Targeted Isolation and Structure Elucidation of Stilbene Glycosides from the Bark of *Lysidice brevicalyx* Wei Guided by Biological and Chemical Screening

Youcai Hu, Shuanggang Ma, Jianbei Li, Shishan Yu,* Jing Qu, Jing Liu, and 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, No. 1 Xiannongtan Street, Beijing 100050, People's Republic of China

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An efficient procedure based on biological and chemical screening has been performed to investigate the antioxidant constituents of the bark of *Lysidice brevicalyx* Wei. The procedure allowed the rapid identification of known compounds and tentative characterization of unknown compounds by online LC/UV/ESIMS^n analyses of the antioxidant fraction. Targeted isolation of the unknown compounds has led to the discovery of seven new stilbene glycosides, named lysidisides L−R (**1**−**7**), together with a known stilbene glycoside, (*E*)-resveratrol 3-*O*-rutinoside (**8**). The structures of these compounds were further determined on the basis of spectroscopic and chemical evidence. The antioxidant activities of compounds **1**−**8** and two related compounds, (*E*)-polydatin (**9**) and lysidiside E (**11**), were evaluated. The known compound (*E*)-polydatin (**9**) showed antioxidant activity at concentrations of 10^{-4} and 10^{-5} mol/L.

As a rich source of compounds with biological and molecular diversity, natural products play an important role in the drug discovery and development process.[1−3] In natural product research, the bioassay-guided isolation approach used to discover bioactive natural products has shown some advantages over the traditional approach focused on extraction, isolation, and structure determination. It is important to establish an efficient procedure for targeted isolation of new and/or bioactive natural products. Hyphenated techniques such as HPLC coupled to UV photodiode array detection (HPLC/UV) and to mass spectrometry (HPLC/MS or HPLC/MS^n) have been extensively used as effective tools for online structure identification of natural products[4,5] and chemical screening of crude plant extracts.[6] Preferably, a procedure that combines biological screening with chemical screening can be carried out for rapid identification of known compounds and targeted isolation of unknown compounds detected from the bioactive fractions. With such an approach, the time-consuming isolation of common natural products is avoided and the success rate of discovering new and/or bioactive compounds is increased dramatically. In this paper, an example of targeted isolation of new and/or bioactive natural products, guided by biological and chemical screening, is illustrated by the isolation of new stilbene glycosides from the antioxidant fraction of the bark of *Lysidice brevicalyx* Wei.

The genus *Lysidice* (Fabaceae) consists of two species, *L. brevicalyx* Wei and *L. rhodostegia* Hance.[7] Bioactive stilbenes, phloroglucinols, flavanoids, and lignans have been reported from the roots of *L. rhodostegia*.[8−14] Subsequently, a total of 24 constituents in the $CHCl_3$ fractions from *L. brevicalyx* were identified or tentatively characterized on the basis of the structural information provided by LC/HRMS, LC/UV/ESIMS^n, and LC/NMR experiments.[15] In order to obtain bioactive and/or novel natural compounds from *L. brevicalyx*, we investigated the antioxidant constituents of the methanol fraction of the bark extract guided by biological and chemical screening. Eight stilbene glycosides including the new lysidisides L−R (**1**−**7**) and a known stilbene glycoside, (*E*)-resveratrol 3-*O*-rutinoside (**8**), were isolated from an antioxidant fraction. The antioxidant capacity of compounds **1**−**8**, (*E*)-polydatin (**9**), and lysidiside E (**11**) was also evaluated.

* To whom correspondence should be addressed. Tel: +8610-63165324, +8610-60212125. Fax: +8610-63017757. E-mail: yushishan@imm.ac.cn.

Table 1. Antioxidant Activity of the Various Fractions of the Bark of *L. brevicalyx*

	restrainability (%)	
fraction	100 μg/mL	10 μg/mL
alcohol extraxt	95	0.5
Fr. A	13	14
Fr. B	95	1
Fr. C	99	10
Fr. C_1	−17	−18
Fr. C_2	102	6
Fr. C_3	100	−5
Fr. C_{2-1}	9	−10
Fr. C_{2-2}	96	−12
Fr. C_{2-3}	100	10

Results and Discussion

Biological and Chemical Screening of the Constituents from the Bark of *L. brevicalyx*. The fractionation and isolation procedures were guided by LC/UV, LC/ESI-MS^n, and antioxidant assays (see Table 1 and Experimental Section). The subfraction C_{2-3}, having the best antioxidant activity among the C_2 subfractions of the methanol fraction, was selected for further analysis by HPLC-DAD/ESIMS^n to obtain detailed structural information (Figure 1 and Supporting Information). The constituents of this subfraction were determined or tentatively characterized based on their retention times, UV spectra, and MS^n fragmentation information (Table 2). HPLC-DAD analyses showed that the UV spectra of peaks 1−6 and 9 exhibited two broad maximum absorption bands at λ 210−220 and 310−320 nm, which were characteristic for the presence of (*E*)-stilbenes. On the other hand, the UV spectra (λ_{max} ~220, ~280 nm) of peaks 7−8 and 10−12 indicated the presence of (*Z*)-stilbenes[16,17] (Figure 2). These characteristic UV spectra can be used to differentiate between (*E*)- and (*Z*)-stilbenes. Moreover, all 12 peaks showed a product ion at *m*/*z* 227 in the negative MS^2 mode, which was characteristic for the stilbene glycosides with the same aglycone core moiety of resveratrol found from the genus *Lysidice*.[12,15] Therefore, peaks 1−12 could be characterized as (*E*)- or (*Z*)-resveratrol glycosides.

