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To cite this version:

Axel Marchal, Pierre Waffo-Téguo, Eric Génin, Jean-Michel Merillon, Denis Dubourdieu. Identification of new natural sweet compounds in wine using centrifugal partition chromatography-gustatometry and fourier transform mass spectrometry. Analytical Chemistry, 2011, 83 (24), pp.9629 - 9637. 10.1021/ac202499a . hal-02645956

HAL Id: hal-02645956
https://hal.inrae.fr/hal-02645956

Submitted on 29 May 2020

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analytical chemistry

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Identification of New Natural Sweet Compounds in Wine Using Centrifugal Partition Chromatography−Gustatometry and Fourier Transform Mass Spectrometry

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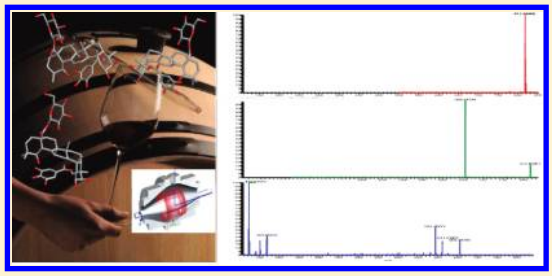
S *Supporting Information*

ABSTRACT: Sweetness contributes notably to the taste-balance of dry wines and increases during oak-barrel aging owing to the release of natural sweeteners from wood. The search for such taste-active molecules, which are sometimes present at very low concentrations in wine or other complex matrixes, requires both reliable purification tools and powerful identification techniques. Here, we report the development of an original inductive method using centrifugal partition chromatography (CPC) and sensorial analysis. This method, called CPC−gustatometry, was implemented to isolate a sweet fraction with only four compounds from a complex oak wood extract. The recently developed Fourier transform mass spectrometry (FT-MS, Orbitrap analyzer) was used jointly with two-dimensional nuclear magnetic resonance (2D ^{1}H and ^{13}C NMR) to obtain the structural elucidation of the purified compounds. The tandem mass spectrometry (MS/MS) spectra obtained with resonant and nonresonant fragmentation modes were compared, thus providing complementary information about the molecular structure. Two oleanane-type triterpenoids substituted with galloyl and glucosyl moieties were identified, one of which exhibits sweet properties. We term these compounds which have never been reported, Quercotriterpenoside I and II.



Wine quality, i.e., the pleasure that a wine brings to the taster, depends on the complexity and the harmony of its aromas and tastes. The mental representation of this quality in the taster's field of consciousness is based on the sensorial perception of the various wine chemicals followed by the neurophysiological and cognitive integration of the perceived stimuli.[1]

The sweet taste, conjuring up the taste of sucrose, paradoxically characterizes the gustatory balance of dry wines and particularly great wines. It softens the acidity, bitterness, and astringency generated by some wine molecules like organic acids and polyphenols.[2] In such dry wines, grape carbohydrates (mainly glucose and fructose) are almost completely transformed by yeasts, so they are present only at very low concentrations and are not perceptible. The perceived sweetness corresponds therefore to "sweetness without sugar" and ensures the consumer's pleasure. However, despite evidence of their sensorial importance, the molecular determinants of sweetness in dry wines have never been elucidated.

Nevertheless, winemakers are convinced that aging in oak wood barrels has a sweetening effect on wine.[3] A similar taste improvement contributes to maturation and the global quality of spirits.

In addition, it is well-known that molecules exhibiting sweet taste are naturally contained in plants, and some of them are now used as sweeteners in the food industry like steviosides.[4−6]

Consequently, nonvolatile compounds with sweet taste may be present in oak wood and then be released in wines and spirits during aging. The search for such compounds is comparable to the study of natural substances with biological and pharmacological properties, a pathway that has led to numerous analytical developments.[7−10] This kind of research involves a dual requirement: purification (only traces of active molecules are present in a complex matrix) and identification (compounds are purified following their biological activity and not their chemical structure, which therefore requires elucidation).[11,12] Consequently, it is necessary to use efficient analytical techniques to meet this dual challenge.

Among these techniques, centrifugal partition chromatography (CPC) is particularly suited for the fractionation of complex extracts, owing to the large amounts that can be injected and the lack of irreversible adsorption with the stationary phase.[13−15] By implementing this technique, we have developed an original taste-guided purification protocol, i.e., fractionation steps of an oak extract have been hyphenated with tasting of the obtained fractions.

Received: September 21, 2011
Accepted: November 1, 2011
Published: November 01, 2011

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However, while the purification of compounds makes it possible to test their organoleptic properties, complementary analytical methods are necessary for structural determination. Fourier transform mass spectrometry has been the subject of promising recent developments (the Orbitrap analyzer).[16,17] Its use in conjunction with multidimensional NMR[18] provides valuable information with a view to determining the molecular structure of natural purified compounds.

The implementation of these different techniques allowed us to isolate and identify new oak wood compounds exhibiting a sweet taste. The methodology developed constitutes a novel and promising approach to study compounds responsible for wine flavor and other taste-active molecules, including novel sweeteners of plant origin.

EXPERIMENTAL SECTION

Taste-Guided Fractionation and Purification. *CPC Apparatus.* The fractionation was performed on an FCPC 200 Kromaton Technologies apparatus (Angers, France) fitted with a rotor made of 20 circular partition disks (1320 partition cells, 130 μL per cell; column capacity of 204 mL; dead volume, 32.3 mL). The rotation speed can be adjusted from 0 to 2000 rpm. The solvents were pumped by a Gilson 321-H1 two-way binary high-pressure gradient pump. The samples were introduced into the CPC column via a high-pressure injection valve (3725(i)038 Rheodyne) equipped with a 10 mL sample loop. The effluent was monitored with a Varian UV Prostar 325 (Les Ulis, France) detector in dual mode equipped with a preparative flow cell. Fractions were collected by a Gilson FC 204 fraction collector. The experiments were conducted at room temperature.

