with BaF_2 windows. IR and VCD spectra of lyoniresinol enantiomers **1a** and **1b** were measured in DMSO-d_6 at a concentration of 50 mM. In all experiments, the photoelastic modulator was adjusted for a maximum efficiency at 1400 cm^{-1}. Calculations were done with the standard ThermoNicolet software, using Happ and Genzel apodization, de-Haseth phase correction, and a zero-filling factor of one. Calibration spectra were recorded using a birefringent plate (CdSe) and a second BaF_2 wire grid polarizer, according to a published procedure [21].

2.10. Density functional theory calculations

The calculation of the IR and VCD spectra began with a thorough analysis of the conformational freedom of the lyoniresinol molecule. This involved exploring the entire conformational energy surface of the (8*R*,8′*R*,7′*S*)-enantiomer (**1a**) and carrying out semi-empirical RM1 [22] calculations with the simulated annealing technique [23], as both implemented in the Ampac package [24], of the relative energies of conformers found in the various local minima of this surface. Energy minima were sought in two stages: (i) a non-local search focused on the main dihedral angles and (ii) a local energy relaxation of the whole degrees of freedom for each of the minima collected at stage (i). Seventy-two conformers within roughly 3 kcal/mol of the lowest energy conformer were kept for further density functional theory (DFT) calculations.

The geometry optimizations, vibrational frequencies, absorption and VCD intensities were calculated with the Gaussian 09 program [25] on the DELL cluster of the MCIA computing center at the University of Bordeaux. Calculations of the optimized geometry of 72 conformers of (8*R*,8′*R*,7′*S*)-lyoniresinol were performed at the density functional theory level using the B3PW91 functional and the 6-311G** basis set. Vibrational frequencies, IR and VCD intensities were calculated at the same level of theory using the magnetic field perturbation method with gauge-invariant atomic orbitals [26]. The spectra were calculated for the isolated molecule in vacuo. For comparison with the experiment, the calculated frequencies were scaled

by 0.968 and the calculated intensities were converted to Lorentzian bands with a half-width of 7 cm^{-1}. The experimental IR spectra of the (+)- and (−)-lyoniresinol as well as the predicted IR spectrum of (8*R*, 8′*R*, 7′*S*)-lyoniresinol are reported in Supplementary Data (Fig. S7).
All spectroscopic data concerning **1a**, **1b** and **2** are available in Supplementary material.

2.11. Sensory analysis of purified compounds

Tasting sessions took place in a specific room air-conditioned at 20 °C and equipped with individual booths and normalized glasses. Compounds were tasted in a hydro-alcoholic solution composed of pure and demineralized water (eau de source de Montagne, Laqueuille, France) and distilled ethanol, as well as in a white non-oaked wine. Compounds were dissolved at 2 mg L^{-1} in a 12% vol. alc. hydro-alcoholic solution. Samples were tasted by five wine-tasting experts. They were asked to describe the gustatory perception of each compound using wine tasting vocabulary and to evaluate the bitterness intensity on a scale from 0 (not detectable) to 5 (strongly detectable).

3. Results and discussion

3.1. Evidence and purification of one lyoniresinol isomer

Owing to differences in their physico-chemical properties, structural isomers and diasteroisomers can be separated in a non-chiral environment. Consequently, ultra high-performance liquid chromatography (U-HPLC) equipped with a classical C18 column was coupled with Fourier Transform mass spectrometry (FTMS) to search for such isomers of lyoniresinol by targeting the *m/z* 419.1712 corresponding to its deprotonated ion $C22H27O8^{-}$. This MS technique is very efficient for screening unknown compounds in complex matrixes. Indeed, its high sensitivity together with the specificity afforded by accurate mass measurement allowed its implementation in full scan mode, whereas the use of triple-quadrupole spectrometers in multiple reaction monitoring (MRM) mode requires preliminary optimization of transitions with pure compounds.