Both peaks 4 (t_R = 21.8 min) and 11 (t_R = 30.1 min) exhibited a $[M - H]^-$ ion at *m*/*z* 389 and yielded the fragment ion at *m*/*z* 227 (i.e., $[389 - 162]^-$), which is characteristic for the loss of a hexosyl unit. However, peaks 4 and 11 showed UV absorptions characteristic for (*E*)- and (*Z*)-stilbenes, respectively. These two

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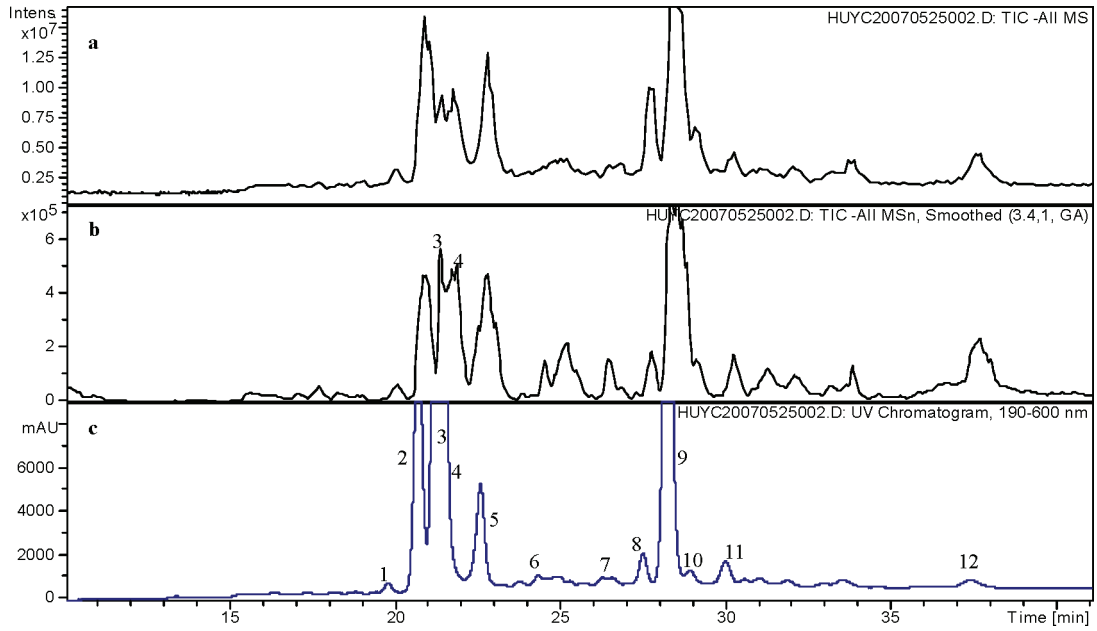


Figure 1. HPLC/ESIMS^n analyses of the antioxidant fraction C_{2-3} from *L. brevicalyx*: (a) total ion chromatogram, all mass; (b) total ion chromatogram, all MS^n; (c) UV chromatogram.

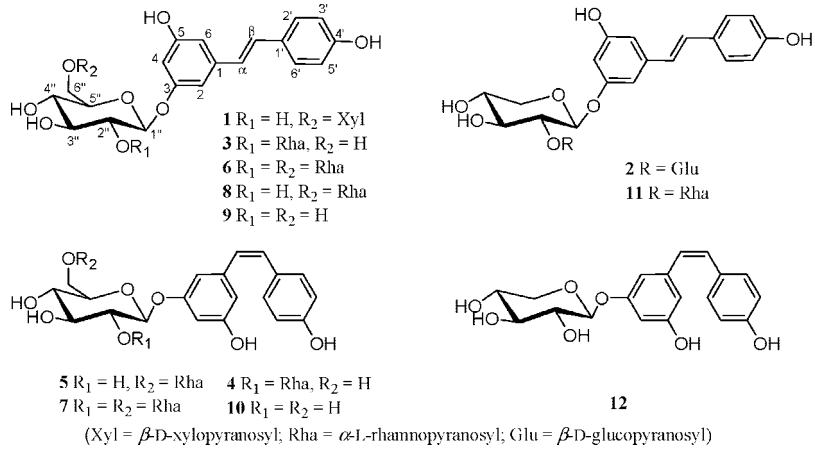
Table 2. MS Characteristics of Compounds from Fraction C_{2-3} of *L. brevicalyx*

peak (t_R, min)	$[M - H]^-$, *m/z*	HPLC/ESIMS^n *m/z*	*E*/*Z* form shown by UV
1 (20.0)	521	MS^2 [521]: 293, 227	*E*
2 (20.6)	535	MS^2 [535]: 307, 227	*E*
3 (21.2)	681	MS^2 [681]: 535, 307, 227	*E*
4 (21.8)	389	MS^2 [389]: 227	*E*
5 (22.6)	535	MS^2 [535]: 389, 227	*E*
6 (24.8)	521	MS^2 [521]: 359, 227	*E*
7 (26.8)	681	MS^2 [681]: 535, 307, 227	*Z*
8 (27.6)	535	MS^2 [535]: 307, 227	*Z*
9 (28.5)	505	MS^2 [505]: 359, 227	*E*
10 (29.1)	535	MS^2 [535]: 389, 227	*Z*
11 (30.1)	389	MS^2 [389]: 227	*Z*
12 (37.6)	505	MS^2 [505]: 359, 227	*Z*

compounds were further identified as (*E*)-polydatin (**9**, peak 4) and (*Z*)-polydatin (**10**, peak 11), respectively, by comparing their retention times with those of authentic samples in HPLC experiments. Likewise, peak 9 (t_R = 28.5 min) and peak 12 (t_R = 37.6 min), showed the same $[M - H]^-$ ion at *m/z* 505 and fragment ions at *m/z* 359 (i.e., $[505 - 146]^-$) and 227 (i.e., $[505 - 146 - 132]^-$) in MS^n analyses and were assigned as lysidisides E (**11**, peak 9) and F (**12**, peak 12), respectively. The (*E*)- and (*Z*)-resveratrol glycosides with the α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl moiety had been isolated from *L. rhodostegia* previously.[13] Accordingly, it was not necessary to carry out further isolation and structure elucidation for the four known compounds (**9**−**12**).

Peak 1 (t_R = 20.0 min) and peak 6 (t_R = 24.8 min), which showed the (*E*)-stilbene UV absorption, shared the same $[M - H]^-$ ion at *m/z* 521. Two key fragment ions at *m/z* 359 (i.e., $[521 - 162]^-$) and *m/z* 227 (i.e., $[521 - 162 - 132]^-$) were observed in the MS^n spectra of peak 6, indicating the presence of pentose and hexose units besides the resveratrol moiety. Peak 6 was presumably a resveratrol glycoside containing one pentose and one hexose unit. Peak 1 yielded a fragment ion at *m/z* 293 (i.e., $[521 - 228]^-$), which was a sugar chain fragment ion consisting of pentosyl and hexosyl units (162 + 132 − H = 293). Peak 1 was also tentatively deduced to be resveratrol glycoside with one pentose and one hexose unit. However, the sequence and/or linkage information on the sugar chains in both peaks 1 and 6 were unknown and likely to be different. A total of four peaks, peaks 2 (t_R = 20.6 min), 5 (t_R = 22.6 min), 8 (t_R = 27.6 min), and 10 (t_R = 29.1 min), exhibiting the same $[M - H]^-$ ion at *m/z* 535, were also tentatively characterized. Peaks 5 and 10, which shared the same fragment ions at *m/z* 389 ([535 − 146 (deoxyhexosyl)]$^-$) and *m/z* 227 ([535