Solvent System Screening. The choice of an appropriate biphasic system of solvents is based on the study of the partition of the compounds of the extract in both phases. In practice, 1 mg of extract is dissolved in a biphasic system (2 mL) in a vial. After shaking, 100 μL of each phase was evaporated to dryness, resolubilized with 1 mL of methanol/water (1:1; v/v), and injected into an analytic HPLC. The partition coefficient K_d was calculated as the ratio of the solute area in each phase. The quaternary Arizona systems (*n*-heptane/ethyl acetate/methanol/water) B, C, D, F, H, J, K, L, and M were tested.[19,20] In optimal conditions in ascending mode where $K_d \approx 1$, the compounds are partitioned equally between the two phases and a satisfactory separation may occur. The Arizona-G system (1:4:1:4, v/v) was chosen on this basis.

Preparation of CPC Solvent. A biphasic system was prepared by mixing *n*-heptane (200 mL), ethyl acetate (EtOAc, 800 mL), methanol (MeOH, 200 mL), and H_2O (800 mL) in a separatory funnel at room temperature, shaking vigorously, and allowing them to settle until the phases became limpid. The resulting two phases were separated just before use.

Preparation of the Prepurified Extract. The oak heartwood used in this work came from staves of *Quercus petraea* trees grown in Caucasia and were seasoned outside, without shelter, for at least 24 months. A total of 500 g of wood chips (30 mm × 20 mm × 5 mm, Seguin-Moreau) were soaked in 2 L of hydro-alcoholic solution (50% ethanol) at 20 °C without stirring. After 20 days, the liquid medium was filtered (0.45 μm) and concentrated *in vacuo* to remove ethanol. The aqueous extract was extracted three times with 200 mL of *n*-heptane. This aqueous layer was then extracted with EtOAc (5 × 250 mL). The combined organic layers were evaporated *in vacuo*, suspended in water, and freeze-dried twice to give 802 mg of prepurified extract (brown-orange powder).

CPC Separation Procedure. In CPC, either the lower phase or upper phase of the biphasic solvent system can be used as the mobile phase. In our experiment, the rotor was filled by injecting 500 mL of the aqueous heavier stationary phase in ascending mode at 10 mL/min and at 300 rpm. Then the rotation speed was increased to 1200 rpm. The mobile phase was pumped into the column in ascending mode at 10 mL/min. When the thermodynamic equilibrium was reached (percentage of retention of the stationary phase, 72.5%), the sample solution containing 700 mg of dry prepurified extract dissolved in 6 mL of a mixture consisting of upper and lower phase (4:2, v/v) was injected through a 10 mL loop. Acquisition began at the moment of injection, and UV detection was carried out at 254 and 280 nm. The fraction collector was set at 1 tube/min. The system was switched to descending mode at tube 68. The aqueous heavier phase was pumped at 10 mL/min. The whole experiment lasted 90 min and was performed at room temperature (20 °C).

Analytic HPLC−UV. After the CPC experiment, the tubes were analyzed by HPLC−UV. A volume of 100 μL of liquid were taken from each tube, evaporated, and dissolved in water/acetonitrile 50/50. After filtration (0.45 μm), 10 μL of the samples obtained were analyzed on an Ultrasphere ODS C18 4.6 mm × 250 mm, 5 μm column (Beckman Coulter, Fullerton, CA) connected to a Spectra System autosampler, quaternary pump, and diode array detector (Thermo Fisher Scientific, Les Ulis, France). The mobile phases were water (A) and acetonitrile (B), each containing 0.1% trifluoroacetic acid. The gradient was 0 min, 10% B; 2 min, 10% B; 19 min, 70% B; 20 min, 95% B; 23 min, 95% B; 24 min, 10% B; 27 min, 10% B with a flow rate of 1 mL/min. Signal detection was carried out at 280 nm.

Constitution of Fractions and Tasting. After HPLC analysis, CPC tubes presenting similar chromatographic profiles were combined, evaporated *in vacuo*, suspended in water, and freeze-dried twice. A total of 15 fractions numbered from I to XV were also obtained and contained various quantities (from 3 to 148 mg) of powder (yellow to dark brown). In total, 3 mg of each fraction were suspended in 600 μL of ethanol and 60 mL of water. The samples were then tasted in normalized glasses by four experts in wine tasting. They described the gustatory perception for each fraction using the vocabulary of wine tasting and evaluated in particular the sweetness intensity (from 0 to 5).

HPLC Purification. Purification was performed on a C18 preparative column (Microsorb 100-5 250 mm × 21.4 mm, 5 μm particle size, Varian) connected to a precolumn (Prontosil C18 5 μm 50 mm × 20 mm, Bischoff chromatography). Solvents (water (A) and acetonitrile (B), both containing 0.05% trifluoroacetic acid) were pumped by a Prostar 218 two-way binary high-pressure gradient pump (Varian). The flow rate was 20 mL/min and eluant B followed this gradient: 0 min, 8%; 5 min, 18%; 13 min, 18%; 26 min, 26%; 35 min, 36%; 50 min, 50%, 52 min, 100%; 57 min, 100%. Initially, 17 mg of fraction X were dissolved in methanol (200 μL), filtered (0.45 μm), and manually introduced into the system. UV detection was carried out at 254 and 280 nm by a Prostar 345 detector (Varian). Chromatographic peaks were manually collected, just after the detector. Purification of the entire fraction X (52 mg) was completed by a total of three successive injections. The four samples obtained (corresponding to the four peaks of fraction X) were evaporated *in vacuo* to remove acetonitrile and freeze-dried twice to give four white powders. These compounds were added in water and dry white wine (10 mg/L, 100 mL) and then tasted by five experts in wine-tasting.

