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Analytical Methods

Identification of isomers of resveratrol dimer and their analogues from wine grapes by HPLC/MS^n and HPLC/DAD-UV

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ABSTRACT

A facile method based on HPLC/(−)ESI-MS^n is established for the analysis of seven isomers of resveratrol dimers and three of their analogues in Xinjiang wine grapes. The structures of these compounds are positively or tentatively determined. Among them, three are tentatively identified as new compounds. MS^n experiments on the $[M-H]^-$ ions provide abundant structural information, especially regarding the relative abundance of the key product ions, *m/z* 333 and 369 (385 in compound 3), which can be utilised to distinguish whether or not the compound identified contains the scaffold of the isomer of a resveratrol dimer. The relative abundance of key product ions remains unchanged as collision energy varies from 0.60 to 0.95 V. All the *trans*-, and *cis*-isomers could be identified by HPLC/DAD-UV spectra. The UV spectra of compounds **2** and **9** tentatively show *cis* and *trans*- configurations, respectively.

1. Introduction

Resveratrol dimers and their analogues belong to a group of compounds called stilbenes, which, along with anthocyanins, catechins, proanthocyanidins, and flavonols, are natural phenolic compounds found in grapes and red wine. Stilbenes have a variety of structural isomers, all of which exhibit various biological properties, such as potent antioxidative properties (He, Jiang, Wu, Pan, & Sun, 2009; He, Wu, Pan, & Jiang, 2008; Kong, Ren, Jiang, Pan, & Sun, 2009; Renaud & Delorgeril, 1992; Zern & Fernández, 2005), platelet aggregation inhibition (Van Wauwe & Goossens, 1983), and antimicrobial (Palma, Taylor, Varela, Cutler, & Cutler, 1999) and anti-ageing activities (Shukitt-Hale, Carey, Simon, Mark, & Joseph, 2006). Thus, the identification of bioactive resveratrol dimers and their analogues from wine grapes is of great importance.

HPLC/ESI-MS^n features high sensitivity, low levels of sample consumption, relatively short analysis times, and results of rich structural information. It has the ability to rapidly screen minor constituents in plant extracts that are difficult to obtain through conventional phytochemical means (Chen, Li, Sun, Pan, & Schlunegger, 2008; Huang, Song, Liu, & Liu, 2007; Tong et al., 2008; Wu, Wang, & Simon, 2003). It, however, cannot provide accurate masses of precursor and fragment, Fourier transform ion cyclotron ion resonance tandem mass spectrometry (FTICR-MS/MS) is used to identify compounds and provide information on the molecular formulas. A combination of these two methods may facilitate the identification of components of wine grape extracts.

To the best of our knowledge, no study has yet been conducted regarding the identification of the isomers of resveratrol dimers and their analogues from Xinjiang wine grapes. This study thus aims to identify these compounds using HPLC/ESI-MS^n, FTICR-MS/MS and HPLC/DAD-UV.

2. Materials and methods

2.1. Materials

The stems of wine grapes (*Vitis Vinifera*) were collected in August 2008 in Shihezi, Xinjiang Province, China. The material was identified by Dr. Yongxiang Cheng (Shihezi University, Shihezi, China). Methanol (chromatographic grade) used for analytical HPLC was purchased from Merck (Darmstadt, Germany). Deionized water (18 MΩ) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

The dried stems (4 kg) of wine grapes (*Vitis Vinifera*) were extracted three times with MeOH (15.0 L × 3) at room temperature. The solvent was evaporated in vacuo to afford a concentrated MeOH extract (350 g), which was then diluted with H_2O (2.0 L) to give the aqueous solution (2.0 L). The aqueous solution was extracted with ethyl acetate (4.0 L × 3). The combined ethyl acetate

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layers were concentrated to dryness in vacuo to provide an ethyl acetate extract (92 g), which was subjected to silica gel CC (1000 g, 5 cm diameter) eluted with light petroleum–ethyl acetate mixtures (100:1–1:10) yielding 21 fractions. Fraction 11 (1.3 g) was separated by RP-18 CC (MeOH–H_2O, 50:50%) to give compounds scirpusin A (**3**) (18 mg), *trans*-ε-viniferin (**5**) (28.1 mg) and *trans*-δ-viniferin (**9**) (10 mg). Compounds (**3**, **5**, **8**) were identified by direct comparison of their ^1H NMR, ^{13}C NMR with those reported in the literatures, respectively (Lin, Li, Li, Yu, & Liang, 1992; Pezet et al., 2003; Shao et al., 2007). *Cis*-ε-viniferin(**4**), *cis*-δ-viniferin(**10**) were obtained after sunlight exposition of methanolic solutions of *trans*-ε-viniferin, *trans*-δ-viniferin, respectively, and used as *cis*-standards. Fraction 10 was dissolved in methanol, membrane-filtered (0.45 mm), and analysed by LC/(−)ESI-MS^n.

2.3. Analytical and preparative high-performance liquid chromatography

An Agilent 1100 analytical HPLC system with a G1312 Binpump, G1314A variable-wavelength detector (VWD), model 7725 injector fitted with a 20 μL sample loop, along with an Agilent ChemStation data system and Waters 600 separations module equipped with a Waters model 2996 diode-array detector were used. A Phase Agilent Extend C8 column (4.6 mm × 150 mm, 5 μm) was used for separation; The mobile phase consisted of methanol (30%, v/v) and water (70%, v/v) to start, the percentage of methanol changed from 30% to 70% over the 40 min with the flow rate of 0.8 mL min^{-1} at room temperature. The detection wavelength was 280 nm.

