Constituents from *Polygonum cuspidatum*

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Two lignan sulfates, a stilbene derivative and a phenol sulfate, together with 10 known compounds, were isolated from an aqueous extract of the root of *Polygonum cuspidatum*. The new compounds were elucidated based on chemical evidence and spectroscopic techniques including two-dimensional NMR methods. They exhibited no inhibition of lipid peroxidation and no cytotoxic and DNA cleavage activities.

Key words natural product; lignan sulfate; *Polygonum cuspidatum*

The dried root of *Polygonum cuspidatum* SIEB. et ZUCC. (Polygonaceae) is a well-known traditional drug in China as well as in Japan. It has been widely used for the treatment of suppurative dermatitis, gonorrhea, favus athlete's foot, and hyperlipidemia in Chinese folk medicine. Various chemical compounds have been isolated from this plant, including anthraquinones,[2,3)] stilbenes,[4,5)] flavonoids,[6)] and other phenols.[3)] Interesting bioactivities of resveratrol and piceid in this plant have been reported.[4,5,7—12)] Recently we reported on stilbene glycoside sulfates isolated from the water-soluble fraction of this plant.[13)]

In our continuous chemical study of this plant, two lignan sulfates (**1**, **2**), a stilbene derivative (**3**), and a phenol sulfate (**4**), together with 10 known compounds (**5**—**14**) were isolated. 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 compounds **1**—**14**. The structures of **1**—**4** were elucidated based on the analysis of spectral data and chemical evidence (Fig. 1), and their ^{1}H- and ^{13}C-NMR data were unambiguously assigned. The known compounds were determined to be gallic acid (**5**), tryptophan (**6**), 2,6-dihydroxybenzoic acid (**7**), (+)-catechin (**8**),[14)] (+)-catechin-5-*O*-*β*-D-glucopyranoside (**9**),[15)] 1-(3-*O*-*β*-D-glucopyranosyl-4,5-dihydroxyphenyl)-ethanone (**10**),[16)] resveratrol (**11**),[5)] piceid (**12**),[5)] tachioside (**13**),[17)] and emodin-8-*O*-*β*-D-glucopyranoside (**14**)[18)] when compared with the authentic samples. Compounds **5**, **6**, **7**, **10**, and **13** have not been previously isolated from the plant.

Results and Discussion

Compound **1** was obtained as an amorphous powder (15 mg, 0.00015%), $[\alpha]_D^{25}$ −17.0° (c=0.13, MeOH). Its FAB-MS showed an $(M+Na)^+$ ion at m/z 545, indicating a molecular weight of 522, compatible with the molecular formula of $C_{22}H_{27}O_{11}SNa$. This conclusion was consistent with elemental analysis and atomic absorption data. The UV spectrum exhibited maxima at 282, 238 (sh), and 205 nm (MeOH). In the IR spectrum, in addition to the obvious evidence for a hydroxyl (3440 cm^{-1}) and aromatic ring (1616, 1518 cm^{-1}), the strong absorption at 1240 and 1059 cm^{-1} was indicative of the presence of an $-OSO_3^-$ group.[19)] The presence of S was confirmed by elemental analysis, while the presence of Na^+ was determined from atomic absorption analysis. Acid hydrolysis of **1** afforded the anion SO_4^{2-}, which was confirmed by precipitation with $BaCl_2$. The ^{1}H- and ^{13}C-NMR data (Table 1) of **1** showed the presence of two aromatic rings and six aliphatic carbon signals, which exhibited the characteristics of an aryl-tetralin-type lignan. The NMR data resemble those of (−)-lyoniresinol and the aglycon of (−)-lyoniresinol-3′-*O*-*β*-D-glucopyranoside,[20,21)] which flank possessed the lyoniresinol skeleton of **1**. The linkage of $-OSO_3^-$ was determined mainly by the comparison of the NMR data of **1** with that of (−)-lyoniresinol. H-2a of **1** (δ 4.04 and 4.08) had obvious downfield shifts compared with that of (−)-lyoniresinol (downfield about 0.5 ppm), and C-2a unusually shifted downfield (from about δ 64 to 70.5), while C-2 shifted 2 ppm downfield. The evidence above suggests that the $-OSO_3^-$ group was linked to C-2a. The circular dichroism (CD) spectrum of **1** was similar to that of (−)-lyoniresinol. A positive Cotton effect at 286 nm suggested that H-1 was β and C-1 was R.[22,23)] Nuclear Overhauser effect spectroscopy (NOESY) correlations observed between H-2 (δ 2.13)/H-2′ (δ 6.51), H-1 (δ 4.23)/H-3 (δ 1.81), and H-6′ (δ 6.51)/8-Me confirmed the deduction. The H-1 preferential axial conformation was evidenced by its coupling constant (J=7.1 Hz). The methoxyl at C-8 appeared at a rather high field (δ 3.42), which was apparently in the shielding zone of the aromatic ring at C-1. The results were in good accordance with the energy minimized conformation, which was obtained from a molecular modeling program (Sybyl 6.5). The evidence above led to the elucidation of compound **1** as sodium (−)-1*R*-3-hydroxymethyl-1-(4′-hydroxy-3′,5′-

