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

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18 **Abstract:**

19 Sweetness contributes notably to the taste-balance of dry wines and increases during
20 oak-barrel aging owing to the release of natural sweeteners from wood. The search for such
21 taste-active molecules, which are sometimes present at very low concentrations in wine or other
22 complex matrixes, requires both reliable purification tools and powerful identification
23 techniques. Here, we report the development of an original inductive method using centrifugal
24 partition chromatography (CPC) and sensorial analysis. This method, called CPC–
25 gustatometry, was implemented to isolate a sweet fraction with only four compounds from a
26 complex oak wood extract. The recently developed Fourier transform mass spectrometry (FT-
27 MS, Orbitrap analyzer) was used jointly with two-dimensional nuclear magnetic resonance (2D
28 ^{1}H and ^{13}C NMR) to obtain the structural elucidation of the purified compounds. The tandem
29 mass spectrometry (MS/MS) spectra obtained with resonant and nonresonant fragmentation
30 modes were compared, thus providing complementary information about the molecular
31 structure. Two oleanane-type triterpenoids substituted with galloyl and glucosyl moieties were
32 identified, one of which exhibits sweet properties. We term these compounds which have never
33 been reported, Quercotriterpenoside I and II.

34 # INTRODUCTION

35

36 Wine quality, i.e., the pleasure that a wine brings to the taster, depends on the complexity
37 and the harmony of its aromas and tastes. The mental representation of this quality in the taster's
38 field of consciousness is based on the sensorial perception of the various wine chemicals
39 followed by the neurophysiological and cognitive integration of the perceived stimuli.[1]
40 The sweet taste, conjuring up the taste of sucrose, paradoxically characterizes the
41 gustatory balance of dry wines and particularly great wines. It softens the acidity, bitterness,
42 and astringency generated by some wine molecules like organic acids and polyphenols.[2] In such
43 dry wines, grape carbohydrates (mainly glucose and fructose) are almost completely
44 transformed by yeasts, so they are present only at very low concentrations and are not
45 perceptible. The perceived sweetness corresponds therefore to "sweetness without sugar" and
46 ensures the consumer's pleasure. However, despite evidence of their sensorial importance, the
47 molecular determinants of sweetness in dry wines have never been elucidated.
48 Nevertheless, winemakers are convinced that aging in oak wood barrels has a
49 sweetening effect on wine.[3] A similar taste improvement contributes to maturation and the
50 global quality of spirits.
51 In addition, it is well-known that molecules exhibiting sweet taste are naturally
52 contained in plants, and some of them are now used as sweeteners in the food industry like
53 steviosides.[4-6]
54 Consequently, nonvolatile compounds with sweet taste may be present in oak wood and
55 then be released in wines and spirits during aging. The search for such compounds is
56 comparable to the study of natural substances with biological and pharmacological properties,
57 a pathway that has led to numerous analytical developments.[7-10] This kind of research involves
58 a dual requirement: purification (only traces of active molecules are present in a complex
59 matrix) and identification (compounds are purified following their biological activity and not
60 their chemical structure, which therefore requires elucidation).[11,12] Consequently, it is necessary
61 to use efficient analytical techniques to meet this dual challenge.
62 Among these techniques, centrifugal partition chromatography (CPC) is particularly
63 suited for the fractionation of complex extracts, owing to the large amounts that can be injected
64 and the lack of irreversible adsorption with the stationary phase.[13-15] By implementing this
65 technique, we have developed an original taste-guided purification protocol, i.e., fractionation
66 steps of an oak extract have been hyphenated with tasting of the obtained fractions.

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

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

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78

79 # MATERIALS AND METHODS

80

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

91 *Solvent System Screening.* The choice of an appropriate biphasic system of solvents
92 is based on the study of the partition of the compounds of the extract in both phases. In practice,
93 1 mg of extract is dissolved in a biphasic system (2 mL) in a vial. After shaking, 100 µL of each
94 phase was evaporated to dryness, resolubilized with 1 mL of methanol/water (1:1; v/v), and
95 injected into an analytic HPLC. The partition coefficient K_d was calculated as the ratio of the
96 solute area in each phase. The quaternary Arizona systems (*n*-heptane/ethyl
97 acetate/methanol/water) B, C, D, F, H, J, K, L, and M were tested.[19,20] In optimal conditions in
98 ascending mode where $K_d \approx 1$, the compounds are partitioned equally between the two phases

99 and a satisfactory separation may occur. The Arizona-G system (1:4:1:4, v/v) was chosen on
100 this basis.