2.4. Mass spectrometry

HPLC/(−)ESI-MS^n experiments were performed using the Agilent HPLC system described above combined with a Bruker Esquire 3000^{plus} ion trap mass spectrometer (Bruker-Franzen Analytik GmbH, Bremen, Germany) equipped with an electrospray ionisation (ESI) source. The ion source was set to 250 °C, while the needle voltage was always set to 4.0 kV. Instrument control and data acquisition were performed using Esquire 5.0 software. To improve the trapping efficiency and act as a collision gas for the MS^n procedure, helium was introduced into the trap at an evaluated pressure 6×10^{-6} mbar. Nitrogen was used as both the drying and nebulizing gas at a back-pressure of 30 psi and a flow rate of 10 L min^{-1}. The mass spectrometer was optimised in the collision energy range of 0.38–1.0 V to maximise the ion current in the spectra.

Off-line FTICR-MS experiments were performed using an Apex III FTICR mass spectrometer with a 7.0 T actively shielded superconducting magnet (Bruker Daltonics, Billerica, MA, USA) combined with an Apollo ESI source operated in the negative ion mode. The solutions were infused at a rate of 3.0 mL min^{-1} with a Cole-Parmer syringe pump. Accurate mass measurements were performed using CF_3COONa as an external calibration compound. MS/MS calibration was performed using a reference standard and its fragment peaks. Through the isolation of the desired precursor ion MS/MS analysis was performed using a correlated sweep. Argon was used as the collision gas and pulsed into the ICR cell. Each spectrum obtained is the average of eight transients, each composed of 512 K points, and acquired using a workstation operating XMASS version 6.1.1.

3. Results and discussion

The HPLC/UV chromatogram and total ion chromatogram (TIC) of the wine grape extracts are shown in Fig. 1. The seven resveratrol dimers and three analogous compounds investigated were numbered as compounds **1**–**10** by retention time. For MS^n analysis, which provides extensive structural information, the negative ion mode of ESI was selected. Fig. 2 shows the structures of the resveratrol dimer and its analogous compounds. The details of each compounds are further described in the following section.

3.1. Identification of standards compounds *3*–*5*, *8* and *10*

Compounds **4**, **5**, **8** and **10**, all of which are isomers of resveratrol dimers, were identified as *cis*-ε-viniferin, *trans*-ε-viniferin, *trans*-δ-viniferin and *cis*-δ-viniferin, respectively. Compound **3**, an analogues of a resveratrol dimer, was identified as scirpusin A. Two key product ions (*m/z* 333 and 369) were observed and considered as characteristics for the structural identification of resveratrol dimers and their analogues. The fragmentations of these reference standard compounds are discussed below in detail.

In the MS/MS spectra of the $[M-H]^-$ ion at *m/z* 469 of compound **3**, the losses of 94 Da (assigned to the phenol moiety), 58 Da ($C_2H_2O_2$), 84 Da (two C_2H_2O), 110 Da (assigned to the pyrocatechol moiety), 18 Da (H_2O), and 136 Da (benzo[c][1,2]dioxete-5-carbaldehyde) were readily observed to yield the product ions at *m/z* 375, 411, 385, 359, 451 and 333, respectively. In the MS^3 experiment of the fragment ion at *m/z* 375 several interesting fragment ions were generated as eliminations of 42 Da (C_2H_2O), 110 Da (assigned to the pyrocatechol moiety) and 18 Da (H_2O). These assignments were supported by an off-line ESI-FTICR-MS^2 experiment with the compositions $C_{28}H_{21}O_7^-$ (measured *m/z* 469.1294, error −0.2 ppm), $C_{26}H_{19}O_5^-$ (measured *m/z* 411.1249, error −2.61 ppm), $C_{24}H_{17}O_5^-$ (measured *m/z* 385.1079, error −0.52 ppm), $C_{28}H_{19}O_6^-$ (measured *m/z* 451.1181, error −0.2 ppm), $C_{22}H_{15}O_6^-$ (measured *m/z* 375.0875, error −0.26 ppm), and $C_{22}H_{15}O_5^-$ (measured *m/z* 359.0926, error −0.28 ppm). It was found that the key product ion at *m/z* 333 was observed in the MS/MS spectra of compound **3**, the relative abundance of which was 4% (the most abundant ion at *m/z* 375 is considered to be as 100%). The relative abundance of the characteristic ion from the loss of benzo[c][1,2]dioxete-5-carbaldehyde (136 Da) of *m/z* 469, is possibly due to hydroxy linkages to the benzene group of 2,3-dihydrobenzofuran of R_1 as shown in Fig. 3. The key product ion at *m/z* 385 (the loss of 84 Da (two C_2H_2O) from *m/z* 469) was observed in the MS/MS spectra of compound **3**, the relative abundance of which was 28% (the most abundant ion *m/z* at 375 is considered to be 100%), which corresponds to the loss of C_2H_2O (42 Da) from resorcinol (R_5) and the loss of other C_2H_2O (42 Da) from pyrocatechol (R_6), as shown in Fig. 3.

Compounds **8** and **10** were a pair of *trans*- and *cis*-isomers, respectively, which have the same MS^n spectra. In the MS/MS spectra of the $[M-H]^-$ ion at *m/z* 453, compound **8** produced five fragment ions at *m/z* 435, 411, 369, 347, and 333, which were generated by the losses of H_2O (18 Da), C_2H_2O (42 Da), two C_2H_2O (84 Da), C_7H_6O (106 Da), $C_7H_4O_2$ (120 Da), respectively. The assignments were supported by their composition of $C_{28}H_{21}O_6^-$ (measured *m/z* 453.1345, error −0.2 ppm), $C_{28}H_{19}O_5^-$ (measured *m/z* 435.1236, error −0.46 ppm), $C_{26}H_{19}O_5^-$ (measured *m/z* 411.1237 error −0.24 ppm), $C_{24}H_{17}O_4^-$ (measured *m/z* 369.1131, error −0.27 ppm), and $C_{22}H_{15}O_5^-$ (measured *m/z* 359.0927, error −0.56 ppm) obtained by off-line FTICR-MS^2. In the MS^3 spectrum fragment ion at *m/z* 369, the neutral losses of two C_2H_2O (84 Da) and C_2H_2O (42 Da) gave corresponding fragment ions at *m/z* 285 and 327. The key product ions at *m/z* 333 were also observed in the MS^2 of compounds **8** and **10**, the relative abundance of which was 36% (the most abundant ion of at *m/z* 369 is considered to be 100%). The loss of $C_7H_4O_2$ (120 Da) from *m/z* 453 in compounds **8** and **10** occurs relatively easily, possibly because no hydroxy groups link to the benzene moiety of 2,3-dihydrobenzofuran of R_3, as shown in Fig. 3. The key product ion at *m/z* 369 was also observed in the MS/MS of compounds **8** and **10**, the relative abundance of which was 100%, this corresponds

