Journal of Chromatography B, 878 (2010) 1–7

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Structural characterization of trace stilbene glycosides in *Lysidice brevicalyx* Wei using liquid chromatography/diode-array detection/electrospray ionization tandem mass spectrometry

Youcai Hu[1], Jing Qu[1], Yuanyan Liu, Shishan Yu*, Jianbei Li, Jinlan Zhang, Dan Du

Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education & Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

ARTICLE INFO

Article history:
Received 6 May 2009
Accepted 24 September 2009
Available online 20 October 2009

Keywords:
Lysidice brevicalyx
Stilbene glycoside
HPLC-DAD/ESI-MS^n
Fragmentation behavior
On-line identification

ABSTRACT

The mass fragmentation patterns of stilbene glycosides isolated from the genus *Lysidice* were investigated by negative ion electrospray ionization tandem mass spectrometry, and the influence of collision energy on their fragmentation behavior is discussed. It is found that the presence of the Y_0^- and B_0^- ions in the MS^2 spectra is characteristic for 1 → 6 linked diglycosyl stilbenes, while the Y_0^-, Y_1^-, and Z_1^- ions are representative ions of 1 → 2 linked diglycosyl stilbenes. These results indicate that ESI-MS^n in the negative ion mode can be used to differentiate 1 → 6 and 1 → 2 linked diglycosyl stilbenes. Based on the fragmentation rules, 9 new trace constituents were identified or tentatively characterized in a fraction of *Lysidice brevicalyx* by using HPLC/HRMS and HPLC-DAD/ESI-MS^n. The results of the present study can assist in on-line structural identification of analogous constituents and targeted isolation of novel compounds from crude plant extracts.

1. Introduction

Stilbene glycosides, which are widely distributed in herbal plants, exhibit various biological activities, including antioxidant [1,2], antiamyloidogenic [3], antibrowning [4], antiplatelet aggregation [5], and hepatoprotective [6] activities. During our previous studies on bioactive constituents from the genus *Lysidice* [7–14], we had obtained stilbenes, phloroglucinols, flavanoids, and lignans. Recently, an efficient procedure based on biological and chemical screening was used to investigate the antioxidant components present in the bark of *Lysidice brevicalyx* Wei, and 12 stilbene glycosides were isolated and/or identified from an antioxidant fraction [15]. The stilbene glycosides found in the genus *Lysidice* possess a same aglycone core moiety as resveratrol, and the sugar moiety is linked at the C-3 of resveratrol. The sugar residues include hexose (glucose), pentose (xylose), and deoxyhexose (rhamnose) units, and each of these units is mainly linked through 1 → 6 and 1 → 2 linkages or by a combination of these.

To further identify novel compounds from *L. brevicalyx* by chemical screening and to assist rapid structural identification of analogous constituents in plants, we investigated the fragmentation patterns of 9 stilbene glycosides by using ESI-MS^n both at the same and different collision energies. The relationship between the MS fragmentation patterns and the structures of the stilbene glycoside is described. Based on these results, we carried out structural identification of similar trace constituents in an antioxidant fraction from the bark of *L. brevicalyx*. As a result, a total of 24 compounds, including 9 new ones, were identified or tentative characterized from the antioxidant fraction. The aim of these studies is to establish a rapid and efficient method for analysis of trace stilbene glycosides.

2. Experimental

2.1. Materials

The barks of *L. brevicalyx* were collected from Guangxi Province, China. Isolation of stilbene glycosides from this plant has been reported earlier [15]. The antioxidant fraction (Fr. C_{2-3}) [15] was subjected to preparative HPLC and a subfraction (Fr. C_{2-3w}) was obtained in which the levels of the main constituents identified earlier were significantly lower and the relative levels of trace constituents was markedly higher.

2.2. ESI-MS^n analyses of pure compounds

Negative ion ESI-MS^n experiments were performed on an Agilent 1100 Series LC/MSD Trap mass spectrometer (Santa Clara, CA,

* Corresponding author. Tel.: +86 10 63165326/60212125; fax: +86 10 63017757.
E-mail address: yushishan@imm.ac.cn (S. Yu).
[1] These authors contributed equally to this work.

1570-0232/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2009.09.033

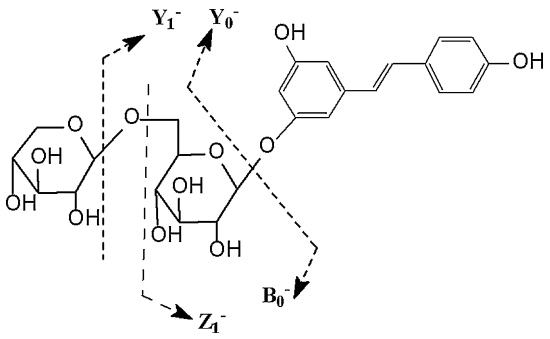


Fig. 1. Nomenclature adopted for stilbene glycosides (illustrated on lysidiside L).

USA). The ESI conditions were as follows: HV capillary voltage, 3.5 kV; drying temperature, 325 °C; drying gas, 6.0 L min^{-1}; nebulizer, 15 psi; capillary exit voltage, 124.8 V; and injection rate, 5 mL min^{-1}. The smart fragmentation function was used (Smart-Frag Ampl: 30–200%). The concentration of each compound was 1 mg mL^{-1}.

Pure compounds were injected into the ESI source by continuous infusion. The $[M-H]^-$ ion was selected for CID fragmentation to produce the MS/MS spectra. Prominent MS/MS ions were selected for further MS^n analyses (n = 3–4). To compare the fragmentation behavior of the compounds tested, the collision energy was set from 0.7 to 1 V in the MS^n analysis.

