Research Article

Rapid Communications in Mass Spectrometry

Received: 27 March 2013 Revised: 16 May 2013 Accepted: 23 May 2013 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2013**, *27*, 1815–1827
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6636

Investigation of monomeric and oligomeric wine stilbenoids in red wines by ultra-high-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry

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RATIONALE: Stilbenoids are secondary plant metabolites responsible for the protection of multiple plant species including grape vine from bacterial and fungal infection. Red wine has been shown to be a major source of these compounds in the human diet, where they display an array of health benefits. Providing a more complete profile of the stilbenoids present in red wine, this study detects 41 stilbenoid compounds, 23 of which have never before been detected in red wine.
METHODS: Red wine extracts were scanned using an ultra-high-performance liquid chromatograph coupled to a hybrid quadrupole time-of-flight mass analyzer. Multiple targeted MS/MS precursor ion scan experiments were performed using electrospray ionization operated in negative mode. Precursor ion masses were scanned for the monomeric and oligomeric stilbenoids, as well as modifications such as O-glycosylation, methoxylation and oxidation products of these compounds. Accurate mass precursor and characteristic product ions afforded partial structural elucidation and assignment of these compounds.
RESULTS: A total of 41 (both known and novel) stilbenoids were detected in extracted red wine. In addition to the well-known monomeric stilbenes, several resveratrol-resveratrol homodimers (*m/z* 453.1344), resveratrol-piceatannol heterodimers (*m/z* 469.1293) and piceatannol-piceatannol homodimers (*m/z* 485.1236) were detected. Modified dimers of resveratrol including O-glycosylated (*m/z* 615.1872), methoxylated (*m/z* 485.1606) and oxidized (*m/z* 471.1449) dimers were also detected. Multiple trimers of resveratrol (*m/z* 679.1978) were detected for the first time in red wine, as well as some known and some novel stilbenoid tetramers (*m/z* 905.2604).
CONCLUSIONS: In summary, 41 stilbenoids were detected in red wine, 23 for the first time. Both monomeric and oligomeric stilbenoids were partially identified and assigned by their accurate mass precursor ions and characteristic stilbenoid fragmentation patterns. Knowledge gained from these experiments contributes to a more complete understanding of the origin of the beneficial properties of red wine.

Stilbenoids are a class of non-flavanoid polyphenolic compounds abundant in several families of plants families including *Vitaceae*. Within *Vitis vinifera*, stilbenoids are often referred to as phytoalexins, compounds that can act as antifungal agents to ward off infection. Because these metabolites are present in multiple constituents of the grapevine, including the stems, leaves and berries, they are regularly transferred into wine during maceration and time on solids.[1] This makes wine a very interesting focus for stilbenoid screening as it is known to be a substantial source of these compounds in the human diet.[2] Not only are stilbenoids known for their role as protective phytochemicals, but they have also been the popularized by the reported health benefits of resveratrol, a stilbenoid, including cardio-protective, antioxidant, and anti-carcinogenic effects.[3]

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Many studies on wine have focused on the monomeric stilbenoids, such as resveratrol, piceatannol, and their glycosides, piceid and astringin.[4–6] While these compounds are the most abundant stilbenes found in wine, they do not represent the full range of stilbenoids that can be present in a wine. More recently, oligomeric products of resveratrol such as ϵ-viniferin, δ-viniferin, and pallidol have been reported using UV, fluorescence and mass spectrometry detection methods.[7–10] However, some studies have shown that a much larger range of stilbenoids can be found within the *Vitis* genus than just these compounds.[11]

High-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) can allow for the separation, detection, and partial identification of trace amounts of natural products within complex samples. Multiple studies have used HPLC/tandem mass spectrometry (MS/MS) to detect stilbenoids in plant and wine extractions.[12–14] Although full identification of unknown compounds is only possible through comparison with authentic standards, or isolation and determination by nuclear magnetic resonance (NMR) spectroscopy, it is possible to partially identify them through accurate mass fragmentation patterns.

In the present study, red wine was screened by selecting the precursor ion masses of potential stilbenoids and their derivatives to gain a profile of which oligomers were present. The partial identification of compounds was facilitated by electrospray ionization quadrupole-time-of-flight (QTOF) tandem mass spectrometry, which can provide MS/MS fragmentation along with precursor and product ion masses with low parts-per-million accuracy. Using characteristic stilbenoid fragmentations, 41 stilbenoid oligomers were partially identified in red wine. This included 23 stilbenoids not previously identified in wine. For structures, see Fig. 1.

EXPERIMENTAL

Reagents and samples

Deionized water was purified with a MilliQ water system (Millipore, Bedford, MA, USA). HPLC grade acetonitrile and diethyl ether were purchased from Fisher Scientific (Waltham, MA, USA). Formic acid was purchased from Sigma Aldrich (St. Louis, MO, USA). The red wines used were Okanagan Valley (VQA, Okanagan Valley) 2010 pinot noir, merlot and cabernet sauvignon, sampled in August 2011, and stored at –20 °C before extraction.

Synthesis of authentic standards of (±)-*trans*-ϵ-viniferin, (±)-*trans*-*δ*-viniferin and (±)-pallidol

The synthesis of *trans*-ϵ-viniferin (**13**), *trans*-δ-viniferin (**16**) and pallidol (**7**) was achieved by employing a modified procedure to that reported by Takaya *et al.*[15] As we wished not to have to purify by preparative HPLC as described by Takaya *et al.*, coupled with the fact that we wished to synthesize large quantities of these dimers, we devised a simple purification procedure that relied upon acetylation prior to purification. Thus, to a stirred solution of *trans*-resveratrol (**1**) (500 mg) and K_2CO_3 (245 mg) in MeOH (120 mL) at room temperature was slowly added an aqueous solution (10 mL) of $K_3Fe(CN)_6$ (575 mg) over 5 min and the mixture was stirred for a further 30 min. The mixture was then concentrated *in vacuo* and loaded directly onto a flash chromatography column and the organics eluted with ethyl acetate. The fraction containing the crude dimers was then concentrated *in vacuo* and then dissolved in CH_2Cl_2 (60 mL)