Chart 1



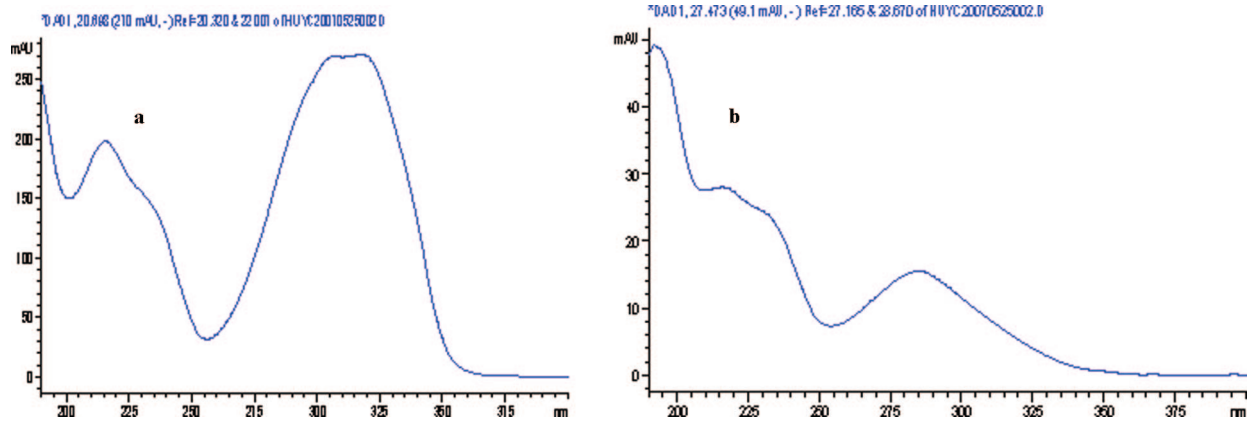


Figure 2. Characteristic UV spectra for (*E*)-stilbene (a) and (*Z*)-stilbene (b).

Table 3. ^{1}H NMR Data for Compounds **1**−**8**[a]

proton	1	2	3	4	5	6	7	8
2	6.63 brs	6.65 brs	6.70 brs	6.25 brs	6.37 brs	6.59 brs	6.36 brs	6.61 brs
4	6.38 brs	6.31 brs	6.28 brs	6.22 brs	6.25 brs	6.30 brs	6.20 brs	6.33 brs
6	6.58 brs	6.56 brs	6.53 brs	6.23 brs	6.31 brs	6.56 brs	6.32 brs	6.59 brs
α	6.86 d (16.5)	6.84 d (16.5)	6.82 d (16.5)	6.33 d (13.0)	6.29 d (12.4)	6.81 d (16.5)	6.26 d (12.0)	6.84 d (16.0)
β	6.99 d (16.5)	7.00 d (16.5)	7.00 d (16.5)	6.40 d (13.0)	6.42 d (12.4)	6.97 d (16.5)	6.42 d (12.0)	6.98 d (16.0)
2′, 6′	7.39 d (8.5)	7.38 d (8.5)	7.38 d (8.5)	7.06 d (8.4)	7.06 d (8.4)	7.39 d (8.5)	7.07 d (8.0)	7.40 d (8.4)
3′, 5′	6.74 d (8.5)	6.74 d (8.5)	6.74 d (8.5)	6.61 d (8.4)	6.62 d (8.4)	6.75 d (8.5)	6.62 d (8.0)	6.75 d (8.4)
1″	4.77 d (7.5)	4.96 d (6.0)	4.92 d (7.5)	4.75 d (7.2)	4.57 d (7.6)	4.92 d (7.0)	4.71 d (7.2)	4.78 d (7.6)
2″	3.20 m	3.48 m (overlap)	3.46 m	3.18 m	3.17 m	3.44 m	3.42 m	3.42 m
3″	3.27 m	3.47 m (overlap)	3.43 m	3.38 m	3.25 m	3.48 m	3.46 m	3.37 m
4″	3.28 m	3.47 m (overlap)	3.16 m	3.15 m	3.08 m	3.16 m	3.10 m	3.35 m
5″	3.49 m	3.78 m	3.35 m	3.43 m	3.42 m	3.50 m	3.28 m	3.29 m
6″	3.95 d (11.0)		3.71 m	3.57 d (10.8)	3.76 d (10.0)	3.82 d (9.5)	3.75 d (10.2)	3.82 d (12.5)
	3.61 dd (11.0, 5.0)		3.45 m	3.45 m	3.46 m	3.48 m	3.32 m	3.45 m
1‴	4.18 d (7.5)	4.46 d (7.5)	5.11 brs	5.06 brs	4.52 brs	5.11 brs	5.06 brs	4.55 brs
2‴	3.00 m	3.00 m	3.37 m	3.33 m	3.39 m	3.36 m	3.34 m	3.73 m
3‴	3.10 m	3.14 m	3.33 m	3.66 m	3.63 m	3.65 m	3.63 m	3.33 m
4‴	3.21 m	3.17 m	3.21 m	3.19 m	3.16 m	3.22 m	3.18 m	3.19 m
5‴	3.68 dd (5.0, 10.0)	3.13 m	3.88 m	3.75 m	3.35 m	3.87 m	3.78 m	3.82 m
	2.98 d (10.0)							
6‴		3.51 m, 3.45 m	1.19 d (6.5)	1.08 d (6.0)	1.09 d (6.4)	1.11 d (6.0)	1.10 d (6.6)	1.08 d (6.4)
1⁗						4.55 s	4.51 s	
2⁗						3.48 m	3.45 m	
3⁗						3.69 m	3.67 m	
4⁗						3.14 m	3.15 m	
5⁗						3.42 m	3.40 m	
6⁗						1.08 d (6.0)	1.09 d (6.6)	

[a] Measured in DMSO-d_6 at 500 MHz for compounds **1**−**3** and **6**, at 400 MHz for compounds **5** and **8**, and at 600 MHz for compounds **4** and **7**.