101 *Preparation of CPC Solvent.* A biphasic system was prepared by mixing *n*-heptane
102 (200 mL), ethyl acetate (EtOAc, 800 mL), methanol (MeOH, 200 mL), and H_2O (800 mL) in a
103 separatory funnel at room temperature, shaking vigorously, and allowing them to settle until
104 the phases became limpid. The resulting two phases were separated just before use.

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

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

126 *Analytic HPLC–UV.* After the CPC experiment, the tubes were analyzed by HPLC–
127 UV. A volume of 100 μL of liquid were taken from each tube, evaporated, and dissolved in
128 water/acetonitrile 50/50. After filtration (0.45 μm), 10 μL of the samples obtained were
129 analyzed on an Ultrasphere ODS C18 4.6 mm × 250 mm, 5 μm column (Beckman Coulter,
130 Fullerton, CA) connected to a Spectra System autosampler, quaternary pump, and diode array
131 detector (Thermo Fisher Scientific, Les Ulis, France). The mobile phases were water (A) and
132 acetonitrile (B), each containing 0.1% trifluoroacetic acid. The gradient was 0 min, 10% B; 2

133 min, 10% B; 19 min, 70% B; 20 min, 95% B; 23 min, 95% B; 24 min, 10% B; 27 min, 10% B
134 with a flow rate of 1 mL/min. Signal detection was carried out at 280 nm.

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

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

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

167 Collision induced dissociation (CID) MS^2 and MS^3 studies were performed in the linear
168 ion trap (LIT). In addition, nonresonant activation was also carried out in the higher collisional
169 energy (HCD) cell situated at the far end of the C-Trap region.[21] In both cases, the product ions
170 were detected in the Orbitrap analyzer in order to generate high mass accuracy data. Indeed, all
171 the ions were detected with mass errors in the 1 ppm range.[22] The resolution setting was 100 000
172 ($m/\Delta m$, fwhm at m/z 400) in the MS and MS^n modes. The maximum injection time was set at
173 100 ms and the automated gain control (AGC) target value, respectively, at 5×10^5 in MS and
174 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
175 experiments.
176 **NMR Experiments.** The experiments were performed on a Bruker Avance 3
177 spectrometer (^{1}H 600 MHz, ^{13}C 150 MHz) equipped with a TXI 5 mm probe and a Bruker
178 Avance DMX 500 spectrometer (^{1}H 500 MHz, ^{13}C 125 MHz) equipped with a TXI 5 mm cryo-
179 probe. All NMR spectra were acquired at 27 °C in methanol-d_4. 1D ^{1}H NMR spectra were
180 recorded with 32k data points, sweep width of 12 ppm, and pulse repetition time of 1.5 s.
181 Correlation spectroscopy (COSY), rotating frame nuclear Overhauser effect spectroscopy
182 (ROESY) (400 ms spin lock), and heteronuclear single-quantum correlation spectroscopy
183 (HSQC)/total correlation spectroscopy (TOCSY) (150 ms spin lock) spectra were performed
184 with 2048-256 data points. HSQC and heteronuclear multiple-bond correlation spectroscopy
185 (HMBC) spectra were acquired using gradient selection. The heteronuclear correlations were
186 optimized for $^1J_{C,H}$ 140 Hz and $^nJ_{C,H}$ 8–12 Hz depending on the sample. Spectral widths were
187 adjusted to the individual samples. ^{1}H and ^{13}C chemical shifts were referenced to solvent signals
188 of methanol-d_4, $\delta(^1H)$ 3.31 and $\delta(^{13}C)$ 49.1.
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190