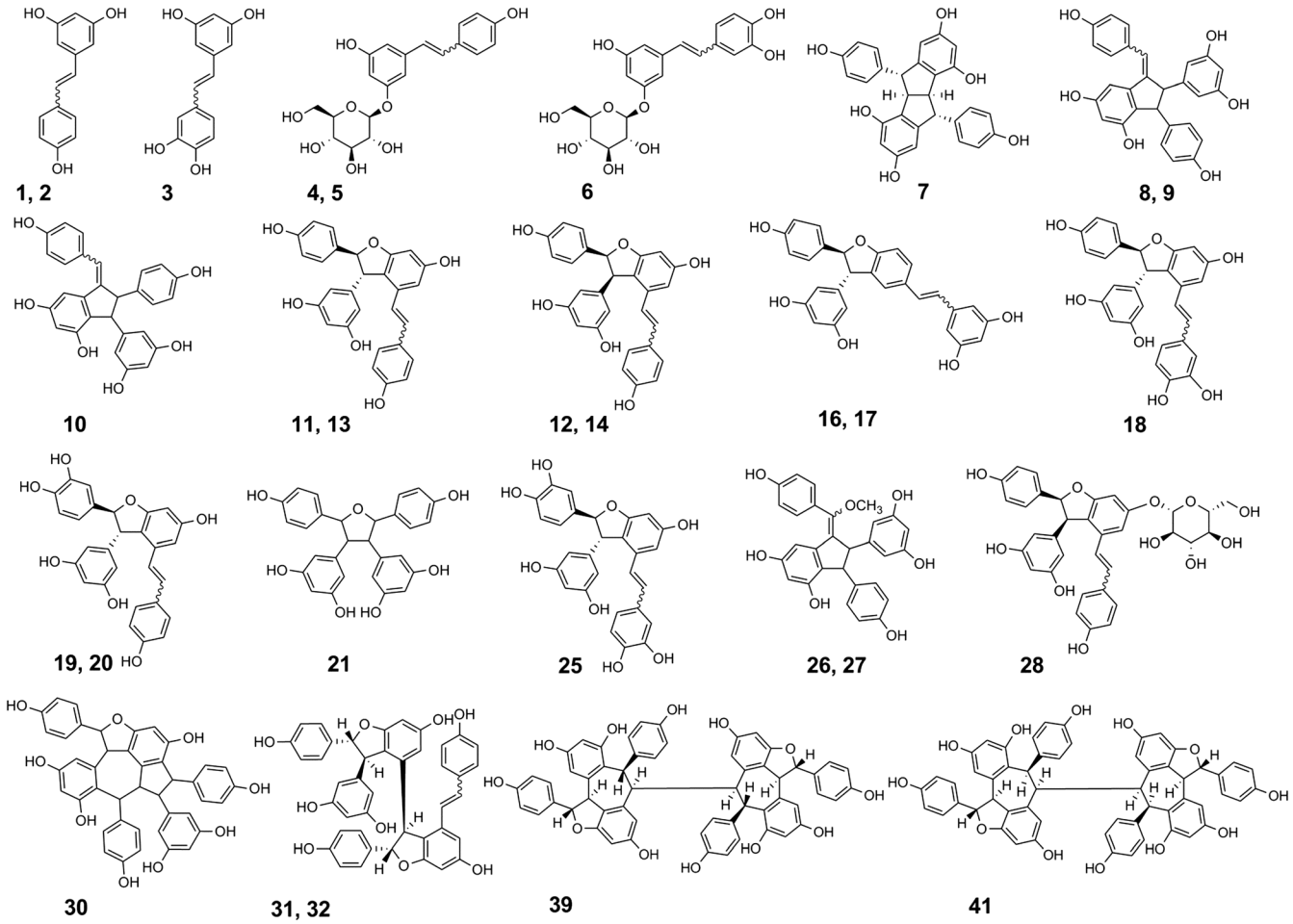

Figure 1. Tentative structures of the major compounds found in red wine extracts studied. **1**, **2** *trans*- and *cis*-resveratrol, **3** *trans*-piceatannol, **4**, **5** *trans*- and *cis*-piceid, **6** *trans*-astringin, **7** pallidol, **8** parthenocissin A, **9** quadrangularin A, **10** ampelopsin D, **11**, **13** *cis*- and *trans*-ϵ-viniferin, **12**, **14** *cis*- and *trans*-ω-viniferin, **16**, **17** *trans*- and *cis*-δ-viniferin, **19**, **20** *trans*- and *cis*-scirpusin A, **21** restrisol A or B, **25** scirpusin B, **26**, **27** parthenostilbenin A and B, **28** ϵ-viniferin glucoside, **30** ampelopsin C, **31**, **32** *E*- and *Z*-miyabenol C, **39** hopeaphenol, **41** isohopeaphenol.

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and DMSO (10 mL). Ac_2O (0.83 mL) and Et_3N (1.23 mL) were then added and the reaction mixture kept at ambient temperature for 24 h. The reaction was then quenched with $NaHCO_3$ (30 mL) and the organics extracted with EtOAc (3 × 30 mL). The combined organics were washed with water (20 mL), dried ($MgSO_4$) and the volatiles were removed *in vacuo*. The acetates were then separated by simple column chromatography (increasing polarity from 20% to 50% ethyl acetate in petroleum spirit) to afford *trans*-ϵ-viniferin pentaacetate and *trans*-δ-viniferin pentaacetate (120 mg, 1:2) and pallidol hexaacetate (150 mg). The two viniferin acetates were further separated by simple recrystallization (MeOH) at ambient temperature to furnish pure *trans*-δ-viniferin pentaacetate (50 mg). The mother liquors were then placed at –20 °C resulting in the precipitation of a mixture of *trans*-δ- and *trans*-ϵ-viniferin pentaacetates (45 mg). The mother liquors were then evaporated to afford pure *trans*-ϵ-viniferin pentaacetate (20 mg). Both the pallidol hexaacetate and the *trans*-ϵ-viniferin pentaacetate had spectral data identical to those previously reported.[16,17]

Deacetylation of the dimers was achieved by dissolving each dimer (20 mg) in MeOH (10 mL) and water (20 mL), followed by the addition of K_2CO_3 (30 mg). The reaction mixture was kept at ambient temperature for 24 h, after which time it was neutralized with diluted HCl. The product was extracted with EtOAc (3 × 20 mL) and the combined extracts washed with water (20 mL), dried ($MgSO_4$) and the volatiles were removed *in vacuo*. The crude product was then subjected to chromatography employing ethyl acetate in petroleum spirit (7:1) as eluent to furnish the target dimers in yields >90%. The proton and carbon NMR spectra of the three dimers were in accordance with those reported in the literature, pallidol,[16] *trans*-ϵ-viniferin,[17–19] *trans*-δ-viniferin.[20–22]

Synthesis of authentic standards of (±)-*cis*-ϵ-viniferin and (±)-*cis*-δ-viniferin

The synthesis of *cis*-ϵ-viniferin (**11**) and *cis*-δ-viniferin (**17**) was achieved by employing a modified procedure to that reported by Yao *et al*.[18] Thus, the *trans*-viniferin (**13** or **16**, 5–10 mg) was dissolved in d_4-MeOH (2 mL) and the solution subjected to UV irradiation (365 nm) for 1 h. After this time a steady state of the *trans*/*cis* dimers had been reached and established by ^{1}HNMR to be (1:1, *trans*/*cis* ϵ-viniferin) and (1:3, *trans*/*cis*-δ-viniferin). The proton and carbon NMR spectra of the *cis* isomers were in accordance with those reported in the literature, *cis*-ϵ-viniferin,[18] *cis*-δ-viniferin.[21] It was further noted that the *cis* isomers isomerize back to the *trans* isomers at ambient temperature over time and as such the samples should be stored in solution at low temperature in the dark.

Sample preparation

Each red wine (100 mL) was extracted with diethyl ether (3 × 100 mL). The solvent was evaporated under vacuum at room temperature and re-suspended in 1 mL of MeOH to afford a concentrated red wine extract. The sample was kept at 4 °C until analysis by UHPLC/ESI-QTOFMS.

UHPLC/ESI-MS/MS

For separation, an Agilent 1290 series UHPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with binary pump, solvent degasser, thermostatted column compartment and a diode-array detector (DAD) was used. The column used for separation was a reversed-phase Zorbax SB AQ (2.1 × 150 mm + 2.1 × 100 mm in series, 1.8 µm; Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of A: water (0.1% formic acid) and B: acetonitrile (0.1% formic acid). The 50 min elution method was 10–18% B over 5 min. A linear gradient was used from 18–40%B over the next 40 min, followed by an increase from 40–100%B in 1 min. A plateau at 100%B for 2 min followed by a decrease from 100–10%B over 1 min was used to clean the column. A 3-min post-run isocratic step at 10%B was used to re-equilibrate the column for the next analysis. The flow rate was held constant at 0.35 mL/min at 40 °C.

MS/MS

MS/MS experiments were performed using the Agilent UHPLC system described above coupled to an Agilent 6530 Series accurate mass QTOF tandem mass spectrometer (Agilent Technologies) operated in high mass resolution (4 GHz) mode. An electrospray ionization source in negative ion mode equipped with Agilent Jet Stream technology was used for ionization. Agilent MassHunter 5.0 software was used for both data acquisition and analysis. Nitrogen was used both as the drying and collision gas. The nebulizer had a pressure of 25 psig with a drying gas flow and temperature of 10 L.min^{-1} and 325 °C, respectively. The nitrogen sheath gas had a temperature of 400 °C and a flow rate of 12 L.min^{-1}.

To optimize the fragmentor and capillary voltages for the best possible ionization of these compounds, a preliminary acquisition in simple MS mode was used. The fragmentor and capillary voltages were fixed at 200 V and 2.25 kV, respectively. A targeted MS/MS acquisition was used (*m/z* 50–1200) with a fixed isolation peak width (1.3 *m/z* units) and collision energy (CE) across all compounds initially. The CE was then varied between 10 and 50 eV and optimal CE values were chosen for each class of compounds to obtain the most characteristic fragments (Table 1).

RESULTS AND DISCUSSION

Three red wine extracts were analyzed to obtain their stilbenoid profiles. In the initial targeted product ion scan experiment, 41 potential compounds (shown in Table 1) exhibiting characteristic neutral losses were isolated. To our knowledge, 26 of these possible oligomeric stilbenes have not yet been identified in wine.