− 146 (deoxyhexosyl) − 162 (hexosyl)]$^-$) in the MS^2 spectra and characteristic UV spectra for (*E*)-/(*Z*)-stilbene, were assigned as (*E*)- and (*Z*)-resveratrol deoxyhexosylhexosides, respectively. Peaks 2 and 8 were also deduced as a couple of (*E*)-/(*Z*)-resveratrol glycosides with one deoxyhexose and one hexose unit, differing only in the linkage position for the sugar chains from those of peaks 5 and 10 by LC-DAD/MS^n analyses. In the same way, peak 3 (t_R = 21.2 min) and peak 7 (t_R = 26.8 min) were deduced as a pair of (*E*)-/(*Z*)-resveratrol glycosides containing one hexose and two deoxyhexose units.

These eight constituents were deduced as (*E*)-/(*Z*)-resveratrol glycosides consisting of two or three sugar units, with unknown sequence and linkage information of the sugar chains. Notably, only one known compound, (*E*)-resveratrol 3-*O*-rutinoside,[18] matched the possible structures containing one deoxyhexose and one hexose unit deduced above by searching the literature, which suggested that at least seven compounds among these eight constituents should be new. Accordingly, it was worth obtaining the eight constituents, determining their exact structures and evaluating their antioxidant activities. Targeted isolation of these eight stilbene glycosides by Sephadex LH-20, ODS, and preparative HPLC led to the identification of seven new stilbene glycosides, lysidisides L−R (**1**−**7**), and a known stilbene glycoside, (*E*)-resveratrol 3-*O*-rutinoside (**8**).

Elucidation of the Structures of Obtained Constituents. Compounds **1** and **2** from peaks 1 and 6, respectively, were obtained as white powders. The molecular formula of both compounds was determined to be $C_{25}H_{30}O_{12}$ by the negative HRESIMS, indicating 11 degrees of unsaturation. Online LC-DAD/MS^n analyses suggested the presence of one pentose and one hexose moiety besides a resveratrol moiety in both **1** and **2**. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **1** (Table 3) showed proton signals for a 1,3,5-trisubstituted aromatic ring at δ 6.38 (1H, brs, H-4), 6.58 (1H, brs, H-6), and 6.63 (1H, brs, H-2), a *para*-disubstituted aromatic ring at δ 7.39 (2H, d, J = 8.5 Hz, H-2′, 6′) and 6.74 (2H, d, J = 8.5 Hz, H-3′, 5′), and two *trans*-olefinic protons at δ 6.86 (1H, d, J = 16.5 Hz, H-α) and 6.99 (1H, d, J = 16.5 Hz, H-*β*). In the HMBC spectrum, H-α (δ_H 6.86) showed correlations to C-2 (δ_C 105.2) and C-6 (δ_C 106.7), while H-*β* (δ_H 6.99) showed correlation to C-1′ (δ_C 127.9). These correlations suggested that **1** possessed an (*E*)-resveratrol aglycone moiety. This was further confirmed by enzyme hydrolysis of **1**, which liberated the (*E*)-resveratrol as aglycone. The observation of two anomeric protons and corre-

Table 4. ^{13}C NMR Data for Compounds **1**−**8**[a]

carbon	1	2	3	4	5	6	7	8
1	139.3 (s)	139.2 (s)	139.4 (s)	138.9 (s)	139.1 (s)	139.3 (s)	139.0 (s)	139.3 (s)
2	105.2 (d)	105.2 (d)	104.1 (d)	106.9 (d)	107.1 (d)	104.7 (d)	106.9 (d)	105.4 (d)
3	158.8 (s)	158.4 (s)	158.7 (s)	158.2 (s)	158.6 (s)	158.6 (s)	158.2 (s)	158.8 (s)
4	102.7 (d)	102.7 (d)	102.5 (d)	102.3 (d)	102.7 (d)	102.4 (d)	102.4 (d)	102.8 (d)
5	158.4 (s)	158.4 (s)	158.4 (s)	158.2 (s)	158.1 (s)	158.5 (s)	158.0 (s)	158.4 (s)
6	106.7 (d)	107.0 (d)	107.5 (d)	109.1 (d)	109.2 (d)	107.0 (d)	109.1 (d)	106.7 (d)
α	125.2 (d)	125.1 (d)	125.2 (d)	127.4 (d)	127.5 (d)	125.1 (d)	127.6 (d)	125.2 (d)
β	128.4 (d)	128.4 (d)	128.7 (d)	126.9 (d)	127.2 (d)	128.6 (d)	127.3 (d)	128.5 (d)
1′	127.9 (s)	127.9 (s)	128.0 (s)	129.98 (s)	130.0 (s)	127.9 (s)	130.0 (s)	127.9 (s)
2′, 6′	127.9 (2 × d)	127.9 (2 × d)	127.9 (2 × d)	129.92 (2 × d)	130.0 (2 × d)	127.9 (2 × d)	130.0 (2 × d)	127.9 (2 × d)
3′, 5′	115.5 (2 × d)	115.5 (2 × d)	115.5 (2 × d)	115.1 (2 × d)	115.0 (2 × d)	115.5 (2 × d)	115.0 (2 × d)	115.5 (2 × d)
4′	157.3 (s)	157.4 (s)	157.4 (s)	157.2 (s)	156.8 (s)	157.3 (s)	156.7 (s)	157.3 (s)
1″	100.6 (d)	99.5 (d)	98.6 (d)	98.3 (d)	100.8 (d)	98.5 (d)	98.5 (d)	100.6 (d)
2″	73.2 (d)	82.2 (d)	76.5 (d)	76.6 (d)	73.1 (d)	76.5 (d)	76.6 (d)	73.3 (d)
3″	76.4 (d)	75.2 (d)	77.5 (d)	77.4 (d)	76.4 (d)	77.3 (d)	77.3 (d)	76.5 (d)
4″	69.4 (d)	68.9 (d)	69.9 (d)	69.5 (d)	69.6 (d)	69.7 (d)	69.8 (d)	69.6 (d)
5″	75.6 (d)	65.2 (t)	76.9 (d)	76.6 (d)	75.4 (d)	75.1 (d)	75.1 (d)	75.4 (d)
6″	68.1 (t)		60.6 (t)	60.3 (t)	66.3 (t)	66.1 (t)	66.2 (t)	66.2 (t)
1‴	103.9 (d)	104.5 (d)	100.5 (d)	100.5 (d)	100.6 (d)	100.5 (d)	100.6 (d)	100.6 (d)
2‴	73.4 (d)	74.7 (d)	70.6 (d)	70.5 (d)	70.7 (d)	70.5 (d)	70.5 (d)	70.7 (d)
3‴	76.4 (d)	76.2 (d)	70.5 (d)	70.4 (d)	70.3 (d)	70.3 (d)	70.3 (d)	70.3 (d)
4‴	69.6 (d)	69.5 (d)	71.9 (d)	71.9 (d)	72.1 (d)	71.9 (d)	71.9 (d)	72.0 (d)
5‴	65.6 (t)	76.9 (d)	68.2 (d)	68.2 (d)	68.3 (d)	68.2 (d)	68.2 (d)	68.3 (d)
6‴		60.5 (t)	18.1 (q)	17.9 (q)	17.8 (q)	18.1 (q)	18.0 (q)	17.8 (q)
1⁗						100.6 (d)	100.6 (d)	
2⁗						70.7 (d)	70.7 (d)	
3⁗						70.4 (d)	70.4 (d)	
4⁗						72.0 (d)	72.1 (d)	
5⁗						68.3 (d)	68.3 (d)	
6⁗						17.8 (q)	17.8 (q)	