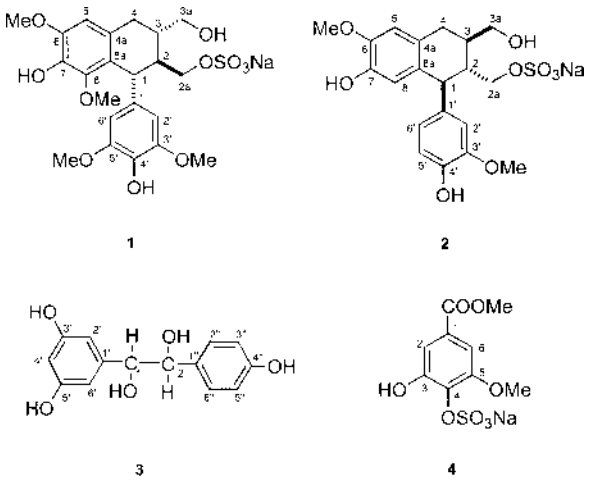

Fig. 1. Structures of Compounds **1**—**4**

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Table 1. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR Data and HMBC Correlations of **1**

No.	Proton[a)]	Carbon[a,b)]	HMBC
1	4.23 d (7.1)	44.3 d	H-2, H-2a, H-2′, H-6′
2	2.13 m	46.9 d	H-1, H-2a, H-3a, H-4
2a	4.08 dd (10.3, 3.6)	70.5 t	H-1
	4.04 dd (10.3, 5.7)		
3	1.81 m	40.1 d	H-1, H-2, H-3a, H-4
3a	3.74 dd (11.2, 3.9)	66.5 t	H-2, H-3, H-4
	3.59 dd (11.2, 6.8)		
4	2.83 dd (15.6, 4.4)	34.6 t	H-3a, H-5
	2.70 dd (15.6, 12.1)		
4a		132.5 s	H-1, H-4, H-5
5	6.78 s	110.5 d	H-4
6		150.0 s	H-5
7		139.2 s	H-5
8		148.7 s	H-1, H-5
8a		127.4 s	H-1, H-4, H-5
1′		141.0 s	H-1, H-2, H-2′, H-6′
2′	6.51 s	108.5 d	H-6′
3′		150.2 s	H-2′
4′		137.4 s	H-2′, H-6′
5′		150.2 s	H-6′
6′	6.51 s	108.5 d	H-2′
6-OMe	3.87 s	58.8 q	
8-OMe	3.42 s	62.3 q	
3′-OMe	3.78 s	58.9 q	
5′-OMe	3.78 s	58.9 q	

a) Measured in D_2O. *b*) The carbon multiplicities were obtained from DEPT experiments.