The dimeric forms of resveratrol that have been previously reported in wine include *cis*-ϵ-viniferin, *trans*-ϵ-viniferin, *cis*-δ-viniferin, *trans*-δ-viniferin, pallidol, parthenocissen A, and quadrangularin A.[7,23,33] In the present study, compounds **7**, **11**, **13**, **16**, and **17** have been unambiguously identified as pallidol, *cis*-ϵ-viniferin, *trans*-ϵ-viniferin, *trans*-δ-viniferin, and *cis*-δ-viniferin, respectively, by comparison with authentic standard compounds. Parthenocissen A and

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Table 1. Fragmentation patterns and tentative assignments of stilbenoid compounds in red wine extract

Peak	RT (min)	Formula $[M–H]^-$	$[M–H]^-$ Calculated	$[M–H]^-$ Experimental	Mass error (ppm)	CE (eV)	MS/MS product ions[a]	Assignment*	MS/MS References[c]
1	20.005	$C_{14}H_{11}O_3^-$	227.0714	227.0725	4.84	22.5	185; **143**	*trans*-Resveratrol	[5,25,27,31]
2	21.96	$C_{14}H_{11}O_3^-$	227.0714	227.0717	1.32	22.5	185; **143**	*cis*-Resveratrol	[5,25,27,31]
3	14.242	$C_{14}H_{11}O_4^-$	243.0663	243.0659	−1.65	25	201; **159**	Piceatannol	[12,25,27,34]
4	11.185	$C_{20}H_{21}O_8^-$	389.1242	389.1235	−1.8	20	**227**	*trans*-Piceid	[5,25,31,34]
5	13.561	$C_{20}H_{21}O_8^-$	389.1242	389.1263	5.4	20	**227**	*cis*-Piceid	[5,25,34]
6	8.209	$C_{20}H_{21}O_9^-$	405.1191	405.1206	3.7	15	**243**; 201; 159	Astringin	[5,25,34]
7	21.459	$C_{28}H_{21}O_6^-$	453.1344	453.1347	0.66	20	359; **265**	Pallidol	[28,34]
8	23.959	$C_{28}H_{21}O_6^-$	453.1344	453.1325	−4.19	20	**359**; 289	Parthenocissin A*	[28]
9	25.225	$C_{28}H_{21}O_6^-$	453.1344	453.1354	2.21	20	**359**; 289	Quadrangularin A*	[28]
10	26.765	$C_{28}H_{21}O_6^-$	453.1344	453.1363	4.19	20	**359**; 289	**Ampelopsin D***	[26]
11	31.448	$C_{28}H_{21}O_6^-$	453.1344	453.1345	0.22	20	435; 411; 369; **359**; **347**; 333; 225	*cis*-ϵ-Viniferin	[13,25,34]
12	32.202	$C_{28}H_{21}O_6^-$	453.1344	453.1332	−2.65	20	435; 411; 369; **359; 347**; 333; 225	*cis*-ω-Viniferin*	[26]
13	33.833	$C_{28}H_{21}O_6^-$	453.1344	453.1376	7.06	20	435; 411; 369; **359**; **347**; 333; 225	*trans*-ϵ-Viniferin	[13,25,34]
14	34.34	$C_{28}H_{21}O_6^-$	453.1344	453.1357	2.87	20	435; 411; 369; **359**; **347**; 333; 225	***trans*-ω-Viniferin***	[26]
15	36.127	$C_{28}H_{21}O_6^-$	453.1344	453.1343	0.22	20	435; 411; 369; 359; 347; 333; 225	**Dimer 1***	–
16	38.789	$C_{28}H_{21}O_6^-$	453.1344	453.1356	1.77	25	435; **411**; **369**; 359; 333	*trans*-δ-Viniferin	[25,34]
17	39.705	$C_{28}H_{21}O_6^-$	453.1344	453.1329	−3.31	25	435; **411**; **369**; 359; 333	*cis*-δ-Viniferin	[25,34]
18	16.228	$C_{28}H_{21}O_7^-$	469.1293	469.1305	2.56	25	451; 427; 385; **375**; 359; 347; 265	**R + P Dimer***	[12]
19	26.482	$C_{28}H_{21}O_7^-$	469.1293	469.1301	1.71	25	451; 427; **385**; **375**; **359**; 347; 333; 241	***cis*-Scirpusin A***	[12,13]
20	27.93	$C_{28}H_{21}O_7^-$	469.1293	469.1307	2.98	25	451; 427; **385**; **375**; **359**; 347; 333; 241	***trans*-Scirpusin A***	[12,13]
21	11.303	$C_{28}H_{23}O_7^-$	471.1449	471.1447	−0.42	20	377; **349**; **255**; 121	**Restrisol A***	[14,25,31]
22	17.626	$C_{28}H_{23}O_7^-$	471.1449	471.1456	1.49	20	**349**, **255**, 121	**Oxidized Dimer 1***	[25]
23	18.251	$C_{28}H_{23}O_7^-$	471.1449	471.1477	5.94	20	387, 377, **349**, **255**, 121	**Oxidized Dimer 2***	[25]
24	19.524	$C_{28}H_{23}O_7^-$	471.1449	471.1463	2.97	20	**349**, **255**, 241, 121	**Oxidized Dimer 3***	[25]
25	23.276	$C_{28}H_{21}O_8^-$	485.1236	485.1248	2.47	25	467; 443; 401; **375**; 363; 357; 333; 265; 241	**P + P Dimer 1***	–
26	19.64	$C_{29}H_{25}O_7^-$	485.1606	485.1635	5.98	15	453; 391; **359**; 289; 255; 187	**Parthenostilbenin A***	[28]

(*Continues*)

Table 1. (Continued)

Peak	RT (min)	Formula $[M–H]^-$	$[M–H]^-$ Calculated	$[M–H]^-$ Experimental	Mass error (ppm)	CE (eV)	MS/MS product ions[a]	Assignment*	MS/MS References[c]
27	21.088	$C_{29}H_{25}O_7^-$	485.1606	485.1594	−2.47	15	453; 391; **359**; 289; 255; 187	**Parthenostilbenin B***	[28]
28	25.202	$C_{34}H_{31}O_{11}^-$	615.1872	615.1878	0.98	20	**453**; 411; 359; 347	ϵ-Viniferin glycoside*	[25]
29	18.392	$C_{34}H_{31}O_{11}^-$	615.1883	615.1872	1.79	15	**453**; 359; 289	**Dimer Glycoside 1***	–
30	30.044	$C_{42}H_{32}O_9$	679.1974	679.1981	1.03	30	**585**; 573; **491**; 479; 385	Trimer 1* (ampelopsin C)	[34]
31	35.372	$C_{42}H_{32}O_9$	679.1974	679.1978	0.59	30	661; 637; **585**; **573**; 555;**451**; 479; 357; **345**	**Trimer 2* (E-miyabenol C)**	[28,34]
32	36.322	$C_{42}H_{32}O_9$	679.1974	679.1984	1.47	30	661; 637; **585**; **573**; 555; 479; **451**; 357; **345**	**Trimer 3* (Z-miyabenol C)**	[28,34]
33	37.661	$C_{42}H_{32}O_9$	679.1974	679.1985	1.62	30	661; 637; **585**; **573**; 555; 479; **451**; 357; **345**	**Trimer 4***	[28]
34	36.866	$C_{42}H_{32}O_9$	679.1974	679.2000	3.83	30	661; 637; **585**; **573**; 555; 479; **451**; 357; **345**	**Trimer 5***	[28]
35	39.152	$C_{42}H_{32}O_9$	679.1974	679.1916	−8.54	30	661; 637; **585**; **573**; 555; 479; **451**; 357; **345**	**Trimer 6***	[28]
36	39.835	$C_{42}H_{32}O_9$	679.1974	679.1998	3.53	30	661; 637; **585**; **573**; 555; 479; **451**; 357; **345**	**Trimer 7***	[28]
37	40.347	$C_{42}H_{32}O_9$	679.1974	679.2001	3.98	30	661; 637; **585**; **573**; 555; 479; **451**; 357; **345**	**Trimer 8***	[28]
38	29.734	$C_{56}H_{41}O_{12}^-$	905.2604	905.2577	−2.98	35	887; **811**; 799; **717**; 705; 699; 611	**Tetramer 1** ***	[12]
39	31.695	$C_{56}H_{41}O_{12}^-$	905.2604	905.2573	−3.42	35	**811**; **717**; 611; **451**; **359**; 265	Tetramer 2 (hopeaphenol)*	[12,34]
40	34.718	$C_{56}H_{41}O_{12}^-$	905.2604	905.2563	−4.53	35	**811**; 793; **717**; 705; 611	**Tetramer 3***	[12,34]
41	37.494	$C_{56}H_{41}O_{12}^-$	905.2604	905.262	1.77	35	**811**; 717; 611; **451**; **359**; 265	**Tetramer 4***	[12]

[a]Major fragments are shown in bold face.
*Tentative assignment was based on accurate mass MS/MS fragmentation pattern and comparison with literature.
[b]Compounds shown in bold face are identified in wine for the first time.
[c]References contain MS/MS fragmentation data for the tentatively assigned compounds.