[a] Measured in DMSO-d_6 at 125 MHz for compounds **1**−**3** and **6**, at 100 MHz for compounds **5** and **8**, and at 150 MHz for compounds **4** and **7**.

sponding anomeric carbons (δ_H 4.77/δ_C 100.6 and δ_H 4.18/δ_C 103.9) in its NMR spectra indicated the existence of two sugar units. Acid hydrolysis of **1**, followed by HPLC analyses,[8,19] indicated the presence of D-xylose and D-glucose (see Experimental Section). The large coupling constants for the anomeric protons (7.5 Hz for both glucose and xylose) indicated a *β*-configuration for these two sugars. Linkage information on the two sugar units was determined on the basis of ^{13}C NMR (Table 4) and 2D-NMR data. The presence of a downfield methylene signal at δ_C 68.1(C-6″) in its ^{13}C NMR spectrum established the attachment of a *β*-D-xylose moiety at C-6″ of *β*-D-glucose, which was further confirmed by the HMBC correlation between H-1‴ (δ_H 4.18) and C-6″ (δ_C 68.1). HMBC correlation between the anomeric proton at δ_H 4.77(H-1″) and C-3 at δ_C 158.8 indicated the attachment of a *β*-D-glucose unit at C-3 of the aglycone. As a result, the structure of **1** was unambiguously determined to be (*E*)-5,4′-dihydroxystilbene 3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside, named lysidiside L.

Compound **2** showed very similar IR, UV, and NMR data to those of **1**, except for the carbon signals of the sugar moieties in the ^{13}C NMR spectra (Tables 3 and 4). Acid hydrolysis of **2**, followed by HPLC analyses,[8,19] indicated the presence of D-xylose and D-glucose. The difference in carbon signals between **1** and **2** was due to the different linkage positions of the sugar moieties. In the HMBC spectrum, correlations between H-1‴ (δ_H 4.46) and C-2″ (δ_C 82.2) and between H-1″ (δ_H 4.96) and C-3 (δ_C 158.4) suggested that the *β*-D-glucose unit was linked to C-2″ of the *β*-D-xylose unit and that the *β*-D-xylose unit was connected to C-3 of the aglycone. On the basis of these findings, **2** was elucidated as (*E*)-5,4′-dihydroxystilbene 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside and was named lysidiside M.

Compounds **3** and **4**, corresponding to peaks 5 and 10, respectively, have the same molecular formula, $C_{26}H_{32}O_{12}$, as deduced from HRESIMS, indicating 11 degrees of unsaturation. Compounds **3** and **4** were tentatively characterized as (*E*)- and (*Z*)-resveratrol deoxyhexosylhexosides, respectively, according to the online LC-DAD/MS^n analyses. The coupling constant of the olefinic protons (16.5 Hz) in the ^1H NMR spectrum of **3** (Table 3) confirmed the presence of an *E*-form of resveratrol,[20] which was consistent with its characteristic UV spectrum. Acid hydrolysis of **3**, followed by HPLC analysis,[8,19] suggested the presence of L-rhamnose and D-glucose. The large coupling constant (7.5 Hz) of the anomeric proton at δ_H 4.92 revealed the *β*-configuration for the glucose, and a broad singlet at δ_H 5.11 indicated the α-configuration for the rhamnose in **3**. In the HMBC spectrum, correlations between H-1‴ (δ_H 5.11) and C-2″ (δ_C 76.5) and between H-1″ (δ_H 4.92) and C-3 (δ_C 158.7) indicated the presence of a rhamnopyranosyl-(1→2)-glucopyranosyl unit located at C-3 of the aglycone. Accordingly, compound **3** was established as (*E*)-5,4′-dihydroxystilbene 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, named lysidiside N.

Compound **4** (from peak 10) showed IR, MS, and NMR data similar to those of **3** (Tables 3 and 4). In the ^1H NMR spectrum, the relatively small coupling constants of the olefinic protons (13.0 Hz) suggested that **4** was a (*Z*)-stilbene,[15] which was consistent with its characteristic UV spectra. Enzyme hydrolysis of **4** gave the aglycone as (*Z*)-resveratrol. The assignment of NMR data was accomplished by HMQC and HMBC analyses. Thus, **4** was determined to be (*Z*)-5,4′-dihydroxystilbene 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, named lysidiside O.

Compound **5** (from peak 8) was obtained as a white powder. Its molecular formula was established as $C_{26}H_{32}O_{12}$, the same as **3** and **4**, on the basis of an $[M - H]^-$ pseudomolecular ion peak at 535.18231 in its HRESI mass spectrum. The IR, MS, and NMR data of **5** were similar to those of **8** (Tables 3 and 4),[18] with the exception of the coupling constants (J = 12.4 Hz) of the olefinic proton signals in its ^1H NMR spectrum, suggesting the presence of a (*Z*)-stilbene moiety. The presence of a downfield methylene signal at δ_C 66.3 in the ^{13}C NMR spectrum of **5** indicated the attachment of a rhamnose unit at C-6″ of glucose, which was further confirmed by the HMBC experiment. Hence, **5** was determined to be (*Z*)-5,4′-dihydroxystilbene 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, named lysidiside P.

Compounds **6** and **7** from peaks 3 and 7, respectively, showed a molecular ion peak at *m*/*z* 681 $[M - H]^-$ in the negative ESIMS.

