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RESEARCH ARTICLE

Sequencing of red wine proanthocyanidins by UHPLC-ESI-Q-ToF

Adéline Delcambre[1,*], Yann André[1], Cédric Saucier[2]

[1]*Enology Laboratory, Department of Chemistry, University of British Columbia, Kelowna, Canada.*
[2]*Research and Formation Center in Enology, University of Montpellier 1, 34093 Montpellier Cedex 5, France.*

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Dimers of proanthocyanidins with four monomeric units and two distinct linkages are detected and tentatively identified for the first time in Merlot red wine variety without sample preparation. These compounds were characterized by electrospray ionization quadrupole time of flight mass spectrometry in negative mode. Fragments ions derived from retro-Diels Alder, heterocyclic ring fission and quinone methide were detected in targeted MS/MS mode and then assigned by using well-known theoretical fragmentation pathways. The sequencing of these compounds was correlated with the theoretical numbers of oligomers established by mathematical relationship taking in consideration the four monomeric units, the interflavan bond and the ether bond. Our analytical method allows the identification of twenty B-type dimers and twelve A-type dimers in red wine.

Keywords: wine, mass spectrometry, proanthocyanidins, fragmentation pathway.

Introduction

Phenolic compounds are considered as secondary metabolites and are widespread in the plant kingdom [1, 2]. These compounds are present in vegetables [3], fruits [4], tea [5] and red wine [6-8]. They are known for their oxidative defense [9], their ability to reduce certain cancers [10, 11], their preventive activity against infectious [12] and degenerative diseases [13,14]. Among these phenolic compounds, the proanthocyanidins (PAs) or flavan-3-ols represent a significant family and they play an important role during wine making [15] and red wine tasting [16]. Four monomeric units [17, 18] are present in the grape and red wine: (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epicatechin-3-O-gallate (ECG) **(Figure 1)**. These monomers give rise to the formation of oligomers and polymers via an interflavan bond between C4 of the top unit and C6 or C8 [4, 19] of the lower unit and sometimes an additional ether bond between C2 of the top unit and C5 or C7 of the lower unit [20, 21]. (-)-epicatechin (EC), (+)-catechin (C) and (-)-epicatechin-3-O-gallate (ECG) are mainly located in grape seeds, whereas the monomeric unit (-)-epigallocatechin (EGC) is only present in grape skins [22, 23].

These compounds present in red wine are involved in the astringency phenomenon [16, 17], the bitterness, the body [24], the wine aging [25] and the organoleptic properties [26].

These proanthocyanidins have been studied by analytical method such as high-performance liquid chromatography (HPLC) [27], mass spectrometry coupled with UHPLC system [28], and nuclear magnetic resonance (NMR) [29].

In the current study, we first describe the theoretical possibilities to form oligomers with A and B-type interflavan bond. In a second part, we describe specific fragmentation pathways allowing the sequencing of proanthocyanidins in red wine using a UHPLC-ESI-Q-ToF.

***Correspondence:**
Enology Laboratory, Department of Chemistry, University of British Columbia, Okanagan Campus, 3333 University Way, Kelowna, British Columbia V1V 1V7, Canada. Phone/Fax. +250-807-9618/+250-807-8004. E-mail: adelinedelcambre@gmail.com