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quadrangularin A have been tentatively identified by comparison with accurate mass fragmentation patterns from the literature.

In addition to the homodimers of resveratrol, several derivatives have been identified in various plants. These include O-glycosylated dimers, methoxylated dimers, piceatannol-resveratrol heterodimers, piceatannol homodimers, and even additional oxidation products of these dimers.[11] Although some of these compounds such as pallidol-3-*O*-glycoside (**29**) and ϵ-viniferin-glycoside (**28**) have been isolated in white wine,[24] we report ten dimers that have not yet been reported in wine but have been described in cell cultures of *Vitis vinifera*.[25] Many of these stilbenoid dimer derivatives have also been identified in other plant species as well as in the leaves and stems of *Vitis vinifera*.[26]

The stilbenoid trimers and tetramers are also well represented in many different species of plants, including *Vitis vinifera*.[11] Although trimeric and tetrameric stilbenoids have been detected in the leaves and stems of grapevine, only the tetramer hopeaphenol (**39**) has been identified in wine.[10] In this study, eight potential trimers (**30–37**) and four potential tetramers (**38–41**) have been found, displaying similar fragmentation patterns to stilbenoid oligomers in the literature. All precursor and product ion masses are supported by accurate mass measurements with mass error below 10 ppm.

Monomers

Monomeric stilbenes are the most abundant stilbenoids found in wine. These were characterized by both MS/MS fragmentation patterns and retention times as compared with authentic standards. Compounds **1** and **2** were a pair of *cis/trans* isomers with an $[M–H]^-$ ion at *m/z* 227, which corresponded to deprotonated resveratrol (Fig. 2). The MS/MS spectra of these two compounds were identical, producing two product ions at *m/z* 187 and 143, which were derived from the loss of one and two C_2H_2O (42 Da) moieties, respectively. The loss of C_2H_2O from the resorcinol ring is characteristic of resveratrol and most other stilbenoids (Fig. 3).[27] The presence of *cis*- and *trans*-resveratrol was confirmed by comparison of retention times of pure standards. A similar fragmentation pattern was observed for *trans*-piceatannol (**3**) with an $[M–H]^-$ ion at *m/z* 243, which yielded fragments at *m/z* 201 and 159 from the subsequent losses of C_2H_2O (42 Da) as above. Piceatannol was also confirmed by retention time and fragmentation pattern through comparison with a pure standard.

Glycosylated monomers

Piceid and astringin are the *O*-glycosides of resveratrol and piceatannol, respectively. The identities of compounds **4** and **5** were confirmed as *trans*- and *cis*-piceid by the detection of an $[M–H]^-$ ion at *m/z* 389. In the MS/MS spectra of **4** and **5**, only one strong product ion at *m/z* 227 corresponding to the loss of glucose (162 Da) was observed. Compound **6** displayed a low intensity signal that corresponded with both retention time and fragmentation of *trans*-astringin, the glycoside of piceatannol. The loss of glucose (162 Da) resulting in an ion at *m/z* 243 was the only fragment visible in the MS/MS spectrum of compound **6** with a $[M–H]^-$ ion at *m/z* 405. Reference standards for *cis/trans*-piceid and *trans*-astringin corresponded to compounds **4**, **5**, and **6**, respectively.

Dimers (R + R)

Two structurally separate groups of resveratrol dimers exist in nature. The 'viniferins' possess a 2,3-dihydrobenzofuran ring system where a major product ion of *m/z* 347 is observed,. The second group are typified by an indane ring system which produces a highly abundant ion at *m/z* 359, such as pallidol.[11] A product ion scan of *m/z* 453.1344 was acquired in order to detect 11 homodimers of resveratrol numbered **7–17** (Fig. 4). The structural identifications of stilbenoid oligomers were based on the presence of characteristic product ions that originate from the neutral losses of 42 Da (C_2H_2O), 94 Da (C_6H_6O), 106 Da (C_7H_6O) and 110 ($C_6H_6O_2$).

In the MS/MS spectrum of compound **7**, the $[M–H]^-$ ion of *m/z* 453 produces two main product ions. The most abundant ion at *m/z* 359 of composition $C_{22}H_{15}O_5^-$ (measured *m/z* 359.0933, error 2.23 ppm) is generated from the loss of a single phenol group (94 Da). The loss of a second phenol group (94 Da) produces $C_{16}H_9O_4^-$ with *m/z* 265 (measured 265.0515, error 3.40 ppm). The identity of compound **7** is

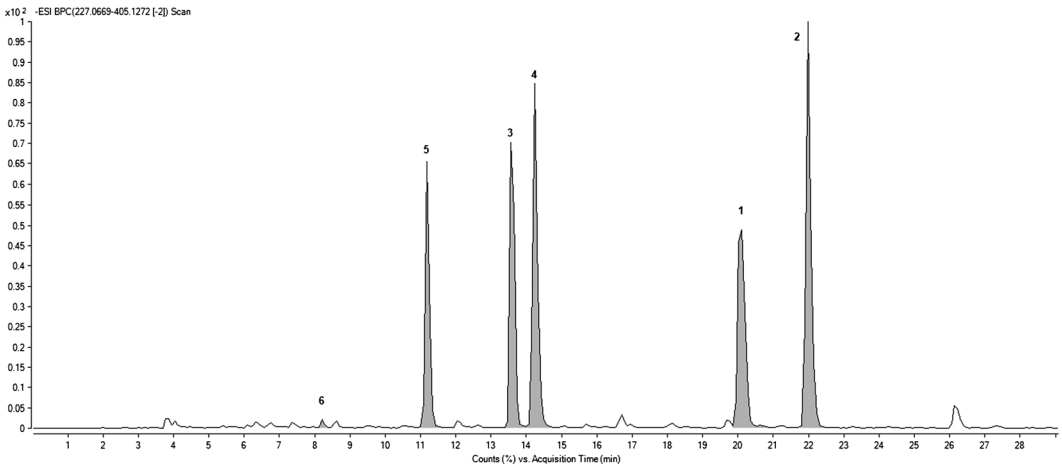

Figure 2. Combined base peak chromatogram of *m/z* 227.0714, 243.0663, 389.1242, and 405.1191 showing compounds **1–6**.

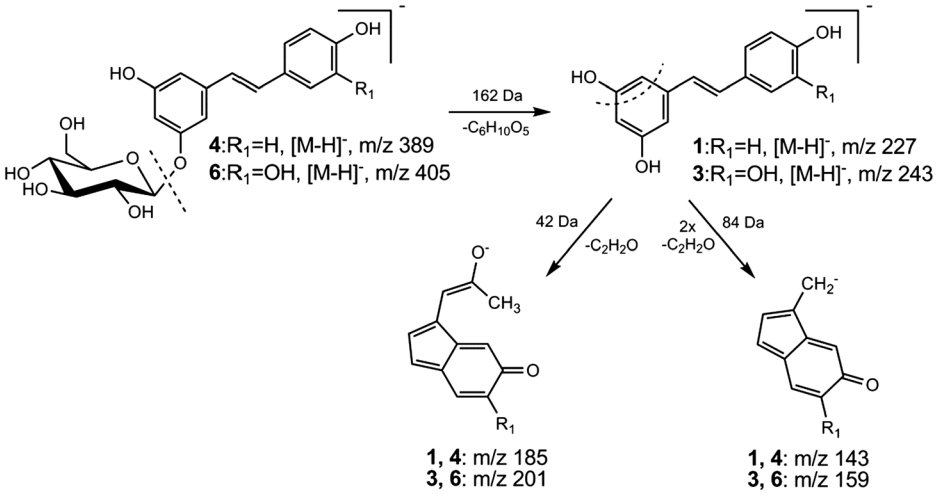

Figure 3. Fragmentation pathway of the stilbene monomers (**1**, **2**, and **3**) and their glycosylated analogues (**4**, **5**, and **6**).

pallidol, a well-known symmetrical resveratrol dimer. The retention time and fragmentation pattern of a pure reference standard matched that observed for compound **7** (Fig. 4).