Table 1. Gustatogram of the AcOEt Prepurified Extract of Oak Wood

fraction	tubes	sensory description	obtained mass (mg)
I	8−13	bitter in attack, dry, "dust flavor"	148
II	14 and 15	"coconut flavor", intense and lingering bitterness	63
III	16−19	slightly sweet (intensity 2), then mushy in final	53
IV	20	slight bitterness	17
V	21−24	slightly sweet in attack (intensity 1), astringent and bitter	43
VI	25−31	sweetness in attack (intensity 2); very intense and lingering bitterness, astringency	57
VII	32−36	not very sapid, bitter in finish	44
VIII	37−39	intense astringency, bitter finish	15
IX	40−47	slight astringency, moderate sweetness (intensity 2)	28
X	48−63	very sapid, intense and lingering sweetness (intensity 4)	59
XI	64−68	bitterness, slight sweetness (intensity 2)	11
XII	69−73	intense sweetness perceived in attack (intensity 3), lingering on the tip of the tongue	27
XIII	74−78	tingly numbness, sweetness in finish (intensity 2), slight bitterness	57
XIV	79−81	sweetness (intensity 3)	7
XV	82−90	no taste	3

FTMS. Direct infusion measurements were performed on an LTQ Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany) mass spectrometer equipped with a heated ESI ion source. The sample was dissolved to 10 ng/μL in CH_3OH/H_2O 1:1 (v/v) and delivered by a syringe pump with a flow rate of 5 μL/min. External calibration of the mass spectrometer was performed using a mixture consisting of caffeine, MRFA peptide, and Ultramark 1621 polymer. The ion source was operated in the negative ion mode at 4 kV. The vaporizer temperature of the source was set at 150 °C, the capillary temperature at 280 °C, the nitrogen sheath gas at 40, and the auxiliary and sweep gas at 0 (arbitrary units). The ion lenses were automatically optimized using the 10 ng/μL solution of purified compounds. A mass range of 200−1000 Th was acquired in full scan MS mode.

Collision induced dissociation (CID) MS^2 and MS^3 studies were performed in the linear ion trap (LIT). In addition, nonresonant activation was also carried out in the higher collisional energy (HCD) cell situated at the far end of the C-Trap region.[21] In both cases, the product ions were detected in the Orbitrap analyzer in order to generate high mass accuracy data. Indeed, all the ions were detected with mass errors in the 1 ppm range.[22] The resolution setting was 100 000 ($m/\Delta m$, fwhm at m/z 400) in the MS and MS^n modes. The maximum injection time was set at 100 ms and the automated gain control (AGC) target value, respectively, at 5×10^5 in MS and 5×10^4 in MS^n. An isolation width of 1 Th was used both for the CID MS^n and the HCD MS/MS experiments.

NMR Experiments. The experiments were performed on a Bruker Avance 3 spectrometer (^{1}H 600 MHz, ^{13}C 150 MHz) equipped with a TXI 5 mm probe and a Bruker Avance DMX 500 spectrometer (^{1}H 500 MHz, ^{13}C 125 MHz) equipped with a TXI 5 mm cryo-probe. All NMR spectra were acquired at 27 °C in methanol-d_4. 1D ^{1}H NMR spectra were recorded with 32k data points, sweep width of 12 ppm, and pulse repetition time of 1.5 s. Correlation spectroscopy (COSY), rotating frame nuclear Overhauser effect spectroscopy (ROESY) (400 ms spin lock), and heteronuclear single-quantum correlation spectroscopy (HSQC)/total correlation spectroscopy (TOCSY) (150 ms spin lock) spectra were performed with 2048-256 data points. HSQC and heteronuclear multiple-bond correlation spectroscopy (HMBC) spectra were acquired using gradient selection. The heteronuclear correlations were optimized for $^1J_{C,H}$ 140 Hz and $^nJ_{C,H}$ 8−12 Hz depending on the sample. Spectral widths were adjusted to the individual samples. ^{1}H and ^{13}C chemical shifts were referenced to solvent signals of methanol-d_4, $\delta(^1H)$ 3.31 and $\delta(^{13}C)$ 49.1.

RESULTS AND DISCUSSION

Use of CPC Off-Line Hyphenated with Gustatometry to Isolate and Purify Sapid Compounds. *CPC Fractionation of Prepurified Extract.* The hydro-ethanolic extract of staves of *Quercus petraea* trees grown in Caucasia was partitioned successively using solvents with increasing polarity: *n*-heptane and EtOAc. The EtOAc prepurified extract was subjected to CPC using Arizona-G system *n*-heptane/EtOAc/MeOH/H_2O (1:4:1:4, v/v) to provide 90 tubes.

The CPC-UV profile (Figure S-1 in the Supporting Information) showed some distinct broad peaks distributed across the chromatogram, confirming the good partitioning of the extract. In order to locate the sapid compounds of the extract, the CPC tubes had to be tasted. Since there were many tubes (90), fractions were constituted by grouping some tubes together. Grouping was done on the basis of the analytical HPLC chromatographic profiles. The HPLC chromatograms obtained from the different tubes presented many peaks but were quite different, confirming the fractioning efficiency of the CPC. When grouping the tubes, samples presenting the same main peaks and similar HPLC chromatographic profiles were pooled together. The 15 (I to XV) fractions thus constituted provided 632 mg of powder after freeze-drying. In comparison to other chromatographic techniques, the yield of this CPC experiment (90%) was very high. This was mainly due to the principle of CPC, which involves no solid support, i.e., no irreversible adsorption. Moreover, CPC offers the possibility of injecting large quantities of extract. Consequently, this technique appears very powerful for the search for nonvolatile compounds with organoleptic properties in complex matrixes.

To locate the targeted compounds, all the fractions were tasted and their sweetness intensity was evaluated. Even if the number of tasters (four) was low owing to the small quantity of product available, their expertise in wine-tasting ensured the pertinence of their judgment.