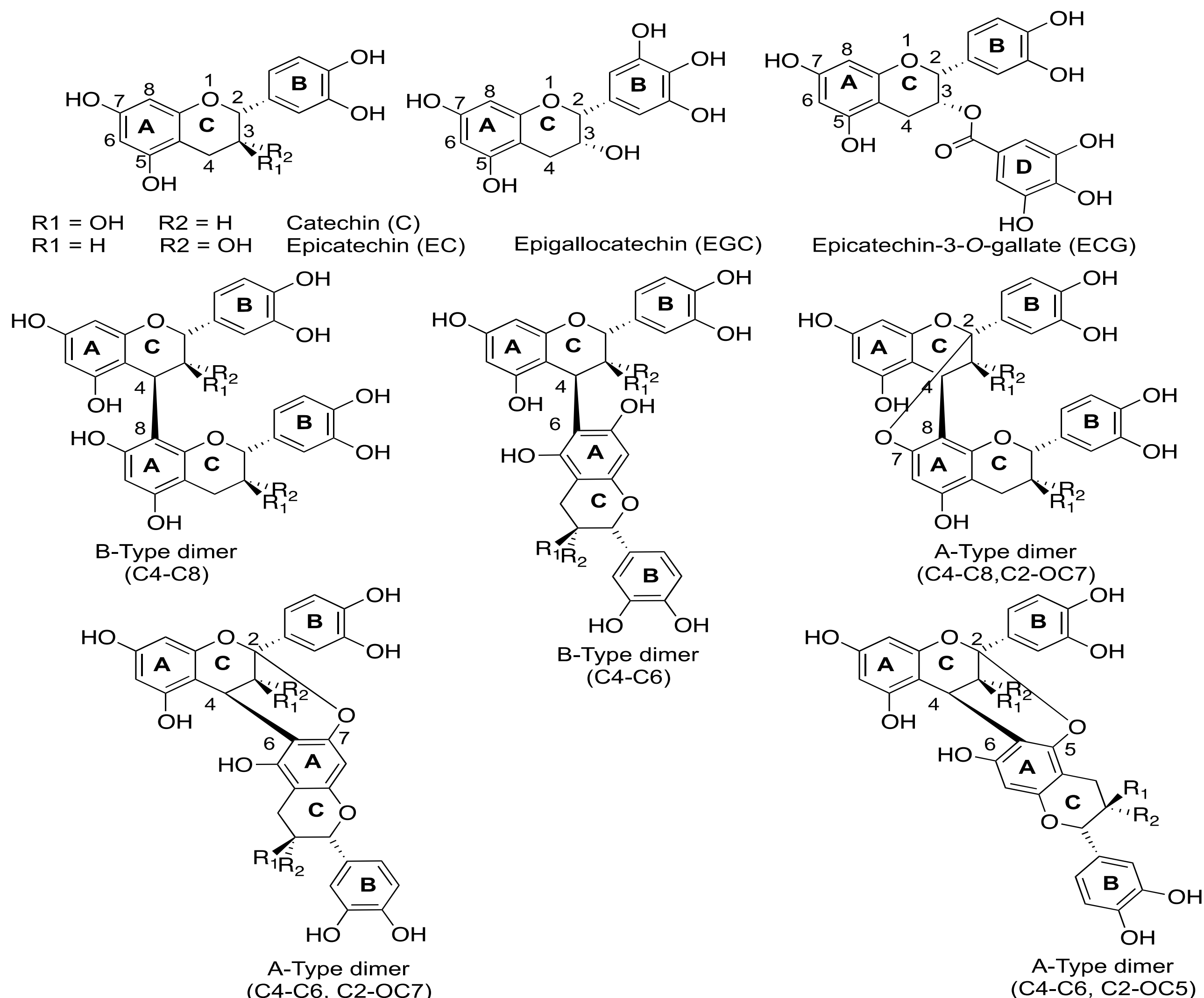

Figure 1. Monomeric units and dimers (A and B-type).

Materials and methods

Chemicals

All chemicals were of analytical reagent grade. Acetonitrile was purchased from Fisher Scientific (Waltham, MA, USA) and formic acid from Sigma Aldrich (St Louis, MO, USA). Deionized water was purified with a MilliQ water system (Millipore, Bedford, MA). Red wine analyzed was from Okanagan Valley (VQA, Okanagan Valley) 2010 Merlot, barrel sample, sampled in May 2010 and was kept in the freezer at - 20°C before analysis. Red wine sample was just filtered with PTFE membrane (VWR).

UHPLC and ESI-MS conditions

Analyses of red wine were carried out using UHPLC-ESI-Q-ToF (Agilent 6530, Series Accurate Mass Q/TOF MS, Agilent Technologies, Santa Clara, CA) in negative mode. The UHPLC system (Agilent 1290 Series, Agilent Technologies, Santa Clara, CA) equipped with an autosampler, a vacuum degaser, a binary pump, a quaternary pump, a thermostated column department and a diode-array detector. Reversed phase UHPLC analyses were performed using a C18 Column (Zorbax SB, 2.1 x 150 mm, 1.8 µm, Agilent Technologies, Santa Clara, CA) maintained at 30ºC, and the flow rate was 0.4 mL.min-1. The binary mobile phase consisted of water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1% formic acid as mobile phase B. The elution conditions were as follows: 0-8 min, 5% B isocratic; 8-32 min, 5-20% B; 32-40 min, 20-100% B; 40-44 min, 100% B isocratic; 44-48 min, 5% B, and followed by two minutes to re-equilibrate the column before the next run. Injection volume was 2 µL with the DAD detector set to an

Table 1. All possibilities to form proanthocyanidins.