Table 5. Antioxidant Activity of the Compounds **1**−**9**, and **11**

compound	restrainability (%)	
	10^{-4} M	10^{-5} M
1	21	4
2	29	5
3	39	9
4	10	11
5	15	0
6	39	5
7	25	8
8	31	11
9	100	29
11	67	6
VE[a]	81	33

[a] As positive control.

The same molecular formula of $C_{32}H_{42}O_{16}$ for the two compounds was established on the basis of HRESIMS, indicating 12 degrees of unsaturation. Comparison of the NMR data of compound **6** with those of **3** and **8** (Tables 3 and 4) revealed that one more rhamnose unit is present in compound **6** compared with **3** and **8**. The presence of a downfield signal at δ_C 76.5 and a downfield methylene signal at δ_C 68.2 in the ^{13}C NMR spectrum of **6** indicated the attachment of the α-L-rhamnose moieties at C-2″ and C-6″ of the β-D-glucose, which was confirmed by HMBC correlations (H-1‴/C-2″, H-1⁗/C-6″). HMBC correlation between the anomeric proton H-1″ (δ_H 4.92) and C-3 (δ_C 158.6) indicated the position of attachment of the β-D-glucose to C-3 of the aglycone. The NMR data for **6** were very similar to those of a known compound, (*E*)-5,4′-dihydroxy-3-methoxystilbene 5-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside,[21] except for the absence of methoxy resonances in the NMR spectra of **6**. Therefore, **6** was determined to be (*E*)-5,4′-dihydroxystilbene 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside, named lysidiside Q.

Compound **7** from peak 7 showed very similar IR, MS, and NMR data to those of **6** (Tables 3 and 4). The coupling constant of the olefinic protons (12.0 Hz) and characteristic UV spectra suggested that **7** was a *Z*-isomer of **6**. The assignment of NMR data was accomplished by HMQC and HMBC analyses. Therefore, **7** was determined to be (*Z*)-5,4′-dihydroxystilbene 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside, named lysidiside R.

The known compound **8** from peak 2 was identified as (*E*)-resveratrol 3-*O*-rutinoside by comparing the UV, IR, MS, and NMR data with those of a reference compound.[18]

Normally, the *Z* configuration of stilbene is not as stable as the *E* configuration;[12] however stilbene glycosides in the *Z* configuration were found in this study and have also been reported as natural products from other plants.[16,19,22−24]

The antioxidant activities of stilbene glycosides **1**−**8** and compounds **9** and **11** (the latter were obtained previously from *L. rhodostegia*) were evaluated in a parallel experiment by measuring their inhibition activity on the liver microsomal lipid peroxidation induced by the Fe^{2+}-Cys system *in vitro*. Vitamin E was selected as the positive control due to its well-known antioxidant activity. As shown in Table 5, only compound **9** showed clear antioxidant activity with inhibition rates of 100 and 29 at concentrations of 10^{-4} and 10^{-5} mol/L, respectively, which were comparable to or stronger than the values for vitamin E. However, the other compounds showed very low-level antioxidant activities. Presumably, compound **9**, a main constituent in fraction C_{2-3}, should contribute to the antioxidant activity of this active fraction.

In conclusion, the antioxidant constituents of the bark of *L. brevicalyx* were investigated with an efficient procedure combining biological screening with chemical screening. Targeted isolation of the unknown compounds from the antioxidant fraction has led to the discovery of seven new stilbene glycosides.

The combination of biological and chemical screening was helpful in finding natural products with interesting structural information and/or bioactivity. This procedure allows us to focus on the unknown compounds from the bioactive fraction. Through this procedure, time-consuming isolation of common natural products could be avoided and the success rate in discovering new and/or bioactive compounds increased dramatically.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 automatic digital polarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by a microscope transmission method. NMR spectra were obtained on an INOVA-500, MP-400, or SX-600 spectrometer with solvent (DMSO-d_6) peaks being used as references. ESIMS data were measured on an Agilent 1100 Series LC/MSD Trap mass spectrometer. HRESIMS data were recorded using a Micromass Autospec-Ultima ETOF spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with a SPD-10A detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Polyamide (30−60 mesh, Jiangsu Linjiang Chemical Reagents Factory, China) and ODS (50 μm, Merck) were used for column chromatography. β-Glucosidase (7.21 U/mg) was bought from Fluka (Sigma-Aldrich CH-9471 Buchs).

Plant Material. Bark from *L. brevicalyx* was collected from Guangxi Province, China, and identified by Professor Songji Wei (Guangxi College of Chinese Traditional Medicine) in September 2006. A voucher specimen was deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

HPLC-DAD/ESIMS^n Analyses of Constituents in Fractions. For the online HPLC-DAD/ESIMS^n analyses, an Agilent 1100 Series liquid chromatography system chromatograph was utilized equipped with a quaternary pump, degasser, column oven, autosampler, and diode array detector operating at 210, 280, and 320 nm, which was coupled to the ion-trap mass spectrometer. The negative ion ESIMS^n experiments were conducted using conditions as follows: dry temperature, 325 °C; drying gas, 6.0 L/min; nebulizer, 15 psi; skimmer, 40 V; injection rate, 5.0 μL/min. HPLC separation was performed on an Agilent XDB-C_{18} column (2.1 × 150 mm, 5 μm) using a mobile phase of MeOH and H_2O (flow rate, 0.8 mL/min; temperature, 25 °C). The mobile phase gradient program was 20:80 (t = 0 min), 30:70 (t = 10 min), 40:60 (t = 25 min), and 50:50 (t = 35 min). Each of the fractions (1.2 mg) was dissolved in 1 mL of 50% MeOH, filtered through a 0.45 μm microporous membrane, and stored at 4 °C until analysis.

