Triterpenoids from *Quercus petraea*; Identification in Wines and Spirits and Sensory Assessment

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16 **ABSTRACT:** Eight new triterpenoids (**1**−**8**), the known genin (**9**), and two known
17 functionalized triterpenoids (**10**−**11**) were isolated from a *Quercus petraea* heartwood extract.
18 The structures of the new compounds were unequivocally elucidated using HRESIMS and
19 1D/2D NMR experiments. Sensory analyses were performed in a non-oaked wine on the pure
20 compounds **1**−**11**. Except compounds **1** and **11**, all molecules exhibited a sweet taste at 5 mg/L
21 that was particularly intense for compounds **3** and **9**. Using LC-HRMS, compounds **1**−**11** were
22 observed in an oak wood extract and in oaked red wine and cognac. They were also semi-
23 quantified in several samples of sessile (*Q. petraea*) and pedunculate (*Q. robur*) oak wood
24 extract. All compounds were found in quantities significantly higher in sessile than in
25 pedunculate oak wood. These results support the hypothesis of their contribution to the increase
26 in sweetness during oak aging and show that they can be used as chemical markers to identify
27 the species of oak used for cooperage.

28 Taste is the sense that enables perception of flavors in food and drink. There are five basic
29 tastes: sweet, salty, sour, bitter, and umami. Sweetness perception is inherited, associated with
30 nutrient-rich food, and is pleasant.[1] Nowadays, the reference compound associated with
31 sweetness is sucrose. However, many other compounds known as sweeteners exhibit a sweet
32 taste without being sugars. Their chemical space is very wide since they belong to different
33 chemical families such as polyols,[2] amino acids,[3,4] proteins,[5,6] terpenes,[7,8] or phenolic
34 compounds.[9–11] Some of them are obtained exclusively by chemical synthesis, while others are
35 naturally present in plants, like steviosides isolated from *Stevia rebaudiana* and mogrosides
36 present in the fruit of *Siraitia grosvenorii*, a Chinese plant also known as "lo han guo".[12]

37 The composition of wine as well as its sensory properties evolve over time and
38 particularly during barrel aging. Several wood species can be used by coopers to manufacture
39 barrels, but the most renowned casks are made from sessile oak (*Q. petraea* L.) and, to a lesser
40 extent, from pedunculate oak (*Q. robur* L.). The sensory modifications that occur during barrel
41 aging are mainly due to the supply of volatile and non-volatile compounds. Indeed, many
42 studies have highlighted wood compounds responsible for the modification of aroma,[13,14] color
43 stabilization,[15] and somatosensory perceptions.[16,17] Besides these well-known phenomena, an
44 increase in wine sweetness also occurs during barrel aging.[18] On a molecular level, this has
45 been partially explained by the recent identification in oak wood of sweet triterpenoids called
46 quercotriterpenosides I, II, III, and VI.[19,20] The strategy developed to isolate these compounds
47 was first based on bio-guided purification methods replacing bio-assays by sensory screening
48 of fractions. A combination of various separation techniques such as liquid/liquid extraction,
49 centrifugal partition chromatography (CPC), and preparative HPLC have allowed these new
50 sweeteners to be purified.

51 Natural biosynthetic pathways generally induce structural diversity among secondary
52 metabolites. For a given molecule, isomers or derivatives can be produced in the same plant

53 and develop similar or more valuable taste properties. Thus, oak wood extracts were screened
54 by LC-HRMS to search for structural analogues to quercotriterpenosides (QTT) on the basis of
55 their putative empirical formulae. Targeted purification of the analogues was guided by LC-
56 HRMS, and the structures of the isolated compounds were assigned by 1D/2D NMR
57 experiments. The objective of the present study was to increase knowledge about oak wood
58 composition by identifying new triterpenoids, to determine their sensory properties, and to
59 compare their abundance in sessile and pedunculate oak wood.
60

61 **RESULTS AND DISCUSSION**

62 Chips of *Q. petraea* heartwood were macerated in an H_2O/EtOH solution (50:50; v/v).
63 The first step consisted of sequential liquid/liquid extraction using EtOAc and *n*-BuOH to pre-
64 purify the crude extract. The resulting enriched EtOAc extract was subjected to CPC using the
65 Arizona solvent system G (*n*-heptane/EtOAc/MeOH/H_2O, 1:4:1:4 v/v), yielding three fractions.
66 Fraction I was still complex, thus a second CPC was performed followed by preparative HPLC,
67 yielding five new triterpenoids (**1**−**3**, **6**−**7**), the known genin (**9**), and a known galloylated
68 triterpenoid (**11**). Chromatographic separation (CPC and preparative HPLC) of fraction II gave
69 the new triterpenoids (**4**, **5**). Solid phase extraction (SPE) of fraction III gave the known
70 triterpenoid (**10**). A CPC experiment was carried out on the *n*-BuOH extract and preparative
71 HPLC allowed the purification of the new triterpenoid (**8**).

72 Compound **1** showed a negative-ion HRESIMS deprotonated ion at *m/z* 655.3485
73 (Figure S1, Supporting Information). Given the isotopic ratio (around 41%), an empirical
74 formula of $C_{37}H_{52}O_{10}$ was attributed using FTMS.[19] To investigate the nature and the sequence
75 of the functional groups, fragmentation was performed on the pure molecule by non-resonant
76 activation in the higher collision dissociation (HCD) mode with a collision energy of 90
77 arbitrary units. The presence of an ion at *m/z* 503.3371 ($[C_{30}H_{47}O_6]^-$) corresponding to the

78 neutral loss of $C_7H_4O_4$ suggested that compound **1** contained one galloyl group (Figure S1,
79 Supporting Information). This observation was confirmed by the presence of an ion at *m/z*
80 169.0134 ($[C_7H_5O_5]^-$) corresponding to gallic acid. Moreover, the empirical formula of the ion
81 at 503.3371 corresponded to a tetrahydroxyoleane-type triterpenoid.
82 The ^{13}C NMR spectrum showed 37 carbon resonances, of which 30 were assigned to the
83 triterpenoid and seven to the galloyl moiety. Inspection of the 1D and 2D NMR spectra [^1H,
84 COSY, ROESY, HSQC-TOCSY, HSQC, and HMBC (Figures S2 and S3, Supporting
85 Information)] indicated the presence of a carbonyl and a carboxylic group at δ_C 180.5, two
86 olefinic carbons at δ_C 123 and 143.4, three oxymethines at δ_C 66.4, 78.6 and 81, an
87 oxymethylene at δ_C 63.7 and six methyl singlets, which suggested a polyhydroxyoleane-type
88 triterpenoid (Table 2). By comparing the ^{13}C NMR data (Table 2) with reported data,[21,22] the
89 triterpenoid skeleton of compound **1** was identified as arjungenin [(2α,3β,19α)-2,3,19,23-
90 tetrahydroxyolean-12-en-28-oic acid].[23] The relative configuration was confirmed by a ROESY
91 experiment (Figure 1). Furthermore, the presence of a two-proton at δ_H 7.1 in the ^1H NMR data
92 (Table 1), and carbon atoms of an ester carbonyl carbon at δ_C 167.5, an aromatic quaternary
93 carbon at δ_C 120.5, and three oxygenated tertiary carbons in the ^{13}C NMR data (Table 2)
94 evidenced the presence of the galloyl group. These observations were in agreement with the
95 MS data. The downfield chemical shift of H-3 (δ_H 5.00) and its HMBC correlation with C-7‴
96 (δ_C 167.5) of the gallate moiety confirmed that galloylation occurred at C-3 of the arjungenin
97 moiety. Thus, the structure of compound **1** was defined as 3-*O*-galloylarjungenin.
98 The molecular formula of compound **2** was established by negative HRESIMS ([M −
99 H]⁻ *m/z* 655.3484) as $C_{37}H_{52}O_{10}$, corresponding to a tetrahydroxyoleane-type triterpenoid with
100 a galloyl moiety (Figure S4, Supporting Information). The ^{13}C and ^1H NMR data of compound
101 **2** were highly comparable to those of compound **1** except for the presence of an oxygen-bearing
102 methylene carbon (δ_C 66.7). The hydroxy group was located at C-24 position rather than C-23

103 based on observed ROE effects (Figure 1) between H-3α (δ_H 3.09) and Me-23 (δ_H 1.27); H-
104 24β (δ_H 4.39) and H-2β (δ_H 3.74); H-24β (δ_H 4.39) and H-25 (δ_H 1.05), suggesting that the
105 triterpenoid part in compound **2** is sericic acid [(2α,3β,19α) 2,3,19,24-tetrahydroxyolean-12-
106 en-28-oic acid].[24,25] The galloyl moiety was located at C-24 instead of C-3 based on the
107 observation of HMBC cross peaks between the protons at δ_H 4.39 (H-24α and H-24β) and C-
108 7‴ at δ_C 167.5 of the galloyl moiety. Thus, the structure of compound **2** was defined as 24-*O*-
109 galloylsericic acid.

110 The molecular formula of compound **3** was deduced as $C_{37}H_{52}O_{10}$ based on HRESIMS
111 ([M − H]$^-$ *m/z* 655.3483), again corresponding to a tetrahydroxyoleane-type triterpenoid with a
112 galloyl moiety (Figure S7, Supporting Information). Compound **3** was closely related to **2** as
113 shown by ^{13}C and ^{1}H NMR data (Table 1 and 2). The ^{13}C NMR spectrum showed 37 carbon
114 resonances, of which 30 were assigned to the triterpenoid and seven to the galloyl moiety. The
115 1D and 2D NMR spectra [^{1}H, ^{13}C, COSY, ROESY, HSQC-TOCSY, HSQC, and HMBC
116 (Figures S8 and S9, Supporting Information)] and comparison with the literature data (Tables
117 1 and 2) indicated that the triterpenoid part in compound **3** is sericic acid.[24,25] The position of
118 the galloyl unit was determined via the HMBC correlation between the proton at δ_H 4.76 (H-3)
119 and C-7‴ (δ_C 167.6) of the galloyl moiety. This suggested that compound **3** is a regioisomer of
120 compound **2**. Thus, its structure was defined as the new 3-*O*-galloylsericic acid.