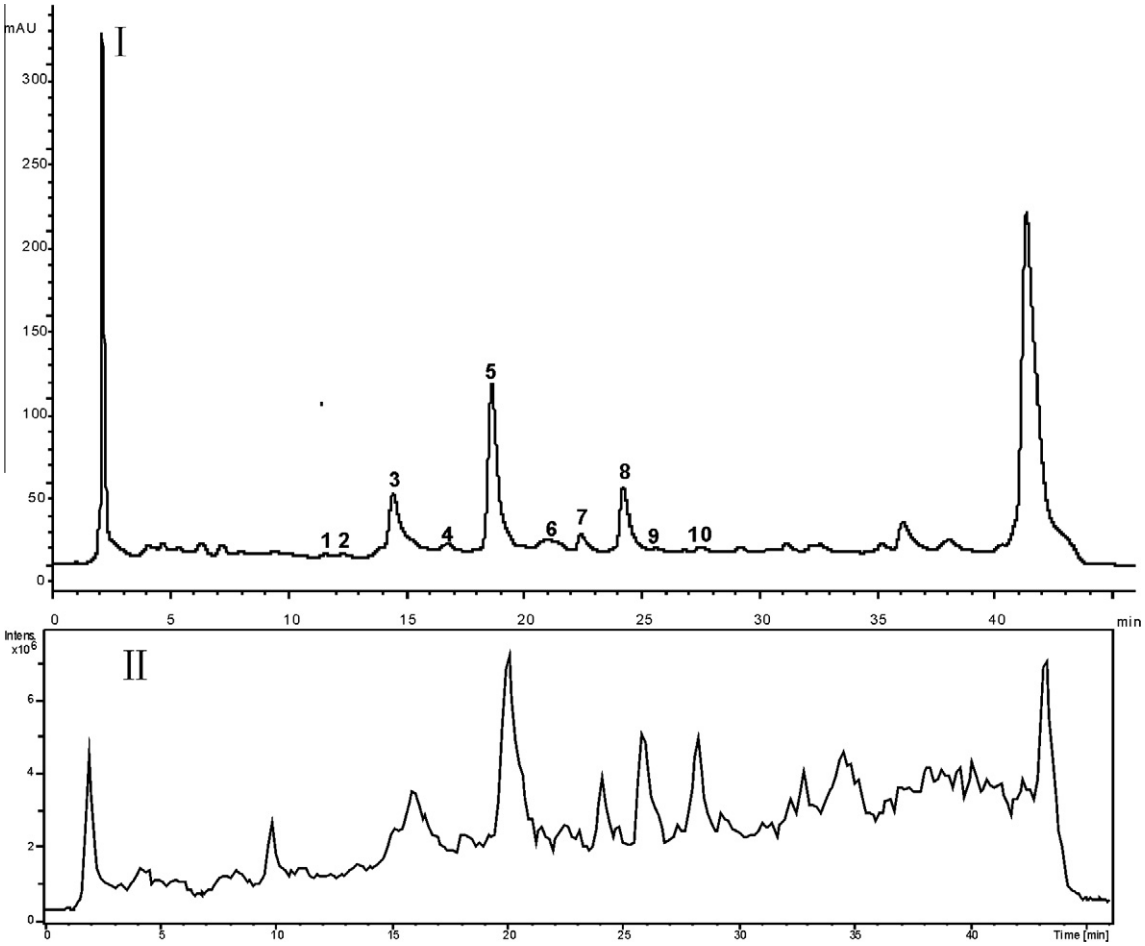


Fig. 1. Hplc-UV separation (280 nm) (I) and TIC (II) of resveratrol dimer and their analogues compounds from ethyl acetate extract of the stems of wine grapes. For chromatography and mass spectrometry conditions, see the experimental section.

to the loss of two C_2H_2O (84 Da) from two molecules of resorcinol (R_5) from compounds **8** and **10**, as shown in Fig. 3.

Compounds **4** and **5** were a pair of *cis*- and *trans*-isomers, respectively, which have the same MS^n spectra. In the MS/MS spectra of the $[M-H]^-$ ion at *m/z* 453, the losses of 94 Da (assigned to the phenol moiety), 106 Da (C_7H_6O), 120 Da ($C_7H_4O_2$) and 84 Da (two C_2H_2O) were observed to yield the product ions at *m/z* 359, 347, 333 and 369, respectively. The fragmentation pathways were supported by ESI-FTICR-MS^n data, and all errors were less than −2.0 ppm. In the MS^3 experiment of the fragment ion at *m/z* 359, several interesting fragment ions were generated from the eliminations of 70 Da (C_2H_2O and CO), 94 Da (phenol), 42 Da (C_2H_2O), thus producing the ions at *m/z* 289, 265 and 317. The key product ions at *m/z* 333 Da was also observed in the MS/MS spectra of compound **4** and **5**, the relative abundance of which was 10% (the most abundant ion of at *m/z* 359 is considered to be 100%). The loss of $C_7H_4O_2$ (120 Da) from compounds **4** and **5**, possibly is relatively difficult, possibly because of the hydroxy group linked to the benzene moiety of 2,3-dihydrobenzofuran of R_2, as shown in Fig. 3. The key product ion at *m/z* 369 was also observed in the MS/MS spectra of compounds **4** and **5**, the relative abundance of which was less than 10% (the most abundant ion at *m/z* 369 is considered to be 100%), which corresponds to the loss of C_2H_2O (42 Da) from the resorcinol (R_5) and the loss of other C_2H_2O (42 Da) from the phenol (R_7) group of compounds **4** and **5** in **3**.