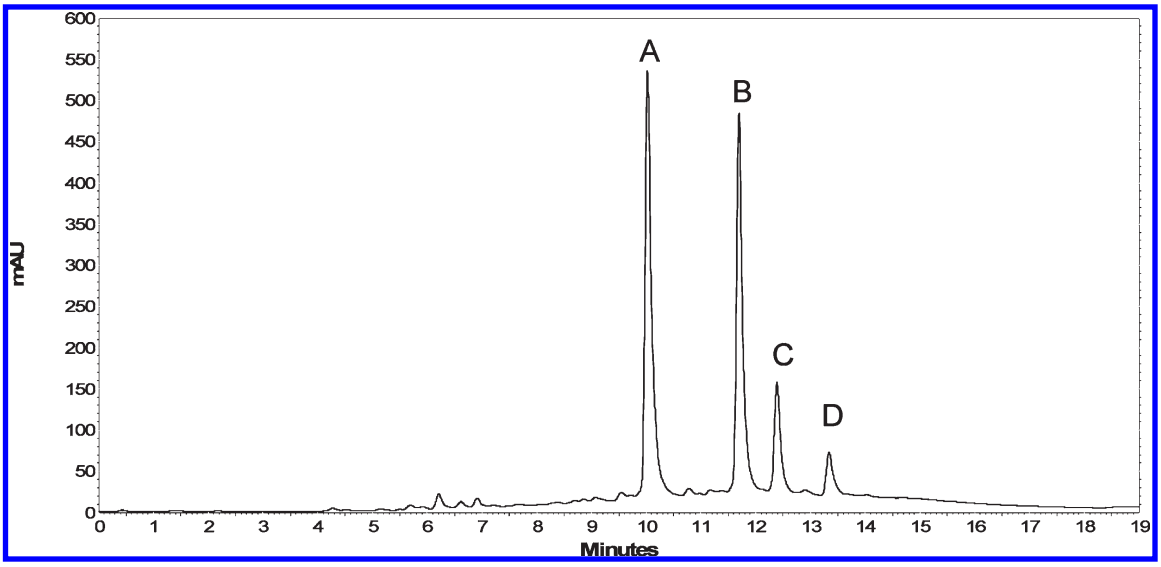

Figure 1. HPLC−UV chromatogram of fraction X: UV chromatogram at 280 nm of fraction X shows four well-separated and symmetric peaks corresponding to four compounds labeled **A**−**D**.

To study volatile compounds, aromaticians have for long hyphenated gas chromatography with olfaction (GC-O) and used the terms "olfactometry" and "olfactogram".[23,24] By analogy, we chose to call our technique "gustatometry". Centrifugal partition chromatography followed by gustatometry allowed the tasters to establish a gustatogram of the extract (Table 1). They used precise sensorial descriptors corresponding to fundamental taste (sweetness, bitterness), tactile sensations (astringency), and multimodal perceptions ("dusty flavor", "coconut flavor"). For most fractions, the taste was composed of multiple sensations, indicating their chemical heterogeneity as revealed by HPLC profiles.

As shown in Table 1, eight fractions developed a characteristic sweet taste with different intensities. The most intense sweetness was perceptible in fraction X (yellow powder, 59 mg). Its HPLC chromatogram (Figure 1) showed only four main peaks, corresponding probably to four compounds (**A**, **B**, **C**, and **D**, in increasing order of retention time).

A fraction containing only four compounds and exhibiting a sweet taste was also obtained from a highly complex extract. This new hyphenation technique termed CPC-G therefore appears powerful for the study of nonvolatile compounds with gustative properties. Because of the need for evaporation of organic solvents prior to sensorial analysis, this hyphenation technique is not online, contrary to GC-O.

Purification and Sensorial Characterization of Fraction X Compounds. Fraction **X** (52 mg) was submitted to preparative HPLC using a water/acetonitrile gradient. The four peaks were well separated and the compounds were obtained as white powders after freeze-drying (**A**, 16 mg; **B**, 4.9 mg; **C**, 1.2 mg; **D**, 0.3 mg). The general yield of this purification step (44%) was lower than for the CPC experiment but was commonly obtained with HPLC. Irreversible adsorption due to the solid phase in the C18 column and the manual collection method could explain this difference. This demonstrates the value of CPC vis-a-vis HPLC for extract fractionation, although the higher chromatographic resolution obtained in HPLC remains useful for final compound purification.

The same procedure was repeated three times and the purified compounds **A**, **B**, and **C** were then tasted at 10 mg/L in water and in dry white wine by five expert tasters. Despite the general sweetness of fraction **X**, its main constituent, compound **A**, was perceived as bitter by the tasters both in water and wine. Compound **B** was tasteless in water and did not modify the taste of wine. On the other hand, compound **C** exhibited a sweet taste in water, significantly enhanced the sweetness of wine, and reduced its bitterness. Therefore, this compound likely contributes substantially to the sweetness perceived in fraction **X**. Compound **D** was not tasted owing to the small quantity available (0.9 mg) and the destructive nature of gustative analysis. HPLC purification and sensorial characterization established the sweet properties of compound **C**, in particular when added in wine.

Molecular Identification of the Isolated Compounds Using FTMS and NMR. Sensory analysis allowed us to determine the taste of each compound purified from fraction **X** and showed in particular that compound **C** exhibits a sweet taste. In order to identify the structure of the isolated compounds, two powerful techniques were used: Fourier transform mass spectrometry (FTMS) and nuclear magnetic resonance (NMR). For this study, we used the example of the sweet compound **C** to illustrate the complementarity of these analytic techniques for identifying unknown natural molecules. The main results for the three others compounds are briefly presented.

Empirical Formula Determination Using FTMS. The mass accuracy, stability, and resolution afforded by FTMS are particularly useful for the empirical determination of the formulas of unknown natural molecules. The full scan spectrum of compound **C** (Figure 2) exhibits an $[M - H]^-$ ion at m/z 817.40082 as the base peak. To calculate the corresponding formula, only elements C, H, and O were considered with the following constraints: $0 < C < 50$, $0 < H < 100$, and $0 < O < 30$. For this quasi-molecular $[M - H]^-$ ion, the neutral formula of $C_{43}H_{61}O_{15}$ was calculated as the first hit with a mass accuracy better than 1 ppm. The second hit was $C_{50}H_{57}O_{10}$ with a mass error of 6.2 ppm far beyond the 3 ppm specifications of our LTQ-Orbitrap instrument. Moreover, the number of carbon atoms did not reflect the relative experimental isotopic abundance of 45%.[25] Additional formulas with very small mass errors were obtained by adding further elements (N, P, S) in the constraints, but none of them