Degree of polymerization ($n > 1$)	Pure A-Type $3^{2n-2} \times 4$	Pure B-Type 2^{3n-1}	Mixed A and B Type	Total $17^{n-1} \times 4$
2	36	32	0	68
3	324	256	576	1156
4	2916	2048	14688	19652
5	26244	16384	291456	334084
6	236196	131072	5312160	5679428
7	2125764	1048576	93375936	96550276

absorbance wavelength of 280 nm. The UHPLC system was connected to an ESI-Q-TOF. This instrument was worked in Extended Dynamic Range of 2 GHz (m/z 3200 Th) high-resolution mode. The parameters of detection were performed as follows: drying gas (N_2) flow rate, 10 $L.min^{-1}$; sheath gas temperature 400 °C; nebulizer pressure 25 psig; drying gas temperature 325 °C; sheath gas flow 12 L.min-1; capillary voltage 3500 V; fragmentor voltage150 V; skimmer 65 V. The collision energy was set at 15 eV to 35 eV following the compounds targeted. All analyses were performed in negative mode, which is more sensible than the positive mode. Indeed, in comparison to the positive mode where sodium or potassium

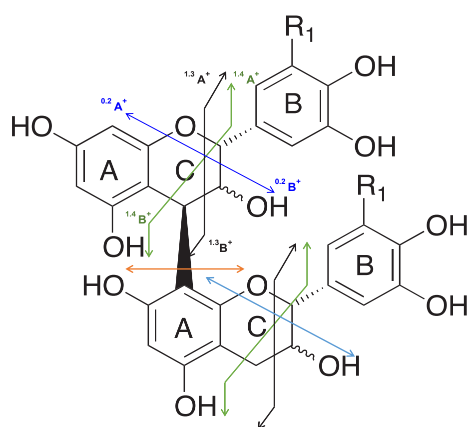

Figure 2. Theoretical fragmentation pathway for B-Type dimer with specific cleavages

salts adducts can be detected, the negative mode produces cleaner spectra without those adducts [30]. Each chromatogram of targeted compounds was obtained by extracted ion current (EIC) in MS/MS mode.

Results

Theoretical models for possible structures for Proanthocyanidins in red wine

By using combinations, considering the number of units and the different ways these units can be linked together, a counting of the possible polymers can be done in order to depict the complexity of the wine matrix. The four monomeric units ((+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin-3-O-gallate) lead to the polymer production. The polymerization occurs via the formation of interflavan linkage. The most common linkage is the B-type linkage between the C4 of the top unit and the C8 of the lower unit (noted C4→C8) or between the C4 of the top unit and the C6 of the lower unit (C4→C6). An additional ether bond between the C2 of the top unit and the C7 or C5 of the lower unit (C2-O-C7 or C2-O-C5) can occur and the linkage is then categorized as A-Type.

In red wine the dimer A2 (two epicatechin linked with C4→C8 interflavan bond and C2-O-C7 ether bond) has been identified by Vivas and Glories [31]. The main oligomers (dimers to pentamers) of A-Type characterized, are linked by C4→C8 bond and an ether bond between C2-O-C7 in grape seeds, plums, cranberries. The combination C4→C8 and C2-O-C5 is not considered, the interflavan linkage C4→C8 cannot create an ether bond C2-O-C5 because the atoms are too far apart. Considering B-type and A-type linkages, five possibilities exist to bond a unit to the next one: C4→C6; C4→C8; C4→C8 and C2-O-C7; C4→C6 and C2-O-C7; C4→C6 and C2-O-C5.

Taking into consideration the four monomeric units and the five possible linkages, the theoretical number of different structures for a given number of monomeric unit can be determined. The **table 1** shows the results up to the heptamer (DP7).