Based on the above analyses of five standard compounds, we can conclude that the fragmentation patterns were similar in MS^n experiments, thus pointing to the consistency of their structural similarities. First, the key product ion at *m/z* 333 was observed in MS/MS spectra of compounds **3**, **4**, **5**, **8** and **10**, but the relative abundances were different (in Fig. 3). These differences are attributed to hydroxyl whether or not links to benzene groups of 2,3-dihydrobenzofuran of compounds **3**, **4**, **5**, **8** and **10**. When a hydroxyl group is linked to the benzene group of 2, 3-dihydrobenzofuran, the relative abundance of compounds **3**, **4** and **5** at *m/z* 333 in the MS^2 spectra was less than 10%; when no hydroxy links were present, as in compounds **8** and **10**, the relative abundance at *m/z* 333 Da was more than 30%. The key product ion at *m/z* 369 (385 in compound **3**) was from the neutral loss of two C_2H_2O (84 Da) in the MS^2 of compounds **3**, **4**, **5**, **8**, and **10**, but the relative abundance of the fragment ion at *m/z* 369 (*m/z* 385 in compound **3**) compared to the most abundant ion was different (see Fig. 3). Since the structure of compounds **8** and **10** contained two resorcinols (R_5), the relative abundance at *m/z* 369 in their MS^2 spectra was more than 90% (the most abundant ion at *m/z* 435 is considered to be 100%). The structure of compound **3** contained resorcinol (R_5) and pyrocatechol (R_6). Thus, its relative abundance at *m/z* 369 in MS^2 was more than 20%. The structure of compounds **4** and **5**, on the other hand, contained resorcinol (R_5) and phenol (R_7), thus, their relative abundance at *m/z* 369 in MS^2 was less than 10%.

3.2. Identification of the unknown compounds *1*, *2*, *6*, *7* and *9*

The full-scan mass spectrum of compound **9** showed a deprotonated ion at *m/z* 453 which had similar MS^2 fragmentation behav-

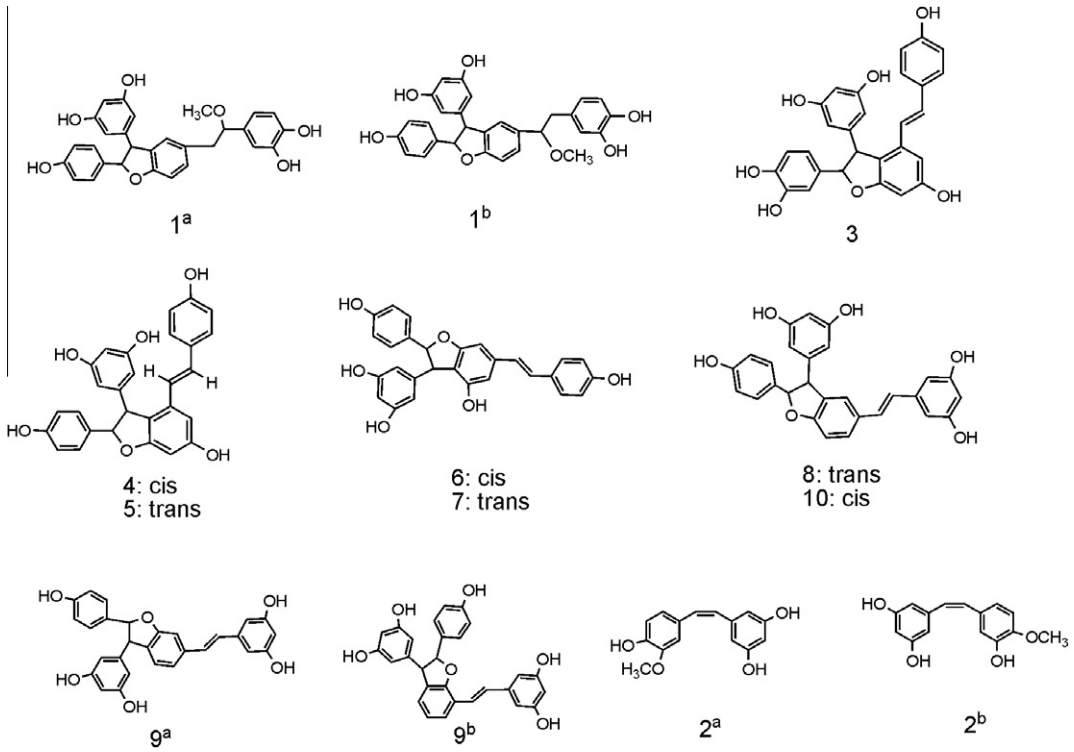
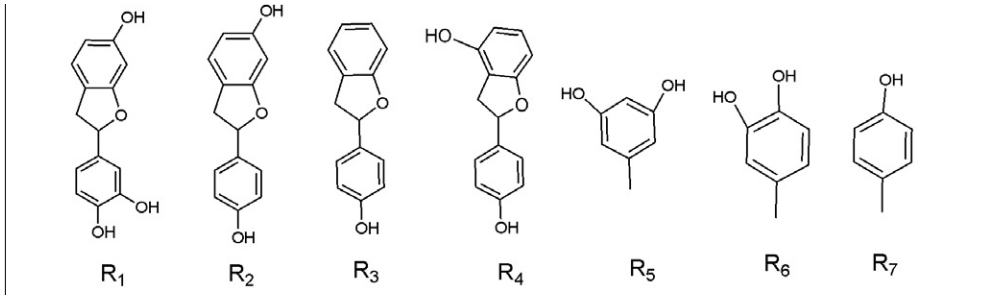


Fig. 2. Structures assigned to seven resveratrol dimers and three analogues compounds (**1**–**10**) studied in the stems of wine grape. **1**^{a} and **1**^{b}, **2**^{a} and **2**^{b}, **9**^{a} and **9**^{b}: proposed structures for compounds **1**, **2** and **9**, respectively.