As shown in Fig. 1, the analysis of a hydro-ethanolic oak wood extract showed two peaks at retention times of 2.69 and 2.85 min in the extracted ion chromatogram (XIC) recorded in a 5 ppm window around *m/z* 419.1712. The gradient was optimized for a distinct separation of the two signals. The analysis of the same sample boosted with a stock solution of pure lyoniresinol resulted in an increase in the intensity of the dominant peak at the retention time

of 2.69 min, establishing that this first peak was lyoniresinol (compound **1**). The existence of a second peak at a retention time of 2.85 min highlighted the existence of an isomer of lyoniresinol in the oak wood extract (compound **2**). Such an isomer has never been reported in oak wood. To purify this new isomer, an LC–HRMS-guided procedure was developed.

After AcOEt extraction to eliminate the most polar compounds and in particular glycosylated lignans, the prepurified AcOEt extract was further fractionated by centrifugal partition chromatography (CPC). An appropriate solvent system was chosen to maximize the differences between the calculated partition coefficients *Kd*. The system *n*-heptane/ethyl acetate/acetonitrile/water 1:4:1.29:4 v/v gave the best results with *Kd* of 1.7 and 1.1 for compounds **1** and **2**, respectively. Nevertheless, considering these values, the two compounds are not likely to be completely separated, but a fraction enriched in the targeted compound might be obtained. CPC fractionation using the 100 mL rotor allows the injection of up to 1 g of AcOEt prepurified extract with a minimum consumption of solvent for 45 min. Two successive injections were performed to process the whole extract. After LC–HRMS analyses, CPC tubes were pooled and freeze-dried to make one enriched fraction of lyoniresinol isomer. This fraction was then submitted to preparative HPLC with an optimized gradient for compound separation. The purity of the isolated lyoniresinol isomer was found to be higher than 95% by LC–HRMS analysis.

3.2. Structural and sensory analysis of lyoniresinol diastereoisomer

To elucidate the chemical structure of this lyoniresinol isomer, 1D and 2D NMR experiments were performed (Supplementary Data, Figs. S1 to S3). ^{1}H and ^{13}C NMR signals were assigned as presented in Supplementary Data (Table S1) and were compared to those reported in the literature for *epi*-lyoniresinol [17]. In particular, the relative configuration of the stereogenic carbons (C-8, C-7′, and C-8′) was established by ROESY. The ROEs correlation between H-2′(H-6′)/H-8 and H-7′/H-8′ indicated that H-7′ and H-8′ are *cis*-orientated (cofacial). Moreover, the absence of a cross peak between H-8/H-8′ suggested that these protons were *trans*-orientated (Supplementary Data, Fig. S3). Thus, these results established that the purified molecule is a diastereoisomer of lyoniresinol (compound **2**) whose relative configuration was concluded to differ from lyoniresinol by the epimerization on C-7′ position, as shown in Fig. 2. This diastereoisomer has already been identified in the bark of *Aegle marmelos* by Ohashi et al. [17], but we report here for the first time its identification in *Quercus* genus.

The specific optical rotation of *epi*-lyoniresinol **2** was measured to be −39 and its analysis by LC–HRMS using a chiral column revealed the presence of two chromatographic

peaks (Supplementary Data, Figure S4). Based on these observations and on literature data showing a specific optical rotation of −140.8 for (−)-*epi*-lyoniresinol [17], *epi*-lyoniresinol **2** appeared to be present in oak wood as a mixture of both its enantiomeric forms but with an excess of the levorotatory form.

Unlike lyoniresinol [27], the presence of *epi*-lyoniresinol in wine has not been described until now. Fig. S5 in Supplementary Data presents extracted ion chromatograms (XIC) obtained in an oaked red wine for an *m/z* ratio specific to lyoniresinol and *epi*-lyoniresinol. Two signals were detected at 2.64 and 2.80 min. These retention times were similar (0.05 min lower) to those measured for oak wood extract in a 5 ppm window, demonstrating the presence of both compounds **1** and **2** in oaked red wine. Consequently, this study is the first to identify **epi**-lyoniresinol in wine.

Owing to the sensory properties of lyoniresinol **1** with a perception threshold of bitterness evaluated at 1.5 mg/L by Marchal et al. 2014 [6], we decided to analyze the taste perception of *epi*-lyoniresinol. Therefore, *epi*-lyoniresinol **2** was tasted at 2 mg/L in a 12% vol. alc. hydro-alcoholic as well as in a non-oaked white wine. Experts described its taste as not bitter and barely slightly sweet in comparison to the control medium solution.