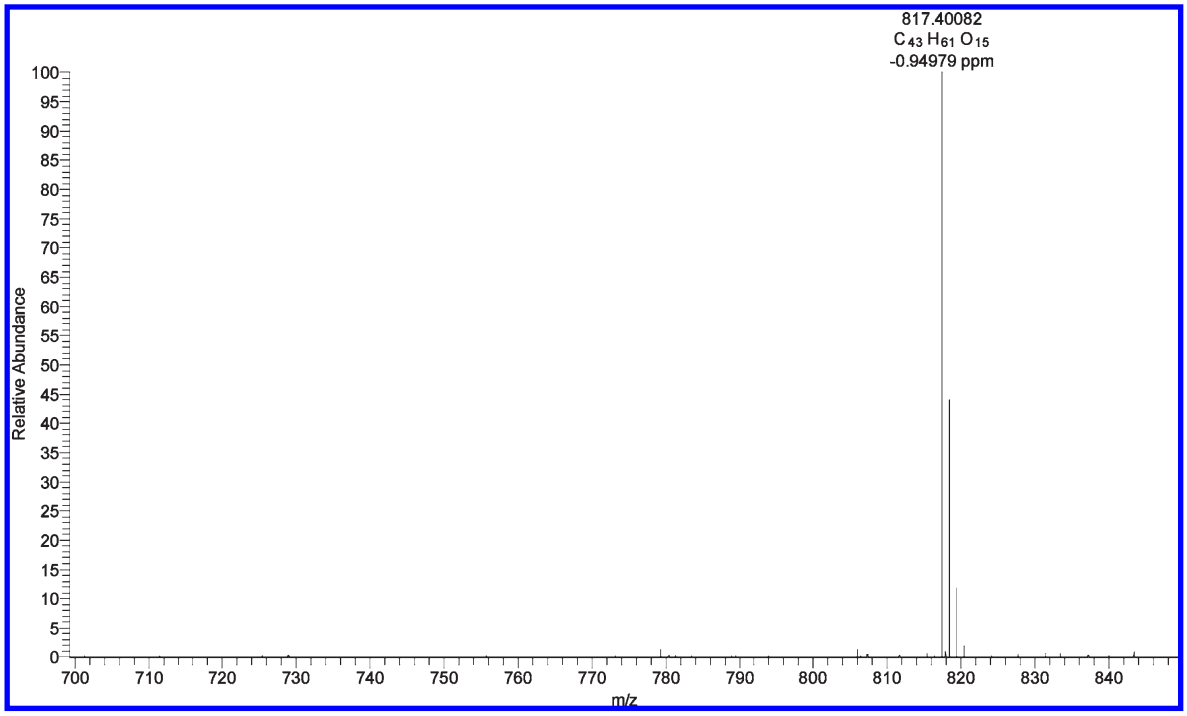

Figure 2. Orbitrap negative ion full scan MS of Quercotriterpenoside I (compound **C**): The spectrum exhibits the quasi-molecular ion at m/z 817.400 82 Th, with an isotopic ratio of 45%, corresponding to the empirical formula $C_{43}H_{61}O_{15}$.

was compatible with the accurate mass MS^n data shown below. Consequently, the empirical formula $C_{43}H_{62}O_{15}$ was assigned to compound **C**. Using the same method, the formulas of compounds **A** and **B** were determined as $C_{22}H_{28}O_8$ and $C_{22}H_{32}O_{12}$, respectively. Moreover, compound **D** was an isomer of compound **C** ($C_{43}H_{62}O_{15}$).

Structural Identification Based on NMR Data. To identify the structure of compound **C**, extensive NMR techniques 1D (^{1}H, ^{13}C) and 2D (COSY, ROESY, TOCSY-HSQC, HSQC, and HMBC) were used.

Among the 43 carbons, 30 were assigned to a triterpenoid aglycone, 6 to a sugar moiety and the remaining 7 to a galloyl group. Six signals of the triterpenoid moiety were assigned to methyl carbons at δ 12.3, 15.7, 16.3, 23.4, 23.5, and 27.4 and the corresponding protons were identified by an HSQC experiment (Table 2). Three oxygen-bearing methine carbons were found at δ 67.9, 76.4, and 80.9, a hydroxymethyl carbon was detected at δ 65.2, and a pair of olefenic carbons was observed at δ 123.1 and 143.5. When coupled with information from the ^{1}H NMR spectrum, i.e., six methyl singlets at δ 0.76, 0.86, 0.95, 0.96, 1.08 and 1.19, three oxygen-bearing methine protons at δ 3.27 (d, J = 4.0), 3.45 (d, J = 9.9), 3.76 (td, J = 9.9, 4.3), a primary alcohol function at δ 4.07 (d, J = 11.3 Hz) and 4.23 (d, J = 11.3 Hz), and an olefinic proton at δ 5.33 (t, J = 3.3 Hz), this data was characteristic of a polyhydroxyolean-12-ene triterpene derivative. After observation of the connectivities in COSY, HSQC, HSQC-TOCSY, and HMBC experiments, the triterpene skeleton of **C** was identified as arjungenin (olean-12-ene-2α,3β,19α,23α-tetrol).[26] The stereochemistry was verified by a ROESY experiment. Moreover, the presence of one singlet of two protons at δ 7.10 (H-2″, H-6″) on the ^{1}H NMR spectrum, and carbon atoms of one carbonyl at δ 166.9 (C-7″), one aromatic quaternary carbon at δ 120.2 (H-1″), two aromatic methines at δ 108.2 (C-2″, C-6″), and three quaternary oxygenated carbons at δ 138.7 (C-4″) and 145.2 (C-3″, C-5″) on the ^{13}C NMR spectrum evidenced the presence of the galloyl group in compound **C**. The downfield chemical shift of H-23 (δ 4.07 and 4.23) of **C** and its correlation with C-7″ (δ 166.9) of galloyl in the HMBC experiment established that galloyl esterified C-23 of the aglycone. The aglycone of compound **C** was thus 23-*O*-galloylarjungenin. Furthermore, the presence of an anomeric proton signal at δ 5.38 (d, J = 8.6 Hz), in addition to several signals between δ 3.31 and 3.82 on the ^{1}H NMR spectrum and the six signals of carbon atoms on the ^{13}C NMR spectrum at δ 94.5 (C-1′), 72.6 (C-2′), 77.2 (C-3′), 69.7 (C-4′), 76.9 (C-5′), and 61.1 (C-6′), confirmed the presence of a terminal glucose unit. The relative stereochemistry of this sugar moiety was determined as β-glucopyranose on the basis of the characteristic $J_{1,2}$ coupling constant of its anomeric proton (J = 8.2 Hz) and typical ^{1}H and ^{13}C NMR shifts.[27] The linkage position of this glucose moiety at C-28 was evidenced by the upfield shift of this carbon from δ 180.1 to δ 176.7 in compound **C** and the signal of the anomeric carbon (C-1′) at δ 94.3. In addition, the HMBC cross peak correlation observed between the anomeric proton (H-1′) and C-28 confirmed the sugar linkage position at C-28. Thus, the structure of compound **C** was concluded to be a new triterpenoid, 23-*O*-galloyl arjungenin 28-*O*-β-glucopyranosyl (23-*O*-galloyl arjunglucoside). Owing to its botanical origin and its chemical nature, we called it Quercotriterpenoside I (**QTT I**, Figure 3).