121 The negative-ion HRESIMS spectrum of compound **4** showed a deprotonated molecular
122 ion [M − H]$^-$ at *m/z* 817.4011 (Figure S10, Supporting Information). Its empirical formula was
123 determined as $C_{43}H_{62}O_{15}$. The presence of an ion at *m/z* 655.3483 ([$C_{37}H_{51}O_{10}$]$^-$), observed after
124 fragmentation by non-resonant activation in the HCD mode and corresponding to the neutral
125 loss of $C_6H_{10}O_5$, suggested that compound **4** contains a hexosyl group (Figure S10, Supporting
126 Information). The presence of a minor peak associated with the ion at *m/z* 665.3908,
127 corresponding to the neutral loss of $C_7H_4O_4$, suggests that compound **4** also contains a galloyl

128 group. This observation is supported by the presence of another minor peak related to ion *m/z*
129 503.3375. It corresponds to the neutral loss of $C_{13}H_{14}O_9$ so the empirical formula ($C_{30}H_{48}O_6$) is
130 assigned to a tetrahydroxyoleane-type triterpenoid. The ^{13}C NMR data of compound **4**
131 displayed resonances for 43 carbons. Examination of the spectrum revealed the same signals as
132 those in compound **2**, together with six more signals between 60 and 100 ppm, suggesting that
133 **4** was a hexosyl derivative of compound **2** (Table 2). The genin moiety was established by
134 analysis of the 1D and 2D NMR spectra (^{1}H, ^{13}C, COSY, ROESY, HSQC-TOCSY, HSQC, and
135 HMBC [Figures S11 and S12, Supporting Information)], which allowed shift assignments
136 (Table 1 and 2). The ^{13}C NMR chemical shifts of the glycoside part suggested that it was a
137 glucose moiety. All the vicinal coupling constants of the hexosyl moiety were of 7-9 Hz
138 magnitude (Table 1), indicating a *β*-glucopyranose structure. The HMBC spectrum showed
139 cross-peaks between C-28 of the sericic acid aglycone and H-1′ of the glucosyl moiety (δ_H
140 5.37). This was confirmed by the upfield shift of C-28 (δ_C 176.9). Thus, compound **4** was
141 defined as 24-*O*-galloylsericoside. It is a new isomer of quercotriterpenoside II,[19] thus **4** was
142 named quercotriterpenoside VII (QTT VII).
143 HRESIMS analysis of compound **5** showed a molecular ion $[M - H]^-$ at *m/z* 817.3996
144 (Figure S13, Supporting Information) accounting for the molecular formula, $C_{43}H_{62}O_{15}$. Like
145 compound **4**, compound **5** is composed of a tetrahydroxyoleane-type triterpenoid with a hexosyl
146 moiety and a galloyl moiety (Figure S13, Supporting Information). The ^{13}C NMR data of
147 compound **5** displayed resonances for 43 carbons. The ^{13}C and ^{1}H NMR data (Table 1 and 2)
148 of compound **5** were closely comparable to those of compound **4**. The position of the galloyl
149 unit was determined by the HMBC correlation between a proton at δ_H 5.32 (H-2) and C-7‴ (δ_C
150 166.9) of the galloyl moiety. This indicated that compound **5** is a regioisomer of compound **4**
151 with the gallate moiety attached to C-2 rather than C-24 of the genin unit. Thus, compound **5** is

152 2-*O*-galloylsericic acid 28-*O*-*β*-D-glucopyranosyl. This is a new compound named
153 quercotriterpenoside VIII (QTT VIII).

154 The molecular formula of compound **6** was established by negative HRESIMS ([M −
155 H]$^-$ *m/z* 817.4011) as $C_{43}H_{62}O_{15}$, again corresponding to a tetrahydroxyoleane-type triterpenoid
156 with a hexosyl and a galloyl moiety (Figure S16, Supporting Information). The fragment ion at
157 *m/z* 313.0562 ([$C_{13}H_{13}O_9$]$^-$) indicated that these moieties are linked. As the fragment ion at *m/z*
158 169.0133 ([$C_7H_5O_5$]$^-$) was observed separately, the galloyl group could be in the terminal
159 position. The ^{13}C NMR spectrum showed 43 carbon signals, of which 30 were assigned to the
160 triterpenoid, six to the hexosyl group and seven to the galloyl unit. Inspection of the 1D and 2D
161 NMR spectra [^{1}H, ^{13}C, COSY, ROESY, HSQC-TOCSY, HSQC, and HMBC (Figures S17 and
162 S18, Supporting Information)] and comparison with the literature data (Tables 1 and 2)
163 indicated that the triterpenoid part in compound **6** is arjungenin [(2α,3β,19α)-2,3,19,23-
164 tetrahydroxyolean-12-en-28-oic acid].[23] The complete assignments of the glycosidic proton
165 system were achieved by COSY and HSQC-TOCSY experiments. The sugar was identified as
166 *β*-glucose (Table 1 and 2) which possessed a deshielded H-6′*α* (δ_H 4.36) and H-6′*β* (δ_H 4.60),
167 indicating the position of the galloyl moiety C-6′. The HMBC experiment showed cross peaks
168 between C-3 (δ_C 87.1) of the arjungenin and H-1′ of the glucopyranose (δ_H 4.46, J = 8.0 Hz),
169 between C-7‴ at δ_C 166.5 of the gallate moiety and H-6′*α* (δ_H 4.36) and H-6′*β* (δ_H 4.60) of the
170 sugar. Thus, compound **6** is 3-*O*-[(6-*O*-galloyl)-*β*-D-glucopyranosyl]arjungenin. This new
171 isomer of the recently described quercotriterpenosides I (QTT I) and III[19,20] was named
172 quercotriterpenoside IX (QTT IX).

173 The molecular formula of compound **7** was deduced via negative HRESIMS ([M − H]$^-$
174 *m/z* 817.4008; Figure S19, Supporting Information) data as $C_{43}H_{62}O_{15}$. The fragment ions at
175 *m/z* 313.0562 ([$C_{13}H_{13}O_9$]$^-$) and 169.0133 ([$C_7H_5O_5$]$^-$) were also observed. These data suggest
176 that compound **7** is composed of a tetrahydroxyoleane-type triterpenoid with a hexose−galloyl

177 moiety (Figure S19, Supporting Information). ^{1}H and ^{13}C NMR data (Table 1 and 2) of
178 compound **7** were close to those of compound **6**, except for the signals of the triterpenoid ring
179 A. The hydroxy group was located at C-24 instead of at C-23 based on the observation of an
180 ROE association between H-3α (δ_H 3.20) and H-23 (1.25); H-24β (δ_H 3.91) and H-2β (3.81);
181 H-24β (δ_H 3.91) and H-25 (0.91). This suggested that the C-4 configuration of the triterpenoid
182 has changed. The structure of the new compound **7** was assigned as 3-*O*-[(6-*O*-galloyl)-β-D-
183 glucopyranosyl]sericic acid and named quercotriterpenoside X (QTT X).
184 The negative-ion HRESIMS spectrum of compound **8** showed a deprotonated molecular
185 ion $[M - H]^-$ at *m/z* 979.4550 (Figure S22, Supporting Information). Its empirical formula was
186 determined as $C_{49}H_{72}O_{20}$. Several fragment ions were observed: *m/z* 817.4030 ($[C_{43}H_{61}O_{15}]^-$)
187 corresponded to the loss of a hexosyl group, 665.3942 ($[C_{36}H_{57}O_{11}]^-$) to the loss of a hexosyl
188 and a galloyl group, and 503.3399 ($[C_{30}H_{47}O_6]^-$) the loss of a galloyl and two hexosyl groups.
189 The fragment ion at *m/z* 755.4028 was intense. It differed by 162.0551 Da from the fragment
190 ion at *m/*z 593.3477 observed in QTT I,[19] presumably corresponded to the loss of a hexosyl
191 group, a decarboxylation, and a dehydration process. Finally, the observed fragment ions at *m/z*
192 313.0571 ($[C_{13}H_{13}O_9]^-$) and at *m/z* 169.0133 ($[C_7H_5O_5]^-$) indicated a hexose−galloyl moiety
193 with one galloyl group in the terminal position. The ^{13}C NMR spectrum showed 49 carbon
194 signals, of which 30 were assigned to the triterpenoid moiety, 12 to the two hexosyl groups and
195 seven to the galloyl moiety. Inspection of the 1D and 2D NMR spectra [^{1}H, ^{13}C, COSY,
196 ROESY, HSQC-TOCSY, HSQC, and HMBC (Figures S23 and S24, Supporting Information)]
197 and comparison with the literature data (Tables 1 and 2) indicated that the triterpenoid part in
198 compound **8** is arjungenin. Two anomeric carbons were detected at δ_C 94.4 and 104.2 in the ^{13}C
199 NMR spectrum and the anomeric proton doublets at δ_H 5. 38 (J = 8.2 Hz) and 4.47 (J = 8.0 Hz)
200 as shown in the HSQC experiment. The complete assignments of each glycosidic proton system
201 were achieved by COSY and HSQC-TOCSY experiments. The sugars were identified as two

