Dimeric Stilbene Glycosides from *Polygonum cuspidatum*

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Two dimeric stilbene glycosides were isolated from an aqueous extract of the root of *Polygonum cuspidatum*. Their structures were established based on chemical evidence and spectroscopic techniques, including extensive 2D NMR methods. One of these glycosides possesses a novel four-membered ring. Both compounds exhibit strong inhibition of lipid peroxidation, but show no cytotoxic, DNA-cleavage activities and no inhibition of protein tyrosine phosphatase 1B (PTP1B).

The dried roots of *Polygonum cuspidatum* Sieb. et Zucc. (Polygonaceae) have been widely used for the treatment of suppurative dermatitis, gonorrhoea, favus athlete's foot and hyperlipemia in Chinese folk medicine. Anthroquinones,[1−4] stilbenes,[5−7] flavonoids,[8] and other phenols[3] have been isolated from this plant. Resveratrol and piceid existing in this plant have been reported to have various bioactivities.[6,7,10−15] Recently we reported ten stilbene glycoside sulfates isolated from the water-soluble fraction of this plant.[9]

Herein, we report the isolation and structural elucidation of two dimeric stilbene glycosides **1** and **2**.

Results and Discussion

The water-soluble fraction of an aqueous acetone extract of the dried roots of *P. cuspidatum* was subjected to a combination of column chromatography on Sephadex LH-20, MCI gel CHP20P, Cosmosil ODS and Toyopearl HW-40F to give stilbene glycosides **1** and **2**. Their structures were elucidated by the spectroscopic analysis and physicochemical evidence, and their ^{1}H and ^{13}C NMR spectroscopic data were unambiguously assigned by 2D NMR analysis.

Compound **1** was obtained as amorphous powder, $[\alpha]_D^{25} = -160.2$ ($c = 0.21$, MeOH), with a molecular formula of $C_{40}H_{44}O_{17}$ as indicated by FAB-MS and elemental analysis. The UV spectrum exhibited maxima at 280, 225 (sh) and 203 nm (MeOH). The IR spectrum showed strong bands for a hydroxyl group (3360 cm^{-1}) and an aromatic ring (1607, 1512 cm^{-1}). FAB-MS showed ions at $m/z = 835$ $[M + K]^+$ and 819 $[M + Na]^+$.

The NMR spectroscopic data of **1** suggested that **1** was a dimeric resveratrol glycoside. The ^{1}H NMR spectrum (Table 1) contained two sets of *ortho*-coupled protons assignable to two *para*-hydroxy phenyl groups in an AA′XX′ type of arrangement [δ = 7.05 (d, $J = 8.1$ Hz, 2 H), 6.53 (d, $J = 8.1$ Hz, 2 H); δ = 6.84 (d, $J = 8.2$ Hz, 2 H), 6.60 (d, $J = 8.2$ Hz, 2 H)], a set of *meta*-coupled protons assignable to a 1,2,3,5-tetrasubstituted phenyl group [δ = 6.70 (br, 1 H) and 6.37 (br, 1 H)], a *trans* double bond [δ = 6.54 (obscured, 1 H) and 6.45 (d, $J = 16.0$ Hz, 1 H)] and two aliphatic protons [δ = 5.81 (d, $J = 9.0$ Hz, 1 H), 4.60 (d, $J = 9.0$ Hz, 1 H)]. The other signals could be assigned to two pyranoses which were D-glucose according to the results of the acidic hydrolysis and the subsequent GC analysis.[16] The β-linkage of the glucoses was determined from the coupling constants of the anomeric protons [δ = 5.28 (d, $J = 7.6$ Hz, 1 H) and 4.74 (d, $J = 7.5$ Hz, 1 H)].[17]