2.3. HPLC/HRMS of constituents of the fraction

High-resolution accurate mass spectra of Fr. C_{2-3w} were recorded on a Thermo Finnigan LTQ Fourier transform (FT) mass spectrometer. The FT-MS conditions in negative ion mode were as follows: sheath gas flow rate, 35 arb; aux gas flow rate, 10 arb; sweep gas flow rate, 5 arb; I spray voltage, 3.50 kV; capillary temperature, 300 °C; capillary voltage, −40.0 kV; and tube lens, −120.0 kV.

HPLC separation was performed on an Agilent XDB-C_{18} column (4.6 × 150 mm, 5 μm) using a mobile phase of acetonitrile and water (flow rate: 0.3 mL min^{-1}). The mobile phase gradient program (acetonitrile/water) for Fr. C_{2-3w} was 11:89 (t = 0 min), 13:87 (t = 11 min), 25:85 (t = 20 min), 18:82 (t = 30 min), and 20:80 (t = 40 min).

2.4. HPLC/ESI-MS^n analyses of constituents of the fraction

For on-line HPLC/MS^n analyses, an Agilent 1100 Series liquid chromatography system was utilized that was coupled to an ion-trap mass spectrometer. The negative ion ESI-MS^n experiments were conducted under the same conditions as those used for the pure compounds described above. HPLC separation was performed on an Agilent XDB-C_{18} column (4.6 × 150 mm, 5 μm) (flow rate: 0.3 mL min^{-1}). The mobile phase for each fraction was the same as that used in the HPLC/HRMS analyses.

3. Results and discussion

3.1. Nomenclature

The nomenclature proposed by Domon and Costello [16] for glycoconjugates was adopted to denote the product ions of stilbene glycosides (Fig. 1). Ions containing the aglycone are labeled Y_n^- or Z_n^-, and the ions formed by the cleavage of one of the glycosidic bonds with the charge being retained on the carbohydrate residue are designated B_n^-.

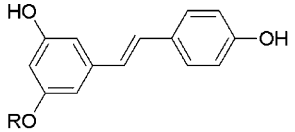
3.2. Fragmentation patterns of stilbene glycosides in ESI-MS^n at the same collision energy

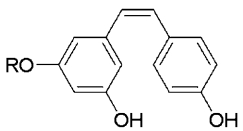
The tested stilbene glycosides could be classified into three types based on their sugar moieties (Fig. 2). Type I compounds are 1 → 6 linked diglycosyl resveratrols; type II compounds are 1 → 2 linked diglycosyl resveratrols; and type III compounds are resveratrol triglycosides with both 1 → 6 and 1 → 2 linkages. All stilbene glycosides form $[M-H]^-$ and $[M+36-H]^-$ ions in their negative ion mass spectra. The major fragment ions are shown in Table 1.

3.2.1. Lysidisides L and P and (E)-resveratrol rutinoside (type I)

Lysidiside L formed an $[M-H]^-$ ion at m/z 521, which produced two prominent ions at m/z 293 (B_0^-) and m/z 227 (Y_0^-) in the MS^2 spectrum. It is assumed that the two product ions are formed by the neutral loss of a resveratrol unit (Δm = 228 Da) and disaccharide unit (Δm = 294 Da), respectively. The ion at m/z 293 gave signals at m/z 149 (B_1^-), 131, and 125 in its MS^3 spectrum, while the ion at m/z 227 produced fragment ions at m/z 185 and 143 (Fig. S1, see supporting information). The proposed fragmentation pathway of lysidiside L in negative ion ESI-MS^n is shown in Scheme 1.

Lysidiside P and (*E*)-resveratrol rutinoside are a pair of (*E*)-/(*Z*)-resveratrol α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosides. Both of these yielded prominent ions at m/z 307 (B_0^-) and m/z 227 (Y_0^-) in their MS^2 spectra, which originated from the neutral loss of a resveratrol unit (Δm = 228 Da) and disaccharide unit (Δm = 308 Da), respectively. The almost uniform ESI-MS^n spectra (Figs. S2 and S3, see supporting information) of lysidiside P and (*E*)-resveratrol rutinoside suggest that the configuration of the C–C double bond has no obvious influence on the fragmentation pathways of the compounds investigated.



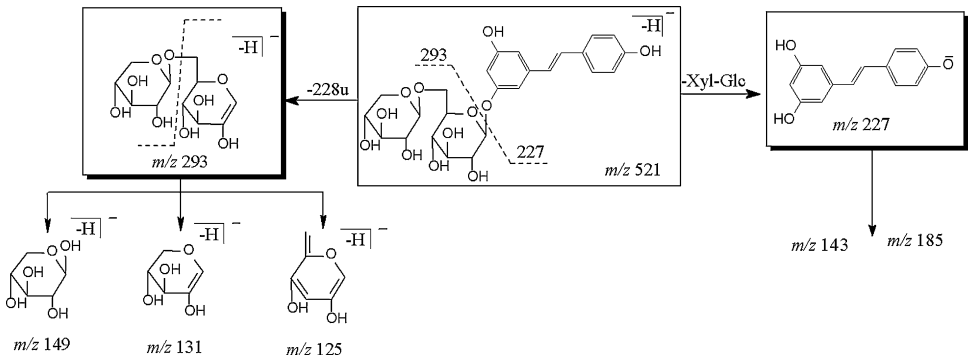


Type I	R = -Glu6-Xyl R = -Glu6-Rha	lysidiside L (E)-resveratrol rutinoside	R = -Glu6-Rha	lysidiside P
Type II	R = -Xyl2-Glu R = -Glu2-Rha R = -Xyl2-Rha	lysidiside M lysidiside N lysidiside E	R = -Glu2-Rha	lysidiside O
Type III	R = -Glu(2,6)-Rha	lysidiside Q	R = -Glu(2,6)-Rha	lysidiside R

Fig. 2. Investigated compounds previously isolated from *L. brevicalyx*.

Table 1
Main fragment ions in negative mode for stilbene glycosides tested.