Compounds	Key structure	Key product ions	MS^n	Key product ions	Key structure
1	R_3	347(100), 333 (39)	MS^3	347(100), 369 (23)	R_5 and R_6
2	/	/	MS^2	/	/
3	R_1	375(100), 333 (4)	MS^2	375(100), 385 (28)	R_5 and R_6
4	R_2	359(100), 333 (10)	MS^2	359(100), 369 (10)	R_5 and R_7
5	R_2	359(100), 333 (10)	MS^2	359(100), 369 (9)	R_5 and R_7
6	R_4	359(100), 333 (8)	MS^2	359(100), 369 (5)	R_5 and R_7
7	R_4	359(100), 333 (5)	MS^2	359(100), 369 (6)	R_5 and R_7
8	R_3	369(100), 333 (36)	MS^2	369(100), 369 (100)	R_5 and R_5
9	R_3	435(100), 333 (45)	MS^2	435(100), 369 (90)	R_5 and R_5
10	R_3	369(100), 333 (31)	MS^2	369(100), 369 (100)	R_5 and R_5

Fig. 3. Summary of key product ions (*m/z* 333 and 369) 10 compounds. *m/z* of the key product ions are given with relative abundance in parentheses (100 for the most abundant ion). R_3:4-(2,3-dihydrobenzofuran-2-yl)phenol.

iours with compounds **8** and **10**. Compound **9** was supported by an off-line ESI-FTICR-MS experiment with a composition of $C_{28}H_{21}O_6^-$ (measured *m/z* 453.1330, error −3.1 ppm). In the MS/MS spectra of the $[M–H]^-$ ion at *m/z* 453, compound **9** generated the losses of H_2O (18 Da), C_2H_2O (42 Da), two C_2H_2O (84 Da), 4-methylenecyclobexa (106 Da), $C_7H_4O_2$ (120 Da), which corresponds to the product ions at *m/z* 435, 411, 369, 347, and 333, respectively. The relative abundance of key fragment ion at *m/z* 333 was 45%, indicating 2,3-dihydrobenzofuran of R_3 was included in the structure of compound **9**. It is possible that no hydroxyl groups were

linked to the benzene ring of 2,3-dihydrobenzofuran. The relative abundance of key fragment ion at *m/z* 369 was 90%, indicating two resorcinols (R_5) contained in the structure of compound **9**. Compound **9** is tentatively proposed to be a new compound, as shown in Fig. 2.

The $[M–H]^-$ ions for compounds **6** and **7** were observed at *m/z* 453, both of which have the same MS^n spectra. According to the UV spectrum in Fig. 4, compounds **6** and **7** appear to be a pair of *cis*- and *trans*-isomers. Their MS^2 spectra exhibited similar fragmentation behaviours compared to compounds **4** and **5**. In the MS/MS spectra of the $[M–H]^-$ ion at *m/z* 453, the loss of 94 Da , 106 Da, 120 Da and 84 Da were observed to yield product ions at *m/z* 359, 347, 333, and 369, respectively. The relative abundance of key product ions at *m/z* 333 was less than 10%. Therefore, similar structures of R_2 were probably included in structures of compounds **6** and **7**. Hydroxy links to the benzene group of 2,3-dihydrobenzofuran appear to exist for both compounds. The relative abundance of key fragment ions at *m/z* 369 was less than 10%, implying that the structure of resorcinol (R_5) and phenol (R_7) were included in the structures of compounds **6** and **7**. According to the above data and those in the literature (Kato, Tokunaga, & Sakan, 2009), compound **7** is (E)-5-(6-(4-hydroxystyryl)-4-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydrobenzofuran-3-yl) benzene-1,3-diol, also known as Gnetin C. compound **6**, on the other hand, is tentatively proposed to be a new compound.

The full-scan mass spectrum of compound **1** gives an $[M–H]^-$ ion at *m/z* 485, which is supported by an off-line ESI-FTICR-MS experiment with the composition of $C_{29}H_{25}O_7^-$ (measured *m/z*