The connectivity of the partial structures was established by ^{1}H-^{1}H COSY and HMBC experiments. The key C−H long-range correlations observed between H-7b and C-2a, H-8b and C-2a, and H-8b and C-1a and C-3a strongly suggested that the 1,2,3,5-tetrasubstituted phenyl was connected to C-8b at the C-2a position. The linkage of the sugar moieties was resolved by the HMBC and ROESY experiments. The long-range correlations between H-1′ and C-3a, and H-1′′ and C-3b suggested that the sugar moieties were connected to the C-3a and C-3b positions of the aglycon, respectively, while the NOE correlations observed between H-1′ and H-4a, and H-1′′ and H-4b and H-2b confirmed the linkage. The coupling constants (9.0 Hz) of H-7b and H-8b suggested that they were in the *threo* form (Figure 1).[18,19]

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Table 1. ^1H and ^{13}C assignment for **1** (D_2O) with ^1H-^1H COSY, HMBC and ROESY correlations

Position	δ_H mult. (*J*/Hz)	δ_C mult.[a]	^1H-^1H COSY	HMBC[b]	ROESY
1a		145.0 s		8a	
2a		122.9 s		4a, 6a, 7b, 8b	
3a		158.9 s		4a, 1′	
4a	6.70 br	104.1 d	6a	6a	1′
5a		158.0 s		4a, 6a	
6a	6.37 br	113.0 s	4a	4a, 7a	8a
7a	6.45 d (16.0)	130.4 d	8a	6a	10a
8a	6.54 obscured	133.2 d	7a	7a, 10a, 14a	6a, 14a
9a		131.5 s		7a, 8a, 11a, 13a	
10a	6.84 d (8.2)	133.4 d	11a	8a, 11a, 14a	7a, 11a
11a	6.60 d (8.2)	117.6 d	10a	10a, 13a	10a
12a		157.4 s		10a, 11a, 13a, 14a	
13a	6.60 d (8.2)	117.6 d	14a	11a, 14a	14a
14a	6.96 d (8.2)	133.4 d	13a	8a, 10a, 13a	8a, 13a
1b		149.2 s		7b	
2b	6.52 obscured	110.0 d	4b, 6b	4b, 6b, 7b	1′′
3b		160.3 s		4b, 1′′	
4b	6.48 obscured	106.4 d	2b, 6b	2b, 6b	1′′
5b		159.2 s		4b, 6b	
6b	6.59 obscured	112.2 d	2b, 4b	2b, 4b, 7b	
7b	5.81 d (9.0)	77.5 d	8b	6b, 8b	10b
8b	4.60 d (9.0)	55.7 d	7b	7b, 10b, 14b	14b
9b		135.8 s		7b, 11b, 13b	
10b	7.05 d (8.1)	133.1 d	11b	11b, 14b	7b, 11b
11b	6.53 d (8.1)	117.1 d	10b	10b, 13b	10b
12b		156.1 s		10b, 11b, 13b, 14b	
13b	6.53 d (8.1)	117.1 d	14b	11b, 14b	14b
14b	7.05 d (8.1)	133.1 d	13b	10b, 13b	8b, 13b
1′	5.28 d (7.6)	102.1 d	2′	2′	4a, 3′, 5′
2′	3.68 m	75.6 d	1′, 3′	3′	4′
3′	3.52 m	78.2 d	2′, 4′	2′, 4′	1′, 5′
4′	3.50 m	71.8 d	3′, 5′	3′, 6′α	2′, 6′α
5′	3.38 m	78.7 d	4′, 6′α, 6′β	4′	1′, 3′
6′α	3.97 br d (11.3)	63.6 t	5′, 6′β		4′
6′β	3.77 m		5′, 6′α		
1′′	4.72 d (7.5)	103.5 d	2′′	2′′	2b, 4b, 3′′
2′′	3.49 m	75.5 d	1′′, 3′′	3′′	4′′
3′′	3.48 m	78.8 d	2′′, 4′′	2′′, 4′′	1′′, 5′′
4′′	3.50 m	71.8 d	3′′, 5′′	3′′	2′′, 6′′α
5′′	3.70 m	78.9 d	4′′, 6′′α, 6′′β	4′′	1′′, 3′′
6′′α	3.81 br d (12.2)	63.0 t	5′′, 6′′β	4′′	4′′
6′′β	3.74 m		5′′, 6′′α		

[a] The carbon multiplicities were obtained from a DEPT experiment. [b] Protons that correlate with the carbon resonance.