Compound		[M+36−H]⁻	[M−H]⁻	MS^n fragment ions				
				Y_0^-	Y_1^-	B_0^-	Z_1^-	Others
Type I	Lysidiside L	557	521	227	/	293	/	149, 131, 125; 185, 143
	(*E*)-resveratrol rutinoside	571	535	227	/	307	/	163, 125
	Lysidiside P	571	535	227	/	307	/	163, 145, 125
Type II	Lysidiside M	557	521	227	359	/	341	227, 185, 157, 143
	Lysidiside N	571	535	227	389	/	371	227
	Lysidiside O	571	535	227	389	/	371	227
	Lysidiside E	541	505	227	359	/	341	463, 227
Type III	Lysidiside Q	717	681	227	535	/	517	647, 517, 453, 389, 371, 307, 227, 163, 125
	Lysidiside R	717	681	227	535	/	517	389, 371, 307, 227, 163, 119



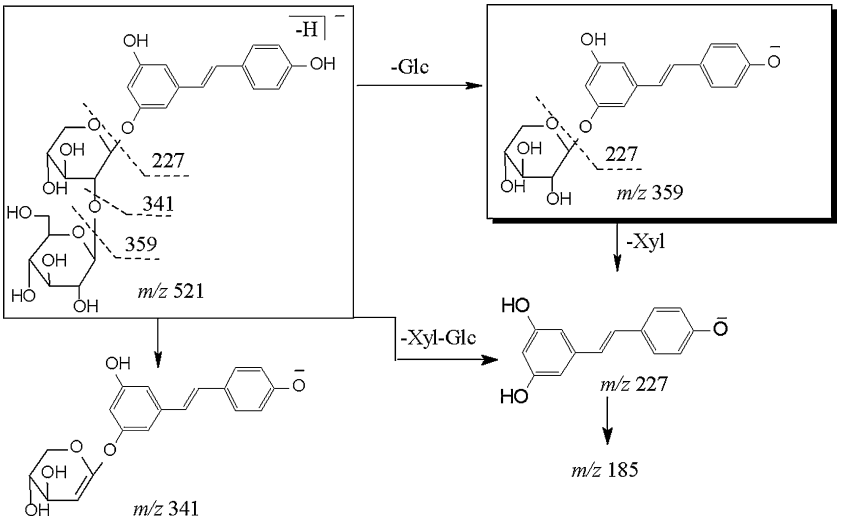
Scheme 1. Proposed fragmentation pathway for lysidiside L in (−)ESI-MS^n.

From the studies described above, it was concluded that the B_0^- and Y_0^- ions, which originate from the neutral loss of a resveratrol unit and disaccharide unit, respectively, are characteristic of 1 → 6 linked diglycosyl stilbenes.

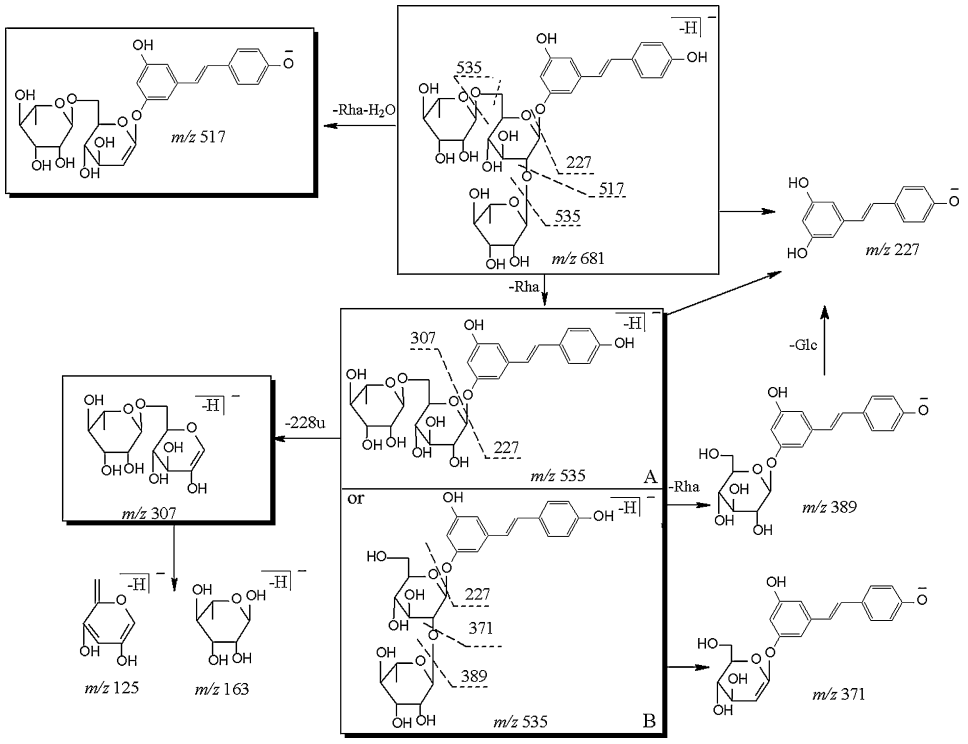
3.2.2. Lysidisides M, N, O, and E (type II)

The fragmentation behaviors of lysidisides M, N, O, and E were completely different from those of type I stilbene glycosides (Table 1). The $[M-H]^-$ ion of lysidiside M at *m*/*z* 521 produced two prominent ions at *m*/*z* 359 (Y_1^-) and 227 (Y_0^-) in the MS^2 spectrum, and these originated from the neutral loss of an outer glucose moiety (Δm = 162 Da) and disaccharide unit (Δm = 292 Da), respectively. It also produced a trace ion at *m*/*z* 341 (Z_1^-) due to the loss of an outer glucose unit and a molecule of H_2O. However, no B_0^- ion was observed in the MS^2 spectrum, which markedly differed from that of type I compounds. The Y_1^- (*m*/*z* 359) ion was subjected to MS^3 analysis to yield an ion at *m*/*z* 227, which resulted in fragment ions at *m*/*z* 185 and 143 (Fig. S4, see supporting information). The proposed fragmentation pathway of lysidiside M in negative ion ESI-MS^n is shown in Scheme 2. Similarly, the prominent ions, Y_1^- and Y_0^- and the trace ion Z_1^- were also observed in the ESI-MS^n spectra of lysidisides N, O, and E (Table 1 and Figs. S5–S7, see supporting information).

