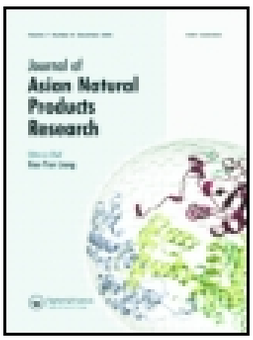

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New benzoic acid glycosides from *Sophora flavescens*

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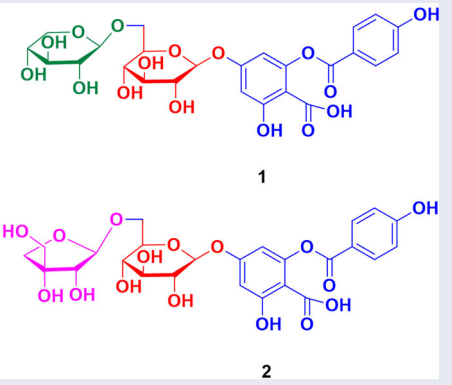
ABSTRACT

Two new benzoic acid derivatives, sophophenoside A (**1**) and sophophenoside B (**2**), were isolated from *Sophora flavescens*. Their structures were elucidated by detailed spectroscopic analysis and chemical methods. Compounds **1** and **2** were assayed for their hepatoprotective activity on the cytotoxic effect of D-galactosamine on HL-7702 cells, and compound **1** exhibited a moderate hepatoprotective activity at a concentration of 10 μM.



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Leguminous; *Sophora flavescens*; benzoic acid; sophophenoside; hepatoprotective effects

1. Introduction

The dried root of *Sophora flavescens* (Leguminous) has been widely reported for its various biological activities, including antiinflammatory, anthelmintic, free radical scavenging, and antimicrobial activity [1–4]. To date, a variety of phytochemical constituents such as quinolizidine alkaloids and flavonoids [5–8], have been isolated from this plant. In the course of searching for active components from this plant,

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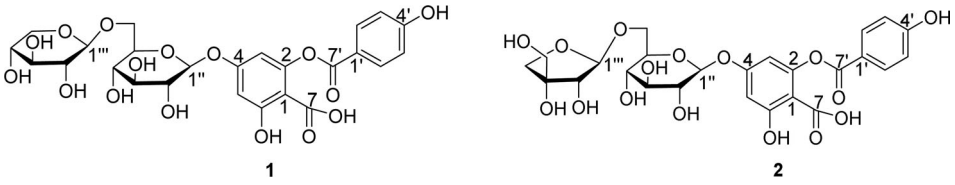

1 2

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

two benzoic acid derivatives were isolated and named sophophenoside A (**1**) and sophophenoside B (**2**) (Figure 1). Additionally, compounds **1** and **2** were evaluated for hepatoprotective effect.

2. Results and discussion

Compound **1** was obtained as a yellow powder. The IR spectrum of **1** showed the presence of hydroxyl groups (3456 cm^{-1}), carbonyl groups (1715 cm^{-1}) and aromatic rings (1515 cm^{-1}). A molecular formula of $C_{25}H_{28}O_{16}$ ($\Omega = 12$) was deduced on the basis of HR-ESI-MS at *m/z* 607.1256 $[M + Na]^+$. The ^1H NMR spectrum of **1** (Table 1) displayed an AA′BB′ system proton signals at δ_H 7.95 (2H, d, $J = 8.5$ Hz) and 6.92 (2H, d, $J = 8.5$ Hz), which suggested the existence of a *p*-substituted phenyl group in **1**. Furthermore, two coupled aromatic meta-proton signals at δ_H 6.56 (1H, d, $J = 2.0$ Hz) and 6.39 (1H, d, $J = 2.0$ Hz) were observed, suggesting the existence of a tetrasubstituted phenyl group in **1**. Additionally, a downfield proton signal at δ_H 10.45 (1H, brs) and two anomeric protons at δ_H 4.98 (1H, d, $J = 7.5$ Hz) and 4.16 (1H, d, $J = 7.5$ Hz) were demonstrated. Detailed analysis of the ^1H, ^{13}C, and 2 D NMR spectroscopic data revealed the presence of two glycosyl moieties, two aromatic rings and two carbonyl carbons. The chemical shift of two carbonyl carbons at δ_C 170.6 and 164.8 revealed that they were ester carbonyl or carboxyl group in ^{13}C NMR spectrum (Table 1). Furthermore, one ester carbonyl at δ_C 164.8 was linked to C-1′ of *p*-substituted phenyl group by the HMBC correlations (Figure 2) between H-2′, 6′ and C-7′. Another tetrasubstituted benzene ring contained three carbon signals (δ_C 152.9, 161.9, 164.2) larger than 150 ppm, indicating that there were three oxygen-containing substitutions on the benzene ring. It was preliminarily concluded that tetrasubstituted benzene ring was like phloroglucinol and the C-1 was linked with another carboxyl group. According to the above conclusions, the NMR data of the aglycone in **1** were similar to those of 2,4-dihydroxy-6-(4-hydroxybenzoyloxyl)-benzoyl acid [9]. Therefore, the aglycone structure of **1** was formed by esterification of two benzoic acids. According to the ^{13}C NMR spectrum and 2 D NMR spectra of **1**, glucopyranosyl moiety at δ_C 100.1 (C-1″), 73.5 (C-2″), 76.7 (C-3″), 69.8 (C-4″), 75.9 (C-5″) and 68.7 (C-6″), and xylopyranosyl moiety at δ_C 104.5 (C-1‴), 73.8 (C-2‴), 76.9 (C-3‴), 69.9 (C-4‴) and 66.1 (C-5‴) were found. Moreover, two sugar moieties were assigned to be *β*-form by the coupling constant of the anomeric proton signal (glu: $J = 7.5$ Hz) and (xyl: $J = 7.5$ Hz). Furthermore, the glucopyranosyl moiety and the xylopyranosyl moiety were proved to be D-glucopyranose and D-xylopyranose based on the GC analysis by acid hydrolysis and chiral derivatization of **1**. In HMBC spectrum, the cross-peaks between H″-1 (δ_H 4.98)/C-4 (δ_C 161.9) demonstrated that