191 RESULTS AND DISCUSSION

192

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

199 The CPC-UV profile (Figure S-1 in the Supporting Information) showed some distinct
200 broad peaks distributed across the chromatogram, confirming the good partitioning of the
201 extract. In order to locate the sapid compounds of the extract, the CPC tubes had to be tasted.
202 Since there were many tubes (90), fractions were constituted by grouping some tubes together.
203 Grouping was done on the basis of the analytical HPLC chromatographic profiles. The HPLC
204 chromatograms obtained from the different tubes presented many peaks but were quite
205 different, confirming the fractioning efficiency of the CPC. When grouping the tubes, samples
206 presenting the same main peaks and similar HPLC chromatographic profiles were pooled
207 together. The 15 (I to XV) fractions thus constituted provided 632 mg of powder after freeze-
208 drying. In comparison to other chromatographic techniques, the yield of this CPC experiment
209 (90%) was very high. This was mainly due to the principle of CPC, which involves no solid
210 support, i.e., no irreversible adsorption. Moreover, CPC offers the possibility of injecting large
211 quantities of extract. Consequently, this technique appears very powerful for the search for
212 nonvolatile compounds with organoleptic properties in complex matrixes.
213 To locate the targeted compounds, all the fractions were tasted and their sweetness
214 intensity was evaluated. Even if the number of tasters (four) was low owing to the small quantity
215 of product available, their expertise in wine-tasting ensured the pertinence of their judgment.
216 To study volatile compounds, aromaticians have for long hyphenated gas
217 chromatography with olfaction (GC-O) and used the terms "olfactometry" and
218 "olfactogram".[23,24] By analogy, we chose to call our technique "gustatometry". Centrifugal
219 partition chromatography followed by gustatometry allowed the tasters to establish a
220 gustatogram of the extract (Table 1). They used precise sensorial descriptors corresponding to
221 fundamental taste (sweetness, bitterness), tactile sensations (astringency), and multimodal
222 perceptions ("dusty flavor", "coconut flavor"). For most fractions, the taste was composed of
223 multiple sensations, indicating their chemical heterogeneity as revealed by HPLC profiles.
224 As shown in Table 1, eight fractions developed a characteristic sweet taste with different
225 intensities. The most intense sweetness was perceptible in fraction X (yellow powder, 59 mg).
226 Its HPLC chromatogram (Figure 1) showed only four main peaks, corresponding probably to
227 four compounds (**A**, **B**, **C**, and **D**, in increasing order of retention time).
228 *Purification and Sensorial Characterization of Fraction X Compounds.* Fraction
229 **X** (52 mg) was submitted to preparative HPLC using a water/acetonitrile gradient. The four
230 peaks were well separated and the compounds were obtained as white powders after freeze-
231 drying (**A**, 16 mg; **B**, 4.9 mg; **C**, 1.2 mg; **D**, 0.3 mg). The general yield of this purification step
232 (44%) was lower than for the CPC experiment but was commonly obtained with HPLC.

233 Irreversible adsorption due to the solid phase in the C18 column and the manual collection
234 method could explain this difference. This demonstrates the value of CPC vis-a-vis HPLC for
235 extract fractionation, although the higher chromatographic resolution obtained in HPLC
236 remains useful for final compound purification.

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

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

255 *Empirical Formula Determination Using FTMS.* The mass accuracy, stability, and
256 resolution afforded by FTMS are particularly useful for the empirical determination of the
257 formulas of unknown natural molecules. The full scan spectrum of compound **C** (Figure 2)
258 exhibits an $[M - H]^-$ ion at m/z 817.40082 as the base peak. To calculate the corresponding
259 formula, only elements C, H, and O were considered with the following constraints: $0 < C <$
260 50, $0 < H < 100$, and $0 < O < 30$. For this quasi-molecular $[M - H]^-$ ion, the neutral formula of
261 $C_{43}H_{61}O_{15}$ was calculated as the first hit with a mass accuracy better than 1 ppm. The second
262 hit was $C_{50}H_{57}O_{10}$ with a mass error of 6.2 ppm far beyond the 3 ppm specifications of our
263 LTQ-Orbitrap instrument. Moreover, the number of carbon atoms did not reflect the relative
264 experimental isotopic abundance of 45%.[25] Additional formulas with very small mass errors
265 were obtained by adding further elements (N, P, S) in the constraints, but none of them was
266 compatible with the accurate mass MS^n data shown below. Consequently, the empirical formula

267 $C_{43}H_{62}O_{15}$ was assigned to compound **C**. Using the same method, the formulas of compounds
268 **A** and **B** were determined as $C_{22}H_{28}O_8$ and $C_{22}H_{32}O_{12}$, respectively. Moreover, compound **D**
269 was an isomer of compound **C** ($C_{43}H_{62}O_{15}$).