Therefore, the typical fragmentation patterns for type II compounds were *O*-glycosidic cleavage and neutral loss of an outer sugar unit, which produced the characteristic Y_1^-, Y_0^-, and Z_1^- ions.



Scheme 2. Proposed fragmentation pathway for lysidiside M in (−)ESI-MS^n.

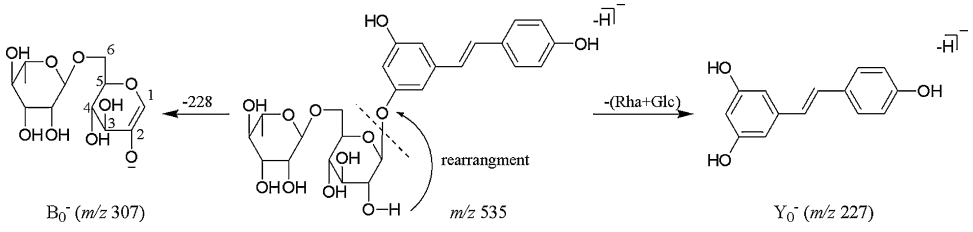


Scheme 3. Proposed fragmentation pathway for lysidiside Q in (−)ESI-MS^n.

3.2.3. *Lysidisides Q and R (type III)*

Lysidisides Q and R are resveratrol triglycosides with both 1 → 6 and 1 → 2 linkages in their sugar moieties. The fragmentation pathways of the two compounds were somewhat similar to those of types I and II compounds. In the MS^2 spectrum of lysidiside Q, a prominent ion was observed at *m*/*z* 535 (Y_1^-), and two trace ions were observed at *m*/*z* 517 (Z_1^-) and 227 (Y_0^-). The Y_1^- ion at *m*/*z* 535 formed fragment ions at *m*/*z* 389, 371, 307, and 227 in its MS^3 spectrum. According to the fragmentation patterns of types I and II compounds described above, the ions at *m*/*z* 389, 371, and 227 were formed from type II stilbene glycosides, and the ions at *m*/*z* 307 and 227 were characteristic for type I stilbene glycosides. This indicated that the Y_1^- ion at *m*/*z* 535 consisted of parts A and B, as shown in Scheme 3. These results are in agreement with the molecular composition of stilbene glycosides with both 1 → 6 and 1 → 2 glycosidic linkage. Lysidiside R, a (Z) isomer of lysidiside Q, showed almost the same ESI-MS^n spectra as lysidiside Q (Table 1 and Fig. S9, see supporting information), indicating that the configuration of the C–C double bond showed no direct effect on the main fragmentation pathways of stilbene glycosides.

Thus, the presence of the Y_0^- and B_0^- species can be employed as a diagnostic measure to detect the presence of the 1 → 6 linked disaccharide moiety in stilbene glycosides. Moreover, the Y_1^-, Y_0^-, and Z_1^- ions are characteristic of 1 → 2 linked diglycosyl stilbenes in negative ion ESI-MS^n. The different fragmentation patterns of these are closely related to the linkage mode of sugar residues. Based on literature reports, the formation of Y_n ions was assumed to occur by rearrangement reactions that involved the migration of *O*-linked hydrogen atoms [17]. Because the hydroxyl group close to the acetal linkage between the aglycone and the sugar part, i.e., the 2-OH group of the inner sugar, is bound to a terminal sugar in types II and III stilbene glycosides, formation of the Y_0^- ion appears to be more difficult than that of type I compounds (Scheme 4). This explains the reason that the formation of other fragments is favored in stilbene glycosides with 1 → 2 linkages and results in distinct spectra for compounds with 1 → 2 and 1 → 6 interglycosidic linkages. The B_0^- ion in the case of stilbene glycosides with 1 → 6 interglycosidic linkages results from the cleavage of a glycosidic bond with the charge being retained on the sugar residue. The double bond formed between C_1 and C_2 of the inner sugar unit has a conjugative effect on the negative ion that sufficiently stabilizes the latter, thereby facilitating its detection. However, the B_0^- ion could not be detected in the ESI-MS^2 spectra of stilbene glycosides with a 1 → 2 interglycosidic linkage due to the absence of a conjugative stabilization effect. Furthermore, because the acidity of the phenolic hydroxyl is greater than that of the alcoholic hydroxyl, the abundance of the Y_0^- ion is greater than that of the B_0^- ion in type I compounds. The above analyses provide a reasonable explanation for the different fragmentation patterns of the stilbene glycosides tested.



Scheme 4. Proposed fragmentation mechanism for stilbene glycosides of type I (illustrated on lysidiside L).