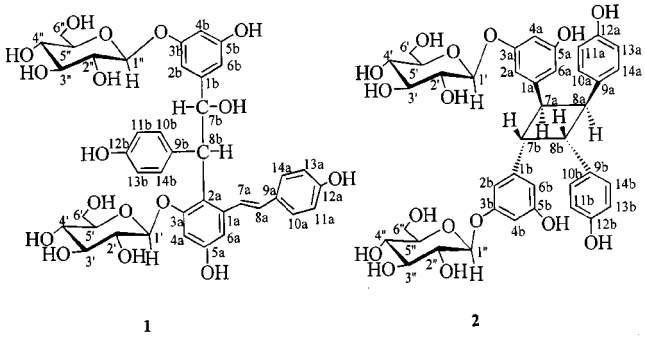

Figure 1. Structures of **1** and **2**

Compound **2** was isolated as a crystalline powder, $[\alpha]_D^{25} = -11.8$ (c = 0.44, MeOH), with a molecular formula of $C_{40}H_{44}O_{16}$ as indicated by the FAB-MS and elemental analysis. The UV spectrum exhibited maxima at 276, 230 (sh) and 203 nm (MeOH). The IR spectrum also suggested the presence of a hydroxyl group (3360 cm^{-1}) and an aromatic ring (1607, 1512 cm^{-1}). FAB-MS exhibited ions at m/z = 819 $[M + K]^+$ and 803 $[M + Na]^+$. The ^1H NMR spectrum of **2** (Table 2) showed two sets of *ortho*-coupled protons in an AA′XX′ type of arrangement assignable to two 1,4-disubstituted phenyl groups [δ = 7.09 (d, J = 8.5 Hz, 2 H), 6.75 (d, J = 8.5 Hz, 2 H), 7.01 (d, J = 8.5 Hz, 2 H), 6.61 (d, J = 8.5 Hz, 2 H)], two sets of *meta*-coupled protons assignable to two 1,3,5-trisubstituted phenyl groups [δ = 6.46 (br, 1 H), 6.41 (br, 1 H), 6.37 (br, 1 H), 6.41 (br, 2 H), 6.35 (t, J = 2.0, 1 H)], and four aliphatic protons (ABCD-type signals, their gravity center appeared at δ = 4.36, 4.34, 4.28 and 4.22, respectively), which could be assigned to four

benzyl sp^3 methine carbons. The rest of the signals were assignable to the two pyranose moieties. The NMR spectroscopic data of **2** suggested that **2** was also a dimeric resveratrol glycoside. The partial structures of four benzyl groups were elucidated of which the ^{1}H and ^{13}C NMR spectroscopic data were unambiguously assigned by an extensive 2D NMR analysis (^{1}H-^{1}H COSY, HMQC and HMBC), and their connectivity were established by ^{1}H-^{1}H COSY analysis, of which the strong correlations between H7a/H-8a, H-7b/H-8b, H-7a/H-7b and H8a/H-8b together with the expected HMBC correlations (Table 2) allowed us to determine the structure of **2** as shown in Figure 1. Compound **2** possesses a four-membered ring structure, which has not been reported in natural stilbene oligomers. The sugar moieties, which were linked to C-3a and C-3b, respectively, according to HMBC and ROESY experiments (Figure 2), were proved to be β-D-glucose using the method described above. The relative stereochemistry of **2** was established from the ROESY spectrum (Table 2), in which the correlations between H-7a and H-8a, and H-6a and H-10a suggested the *cis* configuration of H-7a and H-8a; the correlations between H-7b and H-8b, and H-6b and H-10b suggested the *cis* configuration of H-7b and H-8b, while the correlations between H-7a and H-10b, H-8a and H-6b, H-7b and H-10a, and H-8b and H-2a suggested the *trans* configuration of both H-7a and H-7b, and H-8a and H-8b. The relative configuration of **2** suggested a C_2 symmetry for the molecule. However, the ^{13}C NMR spectroscopic data of the four benzyl substituents did not show the magnetic equivalence between the relevant substituents at positions 7a and 7b, and 8a and 8b. In order to clarify this ambiguity, the conformation of the molecule was calculated using the Sybyl 6.5 program. According to the energy-minimized conformation, the ring is slightly twisted because of the