Table 2. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR Data and HMBC Correlations of **2**

No.	Proton[a)]	Carbon[a,b)]	HMBC
1	4.12 d (10.3)	48.0 d	H-2a, H-8, H-2′, H-6′
2	2.10 ddt (9.9, 10.3, 2.8)	45.4 d	H-1, H-4
2a	4.28 dd (9.9, 2.9)	67.9 t	H-1
	4.06 dd (9.9, 2.8)		
3	2.23 m	39.3 d	H-1, H-3a, H-4
3a	3.97 dd (11.0, 3.3)	65.1 t	H-4
	3.88 dd (11.0, 6.5)		
4	3.02 m	33.8 t	H-3a, H-5
4a		129.6 s	H-1, H-4, H-5, H-8
5	6.88 s	112.8 d	H-4
6		147.5 s	H-5, H-8
7		145.3 s	H-5, H-8
8	6.39 s	117.6 d	H-1
8a		134.1 s	H-1, H-4, H-5, H-8
1′		138.8 s	H-1, H-2′, H-5′, H-6′
2′	6.95 d (1.8)	114.7 d	H-1, H-6′
3′		149.1 s	H-2′, H-5′
4′		145.8 s	H-2′, H-5′, H-6′
5′	6.94 d (8.1)	116.4 d	H-6′
6′	6.79 dd (8.1, 1.8)	123.3 d	H-1, H-2′
6-OMe	4.01 s	56.8 q	
3′-OMe	3.99 s	56.8 q	

a) Measured in CD_3OD+D_2O. *b*) The carbon multiplicities were obtained from DEPT experiments.

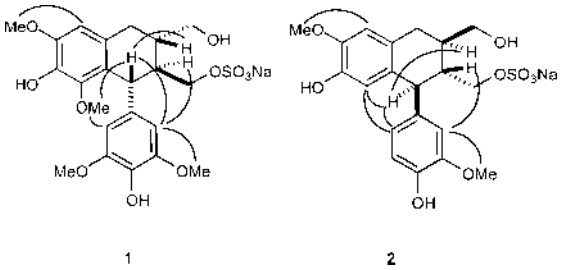

Fig. 2. Significant NOESY Correlations of Compounds **1** and **2**

dimethoxyphenyl)-7-hydroxy-6,8-dimethoxy-1,2,3,4-tetrahydro-2-naphthalenylmethanol sulfate, that is, sodium (−)-lyoniresinol-2a-sulfate.

Compound **2** was obtained as an amorphous powder (26 mg, 0.00026%), $[\alpha]_D^{25}$ +34.0° (c=0.11, MeOH). It exhibited FAB-MS ion at m/z 485 $(M+Na)^+$, indicative of a molecular weight of 462, compatible with a molecular formula of $C_{20}H_{23}O_9SNa$ as determined from elemental analysis and atomic absorption data. The UV spectrum showed maxima at 282, 230 (sh), and 203 nm (MeOH). As in **1**, the strong absorption in the IR spectrum at 1252 and 1063 cm^{-1} suggested the presence of an $-OSO_3^-$ group. The presence of S and Na was determined from further elemental analysis and atomic absorption analysis, respectively. Acid hydrolysis of **2** also afforded the anion SO_4^{2-}. The ^{1}H- and ^{13}C-NMR data (Table 2) of **2** suggested that **2** was another aryl-tetralin-type lignan. The NMR data exhibited the characteristics of (+)-isolaricireinol.[21,24)] The unusual downfield shift of H-2a and C-2a and the upfield shift of C-2 suggest the linkage of the $-OSO_3^-$ group at C-2a. The CD spectrum of **2** resembled that of (+)-isolaricireinol. A negative Cotton effect at 291 nm suggested that H-1 was α and C-1 was *S*. The H-1 also had a preferential axial conformation as evidenced by its coupling constant (J=10.3 Hz). The significant NOESY correlations are shown in Fig. 2, which were in agreement with the minimized energy conformation derived from the Sybyl 6.5 program. Based on the accumulated evidence, compound **2** was determined to be sodium (+)-1*S*-3-hydroxymethyl-1-(4′-hydroxy-3′-methoxyphenyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydro-2-naphthalenylmethanol sulfate, that is, sodium (+)-isolaricireinol-2a-sulfate.