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

273 Among the 43 carbons, 30 were assigned to a triterpenoid aglycone, 6 to a sugar moiety
274 and the remaining 7 to a galloyl group. Six signals of the triterpenoid moiety were assigned to
275 methyl carbons at δ 12.3, 15.7, 16.3, 23.4, 23.5, and 27.4 and the corresponding protons were
276 identified by an HSQC experiment (Table 2). Three oxygen-bearing methine carbons were
277 found at δ 67.9, 76.4, and 80.9, a hydroxymethyl carbon was detected at δ 65.2, and a pair of
278 olefenic carbons was observed at δ 123.1 and 143.5. When coupled with information from the
279 ^{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
280 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),
281 a primary alcohol function at δ 4.07 (*d*, J = 11.3 Hz) and 4.23 (*d*, J = 11.3 Hz), and an olefinic
282 proton at δ 5.33 (*t*, J = 3.3 Hz), this data was characteristic of a polyhydroxyolean-12-ene
283 triterpene derivative. After observation of the connectivities in COSY, HSQC, HSQC-TOCSY,
284 and HMBC experiments, the triterpene skeleton of **C** was identified as arjungenin (olean-12-
285 ene-2α,3β,19α,23α-tetrol).[26] The stereochemistry was verified by a ROESY experiment.
286 Moreover, the presence of one singlet of two protons at δ 7.10 (H-2″, H-6″) on the ^{1}H NMR
287 spectrum, and carbon atoms of one carbonyl at δ 166.9 (C-7″), one aromatic quaternary carbon
288 at δ 120.2 (H-1″), two aromatic methines at δ 108.2 (C-2″, C-6″), and three quaternary
289 oxygenated carbons at δ 138.7 (C-4″) and 145.2 (C-3″, C-5″) on the ^{13}C NMR spectrum
290 evidenced the presence of the galloyl group in compound **C**. The downfield chemical shift of
291 H-23 (δ 4.07 and 4.23) of **C** and its correlation with C-7″ (δ 166.9) of galloyl in the HMBC
292 experiment established that galloyl esterified C-23 of the aglycone. The aglycone of compound
293 **C** was thus 23-*O*-galloylarjungenin. Furthermore, the presence of an anomeric proton signal at
294 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
295 spectrum and the six signals of carbon atoms on the ^{13}C NMR spectrum at δ 94.5 (C-1′), 72.6
296 (C-2′), 77.2 (C-3′), 69.7 (C-4′), 76.9 (C-5′), and 61.1 (C-6′), confirmed the presence of a
297 terminal glucose unit. The relative stereochemistry of this sugar moiety was determined as β-
298 glucopyranose on the basis of the characteristic $J_{1,2}$ coupling constant of its anomeric proton (J
299 = 8.2 Hz) and typical ^{1}H and ^{13}C NMR shifts.[27] The linkage position of this glucose moiety at
300 C-28 was evidenced by the upfield shift of this carbon from δ 180.1 to δ 176.7 in compound **C**

301 and the signal of the anomeric carbon (C-1′) at δ 94.3. In addition, the HMBC cross peak
302 correlation observed between the anomeric proton (H-1′) and C-28 confirmed the sugar linkage
303 position at C-28. Thus, the structure of compound **C** was concluded to be a new triterpenoid,
304 23-*O*-galloyl arjungenin 28-*O*-β-glucopyranosyl (23-*O*-galloyl arjunglucoside). Owing to its
305 botanical origin and its chemical nature, we called it Quercotriterpenoside I (**QTT I**, Figure 3).

306 FTMS of compound **D** gave a quasi-molecular peak at *m*/*z* $[M - H]^-$ 817.402 10 in
307 negative mode, in agreement with the molecular formula of $C_{43}H_{61}O_{15}$. ^1H and ^{13}C NMR data
308 (Table 2) of **D** were closely comparable to those of **QTT I**, except for signals of ring A. This
309 suggested that compound **D** is a regioisomer of **QTT I** where the galloyl unit is transferred from
310 C-23 to C-3. The position of the galloyl unit was confirmed by HMBC NMR, which showed a
311 three-bond correlation between the H-3 signal at δ 4.7 (1 H, d, *J* = 10.3 Hz) and C-7″ at δ 167.1
312 of the galloyl moiety. Moreover, the downfield chemical shift of protons H-24 at δ 3.85 (*d*, *J* =
313 11.6 Hz) and 3.90 (*d*, *J* = 11.6 Hz) according to the primary alcohol and the rOe interactions
314 observed in the ROESY spectrum between H-3 and H-23 suggested that the stereochemistry of
315 C-4 of genin had changed. The structure of **D** was concluded to be 3-*O*-galloyl 4-*epi*-
316 arjunglucoside. This triterpenoid derived from sericoside (4-epi-arjunglucoside) was recently
317 reported as a natural product from the bark of *Terminalia ivorensis*.[28] This compound has never
318 been described in the literature previously, so we named it Quercotriterpenoside II (**QTT II**,
319 Figure 3).