202 β-glucopyranoses (Tables 1 and 2). The unit with the anomeric proton at δ_H 4.47 (J = 8.0Hz)
203 possessed a deshielded H-6′α (δ_H 4.38) and H-6′β (4.61), indicating the location of the galloyl
204 moiety. The HMBC experiment showed cross peaks between C-3 of the arjungenin aglycone
205 moiety and H-1′ of the glucose (δ_H 4.47, J = 8.0 Hz), between C-7‴ (δ_C 166.6) of the gallate
206 group and H-6′α (δ_H 4.38) and H-6′β (δ_H 4.61) of the first glucose moiety, and between C-28
207 at δ_C 177.2 of arjungenin and H-1′ of the second glucose unit (δ_H 5.38). Thus, the structure of
208 the new compound **8** was defined as 3-*O*-[(6-*O*-galloyl)-β-D-glucopyranosyl]-28-*O*-[β-D-
209 glucopyranosyl]arjungenin and named quercotriterpenoside XI (QTT XI) an isomer of QTT
210 IV.[20]

211 The negative-ion HRESIMS spectra of compounds **9−11** showed deprotonated
212 molecular ions $[M - H]^-$ at *m/z* 503.3379, 665.3904, and 655.3486, respectively. Considering
213 the mass accuracy specifications of the spectrometer and the isotopic ratio observed (35, 40,
214 and 43% respectively), the empirical formula $C_{30}H_{48}O_6$ was assigned to compound **9**, $C_{36}H_{58}O_{11}$
215 to compound **10**, and $C_{37}H_{52}O_{10}$ to compound **11**. Supportive GC-MS analyses were performed
216 on the hydrolysate of compound **10** to prove the absolute configuration of the glucose. Analysis
217 of the respective thiazolidine derivative in comparison to two standard sugars, D- and L-glucose,
218 was conducted to identify the sugar moiety of compound **10** as D-glucose. The NMR data (Table
219 S1, Supporting Information) and specific rotation (+37.7, +24, and +14.4, respectively) of each
220 compound were compared to the literature data. This showed that compound **9** was arjungenin,
221 compound **10** was arjunglucoside I, and compound **11** was 23-*O*-galloylarjungenin. These
222 triterpenoids have been identified in the bark of *Terminalia arjuna* (compounds **9−10**)[23] and *T.*
223 *brownii* (compound **11**),[26] and in *Q. robur* (compound **10**).[27] However, the taste of compounds
224 **1−11** has not yet been described.

225 All compounds except compound **5** whose quantities were too low were dissolved in
226 water and in non-oaked white wine (Bordeaux 2013) at 5 mg/L, and the taste of each solution

227 was characterized in comparison to the same water/wine as a reference. QTT I was used as a
228 sweetness standard since its sensory properties have already been characterized, with a
229 detection threshold in white wine of 590 µg/L.[28] In water, compounds **1**−**4** and **7**−**10** exhibited
230 a sweet taste (Table 3). On a 0−5 scale representing relative sweetness intensity assessed as a
231 consensus between the six panelists, compound **1** scored 1/5, compounds **7**, **8** and **10** 2/5,
232 compound **2** 3/5, while compounds **3**, **4**, and **9** were intensively perceived (4/5, 4/5, and 5/5
233 respectively). The same conditions (5 mg/L in water) were applied to QTT I. Its sweetness was
234 assessed as 4/5, which highlights the interest in compounds **3**, **4**, and **9**, for which the taste was
235 perceived as equal or more potent than QTT I. Purified compounds were also dissolved in non-
236 oaked white wine to study their influence on the balance of wine taste (Table 3). The control
237 wine was scored 0/5 for sweetness and 5/5 for acidity. As a reference, wine spiked with QTT I
238 (5 mg/L) was described as sweeter (4/5) and less sour (2/5). Compounds **1**−**2**, **7**, and **11** were
239 described as having a taste close to the reference wine. Compounds **4**, **6**, **8**, and **10** slightly
240 modified the wine taste. Finally, compounds **3** and **9** appeared to be the most interesting ones
241 by decreasing the perception of acidity (scored 2/5 and 1/5, respectively) and increasing that of
242 sweetness in white wine (4/5 and 5/5, respectively). The results suggested that compounds **3**
243 and **9** could have detection thresholds close to or lower than 590 µg/L in wine, which is much
244 lower than that of glucose, i.e. 4 g/L.[29]

245 The comparison of the taste properties and the molecular skeleton of all these
246 compounds, completed by QTT II, QTT III, QTT IV, and QTT VI previously identified and
247 tasted under the same conditions,[19,20] did not reveal any obvious structure-activity relationship
248 (Table 3). The sweet character of the molecules cannot be exclusively due to the presence of
249 some functional groups, their position on the genin, or the configuration of C-4. It seems that
250 all these parameters contribute to the sensory characteristics of the isolated triterpenoids.
251 Molecular representations in three dimensions and the use of *in silico* prediction models of the

252 sweet character could lead to a better understanding of the mechanisms of interaction between
253 triterpenoids and taste receptors.[30,31]

254 During barrel aging, the contact with wine or spirit can trigger the release of oak wood
255 compounds. Nevertheless, the presence of all the molecules constituting oak wood in a beverage
256 aged in barrels is not systematic and depends on their solubility and chemical reactivity. To
257 determine the relevance of the new taste-active compounds identified in this study, their
258 presence in a commercial wine and a commercial cognac aged in oak barrels was therefore
259 investigated.

260 Owing to its mass measurement accuracy, LC-HRMS allows the screening of samples by
261 targeting *m/z* ions characteristic of specified empirical formulae. Jointly with the comparison
262 of retention time, this property can be used to confirm the presence of a given compound in a
263 complex matrix. Figure 2 shows extracted ion chromatograms (XIC) obtained in an oak wood
264 extract (left), in an oaked red wine (middle), and in an oaked cognac (right) for *m/z* ratios
265 specific to compounds **1** to **11**. Similar signals were detected for all compounds in the three
266 matrices. Specificity of mass measurement (<5 ppm) and retention time similarity (<0.08 min)
267 demonstrated that compounds **1−11** were present in oaked wine and cognac. Analyses in the
268 HCD fragmentation mode revealed the same main fragment ions (*m/z* 169.0131, 313.0561,
269 503.3369, 655.3476, 755.4003, 817.4004) in the three matrices for each compound at the same
270 retention time, which confirmed the presence of compounds **1−11** in the analyzed matrices.
271 Moreover, the XIC corresponding to *m/z* 503.3378, 655.3488, 665.3906, 817.4016, and
272 979.4550 also exhibited additional peaks. Some of them correspond to known triterpenoids
273 such as QTT I (*m/z* 817.4016, t_R = 3.13 min), QTT II (*m/z* 817.4016, t_R = 3.60 min), QTT III
274 (*m/z* 817.4016, t_R = 3.37 min), QTT VI (*m/z* 817.4016, t_R = 3.00 min), and QTT IV (*m/z*
275 979.4550, t_R = 3.05 min), but the others implicate the presence of isomers in oak wood, wine,
276 and cognac.

277 Finally, the concentrations of compounds **1** to **11** were estimated in 35 samples of sessile
278 oak and 34 samples of pedunculate oak wood. All the results were expressed in µg/g of
279 equivalent QTT I. As shown in Figure 3, the amounts of the new compounds 3-*O*-
280 galloylarjungenin (**1**) (12.9 µg/g), 24-*O*-galloylsericic acid (**2**) (12.9 µg/g), 3-*O*-galloylsericic
281 acid (**3**) (2.9 µg/g), QTT VII (**4**) (16.7 µg/g), QTT VIII (**5**) (54.8 µg/g), QTT IX (**6**) (0.8 µg/g),
282 QTT X (**7**) (1.2 µg/g), QTT XI (**8**) (9.4 µg/g), and arjungenin (**9**) (15.9 µg/g), arjunglucoside I
283 (**10**) (37.1 µg/g), and 23-*O*-galloylarjungenin (**11**) (4 µg/g) were significantly higher in sessile
284 than in pedunculate oak wood. These results are consistent with previous observations
285 regarding the influence of oak species on the composition of triterpenoids in oak wood.[32,33] A
286 higher prevalence of tetrahydroxyoleane-type triterpenoids, isomers of compounds **4** and **11,**
287 was observed in sessile oak wood than in pedunculate oak wood. These findings could have
288 industrial implications since it is preferable to use oak wood that imparts more sweetness to
289 wines and spirits. The new compounds identified in this study could also be used to assign the
290 botanical species of oak by a chemical method.
291 In conclusion, we have identified eight new oak compounds that are relevant markers
292 of the oak species and whose presence and taste in wine and spirit have been established. Some
293 of them exhibit an intense sweetness that will be characterized in subsequent studies. The
294 findings offer promising perspectives for a better understanding of the molecular phenomena
295 associated with barrel aging and for industrial applications related to oak wood selection in the
296 cooperage industry.
297
298 **EXPERIMENTAL SECTION**
299 **General Experimental Procedures.** Optical rotations were measured with a JASCO P-
300 2000 polarimeter. The sodium emission wavelength was set at 589 nm and the temperature at
301 293 K. The samples were dissolved in MeOH, and the final value resulted from a mean of 10