Compound **3** was obtained as an amorphous powder (10 mg, 0.0001%), $[\alpha]_D^{25}$ +13.5° (c=0.32, MeOH). High resolution (HR)-electron impact (EI)-MS gave an ion at m/z 244.0738 $(M-H_2O)^+$, suggesting a molecular formula of $C_{14}H_{14}O_5$. The UV spectrum showed maxima at 277, 224, and 203 nm (MeOH). The IR spectrum showed strong bands for a hydroxyl (3385 cm^{-1}) and an aromatic ring (1612, 1516 cm^{-1}). The ^{1}H-NMR spectrum of **3** (Table 3) contained three sets of signals, including one set of *ortho*-coupled protons assignable to one *para*-hydroxy phenyl group in an AA′XX′-type arrangement [δ 7.24, 6.66 (2H each, d, J= 8.2 Hz)], a set of protons assignable to a 1,3,5-trisubstituted phenyl group [δ 6.16 (3H, br)], and two aliphatic protons [δ 4.41 (1H, d, J=7.6 Hz), 4.52 (1H, d, J=7.6 Hz)]. The ^{13}C-NMR spectrum (Table 3) exhibited 14 signals. It can be inferred that **3** may be a resveratrol derivative. The analysis of two-dmensional NMR data established the structure **3** to be as shown in Fig. 1. Compared with resveratrol, the double bond was saturated and oxygenated into diol. Based on the coupling constants of the two aliphatic protons (J=7.6 Hz), the two hydroxyls should be in the *threo* form.[25,26)] Thus the structure of **3** was elucidated to be 1-(3′,5′-dihydroxyphenyl)-2-(4″-hydroxyphenyl)-ethane-1,2-diol.

Compound **4** was isolated as an amorphous powder

Table 3. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR Data and HMBC Correlations of **3**

No.	Proton	Carbon[a)]	HMBC
1	4.41 d (7.6)	79.7 d	H-2, H-2′, H-6′
2	4.52 d (7.6)	79.0 d	H-1, H-2″, H-6″
1′		144.9 s	H-1
2′	6.16 br	106.6 d	H-1, H-4′
3′		158.7 s	H-2′, H-4′
4′	6.16 br	102.2 d	H-2′, H-6′
5′		158.7 s	H-4′, H-6′
6′	6.16 br	106.6 d	H-4′
1″		133.3 s	H-1, H-2, H-3″, H-5″
2″	7.24 d (8.5)	129.2 d	H-3″, H-6″
3″	6.66 d (8.5)	115.2 d	H-2″, H-5″
4″		157.3 s	H-2″, H-3″, H-5″, H-6″
5″	6.66 d (8.5)	115.2 d	H-3″, H-6″
6″	7.24 d (8.5)	129.2 d	H-2″, H-5″

a) Measured in CD_3COCD_3. *b*) The carbon multiplicities were obtained from DEPT experiments.