Considering these results and the lower amount of *epi*-lyoniresinol compared to lyoniresinol both in oak wood and oaked wine, *epi*-lyoniresinol appeared unlikely to influence the taste of wine. Consequently, no further investigations were conducted on this compound or on its enantiomeric forms.

3.3. Enantiomeric resolution of lyoniresinol

LC–HRMS analysis of an oak wood extract with a C18 column revealed the existence of two diastereoisomers: lyoniresinol **1** and *epi*-lyoniresinol **2**. Nevertheless, the specific optical rotation of lyoniresinol **1** was reported to be close to zero in oak wood [6] indicating a mixture of two enantiomers **1a** and **1b** (Fig. 2) in the extracted compound. Thus, all sensory studies concerning lyoniresinol until now have been carried out using a racemic mixture purified from oak wood and the properties of each enantiomer have never been individually assessed. Generally, the racemate resolution is performed using HPLC with a chiral column at a preparative scale, but such a column remains very costly. Another approach is to derivatize the enantiomers previously with the same chiral moiety in order to obtain diastereoisomers, which can be separated by non-chiral chromatography. After separation, each diastereoisomer can be hydrolyzed to obtain the targeted enantiomers.

In this study, we decided to resolve the lyoniresinol racemic mixture by separation and hydrolysis of natural derivatized diastereroisomers. Indeed, previous studies have established the presence in oak wood of lyoniside **3** and nudiposide **4**, two native xylopyranoside-derivatives of (+)- and (−)-lyoniresinol, respectively [28], [29]. For this purpose, lyoniside **3** and nudiposide **4** purified as previously described [6] were heated in acidic conditions under inert atmosphere to hydrolyze the acetal formed between the xylose aldehyde and the primary alcohol (C9′) of lyoniresinol, as shown in Fig. 3. TFA was used to acidify the solution since it could be easily removed *in vacuo* from the crude reaction mixture.

The hydrolysis was monitored by LC–HRMS in order to ensure a complete reaction with a total disappearance of lyoniside **3** and nudiposide **4** through xylopyranoside cleavage from the genin, leading to lyoniresinol enantiomers 1a and 1b and xylopyranoside by-products (Supplementary Data, Fig. S6).

Preparative HPLC was performed on each crude reaction mixture to purify **1a** and **1b** by removing all by-products. The percentage yield of the purification of (+)- and (−)-lyoniresinol enantiomers was 44.6% and 43.5%, respectively. Each purified enantiomer was analyzed by chiral liquid chromatography using an analytical chiral column. As shown in Fig. 4, (±)-lyoniresinol **1** injection revealed two peaks at 5.88 min and 7.16 min, while the purified (+)- and (−)-lyoniresinol enantiomers each presented only one peak at 5.92 min and 7.17 min, respectively, confirming the efficiency of the racemic resolution.

3.4. Unambiguous determination of the absolute configurations of lyoniresinol enantiomers

1D and 2D NMR experiments were performed on each enantiomer to confirm their relative configuration. ^{1}H NMR spectra of (+)- and (−)-lyoniresinol enantiomers superimposed on that of (±)-lyoniresinol **1** (Supplementary Data, Fig. S1) and the NMR data (Supplementary Data, Table S1) were identical to those reported in the literature for lyoniresinol [28], [30], [31], [32]. The relative configuration of **1** was established on the basis of ROEs correlations between protons H-2′(H-6′)/H-8′ and H-7′/H-8, suggesting that H-7′ and H-8′ are *trans*-orientated and that H-7′ and H-8 are cofacial (Supplementary Data, Fig. S2).

Specific optical rotation of each enantiomer was measured to be +43 and −45. These values are similar with opposite signs, confirming their enantiomeric relationship. Since the two enantiomers of lyoniresinol had been successfully isolated, we decided to determine their absolute configurations.