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

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

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

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

333 A well-known drawback of the ion trap resonant activation process is the low mass cut
334 off, which prevents product ions from being trapped below one-third of the precursor *m*/*z* value.

335 Even though this one-third ratio can be increased by decreasing the activation Qz parameter of
336 the Matthieu equation, no fragments were observed on the MS^3 spectrum below 180 Th.
337 The nonresonant HCD activation process is not subject to such a low mass cut off
338 limitation.[34] As shown in Figure 4 several intense product ions were observed at *m*/*z* 169.014 24
339 ($C_7H_5O_5$), 151.003 77 ($C_7H_3O_4$), 125.0245 ($C_6H_5O_3$), and 124.016 75 ($C_6H_4O_3$), thus providing
340 further evidence of the presence of the galloyl moiety.
341 Unfortunately, no fragmentation of the triterpenoid ion was observed, with the exception
342 of the small product ion at *m*/*z* 323.11337 ($C_{16}H_{19}O_7$), which could be formed by fragmentation
343 at the B-ring. This fragment confirmed the position of the galloyl moiety on the A-ring of the
344 aglycon. The HCD activation process provided precious structural information and therefore
345 appears to be an interesting fragmentation mode, in addition to the widely used CID. $FTMS^n$
346 results were complementary to the NMR data and confirmed the structure of **QTT I**.
347

348 # CONCLUSIONS

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350 The novel methodology consisting of an off-line centrifugal partition chromatography
351 with gustatometry (CPC–G) hyphenation technique enabled the isolation of sapid compounds
352 from an oak extract and, specifically, a sweet molecule. The power of this technique is based
353 on the fractionation power of CPC with large amounts of extract and the accuracy of subsequent
354 sensorial analysis. Furthermore, FTMS appears to be a powerful tool for structural studies, not
355 only in full scan mode for determining the empirical formulas but also for determining the
356 molecular structure of compounds in conjunction with $^{1}H/^{13}C$ 2D NMR. In this regard, CID and
357 HCD fragmentation experiments provide relevant information that justifies their combination.
358 In this way, a new sweet triterpenoid called Quercotriterpenoside I was identified from an oak
359 extract. These results illustrate the efficiency of the novel method, which allows the purification
360 of taste-active molecules from highly complex mixtures. It could consequently be applied for
361 the discovery of new natural sweeteners in various matrixes.
362
363

364 **Supporting Information**

365 CPC–UV chromatogram at 280 nm of the prepurified oak extract (Figure S-1). This material is
366 available free of charge via the Internet at http://pubs.acs.org.docelec.u-bordeaux.fr.
367