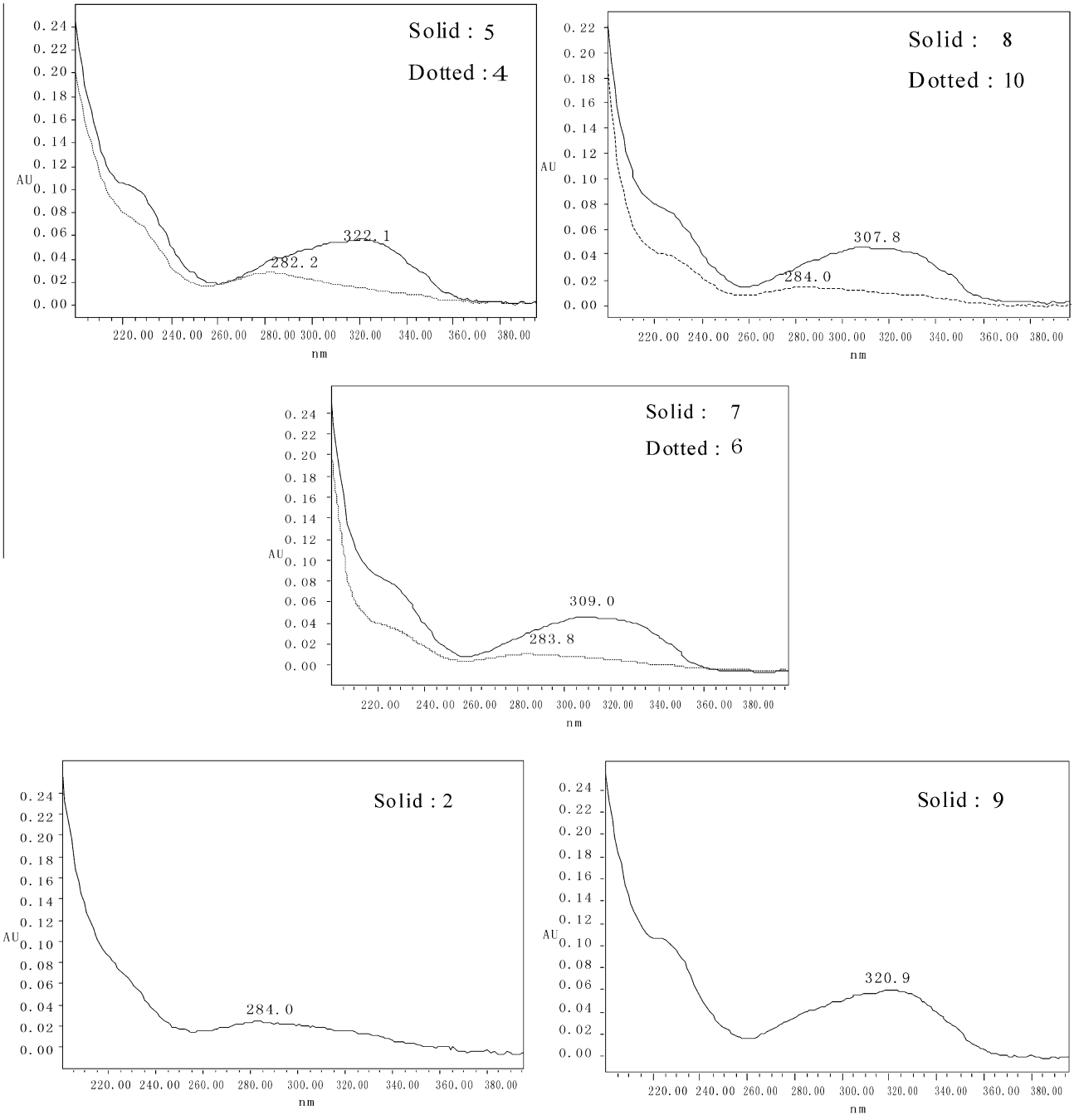


Fig. 4. Hplc/Dad-UV spectra of three pairs of *trans*- and *cis*-isomers (**4** and **5**, **8** and **10**, **6** and **7**) and HPLC/DAD-UV spectra of compounds **2**, **9**.

485.1616, error −2.1 ppm). The mass difference between compound **1** and the dimer of resveratrol is 32 Da, which is consistent with the molecular mass of methanol. In the MS/MS spectrum, the fragmentation of the $[M–H]^-$ ion at *m/z* 485 yielded two series of fragments. The series containing fragment ions at *m/z* 453 and 467, which were generated by the losses of methanol and H_2O (18 Da), indicating the presence of methoxyl group in the structure of compound **1**. The MS^3 spectra showed similar fragmentation behaviours compared to compound **9**. The fragment ion was observed at *m/z* 333, 347, 359, 369, 411, and 435 in the MS^3 spectrum of compound **1**. The fragment originating from the losses of 120 Da, 106 Da, 94 Da, 84 Da, 42 Da, and18 Da were obtained in the MS^3 spectra, indicating the loss of $C_7H_4O_2$, the phenol moiety, two C_2H_2O groups, C_2H_2O, C_6H_6O, and H_2O, respectively. The relative abundance of the key fragment ion at *m/z* 333 was more than 30%. Thus, the structure of R_3 was probably included in compound **1**. The relative abundance of the key product ion at *m/z* 369 was 23%, indicating that resorcinol (R_5) and pyrocatechol (R_6) were possibly included in the structure of compound **1**. Compound **1** is tentatively proposed to be a new compound, the structure of which is shown in Fig. 2. The fragmentation of compound **1** is shown in Fig. 5.

The full-scan mass spectrum of compound **2** gave $[M–H]^-$ ion at *m/z* 257. In the MS/MS spectrum, the fragmentation of the $[M–H]^-$ ion at *m/z* 257 yielded two series of fragments. The first series contained fragment ions at *m/z* 229 and 225, which were generated by the loss of CO (28 Da) and the consecutive loss of methanol (32 Da), respectively, indicating the presence of methoxyl groups in the structure of compound **2**. Fragment ions were observed at *m/z* 211, 185, 141, and 123 in the MS^3 spectrum of the ion at *m/z* 229, indicating the loss of H_2O (18 Da), CO_2 (44 Da), CO_2 (44 Da), and C_7H_6O (106 Da), respectively. These fragmentation behaviours are similar to that of 3,4′,5′-trihydroxy-5-methoxystilbene, 3,5,5′-trihydroxy-4′-methoxystilbene, 3,4′,5-trihydroxy-5′-methoxystilbene, as reported in the literature (Jerkovic, Nguyen, Nizet, & Collin, 2007). Based on the above data, HPLC/DAD-UV spectra, and supplementary information from the literature (Jerkovic et al., 2007; Cardona, Fernandez, Garcia, & Pedro, 1986), compound **2** was tentatively identified to be either (Z)-5-(4-hydroxy-3-methoxystyryl) benzene-1,3-diolor or (Z)-5-(3-hydroxy-4-methoxystyryl) benzene-1, 3-diol. Its structure is shown in Fig. 2.The orientation of the methoxy substitute needs to be identified in the future work.