The absolute configuration of (+)-lyoniresinol has been previously proposed to be (8*R*,8′*R*,7′*S*)-(+)-lyoniresinol by degradation reactions and comparison with known compounds [30], [33]. Nevertheless, the absolute configuration of lyoniresinol has never been confirmed by chiroptical spectroscopic experiments (electronic or vibrational circular dichroism) associated with theoretical calculations. In this study, we used vibrational circular dichroism (VCD) which is a well-established method for determining the absolute configuration and conformation of chiral molecules in solution [34]. The method entails comparison of measured VCD spectra with the spectrum calculated at the density functional theory (DFT) level for a specified absolute configuration [35]. Because enantiomers have a VCD band of opposite sign for each vibrational mode, the VCD spectrum provides a unique rich signature of the absolute configuration.

The VCD spectra of the two enantiomers of lyoniresinol were recorded in DMSO-d_6 at a concentration of 50 mM and are reported in Fig. 5. The two spectra are opposite with respect to the baseline, confirming that the two molecules are enantiomers. Theoretical calculation of the (8*R*,8′*R*,7′*S*)-lyoniresinol (compound **1a** in Fig. 2) was performed to determine the absolute configuration of (+)- and (−)-lyoniresinol. The conformational analysis of **1a** was carried out initially by using semi-empirical RM1 calculations with the simulated annealing technique. Seventy-two conformers of **1a** with energies within 3 kcal/mol of the lowest energy conformer were kept. Subsequently, the 72 RM1 conformations of **1a** were optimized by using DFT at the B3PW91/6-311G** level. Harmonic vibrational frequencies were calculated at the same level to confirm that all structures were stable conformations and to enable free energies to be calculated. The VCD spectrum predicted on the basis of the relative populations of (8*R*,8′*R*,7′*S*)-lyoniresinol was compared to the experimental VCD spectra of (+)- and (−)-lyoniresinol (Fig. 5). The predicted VCD spectrum of (8*R*,8′*R*,7′*S*)-lyoniresinol isomer reproduced fairly well the intensity and the sign of most bands observed in the experimental spectrum of (+)-lyoniresinol, confirming the (8*R*,8′*R*,7′*S*)-(+)-lyoniresinol absolute configuration proposed by Kato [30], [33].

3.5. Gustatory properties of enantiomers and impact on wine taste

To measure the gustatory impact of each lyoniresinol enantiomer on the bitter perception of their racemic mixture 1, enantiomers 1a and 1b were tasted at 2 mg/L in a 12% vol. alc. hydro-alcoholic solution and compared to the control medium solution. The same concentrations were tasted in a non-oaked white wine. Experts described for the first time that lyoniresinol **1a** exhibited a strong bitterness equivalent to the bitterness of (±)-lyoniresinol **1** at

4 mg/L, while lyoniresinol **1b** exhibited no taste. Thus, the bitterness of (±)-lyoniresinol **1** comes from that of (+)-lyoniresinol **1a** while (−)-lyoniresinol **1b** is not involved. Finally, an oaked Margaux wine was analyzed by LC–HRMS equipped with a chiral column to search for the presence of lyoniresinol enantiomers. The XIC for an *m/z* ratio specific to lyoniresinol showed two peaks at 5.94 and 7.15 min corresponding to lyoniresinol enantiomers **1a** and **1b**, respectively (Fig. 6). Analysis of spiked samples confirmed that both (+)-lyoniresinol **1a** and (−)-lyoniresinol **1b** were present in this red oaked wine with a relative abundance similar to that observed in oak wood. In view of these results and the gustatory properties of both enantiomers, it appears that only (+)-lyoniresinol 1a is likely to influence the taste of wine by increasing its bitterness.