302 measurements of 10 s each. NMR experiments were conducted on a Bruker Avance 600 NMR
303 spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz) equipped with a 5-mm TXI probe. All 1D
304 (proton) and 2D (COSY, ROESY, HMBC, and HSQC) spectra were acquired at 300 K in
305 methanol-d_4, which gave as reference the solvent signal (^{1}H δ 3.31; ^{13}C δ 49.00). Data analysis
306 was performed with Bruker Topspin version 3.2. The LC-HRESIMS platform consisted of an
307 HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland), an Accela U-HPLC
308 system with quaternary pumps, and an Exactive Orbitrap mass spectrometer equipped with a
309 heated electrospray ionization (H-ESI) probe (both from Thermo Fisher Scientific, Bremen,
310 Germany). CPC was performed on a Spotprep II LC coupled with a SCPC-100 + 1000 (Armen
311 Instrument, Saint-Avé, France), both controlled by Armen Glider Prep V5.0 software. A Waters
312 Prep 150 LC including a 2545 Quaternary Gradient Module and a 2489 UV/visible detector
313 was used for the last steps of purification. Final purification of compounds was performed by
314 preparative HPLC using columns chosen after LC-HRMS tests. A Hypersil Gold™ C_{18} column
315 (250 × 21.2 mm, 5 µm particle size, Thermo Fisher Scientific) equipped with a preparative
316 guard cartridge (20 × 20 mm, 5 µm particle size, Thermo Fisher Scientific) was used to purify
317 compounds **1**–**3**, **9**, and **11**; a Microsorb 100-5 column (250 × 21.4 mm, 5 µm particle size,
318 Varian, Palo Alto, CA) equipped with a Prontosil C_{18} 5 µm pre-column (50 × 20 mm, Bischoff
319 chromatography, Leonberg, Deutschland) was used to purify compounds **4**–**5** and **8**; and a
320 SunFire™ Prep C_{18} OBD column (250 × 19 mm, 5 µm particle size, Waters, Milford, MA)
321 equipped with a SunFire® preparative C_{18} guard cartridge (20 × 19 mm, 5 µm particle size,
322 Waters) was used to purify compounds **6**–**7**. Compound **10** was purified by SPE using an
323 Oasis® HLB 20 cc Vac Cartridge (1 g sorbent per cartridge, 60 µm particle size, 20/pk, Waters)
324 with a vacuum manifold. For LC-HRMS analyses and quantitation, a Hypersil Gold™ C_{18}
325 column (100 × 2.1 mm, 1.9 µm particle size, Thermo Fisher Scientific) was used as the
326 stationary phase. Concerning hydrolysis procedure, all GC-MS analyses were carried out on an

327 Agilent 5975B Series GC/MSD System equipped with an Agilent 7683B autosampler, and
328 Agilent 6890N GC System using a Phenomenex ZB-35 (60 m × 250 µm × 0.25 µm) capillary
329 column as stationary phase. Sample preparation, extraction, centrifugal partition
330 chromatography, solid phase extraction, hydrolysis procedure, and high-performance liquid
331 chromatography were performed with ultrapure water (Milli-Q purification system, Millipore,
332 France) and HPLC grade solvents (VWR International, Pessac, France). LC-HRMS
333 chromatographic separations were performed with LC-MS grade MeCN and deionized
334 ultrapure water (Optima, Fisher Chemical, Illkirch, France). Two commercial wines were used
335 in this study: a white non-oaked Bordeaux 2013 (100% Sauvignon Blanc, 12.5% v/v) for
336 sensory analysis and a red Pessac-Léognan 2016 (60% Cabernet Sauvignon, 40% Merlot,
337 13.5% v/v) aged in new oak barrels for chemical analysis. A commercial brandy (Cognac XO)
338 aged in oak barrels was also analyzed.

339 **Plant Material.** Oak wood used to isolate compounds was supplied by the cooperage
340 company Seguin-Moreau (Merpins, France). It was sampled in January 2014 from a batch of
341 staves that were to be used to make barrels. The botanical species was assigned to *Q. petraea*
342 according to the method described by Marchal et al.[32] The staves were air-dried for two years
343 according to the cooperage process. They were then reduced to chips (30 mm × 20 mm × 5 mm)
344 by the barrel manufacturer (Seguin Moreau, Merpins, France). A voucher specimen is deposited
345 in the Institute of Vine and Wine Sciences with the reference BCTR-14-07.

346 **Extraction and Isolation.** The oak wood material (2 kg) was macerated in 10 L of
347 H_2O−EtOH solution (50:50; v/v) for 2 weeks at room temperature. Wood chips and particles
348 were filtered (0.45 µm). The liquid medium was concentrated *in vacuo* to remove EtOH and,
349 partly, water. The aqueous solution (800 mL) was extracted with EtOAc (5 × 500 mL). The
350 remaining aqueous phase was extracted with *n*-BuOH (4 × 300 mL). The organic layers of each
351 extraction step were combined, evaporated under reduced pressure, suspended in water, and

352 freeze-dried. The EtOAc extract (26 g), the *n*-BuOH extract (15 g), and the H_2O extract (58 g)
353 were stored under air- and light-protective conditions.
354 After testing the "Arizona" Foucault et Chevolot solvent system,[34] an appropriate system
355 was chosen for the first partition of the different organic extracts. The solvents were pumped
356 by a four-way quaternary high-pressure gradient pump, and an automatic high pressure 30 mL
357 injection valve was used to inject the sample into the system. Separation was performed at room
358 temperature. Each extract was dissolved in 8 mL of the organic/aqueous phase mixture (1:1)
359 and filtered. Separation was conducted in the 1 L rotor at 1200 rpm in ascending mode. The
360 flow rate was set at 25 mL/min during elution (120 min) and 40 mL/min during extrusion (60
361 min). Collection was performed automatically in 25 mL tubes by a Spot Prep fraction collector
362 set to one tube/min during elution and two tubes/min during extrusion. Every five CPC tubes,
363 100 µL were taken, evaporated, and dissolved in 1 mL of H_2O/MeOH 95/5. After filtration
364 (0.45 mm), 5 µL of each sample were injected into LC-HRMS. Consistent with LC-HRMS
365 results, CPC tubes were pooled according to the elution profile of the major compounds,
366 evaporated *in vacuo*, suspended in water, and freeze-dried.
367 The EtOAc extract was fractionated with a CPC using the Arizona solvent system G (*n*-
368 heptane/EtOAc/MeOH/H_2O, 1:4:1:4 v/v). Separation was performed by five consecutive CPC
369 runs with an average of 2.8 g per injection. The CPC fraction I containing compounds **1**−**3**, **6**−**7**,
370 **9**, and **11** was still abundant (441 mg) and chemically complex. A second CPC was carried out
371 using the Arizona solvent system L (*n*-heptane/EtOAc/MeOH/H_2O, 2:3:2:3 v/v), affording six
372 fractions (Fr. A−Fr. F). Compounds **1** (3.6 mg, t_R = 29.8 min), **2** (1.8 mg, t_R = 32.6 min), and **3**
373 (1.6 mg, t_R = 33.9 min) were purified from Fr. B (70 mg); compounds **9** (2.8 mg, t_R = 22.6 min)
374 and **11** (9.1 mg, t_R = 24.1 min) from Fr. D (100 mg) by preparative HPLC (H_2O/CH_3CN both
375 acidified with 0.05% TFA) with a gradient at 20 mL/min as follows: 30% B (0−7 min), 30−35%
376 B (7−22 min), 35% B (22−37 min), 35−100% B (37−39 min). Compounds **6** (2.8 mg, t_R = 16

377 min) and **7** (2.8 mg, t_R = 20 min) were purified from Fr. E (50 mg) using a gradient at 20 mL/min
378 as follows: 28% B (0−5 min), 28−32% B (5−10 min), 32% B (10−15 min), 32−36% B (15−35
379 min), 36% B (35−40 min), 36−100% B (40−41 min). Compounds **4**−**5** were present in the
380 chemically complex CPC fraction II (500 mg). Thus, a second CPC was performed using the
381 derivative Arizona solvent system F with MtBE instead of EtOAc (*n*-
382 heptane/MtBE/MeOH/H_2O, 1:5:1:5 v/v) affording five fractions (Fr. G−Fr. K). Fr. H (110 mg)
383 was purified by preparative HPLC (H_2O/CH_3CN both acidified with 0.05% TFA) with a
384 gradient at 20 mL/min as follows: 25% B (0−7 min), 25−30% B (7−15 min), 30% B (15−17
385 min), 30−43% B (17−55 min), 43−50% B (55−65 min), and 50−100% B (65−70 min) to yield
386 compound **4** (1.5 mg, t_R = 17 min) and **5** (1.2 mg, t_R = 22 min). Finally, compound **10**, which
387 was present in CPC fraction III, was co-eluted with another compound. Preparative HPLC did
388 not allow its purification, regardless of the columns tested, so another approach with SPE was
389 attempted. A series of MeOH/H_2O acidified with TFA (0.05%) solutions (15, 20, 22.5, 25, 35,
390 and 40%) was used to elute compound **10** (12 mg, Fr. 25%). The pure compound solution was
391 evaporated in vacuo and freeze-dried to obtain white amorphous powder.

392 The *n*-BuOH extract was subjected to CPC using the quaternary biphasic Arizona
393 system B with *n*-heptane/EtOAc/MeOH/H_2O (1:19:1:19, v/v). Separation was carried out by
394 four consecutive CPC runs with an injection of 2.3 g approximatively at each run to obtain 6
395 fractions (Fr. L−Fr. Q). Fr. M was purified by preparative HPLC (H_2O acidified with 0.025%
396 TFA /CH_3CN), with a gradient at 20 mL/min as follows: 18% B (0−6 min), 18−26% B (6−18
397 min), 26% B (18−23 min), 26−32% B (23−32 min), 32% B (31−36 min), 32−48% B (36−53
398 min), 48−100% B (53−57 min) affording compound **8** (2.2 mg, t_R = 27 min).

399 For preparative HPLC experiments, a 10-min equilibration phase was applied manually
400 before each injection. Elution was monitored by UV detection at 280 nm and by evaporative
401 light scattering detection (ELSD) for compound **9,** which was not visible at 280 nm.

402 Chromatographic peaks were collected manually just downstream of the detector in 25 mL
403 tubes. Samples obtained after successive injections were pooled, evaporated in vacuo to remove
404 acetonitrile and freeze-dried twice to obtain white amorphous powders.

405 *3-O-Galloylarjungenin (**1**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ +36 (*c* 0.1, MeOH); ^{1}H
406 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2; (−)-
407 HRMS *m/z* 655.3485 (calcd for $C_{37}H_{51}O_{10}^{-}$, 655.3488).