Table 1. ^{1}H (500 M) and ^{13}C NMR (125 M) spectroscopic data of compounds **1** and **2** (DMSO-d_6).

	1		2	
No.	δ_H	δ_C	δ_H	δ_C
1		103.3		103.3
2		152.9		153.0
3	6.39 d (2.0)	103.9	6.38 d (2.0)	103.9
4		161.9		162.0
5	6.56 d (2.0)	101.8	6.53 d (2.0)	101.8
6	12.21 brs (OH)	164.2	12.51 brs (OH)	164.3
7		170.6		170.7
1′		120.3		120.3
2′	7.95 d (8.5)	132.8	7.95 d (9.0)	132.8
3′	6.92 d (8.5)	115.9	6.92 d (9.0)	115.9
4′	10.45 s (OH)	162.9	10.48 s (OH)	162.9
5′	6.92 d (8.5)	115.9	6.92 d (9.0)	115.9
6′	7.95 d (8.5)	132.8	7.95 d (9.0)	132.8
7′		164.8		164.8
1″	4.98 d (7.5)	100.1	5.00 d (7.5)	100.1
2″	3.36 – 3.22 m	73.5	3.25 – 3.22 m	73.5
3″	3.30 – 3.26 m	76.7	3.31 – 3.28 m	76.7
4″	3.28 – 3.23 m	69.8	3.15 – 3.11 m	70.2
5″	3.60 – 3.57 m	75.9	3.59 – 3.56 m	75.9
6″	3.63 – 3.59 m	68.7	3.47 – 3.43 m	68.0
	3.92 d (10.0, 2.0)		3.86 dd (11.0, 2.0)	
1‴	4.16 d (7.5)	104.5	4.82 d (3.0)	109.8
2‴	3.03 – 2.98 m	73.8	3.74 d (3.0)	76.4
3‴	3.10 t (9.0)	76.9		79.2
4‴	3.26 – 3.22 m	69.9	3.90 – 3.86 m	73.8
5‴	3.03 – 2.98 m	66.1	3.33 – 3.29 m	63.8
	3.69 dd (11.0, 5.0)			

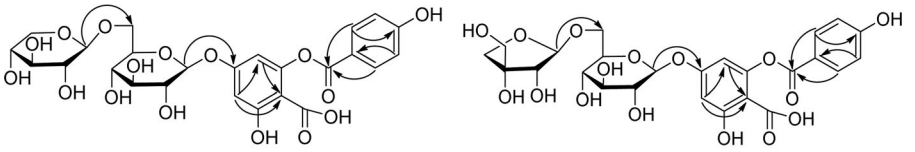

Figure 2. Key HMBC (H→C) correlations of compounds **1** and **2**.

the glucopyranosyl moiety was linked to the C-4 position of the tetrasubstituted phenyl group by oxygen atom. Meanwhile, the xylopyranosyl moiety was directly attached at C-6″ of the glucopyranosyl moiety based on the correlations of H-1‴ with C-6″ in the HMBC experiment. Thus, compound **1** was characterized as 2-[(4-hydroxybenzoyl)oxy)]-4,6-dihydroxybenzoic acid-4-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside and named sophophenoside A.

