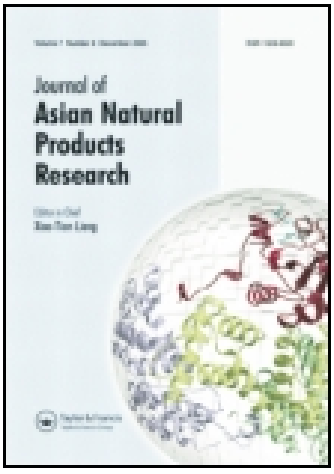

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Phenolic glycosides isolated from the bark of Lysidice brevicalyx Wei

You-Cai Hu [a], Shuang-Gang Ma [a], Shi-Shan Yu [a], Xian-Fu Wu [a] & Yong Li [a]

[a] 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, 100050, China

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ORIGINAL ARTICLE

Phenolic glycosides isolated from the bark of *Lysidice brevicalyx* Wei

You-Cai Hu, Shuang-Gang Ma, Shi-Shan Yu*, Xian-Fu Wu and Yong Li

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 100050, China

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Two new compounds, lysidiside S (**1**) and 7-*O*-(+)-peltogynol-β-D-glucopyranoside (**2**), together with six known phenolic glycosides (**3**–**8**) were isolated from the bark of *Lysidice brevicalyx* Wei. The structures of these compounds were characterized by chemical and spectroscopic methods. The antioxidant activities of compounds **1**–**8** were evaluated, and compound **3** exhibited remarkable antioxidant activity at concentrations of 10^{-4}, 10^{-5}, and 10^{-6} mol/l.

Keywords: *Lysidice brevicalyx*; lysidiside S; 7-*O*-(+)-peltogynol-β-D-glucopyranoside; antioxidant

1. Introduction

Lysidice brevicalyx Wei belongs to the genus *Lysidice* in the family Fabaceae [1]. During our previous studies on bioactive constituents from the genus *Lysidice*, we obtained bioactive stilbenes, phloroglucinols, flavanoids, and lignans [2–9]. Recently, a procedure based on biological and chemical screening has been used to investigate the antioxidant constituents from the bark of *L. brevicalyx*, and seven new stilbene glycosides were obtained from an antioxidant fraction (Fr. C_{2-3}) [10]. As part of our continuing program for targeted isolation of novel bioactive constituents from a natural source, we carried out an investigation of constituents of other subfractions from the title plant. Eight compounds, **1**–**8** (Figure 1), including one new stilbene glycoside (**1**), one new flavanol glycoside (**2**), and six known compounds were isolated. The antioxidant capacity of compounds **1**–**8** was evaluated *in vitro*.

2. Results and discussion

Compound **1** was obtained as an amorphous powder, and the presence of OH (3229 cm^{-1}), conjugated carbonyl esters (1697 cm^{-1}), and aromatic rings (1596 and 1512 cm^{-1}) were indicated by its IR spectrum. The molecular formula of compound **1** was determined to be $C_{28}H_{28}O_{10}$ by negative HR-ESI-MS. Absorption maximum at 210, 310, and 320 nm in its UV spectrum were indicative of a stilbene moiety [10]. The presence of (*E*)-resveratrol moiety in compound **1** was supported by its ^{1}H NMR signals (Table 1) at δ_H 6.33 (1H, br s), 6.58 (1H, br s), 6.64 (1H, br s), 7.33 (2H, d, $J = 8.5$ Hz), 6.73 (2H, d, $J = 8.5$ Hz), 6.81 (1H, d, $J = 16.0$ Hz), and 6.98 (1H, d, $J = 16.0$ Hz). A detailed analysis of the

*Corresponding author. Email: yushishan@imm.ac.cn

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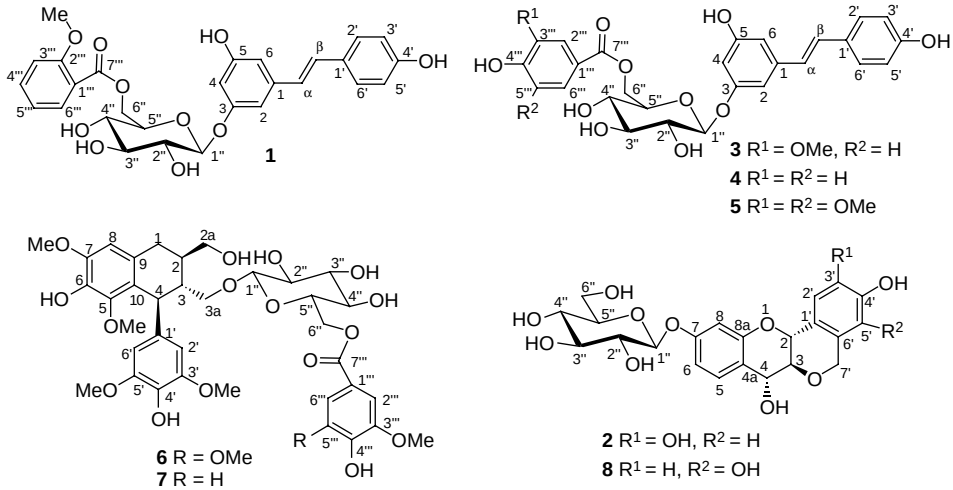