Extraction and Isolation. The extraction and isolation procedures were guided by LC-MS screening and antioxidant assays. The air-dried, powered bark of *L. brevicalyx* (10.0 kg) was macerated for 6 h with 30 L of 95% EtOH and further refluxed for 1 h (20 L × 3). The EtOH extract was evaporated under reduced pressure to yield a dark brown residue (650 g), which was adsorbed on diatomite and eluted successively with $CHCl_3$, EtOAc, and MeOH to yield three fractions (A−C, 135, 137, and 320 g, respectively) after evaporation under vacuum. A portion of antioxidant fraction C (310 g) was applied to a polyamide (2500 g) column and eluted with H_2O, 30% EtOH, and 90% EtOH (35 L each) to yield three corresponding subfractions (C_1−C_3, 203.0, 47.0, and 45.0 g, respectively). The bioactive subfraction C_2 (47.0 g) was chromatographed over Sephadex LH-20 eluting with MeOH to give three fractions (C_{2-1}−C_{2-3}), which were subjected to the antioxidant assay. The active subfraction C_{2-3} was selected for HPLC-MS^n analysis to guide the subsequent isolation procedures. A portion of subfraction C_{2-3} (8.4 g) was subjected to an ODS column (50 μm, 300 g) chromatographic separation and eluted with a gradient of MeOH−H_2O (15:85−90:10) to give five subfractions (C_{2-3-1}−C_{2-3-5}). Subfraction C_{2-3-1} (500 mg), including mainly peaks 1 and 2, was purified by preparative HPLC using 18% CH_3CN−H_2O (5 mL/min) to yield **1** (20 mg, t_R = 38 min) and **8** (198 mg, t_R = 55 min). Subfraction C_{2-3-2} (3.4 g), consisting of a majority of known **9** and a little of **6**, was purified by Sephadex LH-20 (130 g, 1.5 m × 2 cm) to give **6** (62 mg), after eliminating most of the know **9** by crystallization from MeOH. Subfraction C_{2-3-3} (1.2 g), mainly consisting of peaks 5−8, was separated by preparative HPLC using 22% CH_3CN−H_2O (5 mL/min) to yield compounds **3** (553 mg, t_R = 33 min), **2** (21 mg, t_R = 47 min), **7** (6 mg, t_R = 61 min), and **5** (40 mg, t_R = 67 min). Subfraction C_{2-3-4} (2.0 g), mainly consisting of peaks 9 and 10, was recrystallized with

MeOH, and the mother liquor was purified by Sephadex LH-20 (130 g, 1.5 m × 2 cm) to give **4** (18 mg). Subfraction C_{2-3-5} (1.0 g) was not purified further since no desired unknown compounds were found.

Lysidiside L (1): white powder; $[\alpha]^{20}_D$ −71.3 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 210, 310, 323 nm; IR ν_{max} 3311, 2908, 1590, 1512, 1443, 1169, 1039, 839 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) data, see Table 3; ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 4; HRESIMS *m/z* 521.1699 $[M - H]^-$ (calcd for $C_{25}H_{29}O_{12}$, 521.1659); ESIMS *m/z* 521 $[M - H]^-$.

Lysidiside M (2): white powder; $[\alpha]^{20}_D$ −25.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 215, 305, 319 nm; IR ν_{max} 3302, 2917, 1591, 1512, 1443, 1169, 1072, 1036, 836 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) data, see Table 3; ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 4; HRESIMS *m/z* 521.1671 $[M - H]^-$ (calcd for $C_{25}H_{29}O_{12}$, 521.1659); ESIMS *m/z* 521 $[M - H]^-$.

Lysidiside N (3): white powder; $[\alpha]^{20}_D$ −90.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 217, 305, 320 nm; IR ν_{max} 3417, 3329, 2934, 1591, 1513, 1445, 1131, 1046, 912, 837, 812 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) data, see Table 3; ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 4; HRESIMS *m/z* 535.1830 $[M - H]^-$ (calcd for $C_{26}H_{31}O_{12}$, 535.1816); ESIMS *m/z* 535 $[M - H]^-$.

Lysidiside O (4): white powder; $[\alpha]^{20}_D$ −59.0 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 195, 230, 281 nm; IR ν_{max} 3373, 2975, 2933, 1590, 1512, 1442, 1171, 1047, 870, 837 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) data, see Table 3; ^{13}C NMR (150 MHz, DMSO-d_6) data, see Table 4; HRESIMS *m/z* 535.1815 $[M - H]^-$ (calcd for $C_{26}H_{31}O_{12}$, 535.1816); ESIMS *m/z* 535 $[M - H]^-$.

Lysidiside P (5): white powder; $[\alpha]^{20}_D$ −66.7 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 216, 285 nm; IR ν_{max} 3362, 2977, 2924, 1590, 1511, 1441, 1170, 1045, 979, 870, 836 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) data, see Table 3; ^{13}C NMR (100 MHz, DMSO-d_6) data, see Table 4; HRESIMS *m/z* 535.1823 $[M - H]^-$ (calcd for $C_{26}H_{31}O_{12}$, 535.1816); ESIMS *m/z* 535 $[M - H]^-$.

Lysidiside Q (6): white powder; $[\alpha]^{20}_D$ −94.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 215, 307, 320 nm; IR ν_{max} 3374, 2976, 2930, 1592, 1513, 1447, 1133, 1046, 979, 836, 809 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) data, see Table 3; ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 4; HRESIMS *m/z* 681.2361 $[M - H]^-$ (calcd for $C_{32}H_{41}O_{16}$, 681.2395); ESIMS *m/z* 681 $[M - H]^-$.

Lysidiside R (7): white powder; $[\alpha]^{20}_D$ −80.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 205, 285 nm; IR ν_{max} 3302, 2917, 1591, 1512, 1443, 1169, 1071, 1036, 836 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) data, see Table 3; ^{13}C NMR (150 MHz, DMSO-d_6) data, see Table 4; HRESIMS *m/z* 681.2378 $[M - H]^-$ (calcd for $C_{32}H_{41}O_{16}$, 681.2395); ESIMS *m/z* 681 $[M - H]^-$.

(*E*)-Resveratrol 3-*O*-rutinoside (8): white powder; $[\alpha]^{20}_D$ −84.3 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 210, 310, 325 nm; IR ν_{max} 3311, 2908, 1590, 1512, 1443, 1169, 1039, 838 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data were in agreement with reference data for this compound,[18] and see Tables 3 and 4; ESIMS *m/z* 535 $[M - H]^-$.

Absolute Configuration of the Monosaccharides. The acid hydrolysis method and the HPLC analysis were the same as those used in refs 8 and 19. The absolute configurations of the sugars were determined by comparing the retention times of derivatives of sugars obtained from the water layer of the hydrolysis solution with those of standard samples using HPLC, which was performed with an Inertsil SIL-100A column (250 × 4.6 mm, 5 μm, Dikma) eluting with *n*-hexane−EtOH (95:5); flow rate, 1.2 mL/min; detection at 230 nm, 0.04 aufs. The retention times of derivatives of authentic sugars were as follows: D-glucose 39.935 min, D-xylose 31.978 min, and L-rhamnose 28.124 min.