4. Conclusion

This study focused on the use of analytical techniques to highlight the relationship between stereochemistry and the organoleptic properties of lyoniresinol. A diastereoisomer, *epi*-lyoniresinol, was identified for the first time in *Quercus* genus using a CCC isolation procedure guided by LC–HRMS. Moreover, two enantiomers of lyoniresinol were separated by a racemic resolution from natural derivatives. This original approach appears promising for natural products since it is cheaper than chiral separation and more compatible with trends in green chemistry than enantiospecific synthesis. This racemic resolution allowed for the first time the unambiguous determination of the absolute configuration of the two enantiomers by means of VCD measurements associated with DFT calculations. The presence of all these lyoniresinol stereoisomers was established in oaked wine by LC–HRMS analysis. Finally, sensory analyses were carried out to determine their gustatory properties. Whereas (+)-lyoniresinol exhibited strong bitterness, its enantiomer was tasteless and *epi*-lyoniresinol was slightly sweet. These results demonstrate that stereochemistry strongly influences the taste of wine compounds and subsequently the perception of consumers.

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Figures

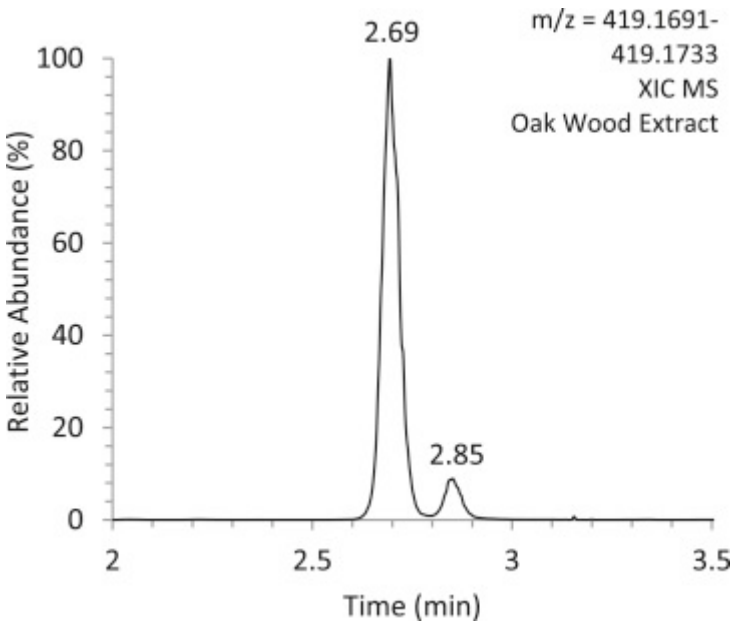

Fig. 1. Negative LC–HRMS XIC of an oak wood extract corresponding to $C_{22}H_{27}O_8^-$ ion (*m/z* 419.1712 with a 5 ppm accuracy).

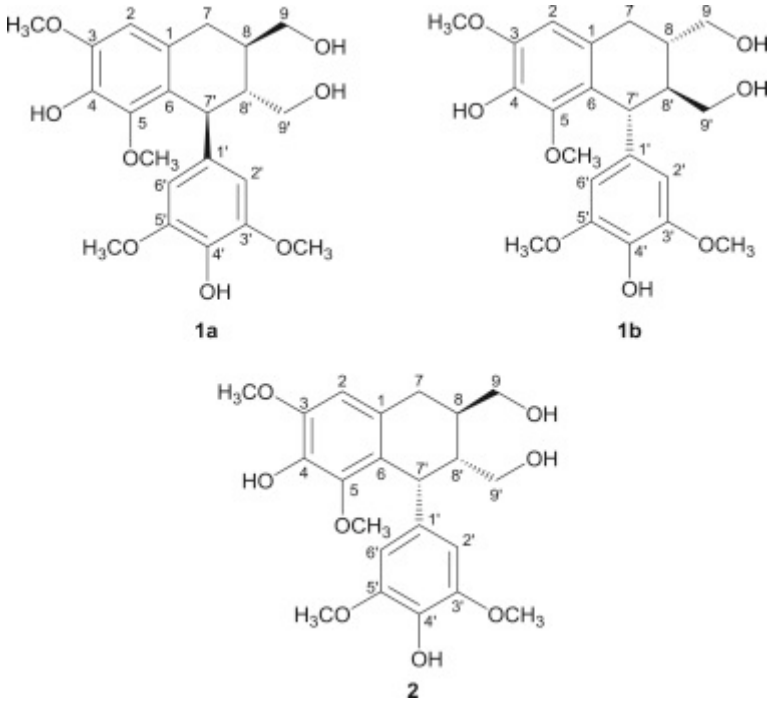

Fig. 2. Chemical structures of lyoniresinol enantiomers **1a** and **1b** (absolute configuration) and *epi*-lyoniresinol **2** (relative configuration).