Figure 1. Structures of compounds **1**–**8**.

NMR spectra of compound **1** with the help of $^{1}H-^{1}H$ COSY and HMBC experiments revealed the presence of a 2-methoxybenzoyl ester and a glucopyranoside unit, besides the resveratrol moiety. The presence of a downfield methylene signal at δ_C 63.9 (C-6″) in its ^{13}C NMR spectrum established the attachment of a 2-methoxybenzoyl ester moiety at C-6″ of glucose, which was further confirmed by the HMBC correlation (Figure 2) from H-6″ at δ_H 4.23 to C-7‴ at δ_C 165.1. HMBC correlation between the anomeric proton at δ_H 4.93 (H-1″) and C-3 at δ_C 158.6 indicated the location of a glucose unit at C-3 of the aglycone. Acid hydrolysis of compound **1**, followed by the HPLC analysis [10,11], indicated the presence of D-glucose. The large coupling constant for the anomeric proton (8.0 Hz) indicated β-configuration for glucose. As a result, the structure of compound **1** was determined to be (*E*)-3,5,4′-trihydroxystilbene 3-*O*-[6-*O*-(2-methoxy)-benzoyl]-β-D-glucopyranoside, named lysidiside S.

Compound **2** was obtained as a pale yellow powder, and its molecular formula, $C_{22}H_{24}O_{11}$, was indicated by HR-ESI-MS. The IR spectrum of compound **2** displayed absorption bands for OH (3401 cm^{-1}) and aromatic (1584 and 1521 cm^{-1}) moiety. The ^{1}H NMR spectrum (Table 2) of compound **2** showed signals due to a 1,3,4-trisubstituted phenyl group and a 1,2,4,5-tetrasubstituted phenyl group. In addition, it showed signals attributed to three oxymethine protons at δ_H 4.67 (H-4), 4.81 (H-2), and 4.83 (H-1″), one methylene proton at δ 4.69 (H-7′), partially overlapped methylene and methine protons at δ_H 3.20–3.70 and signals due to five OH protons between δ_H 4.50 and 5.80, and two phenolic OH protons at δ_H 9.01. Besides the carbon resonance corresponding to the above-mentioned phenyl units, the ^{13}C NMR and DEPT spectra of compound **2** displayed carbon signals attributed to eight oxymethines between δ_C 69.7 and 100.5, and two oxymethylenes at δ 66.99 (C-7′) and 60.7 (C-6″). These spectroscopic data suggested that compound **2** was a flavanol glycoside with an aglycone possessing 16 carbons, similar to mopanolside (**8**) [2]. The proton signals at δ_H 6.46 (1H, s, H-5′) and 6.93 (1H, s, H-2′) indicated the presence of a 1,2,4,5-tetrasubstituted phenyl unit in ring B of compound **2**, which was confirmed by HSQC and HMBC experiments (Figure 3). HMBC correlation between the anomeric proton at δ_H 4.83 and C-7 at δ_C 157.6

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **1**, **3**, and **4** in DMSO-d_6.