408 *24-O-Galloylsericic acid (**2**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ +30 (*c* 0.1, MeOH); ^{1}H
409 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2; (−)-
410 HRMS *m/z* 655.3484 (calcd for $C_{37}H_{51}O_{10}^{-}$, 655.3488).

411 *3-O-Galloylsericic acid (**3**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ +24 (*c* 0.1, MeOH); ^{1}H
412 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2; (−)-
413 HRMS *m/z* 655.3483 (calcd for $C_{37}H_{51}O_{10}^{-}$, 655.3488).

414 *Quercotriterpenoside VII (**4**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ −11 (*c* 0.1, MeOH); ^{1}H
415 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2; (−)-
416 HRMS *m/z* 817.4011 (calcd for $C_{43}H_{61}O_{15}^{-}$, 817.4016).

417 *Quercotriterpenoside VIII (**5**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ −12 (*c* 0.1, MeOH);
418 ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2;
419 (−)-HRMS *m/z* 817.3996 (calcd for $C_{43}H_{61}O_{15}^{-}$, 817.4016).

420 *Quercotriterpenoside IX (**6**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ −14 (*c* 0.1, MeOH); ^{1}H
421 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2; (−)-
422 HRMS *m/z* 817.4011 (calcd for $C_{43}H_{61}O_{15}^{-}$, 817.4016).

423 *Quercotriterpenoside X (7)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ −12 (*c* 0.1, MeOH); ^{1}H
424 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2; (−)-
425 HRMS *m/z* 817.4008 (calcd for $C_{43}H_{61}O_{15}^{-}$, 817.4016).

426 *Quercotriterpenoside XI (**8**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ −21 (*c* 0.03, MeOH);
427 ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Tables 1 and 2;
428 (−)-HRMS *m/z* 979.4550 (calcd for $C_{49}H_{71}O_{20}^{-}$, 979.4544).

429 *Arjungenin (**9**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ +38 (*c* 0.1, MeOH); ^{1}H NMR
430 (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table S1, Supporting
431 Information; (−)-HRMS *m/z* 503.3379 (calcd for $C_{30}H_{47}O_{6}^{-}$, 503.3378).

432 *Arjunglucoside I (**10**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ +24 (*c* 0.1, MeOH); ^{1}H NMR
433 (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table S1, Supporting
434 Information; (−)-HRMS *m/z* 665.3904 (calcd for $C_{36}H_{57}O_{11}^{-}$, 665.3906).

435 *23-O-Galloylarjungenin (**11**)*: white, amorphous powder; $[\alpha]^{25}{}_{D}$ +14 (*c* 0.1, MeOH); ^{1}H
436 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table S1,
437 Supporting Information; (−)-HRMS *m/z* 655.3486 (calcd for $C_{37}H_{51}O_{10}^{-}$, 655.3488).

438 **Hydrolysis Procedure.** Compound **10** (10 mg) was refluxed with 15 mL of 2N HCl for
439 2 h. The mixture was extracted with EtOAc (3 × 15 mL). The aqueous phase was neutralized
440 with 0.5 M KOH and freeze-dried. The dried hydrolysate (1 g) was derivatized with L-cysteine
441 methyl ester hydrochloride (7.5 g/L in pyridine, 4 mL, 60 °C, 1 h), subsequently silylated with
442 *N*,*O*-bis(trimethylsilyl)trifluoroaceamide and chlorotrimethylsilane (BSTFA:TMCS = 99:1,
443 v/v; 500 µL, 60 °C, 1 h) and analyzed by GC-MS. Reference compounds (purity ≥99.5%,
444 Sigma-Aldrich) D-glucose 1.6 mg (t_R = 34.15 min) and L-glucose 0.74 mg (t_R = 34.40 min) were
445 derivatized and analyzed using the same protocol. The following GC-MS parameters were
446 applied: oven 100 °C for 1 min, then 5 °C/min to 250 °C for 15 min, total run time 52 min;
447 injection volume 2 µL; splitless; carrier gas helium; flow rate 1 mL/min; SCAN mode.

448 **Sensory Analysis.** Gustatory analysis was performed in a dedicated room, under normal
449 daylight, and at room temperature (around 20 °C).[35] Samples were poured into clear INAO wine
450 glasses[36] and were tasted by six experts in winetasting (two men, four women, aged from 24 to

451 62 years old). The tasters were informed of the nature and risks of the present study and were
452 asked to give their consent to participate in the sensory analyses. The purified compounds were
453 dissolved individually at 5 mg/L in water (eau de source de Montagne, Laqueuille, France), as
454 well as in a white non-oaked wine (Bordeaux, 2013). The wine experts were asked to describe
455 the gustatory perception of each compound using the vocabulary of winetasting. In particular,
456 sweetness and acidity intensity were evaluated on a scale from 0 (not detectable) to 5 (strongly
457 detectable) compared to a blank solution. QTT I, identified by Marchal et al.,[19] was used as
458 reference. Even though the fractions and compounds were purified from commercial oak wood
459 used for cooperage and observed in oaked wines, the panelists were advised not to swallow but
460 to spit out the samples after tasting.

461 **Quantitation of Compounds 1 to 11 by LC-HRMS.** Quantitation was performed using
462 the LC-HRESIMS platform described in General Experimental Procedures. Samples of oak
463 wood extract (*n* = 35 for sessile oak wood, *n* = 34 for pedunculate oak wood) were provided
464 and prepared by the barrel manufacturer (Seguin Moreau) as described in a previous study.[32]
465 Prior to analysis, each sample was diluted five times with Milli-Q water and filtered through a
466 0.45 μm PTFE syringe filter. The mobile phases were (A) water and (B) MeCN. The flow rate
467 was 600 μL/min, and eluent B varied as follows: 20% B (0−0.5 min); 20−50% B (0.5−4 min);
468 50−98% B (4−4.1 min); 98% B (4.1−6.1 min); 20% B (6.1−6.2 min); 20% B (6.2−7.5 min).
469 The injection volume was 5 μL. Since quantities of compounds were too low to build calibration
470 curves, the results were expressed as μg/L (of oak wood) equivalent QTT I, on the basis of the
471 dilution factor. Oak wood species (sessile or pedunculate) were determined by genetic analysis
472 as described by Guichoux et al..[37,38]

473 **Statistical Analysis.** All values are expressed as mean ± 95% confidence interval (CI).
474 Statistical analysis was performed using the Kruskal−Wallis test and XL-STAT version
475 2010.5.05 (Addinsoft, Paris, France).

476 ## ASSOCIATED CONTENT

477 **Supporting Information**

478 The following data are available as supplementary material:

479 NMR spectroscopic data for compounds **9−11**. HRMS spectra of compounds **1−8** in the full-
480 scan and HCD mode. 1D (^{1}H) and 2D (COSY, ROESY, HSQC and HMBC) NMR spectra of
481 **1−8**.

482

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490

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492

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498 **REFERENCES**

499 (1) Schlienger, J.-L.; Monnier, L. *Médecine Mal. Métaboliques* **2014**, *8*, 346–351.

500 (2) Hufnagel, J. C.; Hofmann, T. *J. Agric. Food Chem.* **2008**, *56*, 1376–1386.

501 (3) Hufnagel, J. C.; Hofmann, T. *J. Agric. Food Chem.* **2008**, *56*, 9190–9199.

502 (4) Sáenz-Navajas, M.-P.; Campo, E.; Avizcuri, J. M.; Valentin, D.; Fernández-Zurbano,
503 P.; Ferreira, V. *Anal. Chim. Acta* **2012**, *732*, 64–72.

504 (5) Gibbs, B. F.; Alli, I.; Mulligan, C. *Nutr. Res.* **1996**, *16*, 1619–1630.

505 (6) Assadi-Porter, F. M.; Maillet, E. L.; Radek, J. T.; Quijada, J.; Markley, J. L.; Max, M.
506 *J. Mol. Biol.* **2010**, *398*, 584–599.

507 (7) Geuns, J. M. C. *Phytochemistry* **2003**, *64*, 913–921.

508 (8) Mogra, R.; Dashora, V. *J Hum Ecol* **2009**, *25*, 117–120.

509 (9) Zehnter, R.; Gerlach, H. *Tetrahedron Asymmetry* **1995**, *6*, 2779–2786.

510 (10) Kinghorn, A. D.; Soejarto, D. D. *Pure Appl. Chem.* **2002**, *74*, 1169–1179.

511 (11) Baek, N.-I.; Chung, M.-S.; Shamon, L.; Kardono, L. B. S.; Tsauri, S.; Padmawinata,
512 K.; Pezzuto, J. M.; Soejarto, D. D.; Kinghorn, A. D. *J. Nat. Prod.* **1993**, *56*, 1532–1538.

513 (12) Kinghorn, A.; Chin, Y.-W.; Pan, L.; Jia, Z. *Comprehensive Natural Products II:*
514 *Chemistry and Biology*; Liu, H.-W. (Ben), Mander, L., Eds.; Elsevier: Oxford, 2010;
515 Vol. 3, pp 269–315.

516 (13) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N. *Aust. New Zealand Wine Ind. J.* **1991**, *6*,
517 73–84.

518 (14) Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. *J. Agric. Food Chem.* **2000**,
519 *48*, 1799–1802.

520 (15) Chassaing, S.; Lefeuvre, D.; Jacquet, R.; Jourdes, M.; Ducasse, L.; Galland, S.;
521 Grelard, A.; Saucier, C.; Teissedre, P.-L.; Dangles, O.; et al. *Eur. J. Org. Chem.* **2010**,
522 *2010*, 55–63.