(30 mg, 0.0003%). Its FAB-MS spectrum gave ions at *m/z* 323 $(M+Na)^+$ and 301 $(M+1)^+$, indicative of a molecular formula of $C_9H_9O_8SNa$, as determined from elemental analysis and atomic absorption data. The UV spectrum showed maxima at 290, 266, and 214 nm (MeOH). The IR spectrum suggested the presence of a carbonyl (1720 cm^{-1}), aromatic ring (1605, 1518 cm^{-1}) and an $-OSO_3^-$ group (1277, 1049 cm^{-1}). The presence of S and Na was determined from further elemental analysis and atomic absorption analysis, respectively. Acid hydrolysis of **4** also afforded the anion SO_4^{2-}. The ^{1}H- and ^{13}C-NMR data of **4** suggested that **4** was a simple phenolic compound. It has one aromatic ring, a carbonyl group, an $-OSO_3^-$ group, and a hydroxyl, a methoxy, and a methyl ester. Full assignment of the signals confirmed that **4** is sodium 3,4-dihydroxy-5-methoxybenzoic acid methyl ester-4-sulfate, a derivative of gallic acid that also occurs in this plant.

Biological assays demonstrated that compounds **1**—**4** exhibited no inhibition of lipid peroxidation and no cytotoxic and DNA cleavage activities.

Experimental

Optical rotations were recorded in CH_3OH using a Perkin-Elmer 241 automatic digital polarimeter. CD spectra were measured with a Jasco-715 Spectropolarimeter. ^{1}H- and ^{13}C-NMR, ^{1}H–^{1}H correlated spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and NOESY spectra were recorded on a Bruker DRX-400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz). The carbon multiplicities were obtained by distortionless enhancement by polarization transfer (DEPT) experiments. FAB-MS and HR-EI-MS were obtained using a Finnigan MAT-90 instrument. UV measurements were 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. Atomic absorption was recorded on a Hitachi Z-5000 spectrometer. 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 *P. 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 the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation The dried roots of the plant (10 kg) were extracted with 60% aqueous acetone 3 times at room temperature. The acetone was evaporated *in vacuo* and some hydrophobic substances precipitated, which were filtered. The filtrate was concentrated to a suitable volume, then chromatographed on a Sephadex LH-20 column eluted with H_2O, aqueous MeOH (10—70%), and 50% acetone successively to give five fractions (Fr.). The sugar eluted by water (Fr. 1) was discarded. Fr. 2 was subjected to MCI gel chromatography eluted with an aqueous MeOH gradient from 10% to 60%. The 10% aqueous MeOH eluate from the MCI column was repeatedly chromatographed on ODS (eluted with 25% MeOH) and Toyopearl HW-40F (eluted with 10% MeOH) to give **4** (30 mg) and **6** (25 mg). The 20% aqueous MeOH eluate was repeatedly chromatographed on Toyopearl HW-40F (eluted with 5% MeOH) and finally purified on ODS (eluted with 10% MeOH) to give **13** (36 mg). The 30% aqueous MeOH eluate from the MCI column was repeatedly chromatographed on ODS (eluted with 25% MeOH) and Toyopearl HW-40F (eluted with 20% MeOH) to give **1** (15 mg) and **7** (16 mg). Fr. 3 was chromatographed on a Toyopearl HW-40F column eluted with an aqueous MeOH gradient from 10% to 60%. The 10% eluate was further purified with Toyopearl HW-40F and MCI gel CHP20P to give **2** (26 mg). The 30% eluate was purified on ODS and MCI gel CHP20P to yield **3** (10 mg). The 40% eluate was also purified on ODS and MCI gel CHP20P to yield **10** (25 mg) and **12** (20 g). Fr. 4 was chromatographed on a Toyopearl HW-40F column and the 40% MeOH eluate was repeatedly chromatographed on an MCI gel column to yield **11** (70 mg), while the 50% MeOH eluate was repeatedly chromatographed on an MCI gel column to yield **8** (40 mg). Fr. 5 was chromatographed on a MCI gel column and the 20% MeOH eluate gave crystal **5** (100 mg), the 30% MeOH eluate yielded crystal **9** (120 mg), and the 50% MeOH eluate gave crystal **14** (1 g).