FTMS of compound **D** gave a quasi-molecular peak at m/z $[M - H]^-$ 817.402 10 in negative mode, in agreement with the molecular formula of $C_{43}H_{61}O_{15}$. ^{1}H and ^{13}C NMR data (Table 2) of **D** were closely comparable to those of **QTT I**, except for signals of ring A. This suggested that compound **D** is a regioisomer of **QTT I** where the galloyl unit is transferred from C-23 to C-3. The position of the galloyl unit was confirmed by HMBC NMR, which showed a three-bond correlation between

Table 2. ^{1}H and ^{13}C NMR Data of Quercotriterpenoside I and II

	QTT I			QTT II		
	assignment	$^{13}C^{a}$	$^{1}H^{b}$	assignment	$^{13}C^{c}$	$^{1}H^{d}$
	1	46.4	0.99 *m*	1	47.9	1.08 *m*
			1.98 *dd* (12.3; 4.3)			2.05 *m*
	2	67.9	3.76 *d* (9.9; 4.3)	2	65.7	3.94 *td* (11 ; 4.6 Hz)
	3	76.4	3.45 *d* (9.9)	3	84.8	4.7 *d* (10.3 Hz)
	4	42.4		4	44.3	
	5	47.8	1.38 *m*	5	56.1	1.09 *m*
	6	17.8	1.48 *m*	6	19.8	1.49 *td* (13.3 ; 5.3 Hz)
			1.39 *m*			1.72 *m*
	7	31.9	1.26 *m*	7	32.8	1.30 *m*
			1.42 *m*			1.46 *m*
	8	39.4		8	40.3	
	9	48	1.85 *t* (9.3)	9	47.9	1.87 *t* (9.0 Hz)
	10	37.7		10	38.1	
	11	23.6	2.02 *m*	11	23.7	2.05 *m*
	12	123.1	5.33 *t* (3.3)	12	123	5.36 *t* (3.4 Hz)
	13	143.5		13	143.2	
	14	41.2		14	44.9	
	15	27.8	0.95 *m*	15	27.8	1.69 *m*
			1.62 *m*			1.79 *m*
	16	26.9	1.7 *m*	16	26.4	1.76 *m*
			2.3 *td* (13.7; 4.3)			2.35 *td* (13.4 ; 3.6 Hz)
	17	45.6		17	45.3	
	18	43.7	3.05 *d* (4.0)	18	43.9	3.08 *d* (3.5 Hz)
	19	80.9	3.27 *d* (4.0)	19	81	3.30 *d* (3.5 Hz)
	20	34.4		20	34.3	
	21	28	1.01 *m*	21	28	1.02 *m*
			1.76 *m*			1.70 *m*
	22	31.7	1.66 *m*	22	32.1	1.66 *m*
			1.76 *m*			1.76 *m*
	23	65.2	4.07 *d* (11.3)	23	21.8	1.06 *s*
			4.23 *d* (11.3)	24	63.9	3.85 *d* (11.6 Hz)
	24	12.3	0.86 *s*			3.90 *d* (11.6 Hz)
	25	15.7	1.08 *s*	25	15.7	1.15 *s*
	26	16.3	0.76 *s*	26	16.5	0.79 *s*
	27	23.4	1.19 *s*	27	23.8	1.33 *s*
	28	176.7		28	177.5	
	29	27.4	0.95 *s*	29	27.2	0.97 *s*
	30	23.5	0.96 *s*	30	23.8	0.96 *s*
	1′	94.5	5.38 *d* (8.2 Hz)	1′	94.4	5.39 *d* (8.2 Hz)
	2′	72.6	3.31 *d* (9.1 Hz)	2′	72.3	3.32 *m*
	3′	77.2	3.35 *m*	3′	77.3	3.35 *m*
glucopyranosyl	4′	69.7	3.36 *d* (8.1 Hz)	4′	69.6	3.37 *m*
	5′	76.9	3.44 *d* (9.1 Hz)	5′	77.1	3.41 *d* (9.2 Hz)
	6′	61.1	3.69 *dd* (11.7; 4.1 Hz)	6′	61	3.69 *dd* (12.1 ; 4.4 Hz)
			3.82 *brd* (11.7 Hz)			3.83 *brd* (11.1 Hz)
	1″		120.2	1″	120.3	
	2″/6″	7.1 *s*	108.2	2″/6″	108.3	7.13 *s*
galloyl	3″/5″		145.2	3″/5″	144.9	
	4″		138.7	4″	138.2	
	7″		166.8	7″	167.1	

[a] Chemical shift (in ppm) in CD_3OD at 125 Hz. [b] Chemical shift (in ppm); multiplicity, (*J*, in Hz) in CD_3OD at 500 Hz. [c] Chemical shift (in ppm) in CD_3OD at 150 Hz. [d] Chemical shift (in ppm); multiplicity, (*J*, in Hz) in CD_3OD at 600 Hz.