Table 2. ^{1}H and ^{13}C assignment for **2** (CD_3OD + D_2O) with ^{1}H-^{1}H COSY, HMBC and ROESY correlations

Position	δ_H mult. (*J*/Hz)	δ_H mult.[a]	^{1}H-^{1}H COSY	HMBC[b]	ROESY
1a		145.8 s		7a, 8a, 7b	
2a	6.41 br	109.6 d	4a, 6a	4a, 6a, 7a	7b, 8b, 1′
3a		159.9 s		2a, 4a, 1′	
4a	6.35 br	103.7 d	2a, 6a	2a, 6a	1′
5a		158.4 s		4a, 6a	
6a	6.41 br	112.1 d	2a, 4a	2a, 4a, 7a	10a, 7b, 8b
7a	4.22 m	49.5 d	8a, 7b	2a, 6a, 7b, 8b	8a, 2b, 6b, 10b
8a	4.36 m	47.4 d	7a, 8b	10a, 14a, 7b, 8b	7a, 6b, 10b
9a		134.0 s		7a, 11a, 13a	
10a	7.09 d (8.5)	131.1 d	11a	8a, 11a, 14a	6a, 11a, 7b, 8b
11a	6.75 d (8.5)	116.5 d	10a	10a, 13a	10a
12a		155.9 s		10a, 11a, 13a, 14a	
13a	6.75 d (8.5)	116.5 d	14a	11a, 14a	14a
14a	7.09 d (8.5)	131.1 d	13a	8a, 10a, 13a	13a
1b		145.7 s		7b, 8b	
2b	6.41 br	110.5 d	4b, 6b	4b, 6b	7a, 8a, 1′′
3b		159.9 s		2b, 4b, 1′′	
4b	6.37 br	103.7 d	2b, 6b	2b, 6b	1′′
5b		158.4 s		4b, 6b	
6b	6.46 br	111.5 d	2b, 4b	2b, 4b	7a, 8a, 10b
7b	4.28 m	48.7 d	8b, 7a	8a, 2b, 6b, 8b	2a, 6a, 10a, 8b
8b	4.34 m	48.7 d	7b, 8a	8a, 10b, 14b	2a, 6a, 10a, 7b
9b		134.0 s		8b, 11b, 13b	
10b	7.07 d (8.5)	131.0 d	11b	8b, 11b, 14b	7a, 6b, 11b
11b	6.73 d (8.5)	116.5 d	10b	10b, 13b	10b
12b		155.9 s		10b, 11b, 13b, 14b	
13b	6.73 d (8.5)	116.5 d	14b	11b, 14b	14b
14b	7.07 d (8.5)	131.0 d	13b	8b, 10b, 13b	13b
1′	4.57 d (7.8)	102.8 d	2′		2a, 4a, 3′, 5′
2′	3.48 m	74.8 d	1′, 3′	3′	4′
3′	3.53 m	77.8 d	2′, 4′	4′	1′, 5′
4′	3.50 m	71.0 d	3′, 5′	3′, 6′	2′, 6′α
5′	3.45 m	78.0 d	4′, 6′α, 6′β	1′, 4′	1′, 3′
6′α	3.86 br d (12.0)	63.3 t	5′, 6′β	4′, 5′	4′
6′β	3.79 m		5′, 6′α		
1′′	4.58 d (7.8)	103.2 d	2′′		2b, 4b, 3′′, 5′′
2′′	3.48 m	74.8 d	1′′, 3′′	3′′	4′′
3′′	3.53 m	77.8 d	2′′, 4′′	4′′	1′′, 5′′
4′′	3.50 m	71.0 d	3′′, 5′′	3′′, 6′′	2′′, 6′′α
5′′	3.45 m	78.0 d	4′′, 6′′α, 6′′β	1′′, 4′′, 6′′β	1′′, 3′′
6′′α	3.90 br d (12.6)	63.3 t	5′′, 6′′β	4′′, 5′′	4′′
6′′β	3.79 m		5′′, 6′′α		