368 **Acknowledgment**

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Figures

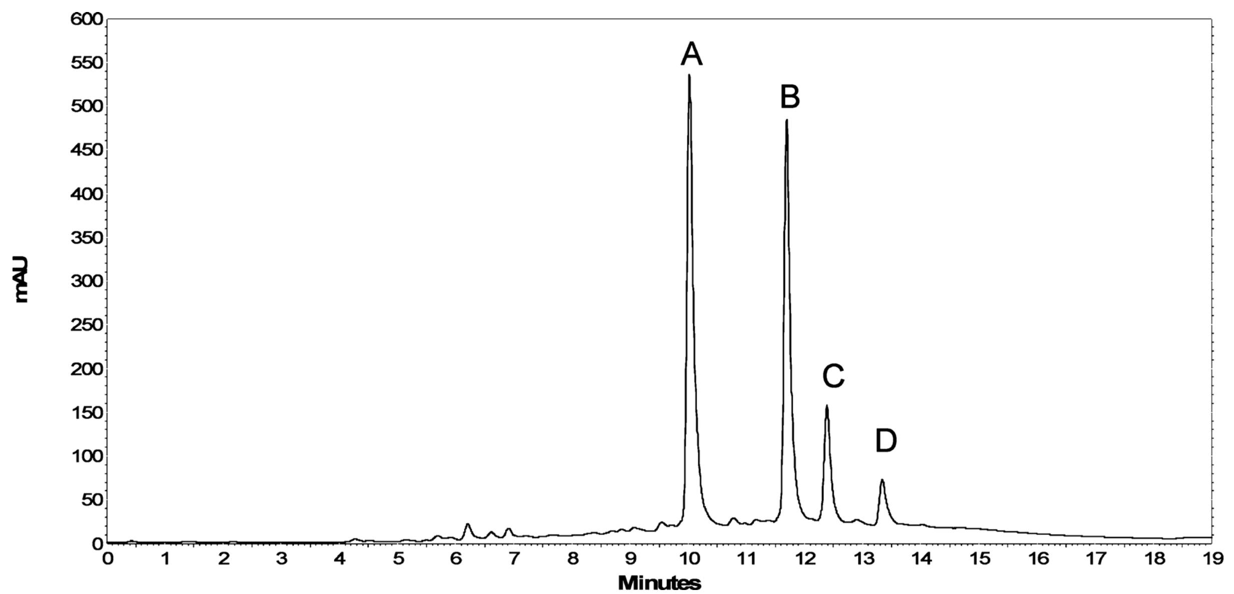

Figure 1. HPLC–UV chromatogram of fraction X: UV chromatogram at 280 nm of fraction X shows four well-separated and symmetric peaks corresponding to four compounds labeled **A–D**.

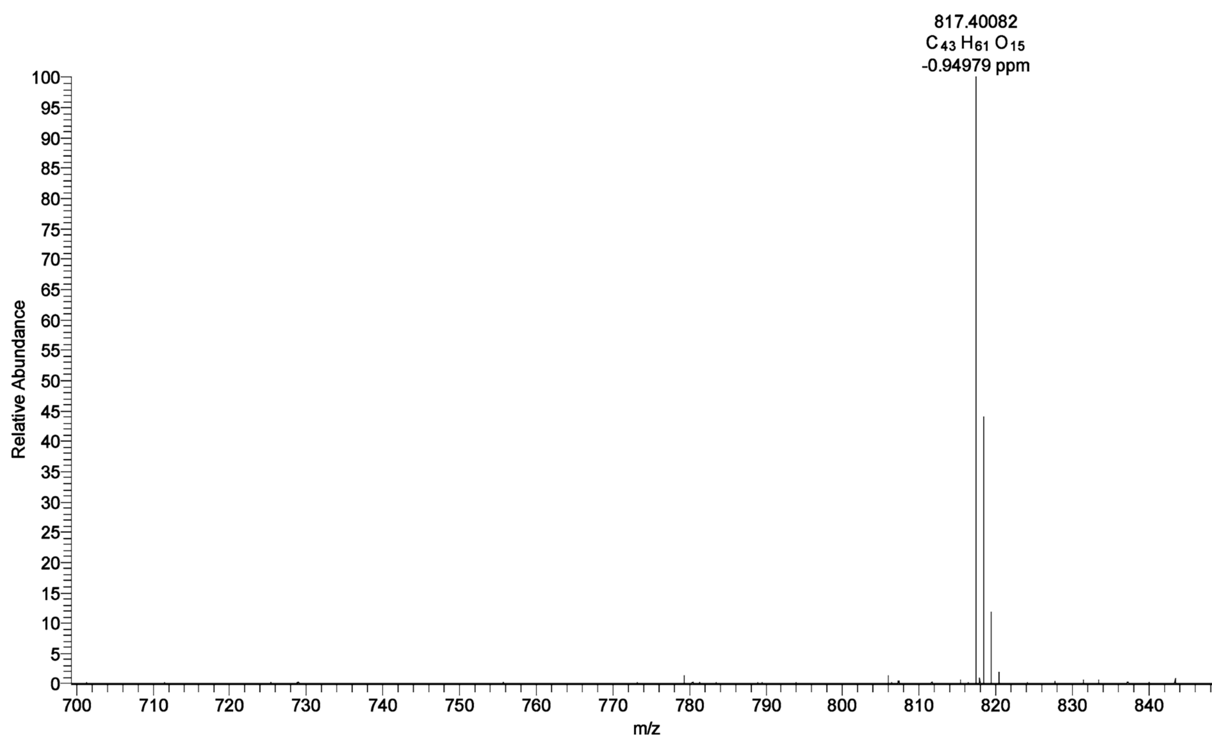

Figure 2. Orbitrap negative ion full scan MS of Quercotriterpenoside I (compound **C**): The spectrum exhibits the quasi-molecular ion at *m*/*z* 817.400 82 Th, with an isotopic ratio of 45%, corresponding to the empirical formula $C_{43}H_{61}O_{15}$.