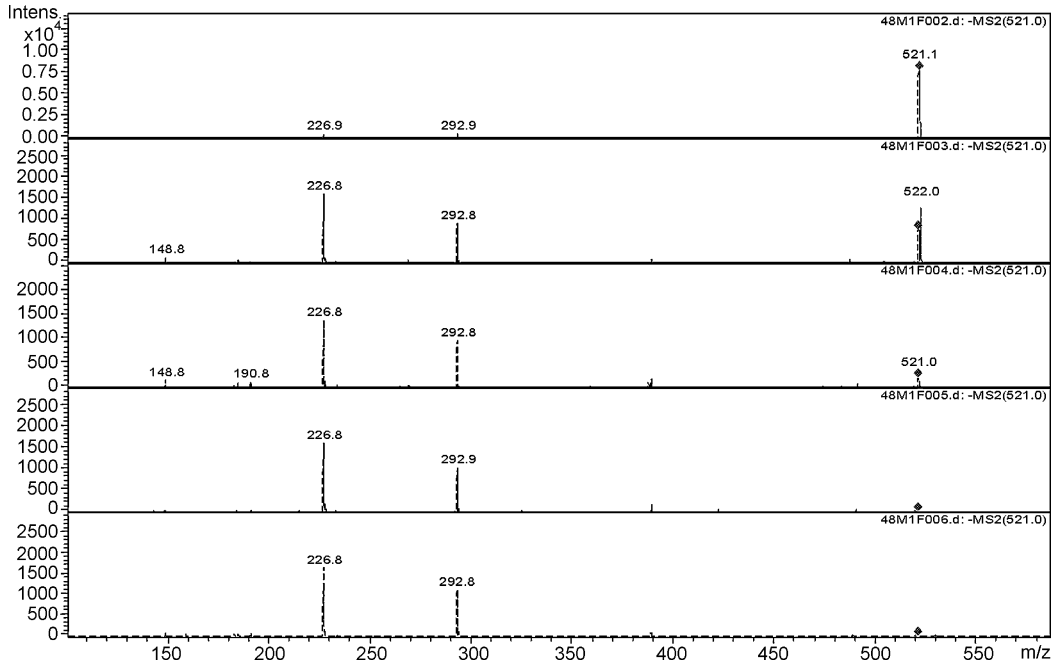


Fig. 3. (−)ESI-MS^2 spectra of lysidiside L at different energy (MS/MS Frag Ampl: (1) 0.4 V; (2) 0.5 V; (3) 0.6 V; (4) 0.7 V; (5) 0.8 V).

3.3. Fragmentation patterns of identical compounds at different collision energies

Lysidiside L was selected as an example for type I stilbene glycosides. In the collision energy range of 0.4–1.0 V, the ion abundance of $[M-H]^-$ at *m*/*z* 521 decreased with an increase in the collision energy and disappeared at a collision energy of 0.8 V. However, no obvious change was observed for characteristic ions such as the B_0^- ion at *m*/*z* 293 and Y_0^- ion at *m*/*z* 227 (Fig. 3). Similar results were observed with other compounds tested, indicating that the changes in the collision energy in this range (0.4–1.0 V) showed no appreciable effect on the fragmentation behavior of stilbene glycosides. As a result, the fragmentation patterns of stilbene glycosides, which have been described above, could be used for the on-line structural identification of trace constituents that possess similar structures.

3.4. Analyses of constituents of a fraction from L. brevicalyx

Twelve compounds in Fr. C_{2-3} have been identified or isolated previously [15]. However, some trace constituents are difficult to deal with because these are present in minute amounts. To increase the relative amount of trace constituents, the main constituents were artificially eliminated, allowing the trace constituents in subfraction Fr. C_{2-3w} (Fig. 4) to be enriched and detected under the conditions used.

By comparing the retention times, UV spectra, and HPLC/HRESIMS and HPLC/ESI-MS^n data with those reported in literature, compounds **4**, **6–8**, **13**, **14**, **16–20**, and **24** were assigned as known compounds that had been identified previously [15]. Moreover, 3 flavonoids were identified as 3′,4′,7-trihydroxylflavone 3-*O*-glucoside (**1**), kaemferol 3-neohesperidoside (**9**), and isorhamnetin 3-neohesperidoside (**11**) based on their UV spectra, $[M-H]^-$

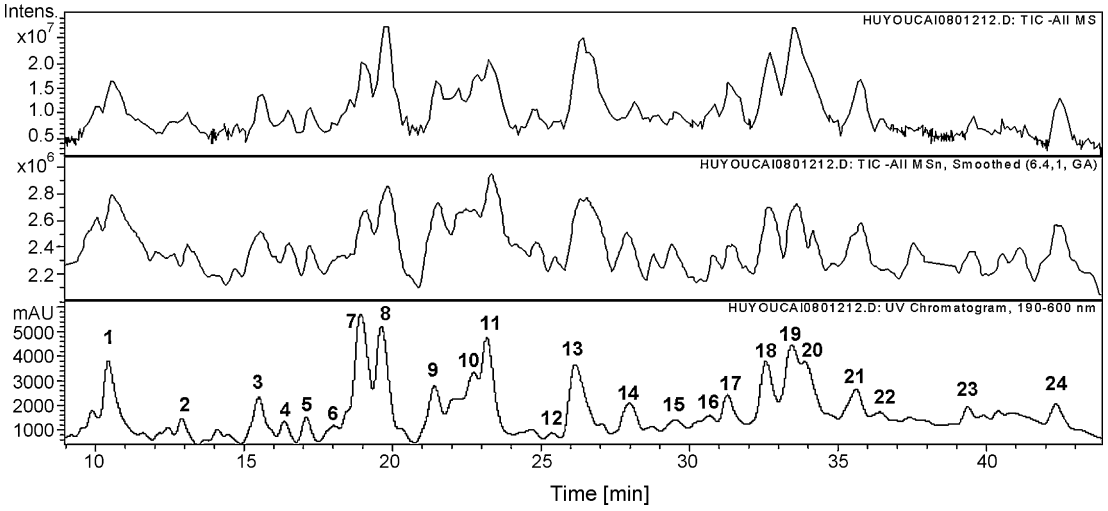


Fig. 4. Liquid chromatogram (LC) and total ion chromatogram (TIC) of Fr. C_{2-3w}.