The three compounds **8**, **9** and **10** displayed identical MS/MS spectra, which can be indicative of a set of structural isomers. Two high-abundance product ions originated from the $[M–H]^-$ ion at *m/z* 453. An ion formed from the loss of phenol (94 Da) at *m/z* of 359 ($C_{22}H_{15}O_5^-$, measured 359.0907, error –4.19 ppm). Further sequential losses of CO (28 Da) and C_2H_2O (42 Da) produced an ion at *m/z* 289 ($C_{19}H_{13}O_3^-$, measured 289.0867, error 1.04 ppm). Minor ions at *m/z* 247, 221, and 205 of abundances less than 1% were observed that could correspond to the losses of C_2H_2O (42 Da), C_3O_2 (68 Da), and two C_2H_2O (84 Da), respectively, from the ion *m/z* 289. These low-abundance ions have been isolated in the MS^3 spectrum of the ion at *m/z* 289 in two compounds which are *cis/trans* isomers that have been isolated in *Parthenocissus laetevirens*.[28] It is therefore proposed that two of these three compounds are the *cis/trans* isomers, quadrangularin A and parthenocissin A, and the other is the regioisomer, ampelopsin D, that was isolated from grapevine leaves.[26,33]

Compounds **11** and **13** are *cis/trans* isomers of *cis/trans*-ϵ-viniferin confirmed by their indistinguishable MS/MS spectra. A $[M–H]^-$ ion of *m/z* 453 formed multiple product ions at 435, 411, 369, 359, 347, and 333. This was in contrast to the indane-type dimers discussed above, which produced few highly abundant product ions. Losses of H_2O (18 Da), C_2H_2O (42 Da), two C_2H_2O (84 Da), phenol (94 Da), 4-methylenecyclohexa-2, 5-dienone (106 Da), and C_8H_8O (120 Da) were supported by accurate mass product ions of $C_{28}H_{19}O_5^-$ (measured *m/z* 435.1237, error 0.23 ppm), $C_{26}H_{19}O_5^-$ (measured *m/z* 411.1257, error 4.62 ppm), $C_{24}H_{17}O_4^-$ (measured *m/z* 369.1126, error 1.63 ppm), $C_{22}H_{15}O_5^-$ (measured *m/z* 359.0927, error 0.56 ppm), $C_{21}H_{15}O_5^-$ (measured *m/z* 347.0936, error 3.17 ppm),

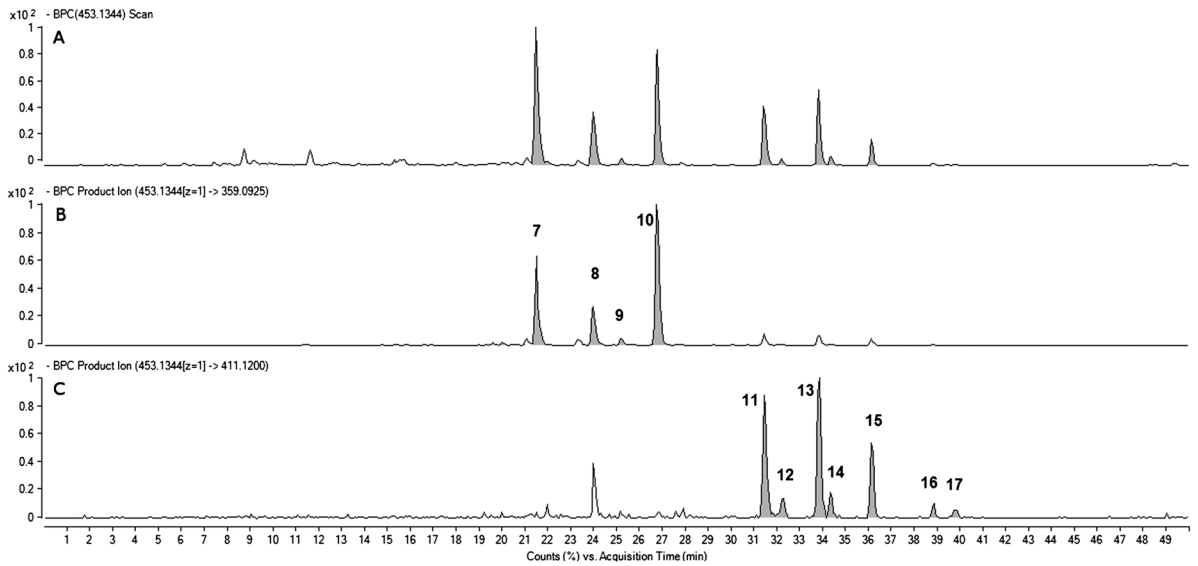

Figure 4. (A) BPC scan of *m/z* 453.1344 showing compounds **7–17**. (B) MS/MS BPC showing the transition *m/z* 453.1344 → 359.0925 displaying the indane-type resveratrol dimers **7–10**. (C) MS/MS BPC of the transition *m/z* 453.1344 → 411.1238 highlighting the 'viniferin'-type dimers **11–17**.

and $C_{20}H_{13}O_5^-$ (measured *m/z* 333.0774, error 1.5 ppm), respectively. The proposed fragmentation pattern for ϵ-viniferin is displayed in Fig. 5. In addition to *cis/trans*-ϵ-viniferin, the compounds **12**, **14**, and **15** also share this exact fragmentation pattern. Two compounds, *cis*- and *trans*-ω-viniferin, isolated in grapevine leaves infected with *Plasmopara viticola* have a near identical structure to ϵ-viniferin differing only in the stereochemistry of the H7a and H8a chiral centers (Fig. 1).[26,33]

For this reason, the fragmentation pattern of these compounds would be indistinguishable from ϵ-viniferin and it is therefore proposed that the identity of two of **12**, **14**, or **15** correspond to *cis/trans*-ω-viniferin.

Compounds **16** and **17** have an $[M–H]^-$ ion at *m/z* 453 indicating a resveratrol dimer. The MS/MS spectra for these compounds are similar to that of ϵ-viniferin with losses of H_2O (18 Da), C_2H_2O (42 Da), C_3O_2 (68 Da), two C_2H_2O (84 Da), and phenol (94 Da), which produce ions at *m/z* 435, 411, 385, 369, and 359, respectively. Accurate mass measurements support the identity of $C_{28}H_{19}O_5^-$ (measured *m/z* 435.1220, error 4.14 ppm), $C_{26}H_{19}O_5^-$ (measured *m/z* 411.1222, error 3.89 ppm), $C_{24}H_{17}O_4^-$ (measured *m/z* 369.1136, error 1.08 ppm), $C_{22}H_{15}O_5^-$ (measured *m/z* 359.0905, error 5.57 ppm), and $C_{20}H_{13}O_5^-$ (measured *m/z* 333.0741, error 8.41 ppm). Although these ions are similar to that of ϵ-viniferin, the ions at *m/z* 411 and 369 have a much higher relative abundance than in the MS/MS spectrum of ϵ-viniferin. Through comparison with authentic reference standards, **16** and **17** were identified as *trans*- and *cis*-δ-viniferin, respectively.