523 (16) Quinn, M. K.; Singleton, V. L. *Am. J. Enol. Vitic.* **1985**, *36*, 148–155.
524 (17) Glabasnia, A.; Hofmann, T. *J. Agric. Food Chem.* **2006**, *54*, 3380–3390.
525 (18) Marchal, A.; Pons, A.; Lavigne, V.; Dubourdieu, D. *Aust. J. Grape Wine Res.* **2013**, *19*,
526 11–19.
527 (19) Marchal, A.; Waffo-Téguo, P.; Génin, E.; Mérillon, J.-M.; Dubourdieu, D. *Anal. Chem.*
528 **2011**, *83*, 9629–9637.
529 (20) Marchal, A.; Génin, E.; Waffo-Téguo, P.; Bibès, A.; Da Costa, G.; Mérillon, J.-M.;
530 Dubourdieu, D. *Anal. Chim. Acta* **2015**, *853*, 425–434.
531 (21) Nandy, A. K.; Podder, G.; P. Sahu, N.; Mahato, S. B. *Phytochemistry* **1989**, *28*, 2769–
532 2772.
533 (22) Jossang, A.; Seuleiman, M.; Maidou, E.; Bodo, B. *Phytochemistry* **1996**, *41*, 591–594.
534 (23) Honda, T.; Murae, T.; Tsuyuki, T.; Takahashi, T. *Chem. Pharm. Bull. (Tokyo)* **1976**,
535 *24*, 178–180.
536 (24) Yeo, H.; Park, S.-Y.; Kim, J. *Phytochemistry* **1998**, *48*, 1399–1401.
537 (25) Ponou, B. K.; Teponno, R. B.; Ricciutelli, M.; Nguelefack, T. B.; Quassinti, L.;
538 Bramucci, M.; Lupidi, G.; Barboni, L.; Tapondjou, L. A. *Chem. Biodivers.* **2011**, *8*,
539 1301–1309.
540 (26) Machumi, F.; Midiwo, J. O.; Jacob, M. R.; Khan, S. I.; Tekwani, B. L.; Zhang, J.;
541 Walker, L. A.; Muhammad, I. *Nat. Prod. Commun.* **2013**, *8*, 761–764.
542 (27) Muccilli, V.; Cardullo, N.; Spatafora, C.; Cunsolo, V.; Tringali, C. *Food Chem.* **2017**,
543 *215*, 50–60.
544 (28) Marchal, A. PhD thesis, Université de Bordeaux II, **2010**.
545 (29) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. *Handbook of Enology*
546 *Vol. 2. The Chemistry of Wine Stabilization and Treatments*; Handbook of Enology;
547 John Wiley & Sons, Ltd, 2012; Vol. 2.

548 (30) Sanz, G.; Thomas-Danguin, T.; Hamdani, E. H.; Le Poupon, C.; Briand, L.; Pernollet,
549 J.-C.; Guichard, E.; Tromelin, A. *Chem. Senses* **2008**, *33*, 639–653.
550 (31) Chéron, J.-B.; Casciuc, I.; Golebiowski, J.; Antonczak, S.; Fiorucci, S. *Food Chem.*
551 **2017**, *221*, 1421–1425.
552 (32) Marchal, A.; Prida, A.; Dubourdieu, D. *J. Agric. Food Chem.* **2016**, *64*, 618–626.
553 (33) Schnell, G.; Schaeffer, P.; Tardivon, H.; Motsch, E.; Connan, J.; Ertlen, D.; Schwartz,
554 D.; Schneider, N.; Adam, P. *Org. Geochem.* **2014**, *66*, 107–124.
555 (34) Foucault, A. P.; Chevolot, L. *J. Chromatogr. A* **1998**, *808*, 3–22.
556 (35) ISO 13299. *Analyse Sensorielle: Recueil, Normes, Agroalimentaire*; AFNOR, 2003.
557 (36) ISO 3591. *Analyse Sensorielle: Recueil, Normes, Agroalimentaire*; AFNOR, 1977.
558 (37) Guichoux, E.; Lagache, L.; Wagner, S.; Léger, P.; Petit, R. J. *Mol. Ecol. Resour.* **2011**,
559 *11*, 578–585.
560 (38) Guichoux, E.; Garnier-Géré, P.; Lagache, L.; Lang, T.; Boury, C.; Petit, R. J. *Mol.*
561 *Ecol.* **2013**, *22*, 450–462.
562

Table 1. ^{1}H NMR Assignments for Compounds 1−8 (600 MHz, methanol-d_4)

position	1	2	3	4	5	6	7	8
	δH (J in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1*α*	1.06, m	0.97, m	1.1, m	0.97, m	1.05, m	0.86, m	0.88, m	0.87, m
1*β*	2, m	1.99, dd (12.9, 4.8)	2.04, m	2.0, m	2.04, m	1.96, dd (12.9, 4.6)	1.96, dd (12.9, 4.4)	1.97, dd (12.8, 3.8)
2	3.97, td (10.7, 4.5)	3.74, td (10.5 ; 4.5)	3.94, td (0.5, 4.5)	3.74, dd (10.9, 4.3)	5.32 brd	3.79, dd (9.7, 6.6)	3.81, m	3.80, m
3	5, d (9.8)	3.09 brd	4.76, d (9.9)	3.06, d (9.9)	3.44, d (10.3)	3.48, d (9.5)	3.20, d (9.2)	3.49, d (9.8)
4								
5	1.53, m	1.05, m	1.11, m	1.05, m	1.09, m	1.33 brd (11.7)	1.05 brd (11.6)	1.34, m
6*α*	1.46, m	1.65, m	1.49, m	1.65, m	1.51, m	1.50, d (12.7)	1.44, d (12.7)	1.39, m
6*β*	1.55, m	1.84, m	1.72, m	1.86, m	1.69, m	brd	1.64, m	1.49, m
7*α*	1.31, m	1.39, m	1.34 brd	1.39, m	1.35, m	1.26, d (13.2)	1.32, d (12.5)	1.29, m
7*β*	1.66, m	1.51, m	1.53 brd	1.49, m	1.50, m	brd	1.51, m	1.62, m
8								
9	1.92, t (8.9)	1.82 brd	1.86, t (9)	1.83, m	1.82, m	1.83, t (9.0)	1.77, m	1.82, m
10								
11*α*	0.98, m	0.97, m	0.97, m	2.02, m	1.97, m	1.99, m	1.94, m	0.98, m
11*β*	2.03, m	2.02, m	2.02, m	brd	brd	brd	2.00, m	2.01, m
12	5.35, t (3.3)	5.34, t (3.6)	5.34, t (3.6)	5.35, t (3.8)	5.33 brd	5.33, t (3.3)	5.32, t (3.3)	5.35, t (3.6)
13								
14								
15*α*	1.03, m	0.94, m	1.06, m	1.02, m	1.03, m	1.02, m	1.02, m	1.01, m
15*β*	1.62, m	1.77, m	1.65, m	1.69, m	1.68, m	1.77, m	1.65, m	1.69, m
16*α*	1.64, m	1.60, m	1.66, m	1.73, m	1.75, m	1.60, m	1.60, m	1.74, m
16*β*	2.31, td (14, 3.5)	2.28, td (13.7, 3.5)	2.29, td (13.4, 3.5)	2.34, td (13.3, 3.3)	2.33, td (12.4, 4.2)	2.30, td (13.9, 3.6)	2.29, td (13.5, 3.7)	2.33, td (13.1, 2.8)
17								
18	3.06 brd	3.05 brd	3.07, m	3.07, d (3.8)	3.06 brd	3.06 brd (3.8)	3.06 brd (3.5)	3.07 d, (3.3)
19	3.26, d (3.7)	3.28, d (3.6)	3.28, d (3.6)	3.29, d (3.8)	3.26, d (3.4)	3.27, m	3.27, d (3.8)	3.29 d, (3.3)
20								
21*α*	1.01, m	1.01, m	1.06, m	1.02, m	1.02, m	1.02, m	1.02, m	1.02, m
21*β*	1.65, m	1.74, m	1.65, m	1.78, m	1.69, m	1.77, m	1.77, m	1.31, m
22*α*	1.64, m	1.62, m	1.64, m	1.68, m	1.67, m	1.77, m	1.77, m	1.68, m
22*β*	1.78, m	1.74, m	1.75, m	1.79, m	1.78, m	1.60, m	1.60, m	1.79, m
23*α*	3.0, d (12.1)	1.27, s	1.05, s	1.29, s	0.71, s	3.28, m	1.25, s	3.29, d (11.2)
23*β*	3.32, d (12.1)					3.70, d (11.6)		3.71, d (11.2)
24*α*	0.85, s	4.01, m	3.87 brd	4.34, d (11.8)	3.50, d (11.6)	0.75, s	3.40, d (11.6)	0.76, s
24*β*		4.39, m	3.90 brd	4.45, d (11.8)	4.13, d (11.6)		3.91, d (11.5)	
25	1.09, s	1.05, s	0.81, s	1.06, s	1.10, s	1.01, s	0.91, s	1.03, s

26	0.81, s	0.76, s	0.81, s	0.74, s	0.75, s	0.76, s	0.76, s	0.75, s
27	1.34, s	1.20, s	1.33, s	1.31, s	1.17, s	1.31, s	1.29, s	1.31, s
28								
29	0.95, s	0.94, s	0.95, s	0.95, s	0.94, s	0.95, s	0.95, s	0.96, s
30	0.96, s	0.99, s	0.97, s	0.96, s	0.95, s	0.97, s	0.97, s	0.97, s
1′				5.37, d (8.5)	5.39, d (8.1)	4.46, d (8.0)	4.49, d (7.8)	5.38, d (8.2)
2′				3.31, m	3.32, m	3.28, m	3.29, m	3.33, m
3′				3.39, d (8.3)	3.35, m	3.43, m	3.44, m	3.36, m
4′				3.34, m	3.36, m	3.43, m	3.46, m	3.35, m
5′				3.34, m	3.42, m	3.64, m	3.66, m	3.42, m
6′*α*				3.68, dd (11.8, 3.9)	3.69, dd (11.0, 4.0)	4.60, dd (11.9, 2.1)	4.62, dd (12.0, 1.9)	3.69, m
6′*β*				3.82, m	3.83, m	4.36, dd (12.0, 5.5)	4.36, dd (12.0, 5.1)	3.83 brd (11.9)
1″								4.47, d (8.0)
2″								3.30, m
3″								3.43, m
4″								3.44, m
5″								3.65, m
6″*α*								4.38, dd (12.1, 5.7)
6″*β*								4.61, dd (12.1, 2.0)
1‴								
2‴, 6‴	7.1, s	7.09, s	7.1, s	7.08, s	7.09, s	7.12, s	7.11, s	7.12, s
3‴, 5‴								
4‴								
7‴								