Theoretical fragmentation in MS/MS for B-Type dimers

The targeted MS/MS mode allowed us to obtain additional information about the proanthocyanidin structures. For all compounds studied theoretical fragmentation pathways were established, the specific fragments provide a unique signature that allows a better characterization and identification. However (-)-epicatechin (EC)

Table 2. Specific fragments obtained in negative mode for B-type dimers

Compounds	Precursor ion	HRF1	HRF2	QM	RDA1	RDA2
EC(C)→ EC(C)	577.1351	125.0244/ 451.1035	413.0878/ 163.0401	287.0561/ 289.0718	151.041/ 425.0878	425.0878/ 151.0410
EC(C)→ECG	729.1461	125.0244/ 603.1144	413.0878/ 315.0510	287.0561/ 441.0827	151.0401/ 577.0988	425.0878/ 303.0510
ECG→ EC(C)	729.1461	125.0244/ 603.1144	565.0988/ 163.0401	439.0671/ 289.0718	303.051/ 425.0878	577.0988/ 151.0401
EGC→ EC(C)	593.1301	125.0244/ 467.0984	163.0401/ 429.0827	303.051/ 289.0718	167.035/ 425.0878	441.0827/ 151.0401
EC(C)→EGC	593.1301	125.0244/ 467.0984	413.0878/ 179.0350	287.0561/ 305.0667	151.0401/ 441.0827	425.0878/ 167.0350
EGC→EGC	609.1250	125.0244/ 483.0933	429.0827/ 179.0350	303.051/ 305.0667	167.035/ 441.0827	441.0827/ 167.0350
EGC→ECG	745.1410	125.0244/ 619.1093	429.0827/ 315.0510	303.051/ 441.0827	167.035/ 577.0988	441.0827/ 303.0510
ECG→EGC	745.1410	125.0244/ 619.1093	565.0988/ 179.0350	439.0671/ 305.0667	303.051/ 441.0827	577.0988/ 167.0350
ECG→ECG	881.1571	125.0244/ 755.1254	565.0988/ 315.0510	439.0671/ 441.0827	303.051/ 577.0988	577.0988/ 303.0510

and (+)-catechin (C) are indistinguishable because they only differ by the stereochemistry of hydroxyl group in position 3 on ring C, the abbreviation EC(C) will be used to describe it. The main cleavages of proanthocyanidins are: retro-Diels Alder (RDA), heterocyclic ring fission (HRF), quinone methide or interflavan bond cleavage (QM) and sometimes benzofuran forming fission (BFF) [30, 32, 33]**(Figure 2)**.

For each compound a targeted MS/MS mode was developed with optimized collision energy accordingly to the mass of the molecule. **Figure 2** describes the different fragmentation patterns occurring for B-type dimers.

Using these fragmentation patterns, the sequence of the proanthocyanidin can be resolved. For instance, a B-type dimer of (epi)catechin and epicatechin-3-O-gallate presents two possible sequences EC(C)→ ECG and ECG→ EC(C). The cleavage HRF1 (fragmentation on top unit) produces the phloroglucinol at m/z = 125.0244 in both situation, which does not resolve the sequence. HRF2 (cleavage on bottom unit), RDA1, RDA2 and QM will produce unique ions that are specific and allow the discrimination between two compounds with a different sequence.

As Li and Deinzer [32] described, the quinone methide can distinguish two B-type dimers with the same exact mass, except for (-)-epicatechin and (+)-catechin series. With EC(C)→ECG, the quinone methide produces the ion at m/z = 287.0561, specific fragments when EC(C) is top unit, and m/z = 441.0827 for the ECG bottom unit. If the sequence is EGC→EC(C) after the QM, the cleavage generates the ion at m/z = 439.0671 identified as ECG unit and the monomeric unit EC(C) at m/z = 289.0718 (bottom unit). For the other sequences EGC→EC(C), EC(C)→EGC, EGC→ECG and ECG→EGC, the quinone methide fragmentation are specific, when EGC, EC(C), and ECG are bottom unit the fragmentation pattern gives the exact mass of the monomeric unit. For the compounds containing the monomeric unit EC(C), the RDA (top and bottom unit) produces the ion at m/z = 151.0401 and the ion at m/z = 425.0878. The m/z = 137.0244 indicates another specific fragment for the monomeric unit EC(C) resulting from a RDA or OC9/C2C3 cleavage.

The same argument can be applied for the compounds including one EGC unit, the ion at m/z = 167.035 and the ion at m/z = 441.0827 are produced after the RDA cleavages. The theoretical fragmentation pathways established can be used to characterize the connection sequence of proanthocyanidins with specific signature. The theoretical fragmentation pathways and specific fragments of these B-type dimers were used to identify them in red wine **(Table 2)**.