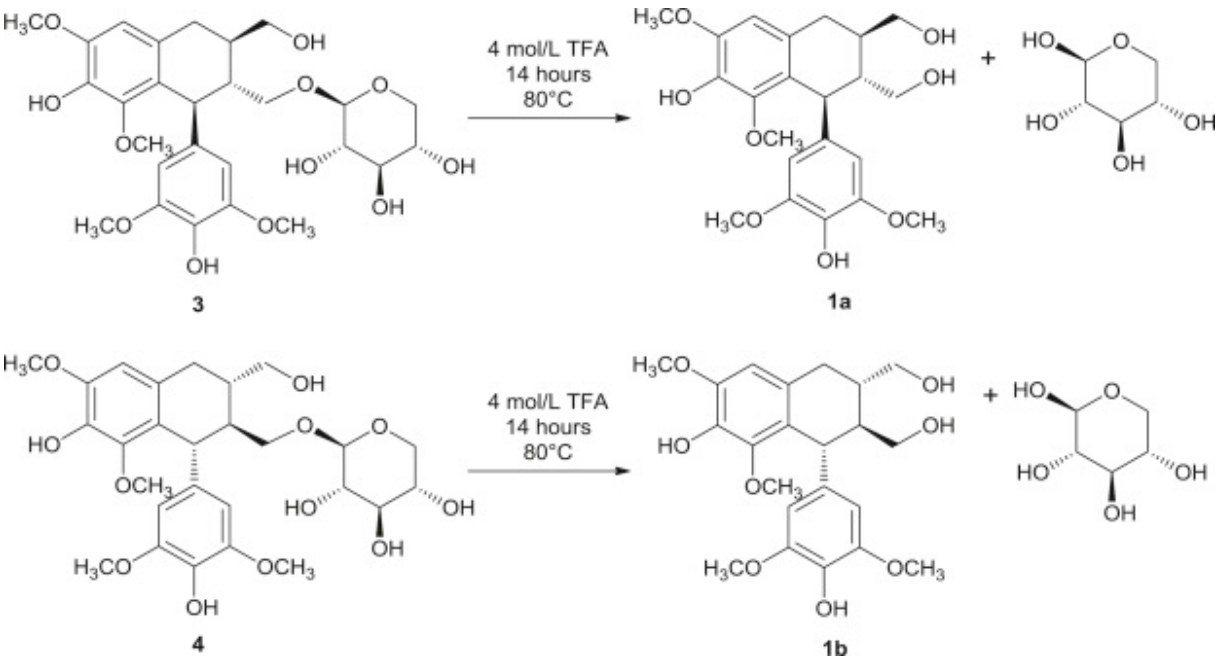

Fig. 3. Release of lyoniresinol enantiomers **1a** and **1b** by acidic hydrolysis of lyoniside **3** and nudiposide **4**, respectively.