3.3. Test the relative abundance of the key product ions in collision energy

Diastereoisomer could be differentiated by the breakdown curves of the collision energy (Chen, He, Mao, Sun, & Pan, 2009). Fig. 6 shows how the fractional abundances of the key product ions of compounds **1**, **3**, **5**, **7**, **8** and **9** changed as the collision energy varied from 0.60 to 0.95 V, along with the collision energy increased, the relative abundance of deprotonated ion at *m/z* 453 wear off, and the relative abundance of key product ions increase

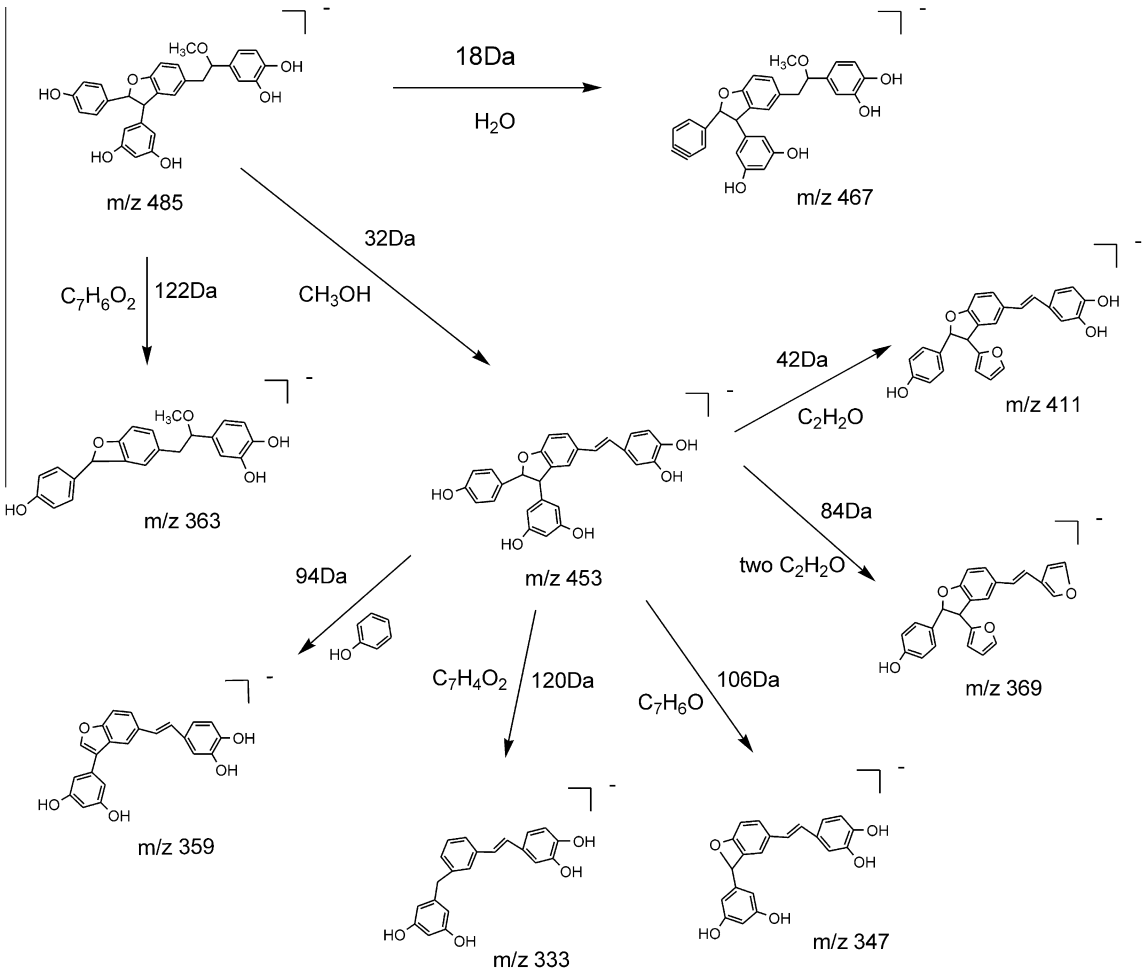


Fig. 5. Fragmentation pathways proposed for $[M–H]^-$ ions of compound **1**.