Identification of B-type dimers in red wine

According to **Table 1** presenting the different possibilities for B-Type dimers, thirty-two theoretical combinations can be detected in red wine. Due to high similarity in term of structure, the only variation being the spatial

arrangement due to stereochemistry and the position of the interflavan bond, proanthocyanidins dimers tend to have identical masses and fragments. For instance, in Table 3, the dimer EC(C)→ECG had three different retention times corresponding to three of the four possibilities: EC→ECG and C→ECG, which can be linked in C4/C6 or C4/C8. The theoretical fragmentation pathway of these four molecules generates the same specific fragments. For the EC(C) dimer series the specific fragmentation pathway allowed the characterization of eight compounds. For the dimer EC(C)→EC(C), compound 1 (*m/z* = 577.1351, rt = 12.726), the fragment ion at *m/z* = 559.1214, was a loss of water from the precursor ion. Neutral loss of phloroglucinol and fragment ion at *m/z* = 451.1022 indicated a HRF on the upper unit. Specific ions at *m/z*= 287.0555 and *m/z* = 289.0718 were detected, thereby indicating a QM cleavage of the interflavan bond. For the RDA fragmentation (bottom and top unit), the ions at *m/z* = 425.0801 and *m/z* = 151.0398 were produced. The formation of the fragment at *m/z* = 401.0779 indicated a loss of water after RDA (upper and lower unit) cleavage produced from *m/z* = 425.0881. Using the same analytical procedure eight B-type dimers of (+)-catechin or (-)-epicatechin were characterized: compounds (1), (2), (3), (4), (5), (6), (7) and (8) **(Table 3)**. Dimers having the sequence EGC→EC(C) showed two peaks: compounds (9) and (10). According to the fragmentation pathway **(Table 2)** the ion at *m/z* = 125.0240 and *m/z* = 467.0972 can be attributed to HRF1 (top unit cleavage). RDA2 fragmentation (bottom unit) generated fragments ions at *m/z* = 151.0387, *m/z* = 441.0961, and then RDA1 (upper unit) lead to the formation of the ion at *m/z* = 425.0889. To confirm the order of the sequence EGC→EC(C), the targeted MS/MS fragmentation produced the specific ions at *m/z* = 303.0498 and *m/z* = 289.0729, that resulted from QM cleavage.

The compounds with EC(C)→EGC sequences were detected and characterized: compounds (11), (12), (13) and (14). Two specific fragmentations, QM and HRF2 fission, allow the discrimination of EC(C)→EGC to EGC→EC(C) dimers. By targeted MS/MS the QM produced the fragment ions at *m/z* = 305.0675 and *m/z* = 287.0561 that are specific to EC(C)→EGC dimer.

Compounds (15) and (16) were EGC→EGC dimers. The fragment ions at *m/z* = 483.0962 and *m/z* = 125.0215 can be attributed to the HRF1 (upper unit cleavage). Furthermore the ions at *m/z* = 441.0369 and 167.0369 can be produced from the RDA (top and bottom unit). The QM fragmentation can also explain the occurrence of the fragment ions at *m/z* = 303.0482 and 305.0664. Compounds (17), (18) and (19) correspond to EC(C)→ECG dimer. Fragment ions at *m/z* = 603.1171, 125.0244 can be attributed to HRF1, *m/z* = 425.09, *m/z* = 303.0545, *m/z* = 577.1197 to the RDA2 and RDA1 cleavages. The specific ions at *m/z* = 711.1359 and *m/z* = 559.1026 were a loss of water from *m/z* = 729.1493 and *m/z* = 577.1197. The QM cleavage can lead to the formation of *m/z* = 441.0841 and the complementary ion at *m/z* = 287.0577. Compound (20) seems to be ECG→ECG dimer. However, the fragments at *m/z* = 729.1514 and *m/z* = 577.0982 can correspond to two successive losses of gallic acid, and then *m/z* = 755.1363 and *m/z* = 125.0183 from HRF1 cleavage. The MS/MS ion at *m/z* = 441.0910 can be attributed to QM fragmentation.

Theoretical fragmentation in MS/MS for A-Type dimers

Compared to the B-type dimers, A-type dimers follow the same the fragmentation pathway, the specific ions produced will differ only by 2 Da.

Identification of A-type dimers in red wine

According to the **Table 1**, thirty-six theoretical A-type dimers can be detected in red wine. For the EC(C)→ EGC series four compounds were observed: (1'), (2'), (3') and (4') **(Table 4)**. Fragment ion at *m/z* = 465.0827 can be generated from HRF1, the complementary ion was a loss of phloroglucinol. The cleavage of interflavan bond (QM) led to the formation of fragments at *m/z* = 305.0667 and then followed by a loss of hydroxyl group to give rise the ion at *m/z* = 289.718.