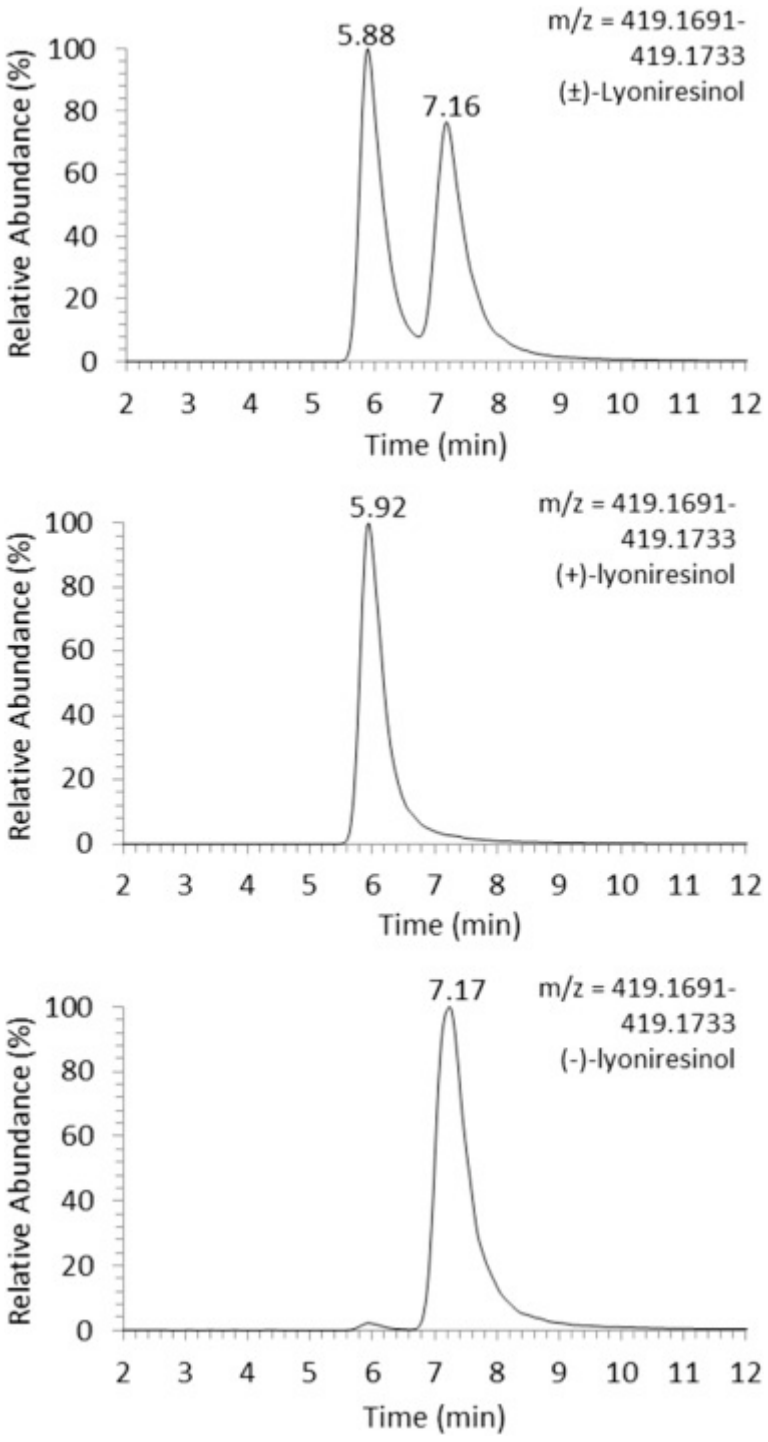

Fig. 4. LC–HRMS chromatograms of (±)-lyoniresinol, (+)-lyoniresinol and (−)-lyoniresinol (from top to bottom) on a chiral column.

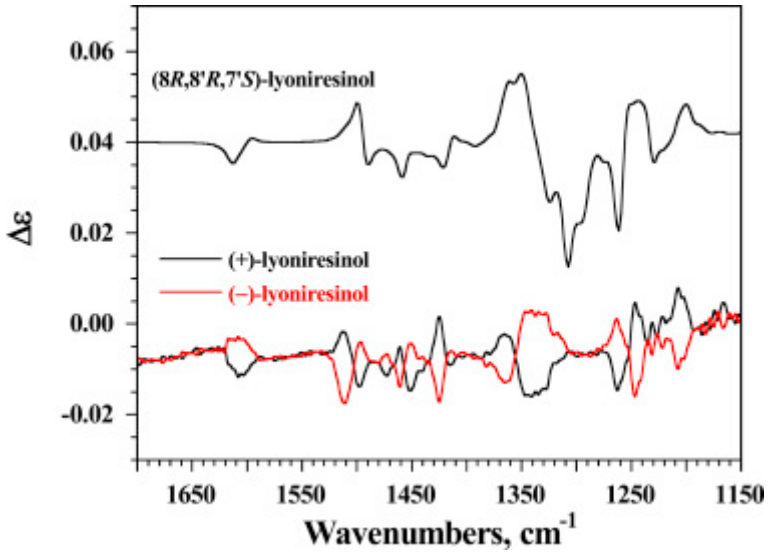

Fig. 5. Comparison of experimental VCD spectra of (+)- and (−)-lyoniresinol recorded in DMSO-d_6 solution (50 mM, 100 μm path length, bottom) with the predicted VCD spectrum of (8*R*,8′*R*,7′*S*)-lyoniresinol isomer calculated using DFT at the B3PW91/6-311G** level (top).