Table 2. ^{13}C NMR Assignments for Compounds 1−8 (150 MHz, methanol-d_4)

	1	2	3	4	5	6	7	8
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1α	46.8, CH_2	46.7, CH_2	47.5, CH_2	46.7, CH_2	43.0, CH_2	45.4, CH_2	44.7, CH_2	45.3, CH_2
1β								
2	66.4, CH	68.1, CH	65.6, CH	67.4, CH	72.2, CH	66.5, CH	66.5, CH	66.7, CH
3	78.6, CH	82.9, CH	84.5, CH	82.9, CH	81.4, CH	87.1, CH	94.1, CH	87.2, CH
4	43.2, C	42.2, C	44.5, C	42.6, C	43.6, C	44.0, C	45.2, C	43.8, C
5	46.5, CH	55.8, CH	55.8, CH	55.9, CH	55.3, CH	45.9, CH	55.3, CH	46.5, CH
6α	17.5, CH_2	20.0, CH_2	19.4, CH_2	20.1, CH_2	18.2, CH_2	17.1, CH_2	17.7, CH_2	17.4, CH_2
6β								
7α	31.9, CH_2	32.9, CH_2	32.9, CH_2	32.8, CH_2	32.3, CH_2	31.8, CH_2	32.1, CH_2	31.7, CH_2
7β								
8	39.2, C	39.0, C	38.3, C	39.5, C	40.3, C	38.8, C	39.1, C	40.3, C
9	48.5, CH	48.0, CH	47.9, CH	48.2, CH	47.4, CH	47.4, CH	47.1, CH	47.5, CH
10	37.5, C	36.6, C	37.4, C	37.7, C	38.2, C	36.7, C	37.4, C	37.6, C
11α	23.3, CH_2	23.2, CH_2	23.4, CH_2	23.3, CH_2	23.0, CH_2	23.0, CH_2	23.0, CH_2	23.4, CH_2
11β								
12	123.0, CH	123.7, CH	123.2, CH	123.2, CH	123.0, CH	123.6, CH	123.3, CH	123.5, CH
13	143.4, C	143.3, C	143.3, C	142.9, C	142.6, C	143.3, C	143.7, C	143.2, C
14	41.3, C	41.5, C	40.5, C	40.7, C	42.2, C	41.0, C	41.4, C	41.4, C
15α	27.9, CH_2	27.8, CH_2	27.8, CH_2	28.0, CH_2	27.6, CH_2	27.6, CH_2	27.7, CH_2	27.9, CH_2
15β								
16α	27.1, CH_2	26.9, CH_2	27.0, CH_2	26.9, CH_2	26.6, CH_2	26.5, CH_2	26.5, CH_2	27.0, CH_2
16β								
17	45.2, C	45.0, C	45.2, C	45.6, C	45.1, C	45.8, C	45.2, C	47.1, C
18	43.8, CH	43.8, CH	43.8, CH	43.6, CH	43.0, CH	43.6, CH	43.6, CH	43.5, CH
19	81.0, CH	81.2, CH	81.1, CH	81.0, CH	80.6, CH	80.6, CH	80.6, CH	81.0, CH
20	34.4, C	34.3, C	34.1, C	34.5, C	34.0, C	34.4, C	34.8, C	34.3, C
21α	28.1, CH_2	27.9, CH_2	27.8, CH_2	28.0, CH_2	27.6, CH_2	27.7, CH_2	27.7, CH_2	27.9, CH_2
21β								
22α	32.5, CH_2	32.5, CH_2	32.3, CH_2	31.7, CH_2	31.4, CH_2	32.4, CH_2	32.4, CH_2	31.8, CH_2
22β								
23α	63.7, CH_2	22.4, CH	21.8, CH	22.3, CH	15.9, CH	62.4, CH_2	21.2, CH	62.5, CH_2
23β								
24α	13.2, CH	66.7, CH_2	63.4, CH_2	66.4, CH_2	64.4, CH_2	12.2, CH	63.0, CH_2	12.7, CH
24β								
25	15.9, CH	15.0, CH	15.0, CH	15.4, CH	15.3, CH	15.3, CH	14.7, CH	15.9, CH
26	16.2, CH	15.8, CH	15.6, CH	16.0, CH	15.9, CH	15.9, CH	15.9, CH	16.4, CH
27	23.5, CH	22.8, CH	23.2, CH	23.4, CH	24.7, CH	23.3, CH	23.0, CH	23.5, CH
28	180.5, C	180.7, C	180.4, C	176.9, C	177.2, C	181.1, C	181.4, C	177.2, C
29	27.1, CH	27.0, CH	26.8, CH	27.1, CH	26.8, CH	26.8, CH	26.8, CH	27.1, CH
30	23.7, CH	23.6, CH	23.3, CH	23.6, CH	23.2, CH	23.2, CH	23.0, CH	23.7, CH
1′				94.4, CH	94.2, CH	104.0, CH	104.2, CH	94.4, CH
2′				72.5, CH	72.4, CH	73.7, CH	73.6, CH	72.7, CH
3′				76.9, CH	77.1, CH	76.2, CH	75.9, CH	77.2, CH
4′				69.6, CH	69.4, CH	70.0, CH	70.0, CH	69.6, CH
5′				77.3, CH	76.5, CH	74.1, CH	74.2, CH	76.9, CH
6′α				60.9, CH_2	60.6, CH_2	62.7, CH_2	62.4, CH_2	61.1, CH_2
6′β								
1″								104.2, CH
2″								74.0, CH
3″								76.7, CH
4″								70.1, CH
5″								74.3, CH
6″α								62.9, CH_2
6″β								
1‴	120.5, C	120.5, C	120.0, C	120.2, C	120.4, C	119.8, C	120.4, C	118.9, C
2‴, 6‴	108.9, CH	108.5, CH	108.9, CH	108.7, CH	108.7, CH	108.9, CH	108.9, CH	108.8, CH
3‴, 5‴	144.8, C	145.1, C	145.1, C	144.9, C	144.6, C	144.7, CH	145.5, C	144.7, C

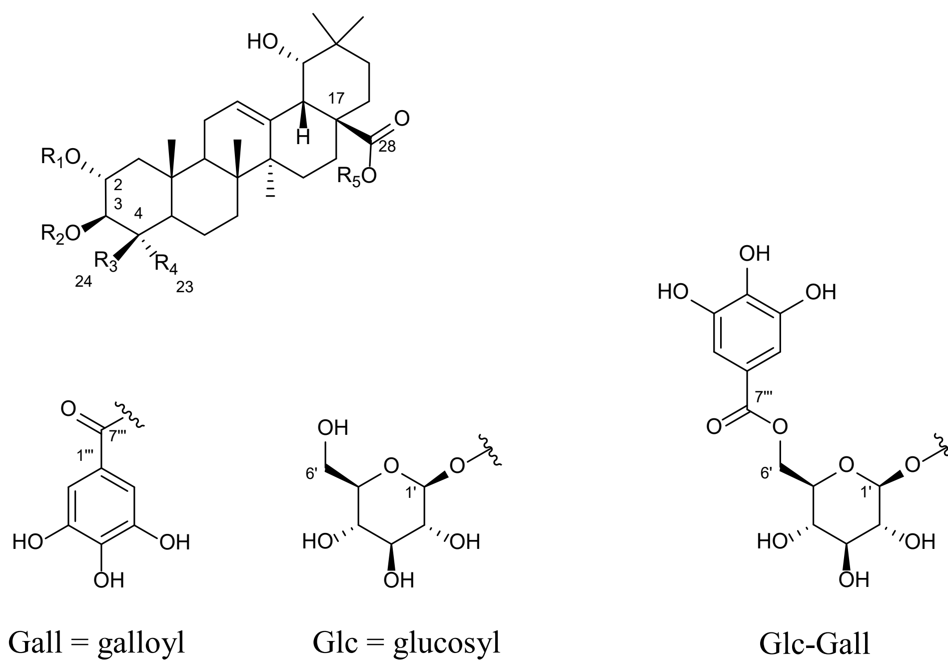
4‴	138.3, C	138.1, C	138.2, C	138.2, C	138.0, C	137.9, CH	139.1, C	138.5, C
7‴	167.5, C	167.5, C	167.6, C	167.1, C	166.9, C	166.5, CH	167.3, C	166.6, C

Table 3. Sensory Description of Isolated Compounds in Water and White Non-Oaked Wine

compound	scale of taste			Gallate position
	water	white wine		
	sweetness	sweetness	acidity	
control	0/5	0/5	5/5	-[a]
QTT I	4/5	4/5	2/5	C-23
1	1/5	0/5	3/5	C-3
2	3/5	1/5	4/5	C-24
3	4/5	4/5	2/5	C-23
4	4/5	2/5	3/5	C-24
5	-	-	-	C-2
6	0/5	2/5	3/5	C-6′
7	2/5	1/5	4/5	C-6′
8	2/5	2/5	2/5	C-6′
9	5/5	5/5	1/5	-
10	2/5	2/5	3/5	-
11	0/5	0/5	4/5	C-23
QTT II[b]	2/5	2/5	3/5	C-3
QTT III[c]	2/5	3/5	3/5	C-3
QTT IV[c]	0/5	1/5	4/5	C-6′
QTT VI[c]	4/5	4/5	3/5	C-2

[a]Not applicable. [b]Marchal et al. (2011). [c]Marchal et al. (2015).