For the EC(C)→EC(C) analogues five peaks can be detected: (5'), (6'), (7'), (8') and (9'). The fragment ions at *m/z* = 289.0718 and *m/z* = 285.0405 resulted from the quinone methide cleavage. Neutral loss of phloroglucinol (*m/z* = 125.0244) and fragment ion at *m/z* = 449.0878 indicated a HRF1 on the upper unit. The retro Diels Alder cleavages (RDA1 and RDA2) produced two specific ions at *m/z* = 423.0722 and *m/z* = 425.0878 respectively. One peak (10') was assigned to EGC→EGC A-type dimer. The MS/MS spectra obtained showed the ions at *m/z* = 305.0667 and *m/z* = 301.0354 characteristic from interflavan bond cleavage. Another specific fragmentation was the RDA1 with the ions at *m/z* = 439.0671.

Compound (11') can be EC(C)→ECG A-type dimer. The quinone methide cleavage led to the formation of two specific fragments at *m/z* = 441.0827 and *m/z* = 285.0405. The ion *m/z* = 441.0827 is characteristic when the gallate unit is bottom unit. Neutral loss of gallate unit produced the dimer at *m/z*= 577.1195.

Compound (12') appears to be EGC→ECG dimer. The heterocyclic ring fission give rise to *m/z* = 427.0671 and

Table 3. Proanthocyanidin B-Type dimers detected and identified by mass spectrometry in red wine sample.

Tentative identification	Retention time (min)	$[M-H]^-$ theoretical	$[M-H]^-$ observed	Error (ppm)	MS/MS fragments
Dimer EC(C) $C_{30}H_{26}O_{12}$					
(1)	12.726	577.1351	577.1351	0.00	289, 287, 125
(2)	13.163	577.1351	577.1349	0.35	289, 287, 125
(3)	13.848	577.1351	577.1339	2.08	289, 287, 125
(4)	17.440	577.1351	577.1342	1.56	289, 287, 125
(5)	18.669	577.1351	577.1342	1.56	289, 287, 125
(6)	19.570	577.1351	577.1355	0.69	289, 287, 125
(7)	23.577	577.1351	577.1344	1.21	289, 287, 125
(8)	28.752	577.1351	577.1339	2.08	289, 287, 125
Dimer EGC→EC(C) $C_{30}H_{26}O_{13}$					
(9)	4.297	593.1301	593.1315	2.36	303, 289, 125
(10)	13.632	593.1301	593.1316	2.53	303, 289, 287, 125
Dimer EC(C)→EGC $C_{30}H_{26}O_{13}$					
(11)	6.081	593.1301	593.1314	2.19	305, 287, 125
(12)	12.516	593.1301	593.1308	1.18	305, 289, 287, 125
(13)	18.300	593.1301	593.1280	3.54	305, 289, 125
(14)	19.320	593.1301	593.1316	2.53	305, 289, 125
Dimer EGC→EGC $C_{30}H_{26}O_{14}$					
(15)	2.073	609.1250	609.1235	2.46	483, 441, 305, 303
(16)	13.023	609.1250	609.1271	3.45	483, 441, 305
Dimer EC(C)→ECG $C_{37}H_{30}O_{16}$					
(17)	22.664	729.1461	729.1493	4.38	603, 441, 289, 287
(18)	23.933	729.1461	729.1502	5.62	603, 441, 289, 287
(19)	33.826	729.1461	729.1487	3.56	603, 441, 289, 287
Dimer ECG→ECG $C_{44}H_{34}O_{20}$					
(20)	27.939	881.1571	881.1637	7.49	729, 577, 439, 441,287

List of abbreviations used: theoretical molecular ion ($[M-H]^-$ theoretical), Molecular ion observed ($[M-H]^-$ observed) in Da. Abbreviations used for molecular species: (-)-epicatechin (EC), (+)-catechin (C), (-)-epigallocatechin (EGC), (-)-epicatechin-3-O-gallate (ECG). The abbreviation EC(C) indicates that the monomeric unit is indistinguishable using the mass spectrometry.

Table 4. Proanthocyanidin A-Type dimers detected and identified by mass spectrometry in red wine sample.