Dimers (R + P)

The MS spectra of compound **18** had a molecular ion at *m/z* 469 which could correspond to a resveratrol-piceatannol heterodimer (Fig. 6). The ion at *m/z* 469 produced four characteristic fragments in the MS/MS spectrum at *m/z* 451, 385, 375, and 359 which were generated from the losses of H_2O (18 Da), two C_2H_2O (84 Da), phenol (94 Da), and resorcinol (110 Da), respectively. The identities of these fragments were again supported by accurate mass measurements of $C_{28}H_{19}O_6^-$ (measured *m/z* 451.1187, error 0.67 ppm), $C_{24}H_{17}O_5^-$ (measured *m/z* 385.1082, error 0.36 ppm), $C_{22}H_{15}O_6^-$ (measured *m/z* 375.0899, error 6.67 ppm), and $C_{22}H_{15}O_5^-$ (measured 359.0918, error 1.95 ppm). Compounds **19** and **20** provide identical MS/MS spectra from the $[M–H]^-$ ion of *m/z* 469, including the ions at *m/z* 451, 385, 375, and 359. Compounds **19** and **20** differ from **18** as they display an ion at *m/z* 241 rather than *m/z* 265. This could indicate that these compounds are structural isomers depending on which section of the dimer originates from piceatannol or resveratrol as the ion at *m/z* 241 ($C_{14}H_9O_4^-$, measured *m/z* 241.0514, error 3.32 ppm) could correspond to a loss of resveratrol (228 Da), while *m/z* 265 ($C_{16}H_9O_4^-$, measured *m/z* 265.0492, error 5.28 ppm) is related to consecutive losses of phenol and catechol or resorcinol (94 + 110 Da). Upon comparison with the literature, compounds **19** and **20** are tentatively identified as *cis*- and *trans*-scirpusin A,[29] which have been identified in grapevine stems,[13] while compound **16** may be an unreported structural isomer of scirpusin A or perhaps ampelopsin A, which also shares this exact mass.

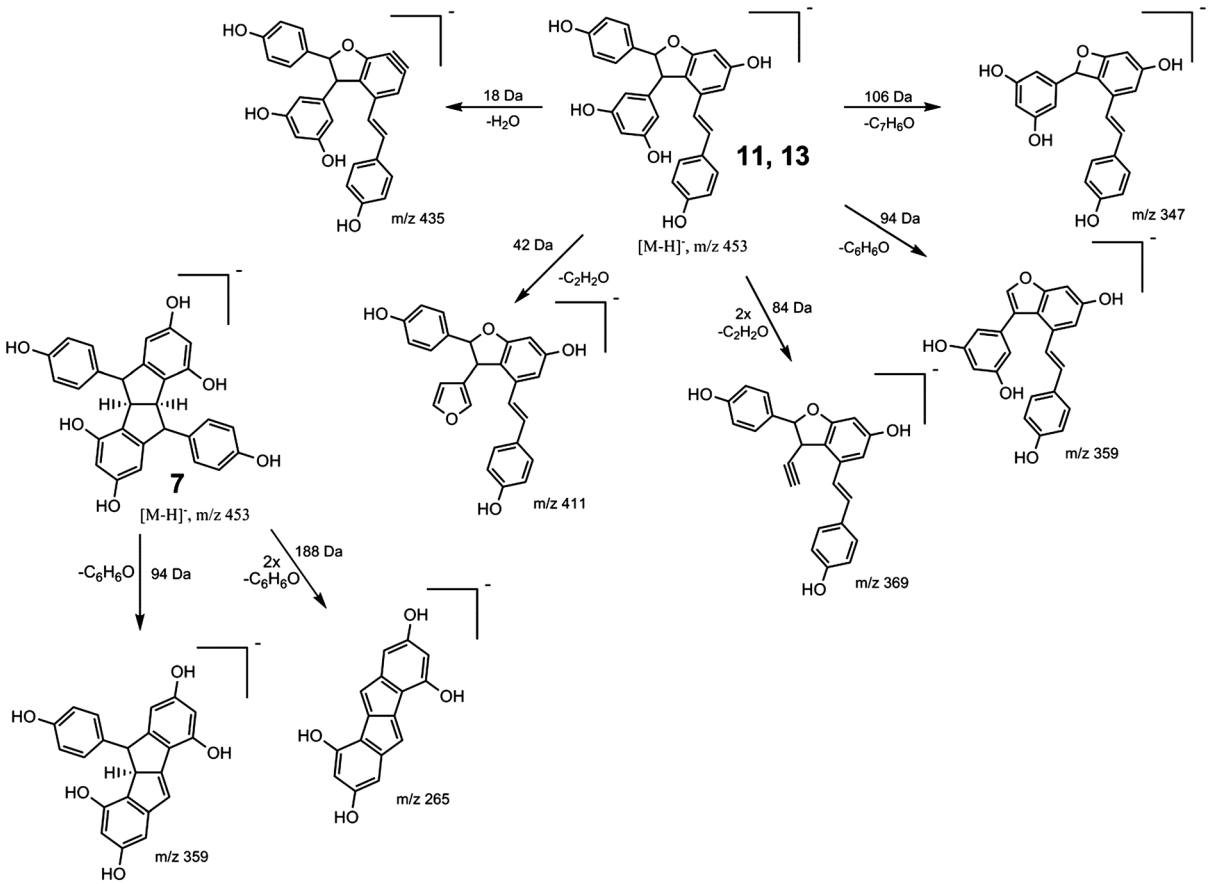

Figure 5. The proposed fragmentation pathways showing the characteristic ions observed for compound **7** (left) and **11**, **13** (right).

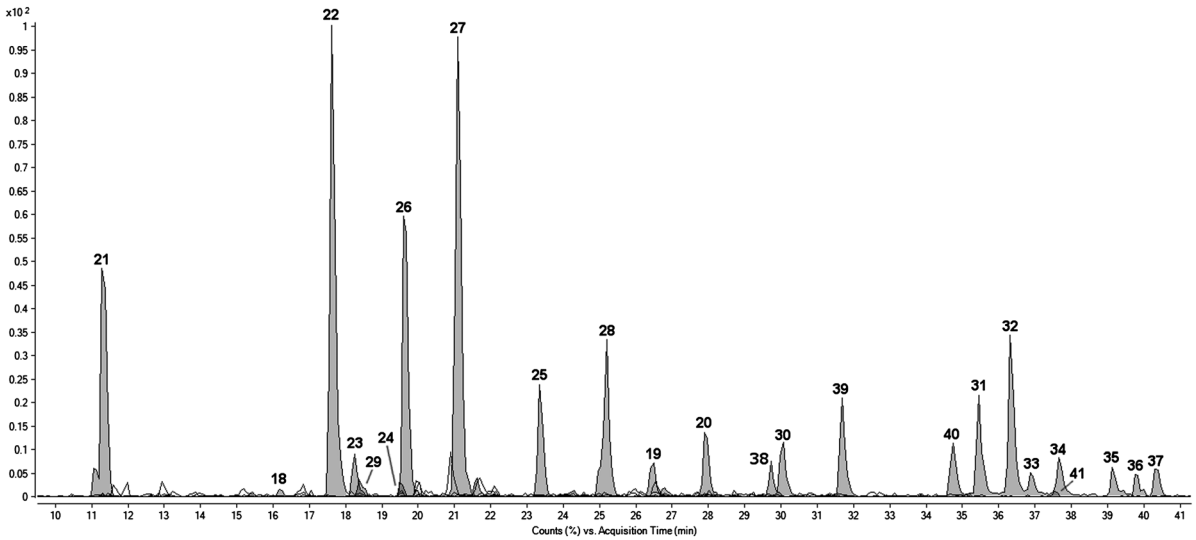

Figure 6. Combined BPC of the stilbenoid oligomers **18–41** found in pinot noir wine extract.

Dimers (P + P)

Compound **25** showed an $[M–H]^-$ ion at *m/z* 485, which indicated a piceatannol dimer (Fig. 6). In the MS/MS spectrum of *m/z* 485, the losses of H_2O (18 Da), two C_2H_2O (42 Da), resorcinol (110 Da), and 2-hydroxy-4-methylenecyclohexa-2,5-dienone (122 Da) yielded product ions at *m/z* 467, 401, 375, and 363. Supporting accurate mass measurements confirmed these ions as $C_{28}H_{19}O_7^-$ (measured *m/z* 467.1153, error 4.92 ppm), $C_{24}H_{17}O_6^-$ (measured *m/z* 401.1012, error 2.99 ppm), $C_{22}H_{15}O_6^-$ (measured *m/z* 375.0895, error 7.19 ppm), and $C_{21}H_{17}O_6^-$ (measured *m/z* 363.0878, error 2.75 ppm), respectively, while the consecutive losses of resorcinol and H_2O (18 +110 Da), C_2H_2O (42 + 110 Da), and another resorcinol (110 + 110 Da) produced the ions at *m/z* 357, 333, and 241. The fragmentation pattern here is very similar to that of ϵ-viniferin, except that instead of losses of phenol and 4-methylenecyclohexa-2,5-dienone groups, compound **25** loses resorcinol and 2-hydroxy-4-methylenecyclohexa-2,5-dienone. Because of this, **25** is tentatively identified as a *cis*- or *trans*-piceatannol homodimer similar to ϵ-viniferin and scirpusin A. The identity of **25** is possibly scirpusin B, which has been detected in various plants including passionfruit.[30]