	1		**3**		**4**	
Position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	139.3		139.3		139.3	
2	104.8	6.64 br s	104.7	6.65 br s	104.6	6.65 br s
3	158.6		158.6		158.6	
4	102.7	6.33 br s	102.5	6.35 br s	102.5	6.36 br s
5	158.4		158.5		158.5	
6	106.8	6.58 br s	106.8	6.58 br s	106.8	6.58 br s
α	125.2	6.81 d (16.0)	125.1	6.81 d (16.5)	125.1	6.84 d (16.5)
β	128.5	6.98 d (16.0)	128.5	7.00 d (16.5)	128.5	7.02 d (16.5)
1′	127.9		127.9		127.9	
2′, 6′	127.9 (2 ×)	7.33 d (8.5)	127.9 (2 ×)	7.33 d (8.5)	127.9 (2 ×)	7.36 d (8.0)
3′, 5′	115.4 (2 ×)	6.73 d (8.5)	115.5 (2 ×)	6.73 d (8.5)	115.5 (2 ×)	6.74 d (8.0)
4′	157.3		157.3		157.3	
1″	100.1	4.93 d (8.0)	100.0	4.94 d (8.0)	99.9	4.95 d (7.5)
2″	73.1	3.22 m	73.1	3.25 m (overlap)	73.1	3.25 m (overlap)
3″	76.2	3.30 m	76.3	3.35 m	76.3	3.32 m
4″	69.9	3.16 m	70.1	3.26 m (overlap)	70.1	3.16 m (overlap)
5″	73.6	3.25 m	73.7	3.72 m (overlap)	73.7	3.75 m (overlap)
6″	63.9	4.49 d (11.0)	64.1	4.58 d (11.5)	64.0	4.54 d (11.0)
		4.23 dd (12.0, 6.5)		4.18 dd (11.5, 7.0)		4.14 dd (12.0, 7.0)
1‴	119.79		120.3		120.2	
2‴	158.3		112.5	7.39 br s	131.5	7.79 d (8.5)
3‴	112.5	7.07 d (7.5)	147.3		115.3	6.75 d (8.5)
4‴	133.5	7.44 dd (1.5, 7.5)	151.7		161.9	
5‴	119.97	6.87 dd (7.5, 7.5)	115.2	6.75 d (8.0)	115.3	6.75 d (8.5)
6‴	130.7	7.60 dd (7.5, 1.5)	123.6	7.46 dd (1.0, 8.0)	131.5	7.79 d (8.5)
7‴	165.1		165.6		165.5	
OMe	55.7	3.75 s	55.5	3.75 s	–	

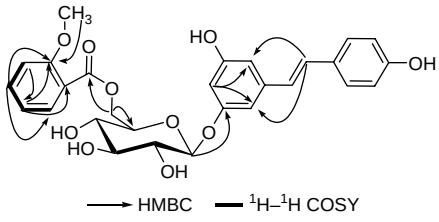

Figure 2. Key HMBC and ^{1}H–^{1}H COSY correlations of compound **1**.

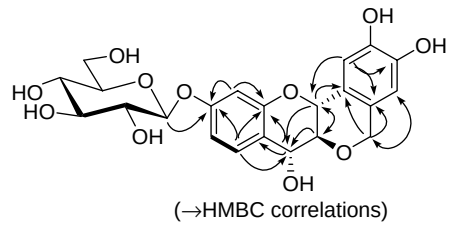

Figure 3. Key HMBC correlations of compound **2**.

indicated the location of a glucose unit at C-7 of the aglycone. Acid hydrolysis of compound **2**, followed by HPLC analysis [2,10,11], indicated the presence of D-glucose. The large coupling constant for the anomeric proton (8.0 Hz) indicated β-configuration for glucose.

The stereochemistry of compound **2** was unequivocally determined on the basis of ^{1}H NMR spectrum and circular dichroism (CD) spectrum. Its ^{1}H NMR spectrum showed an ABX system for: δ_H 4.81 (1H, d, $J = 9.5$ Hz, H-2), 4.67 (1H, dd, $J = 7.5$, 7.5 Hz, H-4), and 3.44 (1H, dd, $J = 9.5$, 7.5 Hz, H-3), which revealed the relative configuration of C-2–C-4, as shown in Figure 3. The absolute configuration of C-2 was determined from the CD spectrum. The negative Cotton effect at 283 nm revealed the *R* configuration for C-2 [12,13]. Thus, the structure of compound **2** was assigned as 7-*O*-(+)-peltogynol-β-D-glucopyranoside.

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compound **2** in DMSO-d_6.

Position	δ_C	δ_H (*J* in Hz)
2	71.8	4.81 d (9.5)
3	77.5	3.44 dd (9.5, 7.5)
4	68.0	4.67 dd (7.5, 7.5)
4a	120.0	
5	129.1	7.35 d (8.5)
6	109.9	6.66 dd (2.0, 8.5)
7	157.6	
8	103.2	6.50 d (1.5)
8a	154.3	
1′	122.6	
2′	112.6	6.93 s
3′	144.3	
4′	145.2	
5′	110.5	6.46 s
6′	125.5	
7′	66.99	4.69 s
1″	100.5	4.83 d (8.0)
2″	73.2	3.21 m
3″	76.5	3.25 m
4″	69.7	3.16 m
5″	77.7	3.32 m
6″	60.7	3.69 m, 3.46 m
4-OH		5.75 d (7.5)
3′-OH		9.00 br s
4′-OH		9.00 br s

Compounds **3** and **4** have been detected in this plant and their structures were assigned on the basis of LC-MS^n and LC-HRMS analysis [9]. However, no spectral NMR data of them were reported earlier. In this paper, the spectral NMR data of compounds **3** and **4** are presented in Table 1. The known compounds **5** [14], **6** [15], **7** [16], and **8** [2] were identified by comparing their spectroscopic data with those of the literature values.