Tentative identification	Retention time (min)	$[M-H]^-$ theoretical	$[M-H]^-$ observed	Error (ppm)	MS/MS fragments
Dimer EC(C) EGC $C_{30}H_{24}O_{13}$					
(1')	2.290	591.1144	591.1148	0.68	465, 305, 289
(2')	12.994	591.1144	591.1172	4.74	465, 305, 289
(3')	14.148	591.1144	591.1177	5.58	465, 305, 301, 289
(4')	18.773	591.1144	591.1140	0.68	465, 305, 289
Dimer EC(C)→EC(C) $C_{30}H_{24}O_{12}$					
(5')	12.988	575.1195	575.1223	4.87	449, 425, 423
(6')	13.425	575.1195	575.1215	3.48	449, 425, 423, 289
(7')	17.674	575.1195	575.1173	3.82	449, 425, 423
(8')	18.762	575.1195	575.1197	0.35	449, 425, 423
(9')	28.992	575.1195	575.1171	4.17	449, 425, 423, 289
Dimer EC(C)→EGC $C_{30}H_{24}O_{14}$					
(11')	624.450	727.1305	727.1324	2.61	575, 441, 289, 285
Dimer EGC→EGC $C_{37}H_{28}O_{17}$					
(12')	24.351	743.1254	743.1278	3.23	441, 427, 305, 289

List of abbreviations used: Molecular ion theoretical ($[M-H]^-$ theoretical), Molecular ion observed ($[M-H]^-$ observed) in Da. Abbreviations used for molecular species: (-)-epicatechin (EC), (+)-catechin (C), (-)-epigallocatechin (EGC), (-)-epicatechin-3-O-gallate (ECG). The abbreviations EC(C) indicate that the monomeric unit is indistinguishable using the mass spectrometry.

m/z = 617.0937. The fragment ion at m/z = 441.0827 indicated an interflavan bond cleavage for this dimer.

Discussion

The first goal of this study was to enumerate all oligomers of proanthocyanidins thanks to mathematical relationships and then to detect them by mass spectrometry directly from a sample of wine.

Regarding dimers, thirty-two theoretical combinations can be obtained for B-type. The use of UHPLC-ESI-Q-ToF and the theoretical fragmentation pathways allowed us to distinguish twenty compounds. For the EC(C)→EC(C) series, eight theoretical compounds were detected, the MS/MS data allowed their total identification. However, the catechin and epicatechin units cannot be discriminated because they only differ by one stereo center in position C3. The use of commercial standards could allow their identification. For the dimer containing gallate unit (ECG), the series ECG→EC(C), EGC →ECG and ECG→ECG were not detected in red wine. Yet our sample was simply filtered, without prefractionation, these compounds could be present under the limit of detection of the spectrometer. Furthermore, in grape and red wine, the epicatechin-3-O-gallate unit is mainly present at the terminal position due to the steric effect.

Concerning A-type dimers the specific fragments obtained were in accordance with the literature [34]. Twelve compounds seem to be in agreement with the theoretical fragmentation pathways established. For EC(C)→ EC(C) analogues, five compounds can be observed and four compounds for EC(C)→EGC series. These results have demonstrated the possibility of sequencing the dimers of A and B type. Thirty-two dimers can be identified in accordance with their specific fragmentation pathways. This method can be adapted to characterize the trimers present in red wine. However as we can see with the mathematical relationship, two hundred fifty-six different trimers could exist for B-type linkage only. The detection

of trimer is possible but it represents a long and fastidious work and the complexity increases exponentially with the degree of polymerization. So far, liquid chromatography coupled with tandem mass spectrometry is the best technique to detect those compounds directly from filtered wine without further preparation. But looking at the theoretical number of molecules it is obvious that we cannot detect everything.

Conclusion

The partial identification of dimers with B and A-type linkage in red wine using an UHPLC-ESI-Q-ToF without sample preparation was reported for the first time. This work has also showed the complexity of oligomers present in red wine with the mathematical relationship established. The targeted MS/MS, high resolution and fragmentation pathways allowed the distinction of twenty among thirty-two possible B-type dimers and twelve among thirty-six A-type dimers. For the A-type dimers, the interflavan bond seems to be an ether bond between C2 and C7 according to the fragment ions detected. The sequencing of these compounds was in accordance with their specific signatures. The results obtained showed the efficiency of the mass spectrometer used. To confirm the sequencing, the same method could be developed with a triple quadrupole or an ion trap. Furthermore, the separation of these compounds could be considered to study the organoleptic properties of A-type dimers.

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