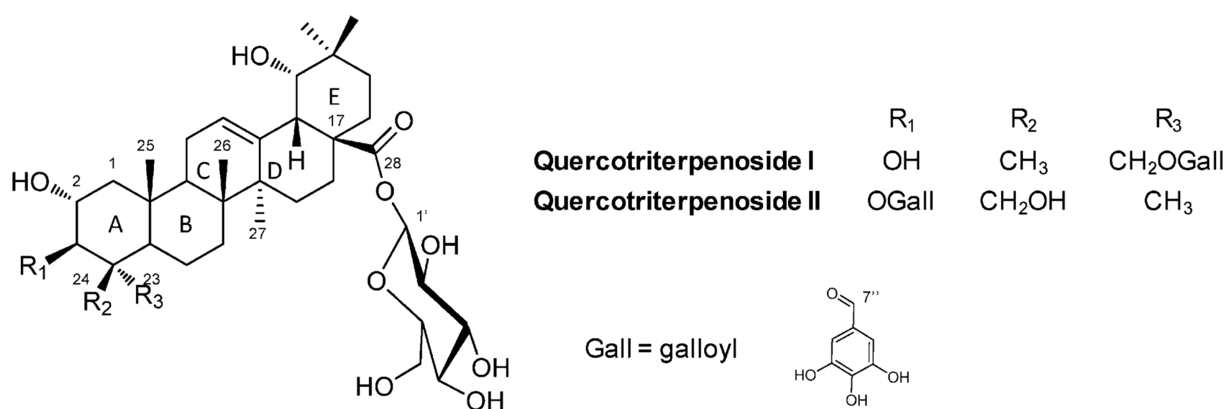

Figure 3. Molecular structure of Quercotriterpenoside I and II.