[a] The carbon multiplicities were obtained from a DEPT experiment. [b] Protons that correlate with the carbon resonance.

steric hindrance between the aromatic rings and the sugar moieties. Thus, the C_2 symmetry does not actually exist in the molecule due to the distortion of the cyclobutane ring, and there is a global chiral center in the molecule, which was further evidenced by the $[\alpha]_D$ data. Glycosides of stilbene oligomers are seldom reported[20] because of the difficulty of isolation and the instability of the structure. The four-membered ring is very rare in stilbene oligomers and also in natural products. To the best of our knowledge, this is the first example in natural stilbene oligomers, although some synthesized products with simpler patterns have been reported.[21−25]

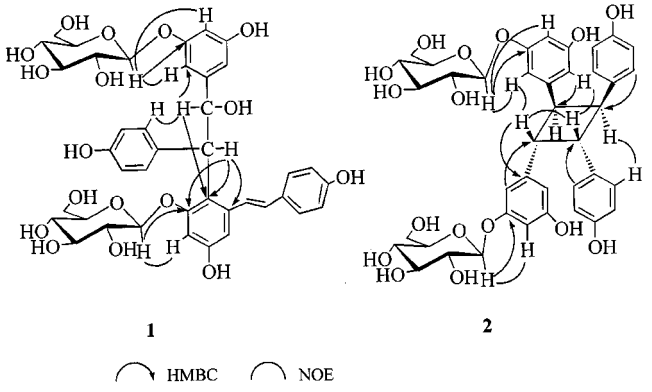

Figure 2. Selected HMBC (from H to C) and ROESY correlations of **1** and **2**

The inhibition of lipid peroxidation induced by $FeSO_4$ and cysteine in homogenate in vitro by compounds **1** and **2** was examined. Compound **1** inhibited 81% of the formation of malondialdehyde (MDA) at a concentration of 2 μM, while 2 μM of **2** inhibited only 36% of the formation of MDA (as a positive control, 2 μM vitamin E inhibited 23% of the formation of MDA). Therefore **1** and **2** possess strong inhibition of lipid peroxidation. This inhibitory activity may be attributed to the phenolic functions in the molecules. The monomeric stilbenes of this plant were also reported to have the inhibitory activity of lipid peroxidation.[11] However, **1** and **2** did not show any cytotoxicity against three human cancer cell lines KB, MCF-7 and A549 in vitro, or DNA-cleavage activity. The two compounds were found to have no inhibition of protein tyrosine phosphatase 1B (PTP1B).

Experimental Section

General Procedures: Optical rotations were recorded in CH_3OH using a Perkin−Elmer 241 automatic digital polarimeter. CD spectra were measured with a Jasco-715 spectropolarimeter. ^1H, ^{13}C NMR, ^1H-^1H COSY, HMQC, HMBC and NOESY spectra were recorded on an INOVA-600 spectrometer. The carbon multiplicities were obtained by DEPT experiments. FAB-MS spectra were obtained using a Finnigan MAT-90 instrument. UV was carried out on a Varian Cary 300 Bio instrument. IR was recorded on a Hitachi 275−50 IR spectrometer. Elemental analysis was carried out on an Elementar Vario EL instrument. Gas chromatography (GC) was run on a HP 1890 gas chromatography. Sephadex LH-20 (Pharmacia), Toyopearl HW40F (Tosoh), MCI-gel CHP20P (Mitsubishi) and Cosmosil ODS (40−60 μm, Nacalai Tesque Inc.) were used for column chromatography.