The *in vitro* antioxidant activities of compounds **1**–**8** were evaluated in a parallel experiment by measuring their inhibition activity on the liver microsomal lipid peroxidation induced by Fe^{2+}-Cysteine system *in vitro* with vitamin E as a positive control. Compounds **1**, **3**–**5**, and **7**–**8** showed clear activities at the concentration of 10^{-4} M (Table 3). Particularly, compound **3** also showed obvious antioxidant activity at concentrations of 10^{-5} and 10^{-6} M.

Table 3. Antioxidant activity of compounds **1**–**8**.

Compound	Restrainability (%)		
	10^{-4} M	10^{-5} M	10^{-6} M
1	115.2	22.9	0
2	41.7	0	0
3	113.8	62.1	15.7
4	115.9	35.3	0
5	84.2	1.5	0
6	36.1	2.1	0
7	93.2	6.1	0
8	80.9	12.2	0
VE[a]	81.5	33.4	0

Note: [a] As positive control.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Perkin-Elmer 241 automatic digital polarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by a microscope transmission method. CD spectra were recorded on a JASCO-712 polarimeter. NMR spectra were obtained on an INOVA-500 spectrometer with solvent peaks being used as references. ESI-MS data were measured with an Agilent 1100 Series LC/MSD Trap mass spectrometer. HR-ESI-MS data were measured using a Micromass Autospec-Ultima ETOF spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with an SPD-10A detector, using YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Polyamide (30–60 mesh, Jiangsu Linjiang Chemical Reagents Factory, Lingjing, China) and ODS (50 μm, Merck, Darmstadt, Germany) were used for column chromatography.

3.2 Plant material

The bark of *L. brevicalyx* was collected from Guangxi Province, China, and identified by Professor Songji Wei in September 2006. A voucher specimen has been deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica.

3.3 Extraction and isolation

The extraction and isolation procedures were successive to those reported previously [10]. The antioxidant fraction C_{2-1} (7.0 g) was submitted to an ODS column (50 μm, 300 g) and purified by preparative HPLC using 25% MeCN–H_2O (5 ml/min) to yield compounds **5** (89 mg, t_R = 38 min) and **6** (72 mg, t_R = 55 min). Fraction C_{2-2} (3.4 g) was submitted to an ODS column (50 μm, 100 g) and purified by preparative HPLC using 20% MeCN–H_2O (5 ml/min) to yield compounds **7** (33 mg, t_R = 33 min) and **8** (90 mg, t_R = 42 min). Subfraction C_3 (38.3 g) was submitted to an ODS column (50 μm, 200 g) and eluted with a gradient of MeOH–H_2O (10:90–80:20), and further separated by Sephadex LH-20 (130 g, 1.5 m × 2 cm, eluted with MeOH) and preparative HPLC (45% MeOH–H_2O; 5 ml/min) to give compounds **1** (24 mg), **2** (61 mg), **3** (6 mg), and **4** (33 mg).

3.3.1 Lysidiside S (*1*)

White powder (24 mg); $[\alpha]_D^{20}$ +53.60 (c = 0.005, MeOH); UV (MeOH) λ_{max} 210, 310, 320 nm; IR ν_{max} 3229, 1697, 1596, 1512, 1463, 1434, 1370, 1246, 1076, 1016, 961, 752 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS *m/z* 523.1612 $[M - H]^-$ (calcd for $C_{28}H_{27}O_{10}$, 523.1604); ESI-MS *m/z* 523 $[M - H]^-$.

3.3.2 7-O-(+)-Peltogynol-β-D-glucopyranoside (*2*)

Pale yellow powder (33 mg); $[\alpha]_D^{20}$ +94.5 (c = 0.06, MeOH); UV (MeOH) λ_{max} 201, 225, 280 nm; IR ν_{max} 401, 2887, 1616, 1584, 1521, 1497, 1281, 1065, 1015, 888, 793 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 2; HR-ESI-MS *m/z* 463.1249 $[M - H]^-$ (calcd for $C_{22}H_{23}O_{11}$, 463.1240); ESI-MS *m/z* 463 $[M - H]^-$.

3.4 Antioxidant assays

The antioxidant assays were performed according to the reported procedures [10].

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 method. The inhibition rate was calculated as $100\% - A_t/(A_p - A_c) \times 100$, 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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