Compound **2** was also isolated as a yellow powder. Its molecular formula of $C_{25}H_{28}O_{16}$ was determined by HR-ESI-MS. The IR spectrum showed the presence of hydroxyl, carbonyl and aromatic groups. Inspection of the ^{1}H and ^{13}C NMR spectra together with the HSQC and HMBC spectroscopic data revealed the presence of 25 carbon signals, due to two aromatic ring groups, a pentose moiety, a hexose moiety, and two carbonyl carbons. The ^{1}H NMR data (Table 1) showed an AA′BB′ system proton signals at δ_H 7.95 (2H, d, $J = 9.0$ Hz) and 6.92 (2H, d, $J = 9.0$ Hz) of a *p*-substituted phenyl group in **2**. Furthermore, the proton signals at δ_H 6.53 (1H, d,

$J = 2.0$ Hz) and 6.38 (1H, d, $J = 2.0$ Hz) implied the presence of a tetrasubstituted phenyl group in **2**. Comparison of the NMR data of **2** with those of **1** inferred that **2** was an analogue of **1** with the same aglycone except for those of the saccharide unit in compound **2**. The ^{13}C NMR spectrum of **2** (Table 1) showed six carbons of hexose moiety at δ_C 100.1 (C-1″), 73.5 (C-2″), 76.7 (C-3″), 70.2 (C-4″), 75.9 (C-5″) and 68.0 (C-6″), and five carbons of pentose moiety at δ_C 109.8 (C-1‴), 76.4 (C-2‴), 79.2 (C-3‴), 73.8 (C-4‴) and 63.8 (C-5‴). Two saccharide moieties were assigned to be β-form (glucose and apiose) by the ^{13}C NMR data and the coupling constants of the anomeric proton signals. Furthermore, acid hydrolysis of **2** yielded D-glucose and D-apiose based on the GC analysis, which suggested that there were a β-D-glucopyranose and a β-*D*-apiofuranose in compound **2**. The HMBC correlations of H-1″ with C-4 (δ_C 162.0) and H-1‴ with C-6″ (δ_C 68.0) indicated that glucopyranose and apiofuranose were linked to the C-4 position of the tetrasubstituted phenyl group and C-6″ position of glucopyranosyl group by oxygen atom, respectively. Thus, compound **2** was established as 2-[(4-hydroxybenzoyl)oxy)]-4,6-dihydroxybenzoic acid-4-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside and named sophophenoside B

All isolated compounds were assayed for their hepatoprotection activity on the cytotoxic effect of D-galactosamine on HL-7702 cells. Compound **1** exhibited a moderate hepatoprotection activity and the cell survival rate was 61% (10^{-5} mol/L), using bicyclol (survival rate: 59%, 10^{-5} mol/L) as a positive control.

3. Experimental

3.1. General experimental procedures

The optical rotations were measured on a Jasco P-2000 polarimeter (Jasco, Easton, Japan). IR spectra were recorded on an IMPACT 400 (KBr) spectrometer (Nicolet, Madison, U.S.A.). UV spectra were recorded on HP 8453 UV-Visible spectrophotometer (Agilent Technologies, CA, U.S.A.). ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), HSQC, and HMBC, spectra were run on an INOVA-500 spectrometer (Bruker-Biospin, Billerica, U.S.A.). High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent Technologies, Waldbronn, Germany). Column chromatography was performed with Macroporous resin (Diaion HP-20; Mitsubishi Chemical Corp. Tokyo, Japan), Rp-18 (50 μm; YMC, Kyoto, Japan), and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden). Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with a SPD-20A detector (Shimadzu, Tokyo, Japan), using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm; YMC, Kyoto, Japan).

3.2. Plant material

The root of *Sophora flavescens* was collected in Weichang, Hebei Province, China, in July 2010. The plant material was identified by associate Prof Lin Ma (Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences). A voucher specimen (ID number: ID-S-2438) was deposited in Department

of Natural Medicinal Chemistry, Institute of Materia Media, Chinese Academy of Medical Sciences, and Peking Union Medical College, Beijing, China.