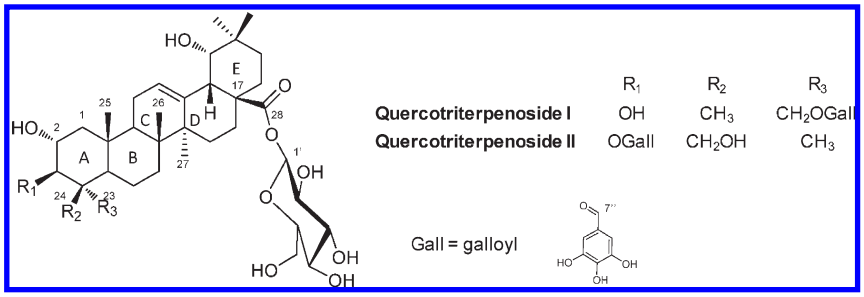

Figure 3. Molecular structure of Quercotriterpenoside I and II.

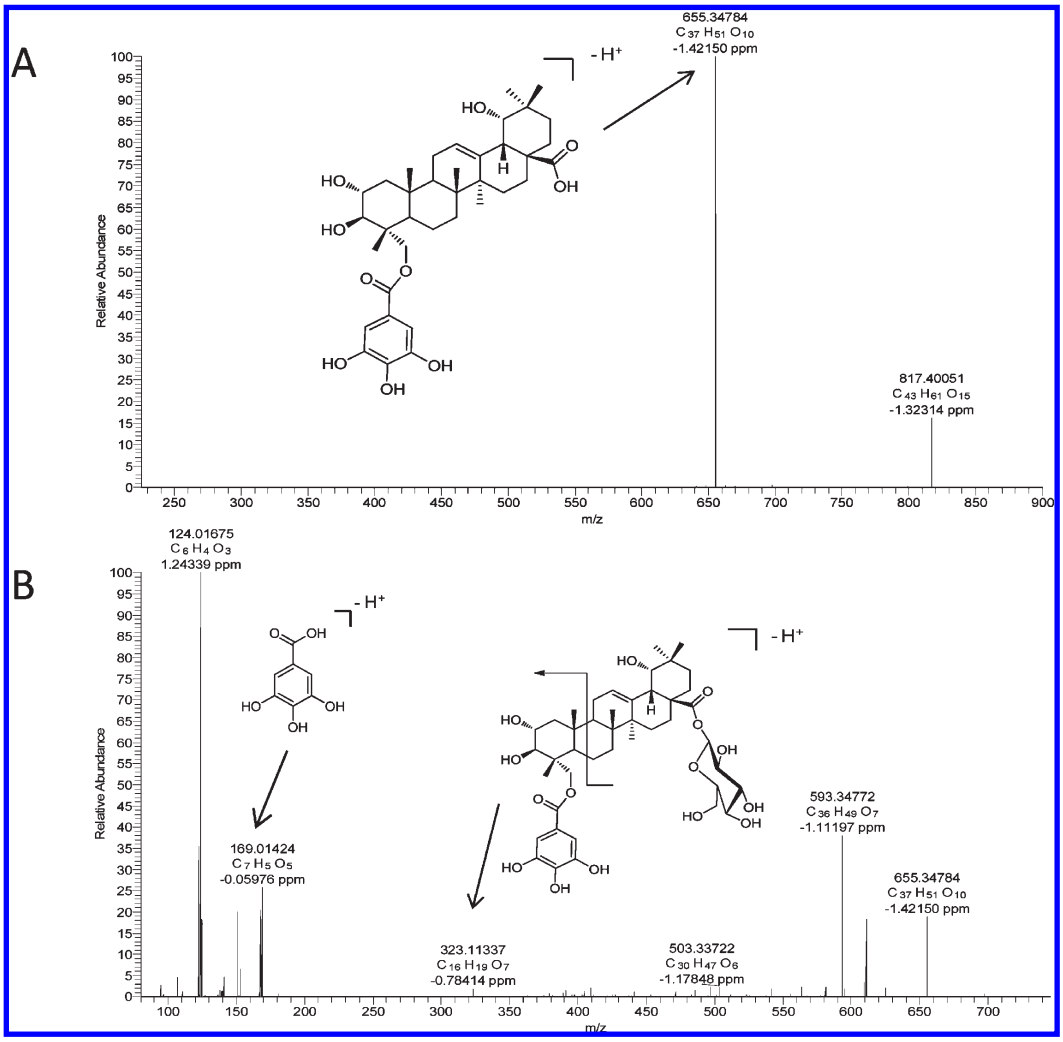

Figure 4. MS/MS spectra of the $[M - H]^-$ ion of Quercotriterpenoside I: (A) LIT CID MS/MS at 22% resonant collision energy, (B) HCD MS/MS at 150 V collision energy. The fragment at m/z 655.347 84 corresponds to the loss of glucosyl moiety. More ions were observed in HCD mode (B), in particular ions at m/z 323.113 37 and m/z 169.01424 corresponding, respectively, to a fragment of QTT I (opening of ring B) and to the gallate ion.

the H-3 signal at δ 4.7 (1 H, d, J = 10.3 Hz) and C-7″ at δ 167.1 of the galloyl moiety. Moreover, the downfield chemical shift of protons H-24 at δ 3.85 (d, J = 11.6 Hz) and 3.90 (d, J = 11.6 Hz) according to the primary alcohol and the rOe interactions observed in the ROESY spectrum between H-3 and H-23 suggested that the stereochemistry of C-4 of genin had changed. The structure of **D** was concluded to be 3-*O*-galloyl 4-*epi*-arjunglucoside. This triterpenoid derived from sericoside (4-epi-arjunglucoside) was recently reported as a natural product from the bark of *Terminalia ivorensis*.[28] This compound has never been described in the literature previously, so we named it Quercotriterpenoside II (**QTT II**, Figure 3).