Enzymatic Hydrolysis of 1−7. A solution of each compound in H_2O (1 mL) was individually hydrolyzed with β-glucosidase (10 mg) at 37 °C for 16 h. The reaction mixtures were extracted separately with EtOAc (3 × 3 mL). The combined EtOAc extracts were concentrated under vacuum to yield dark brown residues, which were identified separately as (*E*)-resveratrol for compounds **1**−**3** and **6** and as (*Z*)-resveratrol for compounds **4**, **5**, and **7**, by comparative HPLC-DAD analysis with authentic samples.

Antioxidant Assays. The antioxidant assays were performed according to the reported procedures.[25] Vitamin E was selected as the positive control. The activities were determined by measuring the content of malondialdehyde (MDA), a compound produced during microsomal lipid peroxidation induced by Fe^{2+}-cysteine. MDA was detected using the thiobarbituric acid (TBA) method. Briefly, 1.0 mg of microsomal protein in 1 mL of 0.1 M PBS buffer (pH 7.4) was incubated with 0.2 μM cysteine and the test samples at 37 °C for 15 min. Lipid peroxidation was initiated by addition of 0.05 mM $FeSO_4$. After incubation, 1 mL of 20% trichloroacetic acid was added to terminate the reaction. The mixture was centrifuged for 10 min at 3000 rpm The supernatant was removed and reacted with 0.67% TBA for 10 min at 100 °C. After cooling, the MDA was quantified by UV/vis (absorbance at 532 nm), from which the inhibition rate (*IR*) was calculated as $IR\ [\%] = 100\% - A_t/(A_P - A_c) \times 100$, where A_p, A_t, and A_c refer to the absorbance of Fe^{2+}-cysteine, test compound, and control (solvent only), respectively.

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Supporting Information Available: HPLC/ESIMS^n spectra and UV spectra for each of peaks **1**−**12** of fraction C_{2-3}. 1D NMR spectra of new compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Cragg, G. M.; Newman, D. J.; Snader, K. M. *J. Nat. Prod.* **1997**, *60*, 52–60.
(2) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022–1037.
(3) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461–477.
(4) Li, B.; Abliz, Z.; Tang, M. J.; Fu, G. M.; Yu, S. S *J. Chromatogr. A.* **2006**, *1101*, 53–62.
(5) Li, W. K.; Sun, Y. K.; Liang, W. Z.; Fitzolff, J. F.; Breemen, R. B. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 978–982.
(6) Hostettmann, K.; Wolfender, J. L.; Rodriguez, S. *Planta Med.* **1997**, *63*, 2–10.
(7) *China Flora Editing Group of China Science Academy*; Flora of China, Science Press: Beijing, China, 1988; Vol. 39, pp 204−206.
(8) Gao, S.; Feng, N.; Yu, S. S.; Yu, D. Q.; Wang, X. L. *Planta Med.* **2004**, *70*, 1128–1134.
(9) Gao, S.; Fu, G. M.; Fan, L. H.; Yu, S. S.; Yu, D. Q. *J. Integ. Plant. Biol.* **2005**, *47*, 759–763.
(10) Gao, S.; Yu, S. S.; Yu, D. Q. *Chin. Chem. Lett.* **2004**, *15*, 313–315.
(11) Gao, S.; Fu, G. M.; Fan, L. H.; Yu, S. S.; Yu, D. Q. *Chin. J. Nat. Med.* **2005**, *3*, 144–147.
(12) Gao, S.; Liu, J.; Fu, G. M.; Hu, Y. C.; Yu, S. S.; Fan, L. H.; Yu, D. Q.; Qu, J. *Planta Med.* **2007**, *73*, 163–166.
(13) Hu, Y. C.; Wu, X. F.; Gao, S.; Yu, S. S.; Liu, Y.; Qu, J.; Liu, J.; Liu, R. B. *Org. Lett.* **2006**, *8*, 2269–2272.
(14) Wu, X. F.; Hu, Y. C.; Gao, S.; Yu, S. S.; Pei, Y. H.; Tang, W. Z.; Huang, X. Z. *J. Asian Nat. Prod. Res.* **2007**, *9*, 471–477.
(15) Qu, J.; Hu, Y. C.; Li, J. B.; Wang, Y. H.; Zhang, J. L.; Abliz, Z.; Yu, S. S.; Liu, Y. B. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 755–765.
(16) Teguo, P. W.; Decendit, A.; Krisa, S.; Deffieux, G.; Vercauteren, J.; Merillon, J. M. *J. Nat. Prod.* **1996**, *59*, 1189–1191.
(17) Teguo, P. W.; Fauconneau, B.; Deffieux, G.; Huguet, F.; Vercauteren, J.; Merillon, J.-M. *J. Nat. Prod.* **1998**, *61*, 655–657.
(18) Wanjala, C. C. W.; Majinda, R. R. T. *Fitoterapia* **2001**, *72*, 649–655.
(19) Oshima, R.; Kumanotani, J. *Chem. Lett.* **1981**, *10*, 943–946.
(20) Xiao, K.; Xuan, L.; Xu, Y.; Bai, D. *J. Nat. Prod.* **2000**, *63*, 1373–1376.
(21) Rahman, A.; Naz, H.; Fadimatou; Makhmoor, T.; Yasin, A.; Fatima, N.; Ngounou, F. N.; Kimbu, S. F.; Sondengam, B. L.; Iqbal Choudhary, M. *J. Nat. Prod* **2005**, *68*, 189–193.
(22) Xu, M. L.; Zheng, M. S.; Lee, Y. K.; Moonl, D. C.; Lee, C. S.; Woo, M. H.; Jeong, B. S.; Lee, E. S.; Jahng, Y. D.; Chang, H. W.; Lee, S. H.; Son, J. K. *Arch. Pharm. Res.* **2006**, *29*, 946–951.
(23) Xiao, K.; Xuan, L. J.; Xu, Y. M.; Bai, D. L. *J. Integ. Plant Biol.* **2002**, *44*, 1491–1494.
(24) Fan, W. Z.; Tezuka, Y.; Kadota, S. *Chem. Pharm. Bull.* **2000**, *48*, 1055–1061.
(25) Dai, S. J.; Wu, Y.; Wang, Y. H.; He, W. Y.; Chen, R. Y.; Yu, D. Q. *Chem. Pharm. Bull.* **2004**, *52*, 1190–1193.

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