3.3. Extraction and isolation

Powdered *S. flavescens* (40.0 kg) was extracted with 70% EtOH under reflux (3 × 2 h). The EtOH extract was concentrated under reduced pressure to give a residue (4.0 kg), which was suspended in H_2O (4 L) and partitioned with, consecutively, petroleum ether (PE) (3 × 4 L), EtOAc (3 × 4 L), and n-BuOH (3 × 4 L). After evaporation of the n-BuOH solvent under reduced pressure, the extract (760.0 g) was suspended in H_2O (2 L) and subjected to column chromatography over macroporous resin, eluting successively with H_2O (A), 15% EtOH (B), 30% EtOH (C), 50% EtOH (D), 75% EtOH (E), and 95% EtOH (E, 30 L each). The 50% EtOH was removed under reduced pressure and the fraction (194.0 g) was subjected to chromatography over Sephadex LH-20 with H_2O–MeOH in gradient as the mobile phase to yield 25 fractions (Fr.D1–Fr.D25) on the basis of HPLC-DAD analysis.

Fr.D7 was purified by preparative RP-HPLC using MeOH–H_2O (40:60, 5.0 ml/min, 254 nm) as the mobile phase to yield **1** (11.0 mg, t_R: 35.0). Fr.D8 was subjected to Sephadex LH-20 column and eluted with MeOH–H_2O in gradient as the mobile phase to afford 15 fractions (fr.D-1–15). fr.D-10 was further purified using preparative RP-HPLC with MeOH–H_2O (40:60, 5.0 ml/min, 254 nm) as the mobile phase to yield **2** (9.0 mg, t_R: 37.0).

3.3.1. Sophophenoside A (1)

Yellow powder; $[\alpha]_D^{25}$ −47.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max}: 254, 300 nm; IR ν_{max}: 3456, 2921, 1715, 1663, 1613, 1515, 1444, 1366, 1290, 1088, 1047, 974, 849, 762 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectral data see Table 1; ESI-MS: *m/z* 583.3 $[M - H]^-$; HR-ESI-MS: *m/z* 607.1256 $[M + Na]^+$ (calcd for $C_{25}H_{28}O_{16}Na$, 607.1270).

3.3.2. Sophophenoside B (2)

Yellow powder; $[\alpha]_D^{25}$-64.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max}: 254, 300 nm; IR ν_{max}: 3369, 2892, 1727, 1608, 1514, 1446, 1268, 1166, 1078, 1023, 826, 763, 693 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectral data see Table 1; ESI-MS: *m/z* 583.3 $[M - H]^-$; HR-ESI-MS: *m/z* 607.1294 $[M + Na]^+$ (calcd for $C_{25}H_{28}O_{16}Na$, 607.1270).

3.4. Hepatoprotective effects

Compounds were tested for hepatoprotective effects using an MTT assay in HL-7702 cells [10]. Each cell suspension of 1×10^5 cells in 1 ml of Dulbecco's modified Eagle's medium containing fetal calf serum (10%), penicillin (100 units/ml) and streptomycin (100 μg/ml) was placed in a 96-well microplate and precultured for 24 h at 37 °C under a 5% CO_2 atmosphere. Fresh medium containing bicyclol and the test samples was added, and the cells were cultured for 1 h. The cultured cells were exposed to

25 mM D-galactosamine for 24 h. The medium was then changed to fresh medium containing 0.5 mg/ml MTT. After an incubation of 4 h, the medium was removed, and DMSO was added to dissolve formazan crystals. The optical density (OD) of the formazan solution was measured on a microplate reader at 492 nm. Inhibition (%) was obtained by the following formula:

$$\text{Inhibition}(\%) = [(\text{OD}(\text{sample}) - \text{OD}(\text{control}))/(\text{OD}(\text{normal}) - \text{OD}(\text{control}))] \times 100$$

3.5. Acid hydrolysis of compounds

Compound **1** (2 mg) was dissolved in 1 mol/L HCl–dioxane (1:1, 5 ml) and maintained at 60 °C for 6 h. After drying in a vacuum, the residue was partitioned in H_2O (5 ml) and extracted thrice with EtOAc (5 ml). The aqueous solution was evaporated in vacuum to obtain the monosaccharide residue. These monosaccharide residues were processed using a reported method [11, 12]. Compound **2** was operated using the same method. The configurational assignments of xylose, glucose and apiose were established by comparing the retention times of their chiral derivatives with those of standard substances, which were prepared using the identical procedure (D-xylose 19.1 min, D-glucose 29.4 min, D-apiose 20.3 min).

Disclosure statement

No potential conflict of interest was reported by the authors.

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