Glycosylated dimers

The MS/MS spectrum of compound **28** contained a $[M–H]^-$ ion at *m/z* 615 which represented a resveratrol dimer with O-glycosylation. The loss of glucose (162 Da) formed the most abundant ion $C_{28}H_{21}O_6^-$ (measured *m/z* 453.1371, error 5.96 ppm), which confirmed the presence of a glycosylated resveratrol dimer. The fragmentation pattern below *m/z* 453 appeared identical to that of ϵ-viniferin with characteristic benzofuran-type dimer fragments at *m/z* 359 and 347 due to loss of phenol (94 Da) and 4-methylenecyclohexa-2,5-dienone (106 Da) from the aglycone ion at *m/z* 453. Additional ions were found below 1% relative abundance at *m/z* 435, 411, and 333 from losses of H_2O (18 Da), C_2H_2O (42 Da), and C_8H_8O (120 Da), respectively. Because ϵ-viniferin-*O*-glycoside has been reported in wine in previous works,[24] and has nearly identical fragmentation patterns, compound **28** is tentatively identified as *cis*- or *trans*-ϵ-viniferin-*O*-glycoside.

Compound **29** has an MS/MS spectrum again containing the ion corresponding to a glycosylated resveratrol dimer at *m/z* 615. Similar to the fragmentation of compound **28**, the most abundant product ion is generated from the loss of glucose (162 Da) resulting in an ion representative of a dimer of resveratrol $C_{28}H_{21}O_6^-$ (measured *m/z* 453.1366, error 4.86 ppm). From here, ions at *m/z* 359 and 289 are formed, produced from the loss of phenol (94 Da) and consecutive loss of two phenols (94 + 94 Da) and CO (28 Da) from *m/z* 453, respectively. Without taking into account the loss of glucose, the fragmentation pattern of compound **29** is comparable to that of compounds **8**, **9** and **10** which are indane-type dimers. Compound **29** could therefore be a glycosylated derivative of either parthenocissin A, quadrangularin A, or ampelopsin D.

Methoxylated dimers

The $[M–H]^-$ ions of compounds **26** and **27** were observed at *m/z* 485, representative of a pair of potential methoxylated dimers. These compounds produced identical MS/MS spectra, indicating a pair of isomers. The precursor ion generated four main product ions at *m/z* 453, 391, 359, and 255. The ion $C_{28}H_{21}O_6^-$ (measured *m/z* 453.1338, error 1.32 ppm), corresponding to a resveratrol dimer, had a mass difference from the $[M–H]^-$ ion of 32 Da, which could be methanol, confirming the presence of a methoxylated dimer. The characteristic stilbenoid loss of a phenol moiety (94 Da) along with the consecutive loss of methanol and phenol (32 + 94 Da) produced the ions $C_{23}H_{19}O_6^-$ (measured *m/z* 391.1182, error 1.28 ppm) and $C_{22}H_{16}O_5^-$ (measured *m/z* 359.0914, error 3.06 ppm), respectively. Loss of 4-(methoxymethylene)cyclohexa-2,5-dienone and phenol (136 + 94 Da) formed the most abundant ion $C_{15}H_{11}O_4^-$ (measured *m/z* 255.0655, error 3.14 ppm). This fragmentation pattern indicated that these compounds were a pair of

methoxylated indane-type resveratrol dimers. In addition to the main product ions, ions at *m/z* 237, 227, 213, 211, and 187 were found below 1% abundance. The fragmentation patterns of compounds **26** and **27** are identical to a methoxylated dimer found in *Parthenocissus laetevirens* that was tentatively identified as parthenostilbenin A or B.[28] Because of this, it is possible that compounds **26** and **27** are the *cis/trans* methoyxlated dimers known as parthenostilbenin A and B.

Oxidized dimers

Five compounds numbered **21–24** were detected when a product ion scan of *m/z* 471.1449 was performed. Compound **21** produced four main product ions other than the $[M–H]^-$ ion at *m/z* 471. The ion at *m/z* 377 ($C_{22}H_{18}O_6^-$, measured *m/z* 377.1024, error 1.59 ppm) was related to the characteristic loss of phenol (94 Da), and consecutive loss of phenol and carbon monoxide (122 Da) formed the ion at *m/z* 349 ($C_{21}H_{18}O_5^-$, measured *m/z* 349.1089, error 2.29 ppm). Neutral loss of two phenols (188 Da) and a carbon monoxide (28 Da) resulted in the most abundant ion at *m/z* 255 ($C_{15}H_{12}O_4^-$, measured *m/z* 255.0667, error 1.84 ppm). Further loss of $C_8H_6O_2$ (134 Da) from *m/z* 255 produced another highly abundant ion at *m/z* 121 ($C_7H_6O_2^-$, measured *m/z* 121.0290, error 3.30 ppm). The detection of a oxidized resvertrol dimer with this fragmentation pattern has been reported in a number of studies to date.[14,31,32] These previous findings have tentatively identified compound **21** as one of the isomers, restrisol A or B.

The three compounds **22**, **23**, and **24** showed comparable fragmentation patterns with an $[M–H]^-$ ion of *m/z* 471. The difference between these three compounds and compound **19** is the absence of the $[M–phenol]^-$ ion at *m/z* 377 and a greater abundance of the ion at *m/z* 349 resultant from the loss of a phenol-CO (122 Da) group. As with compound **19**, additional loss of $C_8H_8O_2$ (134 Da) produces a highly abundant ion at *m/z* 121. Although compounds that fit this description have been found in the literature, they have not been characterized.[25] Additional studies need to be performed about the oxidation products of resveratrol and its dimers including NMR studies.

Trimers

A total of eight peaks were obtained when *m/z* 679 was selected, which corresponded to trimers of resveratrol. Limited information is available in the literature about these compounds, and even less about their fragmentation behaviour is known. These potential trimers exhibit two noticeably different fragmentation patterns. Compound **30** had a ion at *m/z* 679 which produced three main fragments at *m/z* 585, 491, and 385. These ions were generated from the characteristic stilbene losses of phenol (94 Da), two phenols (94 + 94 Da), and consecutive loss of two phenol (94 + 94 Da) and one 4-methylenecyclohexan-2,5-dienone (106 Da), respectively. Minor ions at *m/z* 573 and 479 which originate from the initial loss of 4-methylenecyclohexan-2,5-dienone (106 Da) follow by a subsequent loss of phenol (94 Da).

Meanwhile, compounds **31–37** show very similar fragmentation patterns that cannot be distinguished, indicative of a collection of trimeric isomers. Compound **31** will serve as a model to explain the fragmentation behaviour common to these seven peaks (Fig. 7). The $[M–H]^-$ ion at *m/z* 679 indicated a resveratrol trimer. The MS/MS spectra of **31** generated many product ions at *m/z* 661, 637, 585, 573, 555, and 479, which were a result of the neutral losses of H_2O (18 Da), C_2H_2O (42 Da), phenol (94 Da), and consecutive loss of 4-methylenecyclohexan-2,5-dienone (106 Da) with H_2O (18 + 106 Da) and phenol (106 + 94 Da), respectively. Supported by accurate mass data, these ions were identified as $C_{42}H_{30}O_8^-$ (measured *m/z* 661.1864, error 0.60 ppm), $C_{40}H_{30}O_8^-$ (measured *m/z* 637.1926, error 9.10 ppm), $C_{36}H_{26}O_8^-$ (measured *m/z* 585.1572, error 2.91 ppm), $C_{35}H_{26}O_8^-$ (measured *m/z* 573.1561, error 1.05 ppm), $C_{35}H_{24}O_7^-$ (measured *m/z* 555.1455, error 1.08 ppm), and $C_{29}H_{20}O_7^-$ (measured *m/z* 479.1169, error 6.89 ppm), respectively. Further fragmentation is produced when CO (28 Da), CO-phenol (122 Da) and CO-4-methylenecyclohexan-2,5-dienone (106 + 28 Da) are lost from the ion at *m/z* 479 to form *m/z* 451, 357, and 345. The proposed fragmentation pathway is depicted in Fig. 7. This fragmentation pattern is characteristic of miyabenol C, which has been observed in *Parthenocissus laetevirens* with an authentic reference standard and fragmentation information available.[28] Other studies have identified multiple isomers in *Vitis vinifera*.[25] However, it was only very recently that a trimeric stilbene, ampelopsin C, was identified in wine by LC/NMR.[33]