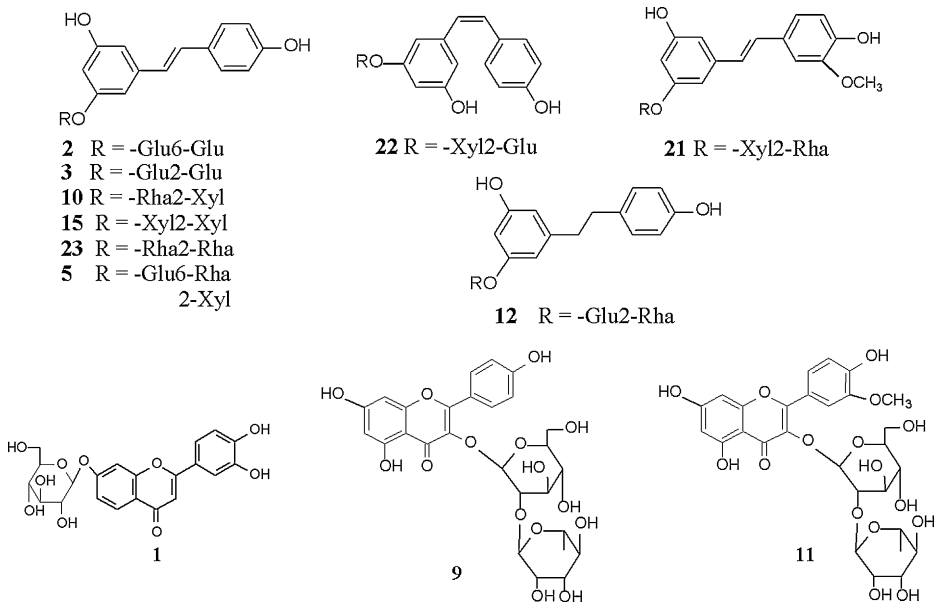


Fig. 5. Structures of new compounds proposed in Fr. C_{2-3w} from *L. brevicalyx*.

ions, and fragment ions in HPLC/HRRSIMS and HPLC/ESI-MS^n [18–20]. For unknown constituents, the structures (Fig. 5) were characterized based on their UV spectra and HPLC/HRESIMS and HPLC/ESI-MS^n fragmentation behaviors (Table 2 and Figs. S11–S19, see supporting information). When several analogues equally matched the spectral data, one of these was preferentially selected based on its biosynthesis. Here, compounds **2** (type I), **3**, **22** (type II), and **5** (type III) are taken as examples to discuss details of the elucidation procedure.

Compound **2** exhibited the characteristic UV spectrum of (*E*)-stilbenes [15,21,22] in HPLC/DAD analysis. The HPLC/MS analysis showed an $[M-H]^-$ ion at *m/z* 551, suggesting a molecular weight of 552 Da. The molecular formula of **2** was established as $C_{26}H_{32}O_{13}$ on the basis of HPLC-HRESIMS, which gave an $[M-H]^-$ ion at *m/z* 551.1763. In the HPLC/ESI-MS^2 spectrum of compound **2**, a clear Y_0^- ion was observed at *m/z* 227 (deprotonated aglycone), suggesting that the aglycone of **2** was resveratrol [15]. Another prominent ion at *m/z* 323 (B_0^-), formed by neutral loss of a resveratrol unit, indicated the presence of two hexosyl units in the structure of compound **2**. According to the ESI-MS^n analysis for type I stilbene glycosides the coexistence of the Y_0^- and B_0^- ions, indicated that the two hexose units in compound **2** are linked by a $1 \rightarrow 6$ linkage. Considering the fact that the glycosides isolated from the genus *Lysidice* mainly contain glucose, xylose, and rhamnose units, the hexose present in compound **2** was probably glucose. Therefore, the existence of an (*E*)-resveratrol unit and a glucopyranosyl-$(1 \rightarrow 6)$-glucopyranose unit in compound **2** was tentatively established. The attachment of a glucose unit at C-3 of the aglycone was deduced on the basis of our previous discovery that the glycosides were attached to resveratrol through the oxygen at the C-3 position in all stilbene glycosides isolated from the genus *Lysidice*.

Table 2
Characteristics of the constituents **1–24** by HPLC/HRMS and HPLC/MS^n in (−)ESI mode.