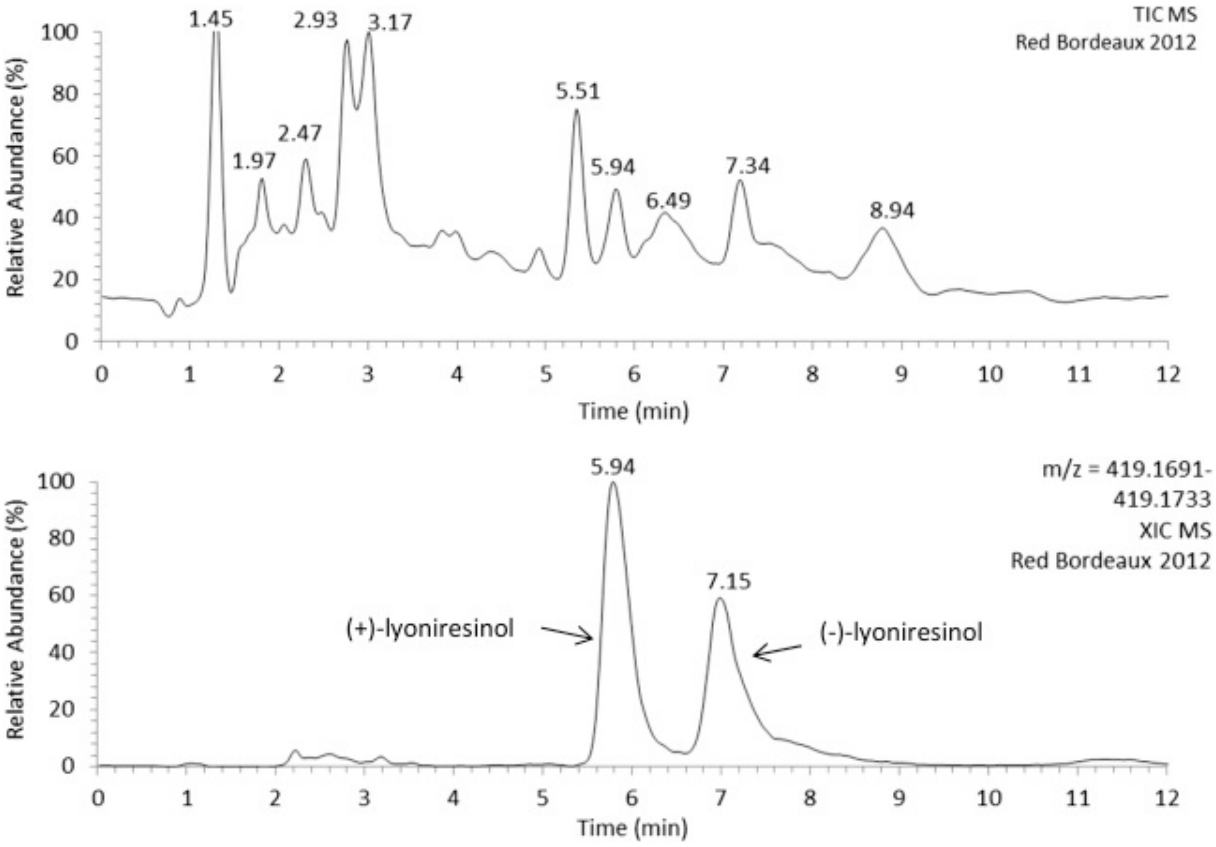

Fig. 6. Negative LC–HRMS TIC (top) and XIC corresponding to $C_{22}H_{27}O_8^-$ ion (bottom) recorded in an oaked Margaux red wine analyzed with a chiral column.