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{25}$ −17.0° (c=0.13, MeOH); UV λ_{max} (MeOH, nm): 282, 238 (sh), 205. IR (KBr) cm^{-1}: 3440, 1616, 1518, 1502, 1460, 1325, 1240, 1219, 1111, 1059. ^{1}H- and ^{13}C-NMR data, see Table 1. CD (c=6.67×10^{-4}, MeOH) $[\theta]^{20}$ (nm): −24852 (243), −10482 (272), +3458 (286). FAB-MS *m/z* 545 $(M+Na)^+$, 523 $(M+1)^+$. *Anal.* Calcd for $C_{22}H_{27}O_{11}SNa$: C, 50.57; H, 5.21; S, 6.14. Found: C, 50.70; H, 5.25; S, 6.07.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{25}$ +34.0° (c=0.11, MeOH). UV λ_{max} (MeOH, nm): 282, 230 (sh), 203. IR (KBr) cm^{-1}: 3423, 1647, 1603, 1514, 1450, 1371, 1252, 1219, 1126, 1063, 1022, 949. ^{1}H- and ^{13}C-NMR data, see Table 2. CD (c=6.67×10^{-4}, MeOH) $[\theta]^{20}$ (nm): +2812 (241), +2973 (275), −6491 (291). FAB-MS *m/z* 485 $(M+Na)^+$. *Anal.* Calcd for $C_{20}H_{23}O_9SNa\cdot 2H_2O$: C, 48.19; H, 4.65; S, 6.43. Found: C, 48.30; H, 4.70; S, 6.33.

Compound **3** was obtained as an amorphous powder, $[\alpha]_D^{25}$ +13.5° (c=0.32, MeOH). UV λ_{max} (MeOH, nm): 277, 224, 203. IR (KBr) cm^{-1}: 3385, 1612, 1516, 1456, 1334, 1238, 1159, 1047, 1005, 837. ^{1}H- and ^{13}C-NMR data, see Table 3. EI-MS *m/z* 244 $(M-D_2O)^+$ (15.4), 228 (1.8), 215 (9), 140 (100). HR-EI-MS *m/z* 244.0738 [Calcd for $C_{14}H_{14}O_5(M-H_2O)^+$: 244.0735].

Compound **4** was obtained as an amorphous powder. UV λ_{max} (MeOH, nm): 290 (sh), 266, 214. IR (KBr) cm^{-1}: 3454, 1720, 1605, 1518, 1435, 1342, 1277, 1230, 1201, 1086, 1049, 1005. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.10 (1H, d, J=1.9, 2-H), 7.04 (1H, d, J=1.9, 6-H), 3.79 (3H, s, 5-OMe), 3.77 (3H, s, COOMe). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 166.2 (s, COO–), 147.8 (s, 5-C), 145.2 (s, 3-C), 139.3 (s, 4-C), 119.3 (s, 1-C), 110.5 (d, 2-C), 104.6 (d, 6-C), 55.9 (q, 5-OMe), 51.7 (q, COOMe). FAB-MS *m/z* 323 $(M+Na)^+$, 301 $(M+1)^+$. *Anal.* Calcd for $C_9H_9O_8SNa\cdot H_2O$: C, 33.97; H, 3.48; S, 10.08. Found: C, 34.10; H, 3.41; S, 9.98.

Acid Hydrolysis of Compounds 1, 2, and 4 Solutions of **1**, **2**, and **4** (2 mg each) in 2 N HCl was heated (90 °C) for 2 h, after removing HCl by evaporation i*n vacuo*, and the mixtures were diluted with H_2O and extracted with EtOAc. A sample of each aqueous layer gave a positive sulfate test with $BaCl_2$.

Bioassay Procedures Cytotoxity was evaluated against three human cancer cell lines KB, Hela, and A549, using previously described MTT protocols.[27] The DNA cleavage activity was carried out according to a modification of the Hecht procedure.[28] The inhibition of lipid peroxidation was assessed using a previously described protocol.[29]

References and Notes

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