Chart 1. Structure of Isolated Compounds



Gall = galloyl Glc = glucosyl Glc-Gall

Compound	R_1	R_2	R_3	R_4	R_5
1	H	Gall	CH_3	CH_2OH	H
2	H	H	CH_2OGall	CH_3	H
3	H	Gall	CH_2OH	CH_3	H
4	H	H	CH_2OGall	CH_3	Glc
5	Gall	H	CH_2OH	CH_3	Glc
6	H	Glc-Gall	CH_3	CH_2OH	H
7	H	Glc-Gall	CH_2OH	CH_3	H
8	H	Glc-Gall	CH_3	CH_2OH	Glc
9	H	H	CH_3	CH_2OH	H
10	H	H	CH_3	CH_2OH	Glc
11	H	H	CH_3	CH_2OGall	H

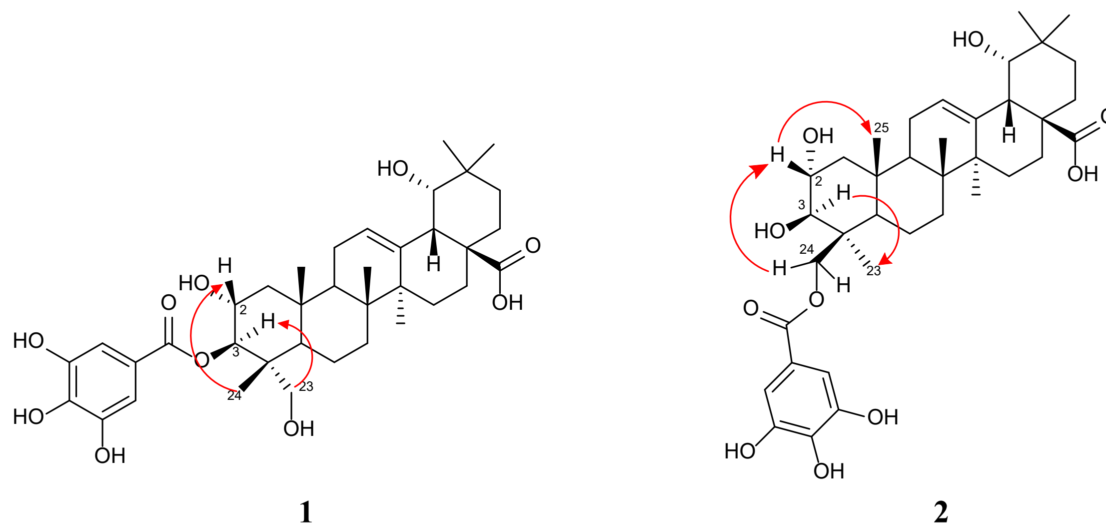


Figure 1. Selected ROESY correlations of compounds **1** and **2**.

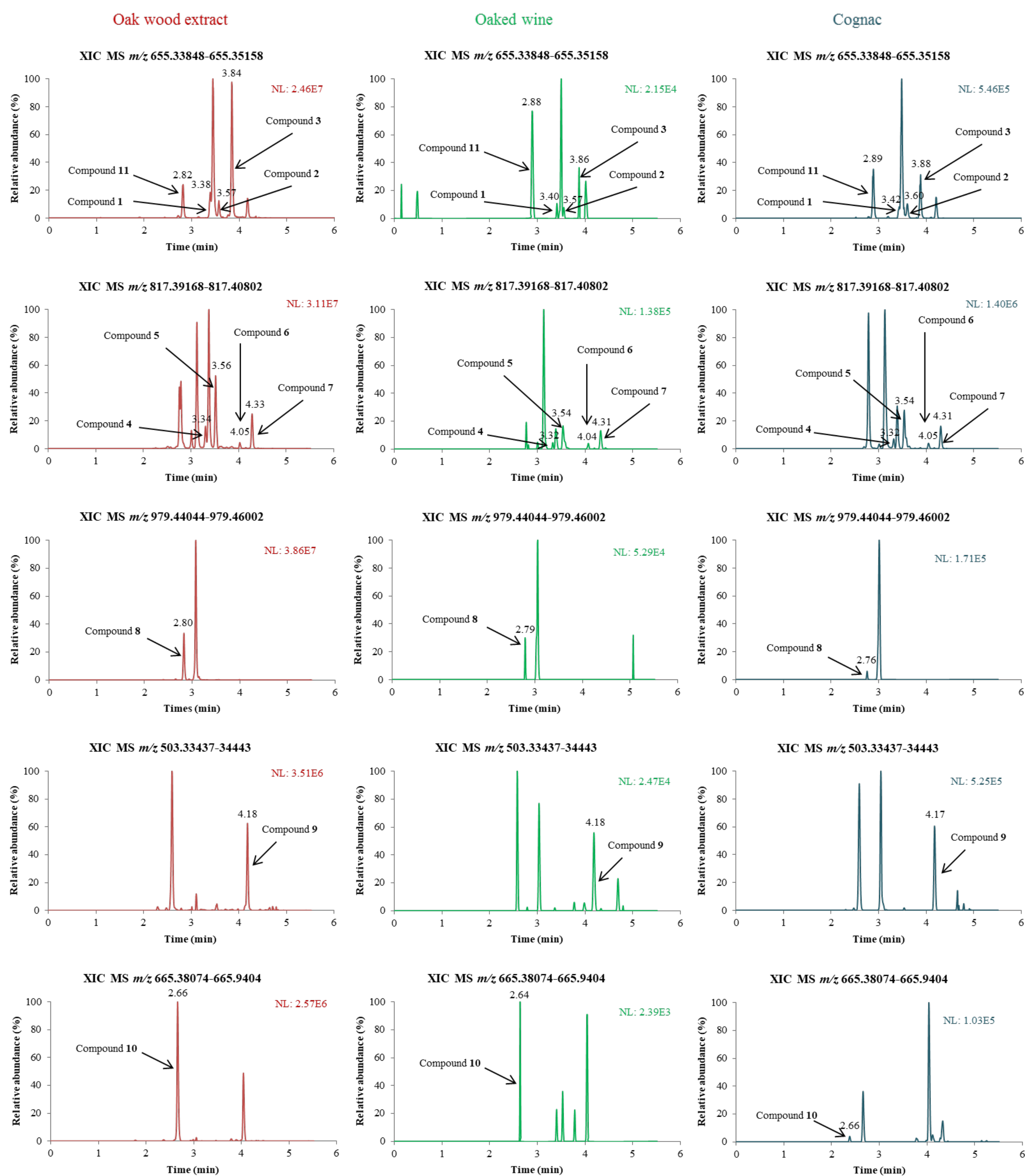


Figure 2. Negative LC-HRESIMS extracted ion chromatograms of an oak wood extract, an oaked wine, and a cognac (left to right) corresponding to $[M-H]^-$ ions of compounds **1** to **11** (top to bottom).

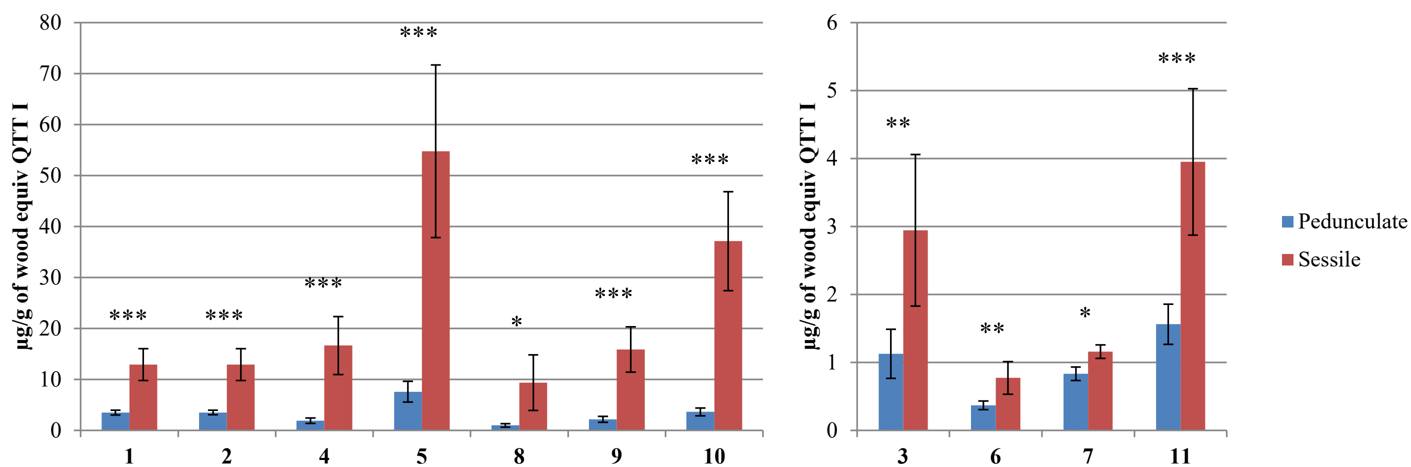


Figure 3. Concentrations (in µg/g equiv. QTT I) of compounds **1** to **11** in sessile and pedunculate oak wood extract. Data are mean ± CI, n = 35 for sessile oak wood, n = 34 for pedunculate oak wood. *$p < 0.05\%$, **$p < 0.01\%$ and ***$p < 0.001\%$, Kruskal−Wallis test.

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