Plant Material: The roots of *Polygonum Cuspidatum* Sieb. et Zucc. were collected from Sichuan province, People's Republic of China in October, 1997, and were identified by the author. A voucher specimen (No. PC001) is deposited at Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation: The slices of the dried roots of *P. cuspidatum* (10 Kg) were extracted with 60% aqueous acetone (15 liters × 3, 24 h each) at room temperature. The acetone was evaporated in vacuum and some hydrophobic substances precipitated; these were removed by filtration. The filtrate was concentrated to a suitable volume and then chromatographed on Sephadex LH-20 column, eluting with H_2O, aqueous MeOH (10−70%) and 50% acetone successively to give five fractions. The fraction eluted by 30% MeOH (2 g) was subjected to MCI gel chromatography eluting with a gradient of aqueous MeOH (from 10 to 60% MeOH). The 40% aqueous MeOH eluate from the MCI column was repeatedly chromatographed on ODS (eluting with 30% MeOH) and Toyopearl HW-40F (eluting with 50% MeOH) to give **1** (5 mg). The 45% aqueous MeOH eluate from the MCI column was repeatedly chromatographed on Toyopearl HW-40F (eluting with 50% MeOH) and finally purified on ODS (eluting with 30% MeOH) to yield **2** (8 mg).

Compound 1: Amorphous powder, $[\alpha]_D^{25} = 160.2$ ($c = 0.21$, MeOH). UV (MeOH) $\lambda_{max} = 282-230$ (sh), 203 nm. IR (KBr): $\tilde{\nu}_{max} = 3380, 1606, 1512, 1452, 1308, 1244, 1175\ cm^{-1}$. For ^1H and ^{13}C NMR spectroscopic data see Table 1. FAB-MS: $m/z = 835$ [M + K]$^+$, 819 [M + Na]. $C_{40}H_{44}O_{17}\cdot H_2O$: calcd. C 58.96, 5.69; found C 58.82, H 5.64.

Compound 2: Crystalline powder, $[\alpha]_D^{25} = -11.8$ ($c = 0.44$ MeOH). UV (MeOH) $\lambda_{max} = 276-225$ (sh), 203 nm. IR (KBr): $\tilde{\nu}_{max} = 3373, 1599, 1603, 1514, 1452, 1238, 1171, 1074\ cm^{-1}$. For ^1H and ^{13}C NMR spectroscopic data see Table 1. CD ($c = 2.0 \times 10^{-4}$ MeOH) $[\theta]^{20}$ (nm) = +11303 (234), +20111 (240), −3000 (272), +3869 (288). FAB-MS: $m/z = 819$ [M + K]$^+$, 803 [M + Na]. $C_{40}H_{44}O_{16}$ (780.8): calcd. C 61.53, H 5.68; found C 61.45, H 5.65.

Acid Hydrolysis of 1 and 2: 2 mg of **1** (or **2**) was refluxed in 2 mL 7% HCl/EtOH (3:7) for 4 h. The mixture was diluted with H_2O and extracted with Et_2O. The aqueous layer was neutralized with 1 N NaOH and then a sample was subjected to TLC analysis on Kieselgel 60 F_{254} (Merck) [using $CHCl_3$/MeOH/H_2O (30:12:4), 9 mL and HOAc, 1 ml] and paper chromatography [using *n*BuOH/HOAc/H_2O (4:1:5)] with standard sugars; in each case the presence of glucose was established. The aqueous layer was then passed through an Amberlite IRA-60E column, the aqueous eluate was concentrated and treated with thiazolidine as described previously.[16] Only the D-glucose derivative was detected by GC in each case. (GC conditions: column, Supelco SPB^{-1}, 0.25 mm ′ 27 m, column temperature 230 °C; carrier gas, N_2; t_R, D-glucose derivative 17.9 min, L-glucose derivative 17.3 min).

Biological Assays: The inhibition of lipid peroxidation assay was evaluated using the previous described protocol.[26] Cytotoxicity was carried out according to previously established MTT protocols against three human cancer cell lines KB, MCF-7 and Hela.[27] A modification of the Hecht procedure was adapted to evaluate the inhibition of DNA-cleavage activity.[28] The inhibition of protein tyrosine phosphatase 1B (PTP1B) was assayed according to the literature.[29]

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