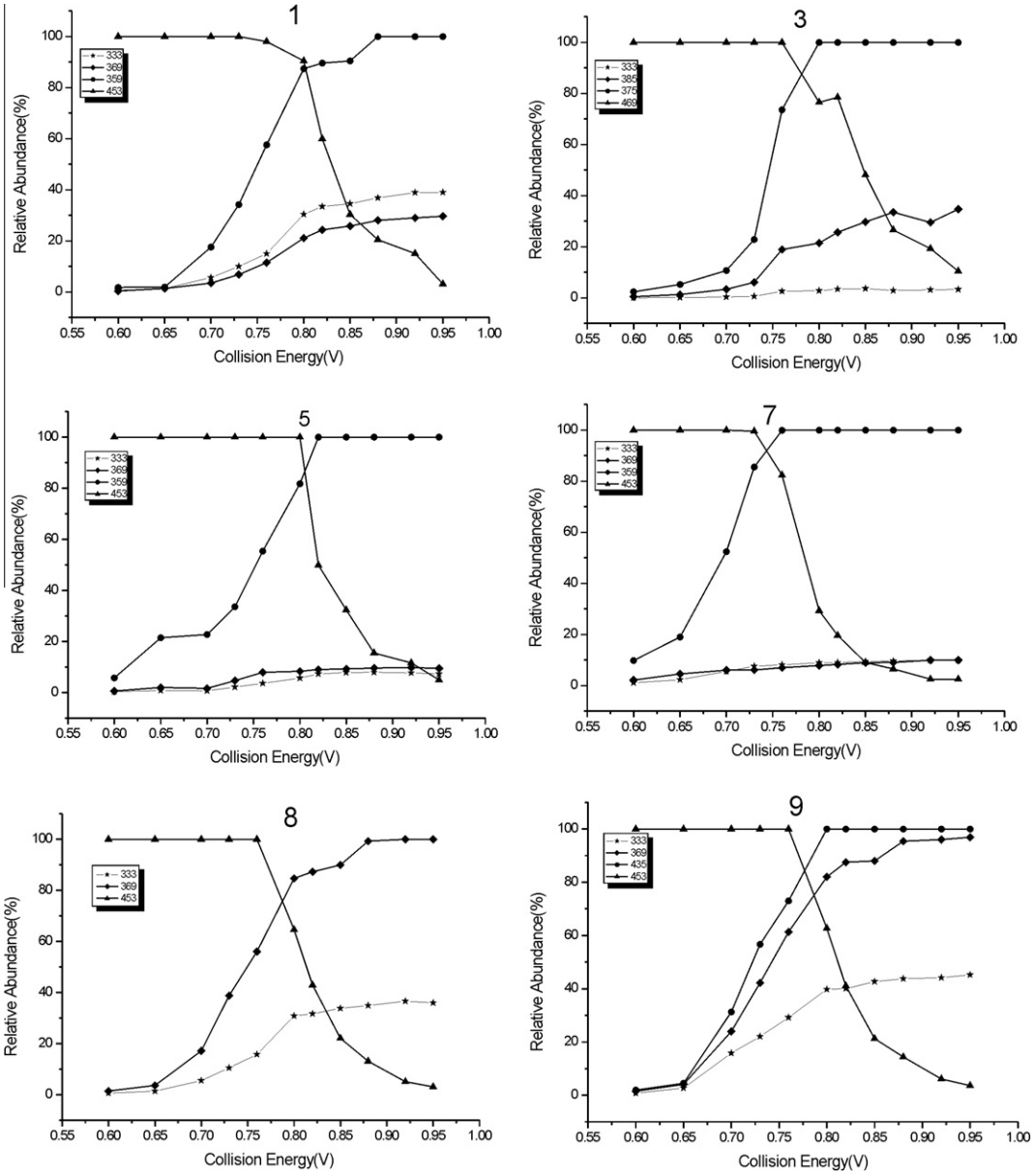


Fig. 6. Breakdown curves of selected product ions for compounds **1**, **3**, **5**, **7**, **8** and **9**.

gradually. However, the relative abundance of the key product ions at *m/z* 333 and 369 (*m/z* 385 in compound **3**) compared with most abundant ion kept unchanged as the collision energy varied from 0.60 to 0.95 V. The relative abundance of the key product ions of compounds **1**, **3**, **5**, **7**, **8** and **9**, alonging with the collision energy variety, were delineated in detail below. The relative abundance of the key product ions at *m/z* 333 compared with most abundant ion at *m/z* 359 (375 in compound **3**) was less than 10% in Fig. 6 (**3**, **5**, **7**), the relative abundance of the key product ion at *m/z* 333 compared with most abundant ion at *m/z* 359 (*m/z* 435 in compound **9** and *m/z* 369 in compound **8**) was more than 30% in Fig. 6 (**1**, **8**, **9**); in addition the relative abundance of the key product ion at *m/z* 369 compared with most abundant ion at *m/z* 359 (435 in compound **9**) was more than 90% in Fig. 6 (**8**, **9**), the relative abundance of the key product ions at *m/z* 369 (385 in compound **3**) compared with most abundant ion at *m/z* 347 (375 in compound **3**) was more than 20% in Fig. 6 (**1**, **3**), the relative abundance of the key product ions at *m/z* 369 compared with most abundant ion at *m/z* 359 was less than 10% in Fig. 6 (**5**, **7**). Therefore, isomers of resveratrol dimer were differentiated by the relative abundance of key product ions.

3.4. Differentiation of the trans- and cis-isomers by HPLC/DAD-UV

No differences between the *trans*- and *cis*-isomers among the relative abundances of key product ions were observed. The HPLC/DAD-UV spectra between the *trans*- and *cis*-isomers, however, were quite different (Chen et al., 2009). As a result, the UV spectra between the *trans*- and *cis*-isomers was quite different (Fig. 4). The *trans*-isomers (compounds **5**, **7** and **8**) had different adsorption wavelengths and stronger intensities than the *cis*-isomers (compounds **4**, **6** and **10**). This difference may be attributed to the more stable the conjugations of the trans-isomers compared to that of the *cis*-isomers. Based on the above HPLC/DAD-UV spectra, compounds **2** and **9** were tentatively assigned to as *cis*- and *trans*-configuration, respectively, as shown in Fig. 4.

4. Conclusions

The results illustrated the potential advantage of the method of HPLC/(-)ESI-MS^n and HPLC/DAD-UV for identification isomers of resveratrol dimers and their analogues from wine grapes. Based on the fragmentation behaviours, ten compounds were analysed, among them, three compounds are tentatively identified as new compounds. The relative abundance of two diagnostic product ions at *m/z* 333 and 369 (385 in compound **3**) were utilised for the key structure identification of a resveratrol dimer. Three pairs of the *trans*- and *cis*-isomers could be identified by their HPLC/DAD-UV spectra. Based on their UV spectra, compounds **2** and **9** were tentatively identified as *cis* and *trans*- configuration, respectively.

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