Tetramers

The two compounds **39** and **41** were determined to be resveratrol tetramers by the $[M–H]^-$ mass at *m/z* 905. Based on very similar fragmentation patterns, it is proposed that these compounds are isomers of each other. The MS/MS spectra of the ion at *m/z* 905 produced six characteristic stilbenoid product ions of *m/z* 811, 717, 611, 451, 359, and 265, supported by accurate mass ions at $C_{50}H_{36}O_{11}^-$ (measured *m/z* 811.2193, error 0.99 ppm), $C_{44}H_{30}O_{10}^-$ (measured *m/z* 717.1720, error 6.41 ppm), $C_{37}H_{24}O_9^-$ (measured *m/z* 611.1322, error 4.09 ppm), $C_{28}H_{20}O_6^-$ (measured *m/z* 451.1171, error 3.77 ppm), $C_{22}H_{16}O_5^-$ (measured *m/z* 359.0915, error 3.62), and $C_{16}H_{10}O_4^-$ (measured *m/z* 265.0494, error 6.79 ppm), respectively. The first three ions originate from the consecutive losses of one (94 Da) and two (94 + 94 Da) phenol groups followed by the loss of 4-methylenecyclohexan-2,5-dienone (106 Da). Alternatively, minor fragments at *m/z* 799 and 705 indicate that the loss of one 4-methylenecyclohexan-2,5-dienone (106 Da) by itself as well as with a phenol (94 Da) is also occurring. The ion at *m/z* 451 and its minor counterpart at *m/z* 453 are the result of the loss of a dimer (454 Da) and dehydrodimer (452 Da), respectively. This suggests a symmetrical tetramer splitting into two dimers. Neutral loss from the divided tetramer occurs as additional losses of one (94 Da) and two (94 + 94 Da) phenols produce the observed fragmentation at *m/z* 359 and 265. Although information about tetrameric stilbenes is scarce, the most well-known tetramer of resveratrol is hopeaphenol, which has been found in multiple plants and wine.[10] A known isomer of hopeaphenol, isohopeaphenol, has also been detected in many plants.[26] It is therefore proposed that compounds **39** and **41** are isomers of hopeaphenol.

Compounds **38** and **40** produced similar MS/MS spectra to the other tetramers. An $[M–H]^-$ ion at *m/z* 905 followed by the major product ions at *m/z* 811, 717, and 611 resulting from the consecutive loss of one (94 Da) and two (94 + 94 Da) phenols and 4-methylenephenol (106 Da). Minor ions at *m/z* 887, 799, 793, and 705 corresponded to the loss of H_2O

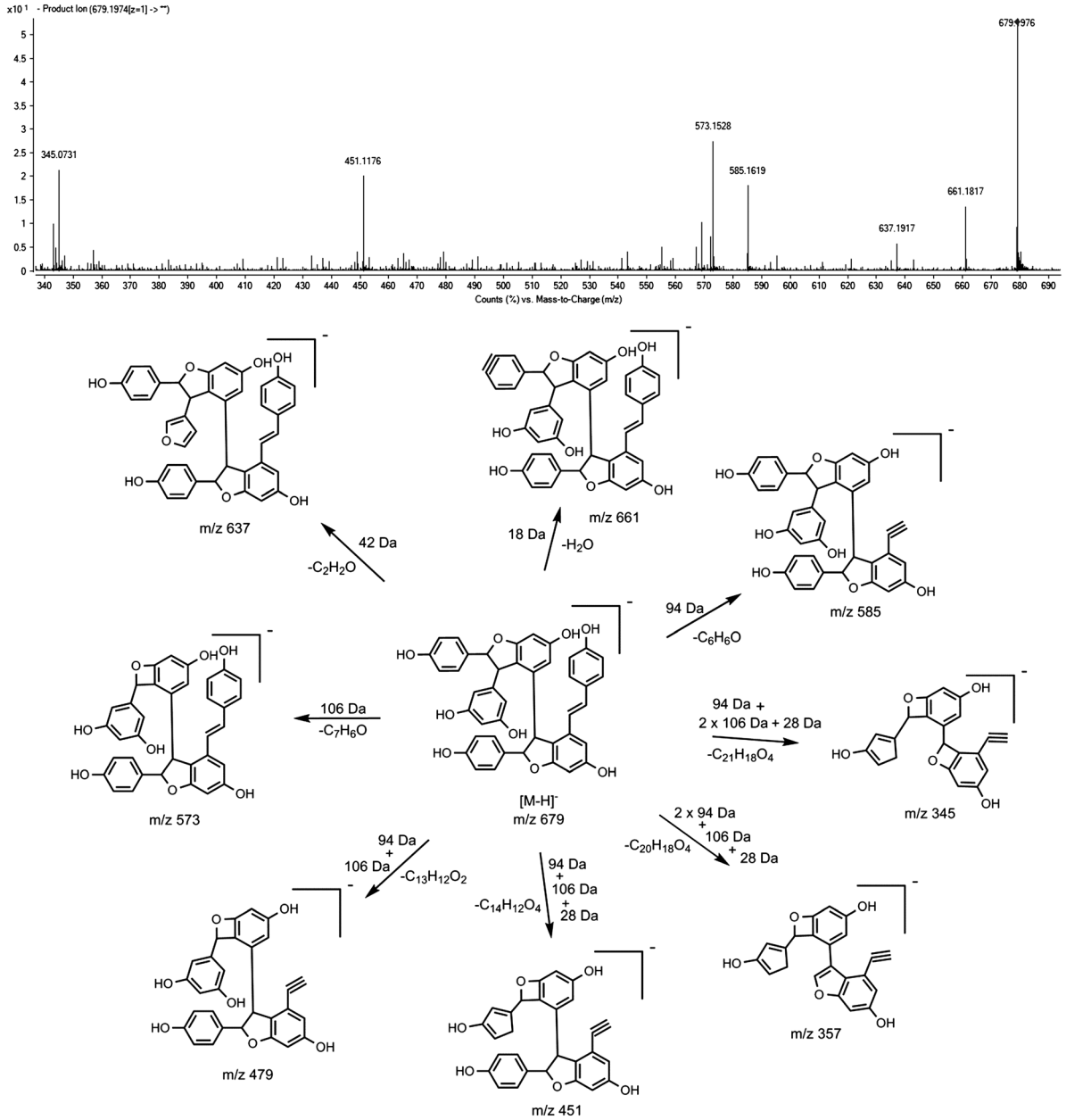

Figure 7. MS/MS spectra of compound **31** with precursor ion *m/z* 679.1974 with the proposed fragmentation pathway for the compounds **31–37**.

(18 Da), 4-methylenecyclohexan-2,5-dienone (106 Da), phenol and subsequent loss of H_2O (94 + 18 Da) and 4-methylenecyclohexan-2,5-dienone (94 + 106 Da). Although this compound shares much of the same fragmentation behaviour as the other three tetramers, no ions above 1% abundance were observed below *m/z* 611. These compounds may be non-symmetrical tetramers of resveratrol.

CONCLUSIONS

The present study used MS/MS analysis to detect and partially identify multiple monomer and oligomer stilbenoids in extracted red wine samples. This was facilitated by accurate mass ESI-QTOF which provided sufficient resolution to identify both precursor and product ions that were characteristic of stilbenoids known in the literature and some potentially new compounds.

A total of 41 stilbenoid compounds were detected, 6 of which were monomers, up to as many as 23 dimers including their many derivatives (glycosylated, methyoxylated and oxidized), as well as resveratrol and piceatannol homo- and heterodimers, 8 trimers and 4 tetramers. Some of these compounds have previously been identified in wine; however, in this study 23 were detected for the first time in red wine.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Acknowledgements

The authors would like to thank their sponsors for important funding: UBC (Okanagan campus-Office of the Provost – International grant), NSERC (Discovery Grant) , CFI (Leader Opportunity Fund grant).This project was also supported in part by the School of Agriculture, Food and Wine at The University of Adelaide, and by Australia's grape growers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government. QM thanks the Faculty of Science for a PhD scholarship.

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