Peak	Formula	LC/HRMS $[M-H]^-$ *m/z*	Calculated *m/z*	$[M-H]^-$ *m/z*	LC/ESI-MS^n *m/z*
1	$C_{21}H_{20}O_{10}$	431.09750	431.09727	431	MS^2 [431]: 269; MS^3 [269]: 226, 201, 133
2	$C_{26}H_{32}O_{13}$	551.17633	551.17592	551	MS^2 [551]: 471, 323, 263, 227; MS^3 [227]: 183, 143
3	$C_{26}H_{32}O_{13}$	551.17627	551.17592	551	MS^2 [551]: 491, 389, 371, 251, 227; MS^3 [227]: 185, 157
4	$C_{25}H_{30}O_{12}$	521.16504	521.16535	521	MS^2 [521]: 293, 249, 185, 227; MS^3 [227]: 185, 157
5	$C_{31}H_{40}O_{16}$	667.22321	667.22326	667	MS^2 [667]: 535, 521, 439, 227; MS^3 [535]: 307, 389, 227
6	$C_{26}H_{32}O_{12}$	535.18103	535.18100	535	MS^2 [535]: 307, 183, 157, 227; MS^3 [227]: 185, 183, 157
7	$C_{20}H_{22}O_8$	389.12418	389.12309	389	MS^2 [389]: 227; MS^3 [227]: 185, 157
8	$C_{32}H_{42}O_{16}$	681.23956	681.23891	681	MS^2 [681]: 535,577, 517, 306, 227; MS^3 [535]: 389, 227, 156
9	$C_{27}H_{30}O_{15}$	593.15051	593.15010	593	MS^2 [593]: 447,431, 285; MS^3 [447]: 357, 327, 285, 284, 255, 179
10	$C_{25}H_{30}O_{11}$	505.17166	505.17044	505	MS^2 [505]: 373, 227; MS^3 [373]: 227
11	$C_{28}H_{32}O_{16}$	623.16107	623.16066	623	MS^2 [623]: 477, 461, 315; MS^3 [477]: 434, 357, 315, 314, 285, 243, 191
12	$C_{26}H_{34}O_{12}$	537.19714	537.19665	537	MS^2 [537]: 391, 229; MS^3 [229]: 123
13	$C_{25}H_{30}O_{12}$	521.16510	521.16535	521	MS^2 [521]: 359, 227; MS^3 [227]: 185, 183, 159
14	$C_{19}H_{20}O_7$	359.11453	359.11253	359	MS^2 [359]: 227; MS^3 [227]: 185, 159
15	$C_{24}H_{28}O_{11}$	491.73453	491.73345	491	MS^2 [491]: 467, 359, 341, 227; MS^3 [227]: 185, 159, 157
16	$C_{32}H_{42}O_{16}$	/	681.23891	681	MS^2 [681]: 591, 567, 535, 429, 385, 227; MS^3 [535]: 389, 323, 227
17	$C_{26}H_{32}O_{12}$	535.18127	535.18100	535	MS^2 [535]: 493, 307, 251, 227; MS^3 [227]: 185, 157
18	$C_{25}H_{30}O_{11}$	505.17059	505.17044	505	MS^2 [505]: 359, 341, 227; MS^3 [227]: 185, 183, 159
19	$C_{26}H_{32}O_{12}$	535.18179	535.18100	535	MS^2 [535]: 389, 227; MS^3 [227]: 185, 159
20	$C_{20}H_{22}O_8$	/	389.12309	389	MS^2 [389]: 227; MS^3 [227]: 199, 185, 159
21	$C_{26}H_{32}O_{12}$	535.18176	535.18100	535	MS^2 [535]: 488, 389, 371, 241, 257; MS^3 [257]: 242, 241, 213, 172
22	$C_{25}H_{30}O_{12}$	521.16461	521.16535	521	MS^2 [521]: 359, 341, 227; MS^3 [227]: 185, 159
23	$C_{26}H_{32}O_{11}$	519.18567	519.18609	519	MS^2 [519]: 373, 355, 227; MS^3 [227]: 185, 159
24	$C_{25}H_{30}O_{11}$	505.17047	505.17044	505	MS^2 [505]: 359, 341, 227; MS^3 [227]: 185, 159

As a result, compound **2** was tentatively characterized as (*E*)-resveratrol 3-*O*-glucopyranosyl-(1 → 6)-glucopyranoside.

The UV spectrum of peak **3** exhibited maximum absorption bands at λ 205–220 and 305–320 nm, indicating that the basic skeleton was (*E*)-stilbene [15,21,22]. HPLC-HRESIMS and HPLC-ESIMS analyses showed that compound **3** exhibited the same molecular formula as compound **2**, i.e., $C_{26}H_{32}O_{13}$. The HPLC/ESI-MS^2 spectrum of **3** revealed an abundant Y_1^- ion at *m/z* 389 ($[M-162-H]^-$) and Y_0^- ion at *m/z* 227 ($[M-323-H]^-$), which were formed by the sequential loss of glucose units. Furthermore, a weak Z_1^- ion at *m/z* 371 was also observed. These results suggested the presence of two glucose units in compound **3**. According to the ESI-MS^n analysis for type II stilbene glycosides the coexistence of the Y_1^-, Y_0^-, and Z_1^- ions indicated that the two glucoses in compound **3** were linked by a 1 → 2 glycosidic linkage. Thus, compound **3** was deduced as (*E*)-resveratrol 3-*O*-glucopyranosyl-(1 → 2)-glucopyranoside.

The UV spectrum of peak **22** revealed the presence of a (*Z*)-stilbene core in this compound [15,21,22]. The molecular formula of compound **22** was determined as $C_{25}H_{30}O_{12}$ on the basis of HPLC-HRESIMS, which gave an $[M-H]^-$ ion at *m/z* 521.1646. The HPLC/ESI-MS^n spectrum of **22** was very similar to that of lysidiside M, including the characteristic product ions at *m/z* 521 ($[M-H]^-$), 359 ($[M-162-H]^-$, Y_1^-), 227 ($[M-162-132-H]^-$, Y_0^-) and 341 ($[M-162-18-H]^-$, Z_1^-). These data indicated that the sugar moieties of **22** are same to those of Lysidiside M, including the presence of xylose and glucose linked by 1 → 2 linkage. On the basis of the UV spectrum revealing the (Z)-stilbene core and characteristic product ions in the LC/ESI-MS^n spectra, **22** was identified as (*Z*)-resveratrol 3-*O*-glucopyranosyl-(1 → 2)-xylopyranoside.

Peak **5** had the molecular formula $C_{26}H_{32}O_{11}$, which was deduced by HPLC/HRESIMS analysis of the ion at *m/z* 667.2232. Two characteristic ions were observed at *m/z* 535 ($[M-132-H]^-$, Y_1^-) and 227 (Y_0^-) in the MS^2 spectrum, indicating the presence of a terminal xylose unit and a resveratrol unit. The Y_1^- ion at *m/z* 535 produced fragment ions identical to those of (*E*)-resveratrol rutinoside at *m/z* 307 and 227, suggesting the presence of an (*E*)-resveratrol rutinoside unit. Based on the ESI-MS^n analysis of type III stilbene glycosides and the characteristic UV spectrum of the (*E*)-stilbene core, compound **5** was deduced as (*E*)-resveratrol 3-*O*-[xylopyranosyl-(1 → 2)-rhamnopyranosyl-(1 → 6)]-glucopyranoside.

Similarly, compounds **10**, **12**, **15**, **21**, and **23** (Fig. 5) were deduced as (*E*)-resveratrol 3-*O*-xylopyranosyl-(1 → 2)-rhamnopyranoside (**10**), 5,4′-dihydroxy-dihydrostilbene 3-*O*-rhamnopyranosyl-(1 → 2)-glucopyranoside (**12**), (*E*)-resveratrol 3-*O*-xylopyranosyl-(1 → 2)-xylopyranoside (**15**), (*E*)-isorhapontigenin 3-*O*-rhamnopyranosyl-(1 → 2)-xylopyranoside (**21**), and (*E*)-resveratrol 3-*O*-rhamnopyranosyl-(1 → 2)-rhamnopyranoside (**23**).