Additionally, two known compounds, lyoniresinol (**A**) and whisky lactone precursor (**B**), were isolated and identified by comparing the observed ^{1}H and ^{13}C NMR data with the literature values.[29−32]

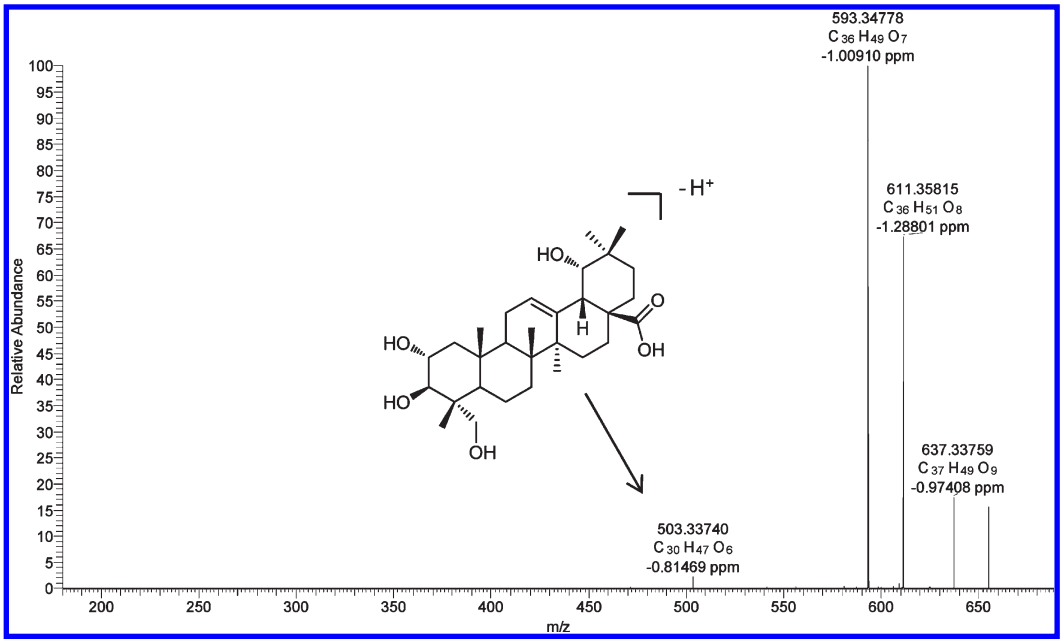

Figure 5. LIT MS^3 spectrum of the product ion at m/z 655, 30% resonant collision energy. The fragment at m/z 503.337 40 corresponds to the genin ion of **QTT I** after the loss of galloyl and glucopyranosyl moieties, confirming the triterpenoide nature of the molecule.

Value of Coupling $FTMS^n$ with CID and HCD Activations for Structural Confirmation. Fragmentation experiments in FTMS can provide valuable information to confirm the molecular structure proposed by NMR data interpretation, as illustrated with **QTT I** (**C**).

The resonant CID MS/MS spectrum of the precursor ion at m/z 817 (Figure 4) yielded one major fragment at m/z 655.347 84 which was attributed to the facile loss of a glucopyanosyl part[33] and confirmed the presence of such a functional group. No other fragment was observed.

The next stage in the fragmentation process was the loss of water and carbon dioxide, as shown in the MS^3 spectrum in Figure 5. The small product ion at m/z 503.3374 ($C_{30}H_{47}O_6$) was attributed to the loss of a galloyl moiety, thus corresponding to the free triterpenoid ion.

A well-known drawback of the ion trap resonant activation process is the low mass cut off, which prevents product ions from being trapped below one-third of the precursor m/z value. Even though this one-third ratio can be increased by decreasing the activation Qz parameter of the Matthieu equation, no fragments were observed on the MS^3 spectrum below 180 Th.

The nonresonant HCD activation process is not subject to such a low mass cut off limitation.[34] As shown in Figure 4 several intense product ions were observed at m/z 169.014 24 ($C_7H_5O_5$), 151.003 77 ($C_7H_3O_4$), 125.0245 ($C_6H_5O_3$), and 124.016 75 ($C_6H_4O_3$), thus providing further evidence of the presence of the galloyl moiety.

Unfortunately, no fragmentation of the triterpenoid ion was observed, with the exception of the small product ion at m/z 323.11337 ($C_{16}H_{19}O_7$), which could be formed by fragmentation at the B-ring. This fragment confirmed the position of the galloyl moiety on the A-ring of the aglycon. The HCD activation process provided precious structural information and therefore appears to be an interesting fragmentation mode, in addition to the widely used CID. $FTMS^n$ results were complementary to the NMR data and confirmed the structure of **QTT I**.

CONCLUSIONS

The novel methodology consisting of an off-line centrifugal partition chromatography with gustatometry (CPC−G) hyphenation technique enabled the isolation of sapid compounds from an oak extract and, specifically, a sweet molecule. The power of this technique is based on the fractionation power of CPC with large amounts of extract and the accuracy of subsequent sensorial analysis. Furthermore, FTMS appears to be a powerful tool for structural studies, not only in full scan mode for determining the empirical formulas but also for determining the molecular structure of compounds in conjunction with $^1H/^{13}C$ 2D NMR. In this regard, CID and HCD fragmentation experiments provide relevant information that justifies their combination.

In this way, a new sweet triterpenoid called Quercotriterpenoside I was identified from an oak extract. These results illustrate the efficiency of the novel method, which allows the purification of taste-active molecules from highly complex mixtures. It could consequently be applied for the discovery of new natural sweeteners in various matrixes.

ASSOCIATED CONTENT

Supporting Information. CPC−UV chromatogram at 280 nm of the prepurified oak extract (Figure S-1). This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

The authors would like to acknowledge the Conseil Interprofessionnel des Vins de Bordeaux, Laffort Oenologie, Seguin-Moreau

cooperage and Rémy-Martin for funding this project. The authors thank Valérie Lavigne-Cruège and Alexandre Pons for sensorial analysis and Jonathan Bisson for the program cpc_plotter.

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