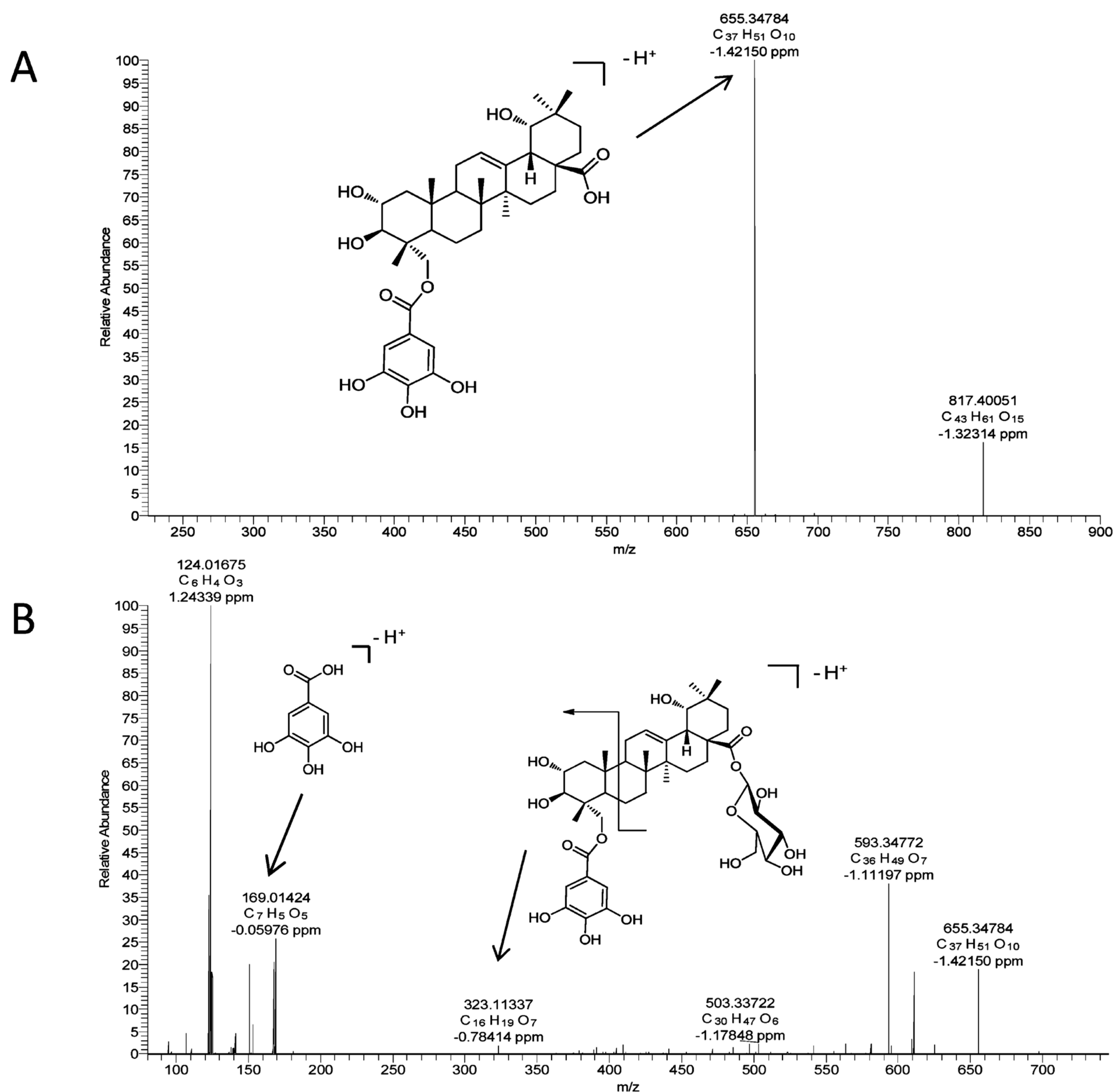

Figure 4. MS/MS spectra of the [M – H]⁻ ion of Quercotriterpenoside I: (A) LIT CID MS/MS at 22% resonant collision energy, (B) HCD MS/MS at 150 V collision energy. The fragment at *m*/*z* 655.347 84 corresponds to the loss of glucosyl moiety. More ions were observed in HCD mode (B), in particular ions 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.

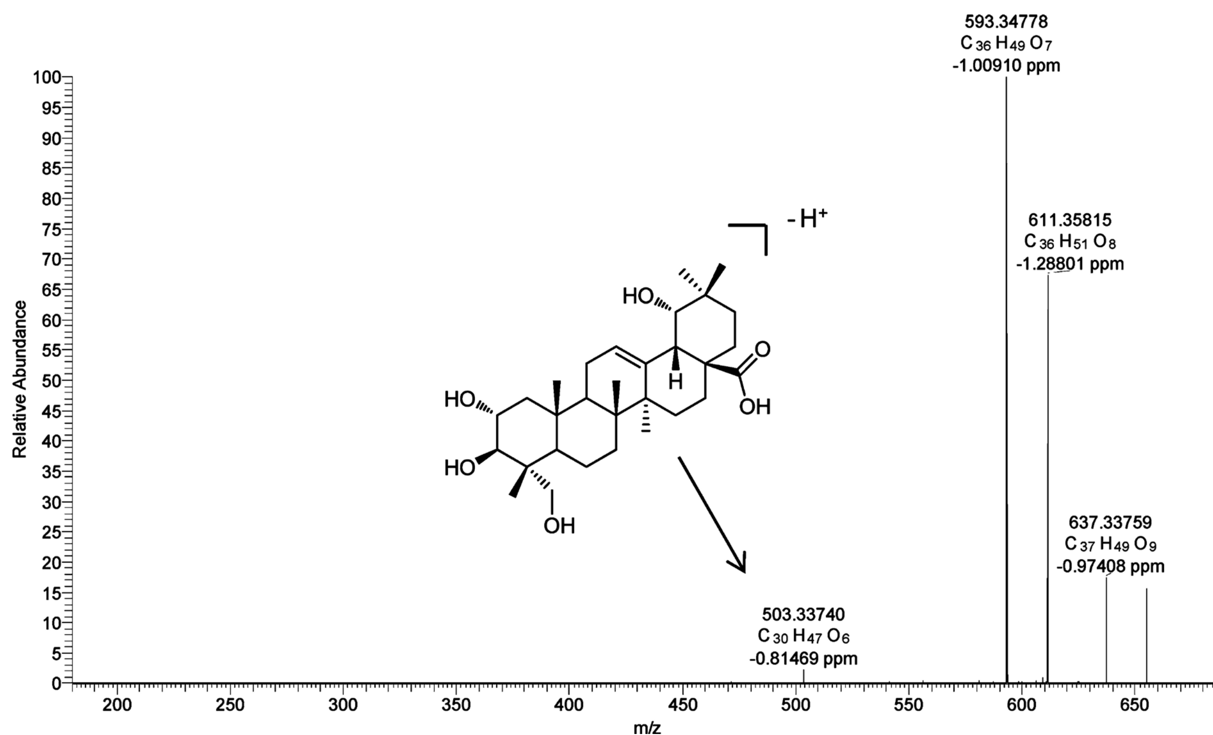

Figure 5. LIT MS^3 spectrum of the product ion at *m*/*z* 655, 30% resonant collision energy. The fragment at *m*/*z* 503.337 40 corresponds to the genin ion of **QTT I** after the loss of galloyl and glucopyranosyl moieties, confirming the triterpenoide nature of the molecule.

Tables

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

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.

Graphical abstract