4. Conclusions

The results reported here illustrate the characteristic fragmentation patterns of stilbene glycosides in ESI-MS^n experiments. The predominant fragmentation pathway for stilbene glycosides with the 1 → 6 interglycosidic linkage involved the formation of the Y_0^- and B_0^- ions, while the characteristic ions of stilbene glycosides with 1 → 2 interglycosidic linkage were Y_0^-, Y_1^-, and Z_1^- ions. The different fragmentation patterns of these compounds were explained in reasonable terms and were closely related to the linkage mode of the sugar residue. According to these fragmentation patterns, 9 new trace stilbene glycosides were structurally characterized from a fraction of *L. brevicalyx* based on the HPLC-DAD/ESI-MS^n and HPLC/HRESIMS analyses. Since the structures of these glycosides were only elucidated on the basis of MS and UV data and were not confirmed by NMR, further experiments are required to distinguish the stereoisomers and positional isomers of the new stilbene glycosides in the fractions. Unfortunately, our attempts to obtain sufficient amounts of samples for NMR experiments failed. Considering the fact that the stilbene glycosides isolated from the genus *Lysidice* possess the same aglycone core moiety as resveratrol and contain β-D-glucose, β-D-xylose, and/or α-L-rhamnose units in the sugar moieties attached at C-3 of the aglycone, the structures of trace stilbene glycosides in fraction Fr. C_{2-3w} were assigned as described above (Fig. 5). Despite the limitations that accompany structural determination only based on UV and MS, the results of the present study can be useful for the online structural identification of analogous constituents and could aid in the targeted isolation of novel compounds from crude plant extracts.

Acknowledgments

The project was supported by the National Fund for Distinguished Young Scholars (no. 30625040), the National Natural Science Foundation of China (no. 20672145), and National Key Basic R&D (973) Project (no. 2004CB518906).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2009.09.033.

References

[1] R. Atta ur, H. Naz, T. Fadimatou, A. Makhmoor, N. Yasin, F.N. Fatima, S.F. Ngounou, B.L. Kimbu, M.I. Sondengam, Choudhary, J. Nat. Prod. 68 (2005) 189.
[2] C.X. Zhou, L.D. Kong, W.C. Ye, C.K. Cheng, R.X. Tan, Planta Med. 67 (2001) 158.
[3] C. Riviere, T. Richard, L. Quentin, S. Krisa, J.M. Merillon, J.P. Monti, Bioorg. Med. Chem. 15 (2007) 1160.
[4] H. Li, K.W. Cheng, C.H. Cho, Z. He, M. Wang, J. Agric. Food Chem. 55 (2007) 2604.
[5] F. Orsini, F. Pelizzoni, L. Verotta, T. Aburjai, C.B. Rogers, J. Nat. Prod. 60 (1997) 1082.
[6] H. Yang, S.H. Sung, Y.C. Kim, J. Nat. Prod. 68 (2005) 101.
[7] S. Gao, N. Feng, S.S. Yu, D.Q. Yu, X.L. Wang, Planta Med. 70 (2004) 1128.
[8] S. Gao, G.M. Fu, L.H. Fan, S.S. Yu, D.Q. Yu, J. Integ. Plant. Biol. 47 (2005) 759.
[9] S. Gao, S.S. Yu, D.Q. Yu, Chin. Chem. Lett. 15 (2004) 313.
[10] S. Gao, G.M. Fu, L.H. Fan, S.S. Yu, D.Q. Yu, Chin. J. Nat. Med. 3 (2005) 144.
[11] S. Gao, J. Liu, G.M. Fu, Y.C. Hu, S.S. Yu, L.H. Fan, D.Q. Yu, J. Qu, Planta Med. 73 (2007) 163.
[12] Y.C. Hu, X.F. Wu, S. Gao, S.S. Yu, Y. Liu, J. Qu, J. Liu, R.B. Liu, Org. Lett. 8 (2006) 2269.
[13] X.F. Wu, Y.C. Hu, S. Gao, S.S. Yu, Y.H. Pei, W.Z. Tang, X.Z. Huang, J. Asian Nat. Prod. Res. 9 (2007) 471.
[14] J. Qu, Y.C. Hu, J.B. Li, Y.H. Wang, J.L. Zhang, Z. Abliz, S.S. Yu, Y.B. Liu, Rapid Commun. Mass Spectrom. 22 (2008) 755.
[15] Y.C. Hu, S.G. Ma, J.B. Li, S.S. Yu, J. Qu, J. Liu, D. Du, J. Nat. Prod. 71 (2008) 1800.
[16] B. Domon, C.E. Costello, Glycoconj. J. 5 (1988) 397.
[17] F. Cuyckens, R. Rozenberg, E.D. Hoffmann, M. Claeys, J. Mass Spectrom. 36 (2001) 1203.
[18] E. Lamer-Zarawska, J. Grimshaw, Pol. J. Chem. 65 (1991) 63.
[19] M.S. Kamel, K. Ohtani, H.A. Hasanain, M.H. Mohamed, R. Kasai, K. Yamasaki, Phytochemistry 53 (2000) 937.
[20] R. Norbaek, T. Kondo, Phytochemistry 51 (1999) 1113.
[21] P.W. Teguo, A. Decendit, S. Krisa, G. Deffieux, J. Vercauteren, J.M. Merillon, J. Nat. Prod. 59 (1996) 1189.
[22] P.W. Teguo, B. Fauconneau, G. Deffieux, F. Huguet, J. Vercauteren, J.M. Merillon, J. Nat. Prod. 61 (1998) 655.