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ISOLATION AND STRUCTURE ELUCIDATION OF A NEW FLAVONOL GLYCOSIDE FROM *Sophora japonica*

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*A new flavonol glycoside, quercetin-3-*O*-(4″-galloyl)-α-L-rhamnopyranoside (**1**), along with two known ones, kaempferol-3-*O*-β-D-glucopyranoside (**2**) and quercetin-3-*O*-rutinoside (**3**), were isolated from the root barks of* Sophora japonica. *The chemical structures of the isolated compounds were elucidated by spectroscopic analysis, including extensive 1D and 2D NMR and MS techniques.*

Keywords: *Sophora japonica*, Leguminosae, flavonol glycoside, spectroscopic analysis.

Sophora japonica, a deciduous tree species belonging to the Leguminosae family, is native to eastern Asian countries such as China, Japan, and Korea. The flowers, buds, leaves, and barks of this species have long been used in traditional Chinese medicine as hemostatic agents [1, 2]. *S. japonica* has also been reported to have antifertility and antitumor potential [3]. Previous studies of *S. japonica* fruits, flowers, and leaves led to the isolation of alkaloids, amino acids, fatty acids, flavonoids, phospholipids, polysaccharides, sterols, and triterpenes [4–6]. However, phytochemical investigations of root barks of *S. japonica* have never been carried out to date. In the current paper, we report the isolation, purification, and elucidation of a new flavonol glycoside [quercetin-3-*O*-(4″-galloyl)-*α*-L-rhamnopyranoside (**1**)], together with two known ones [kaempferol-3-*O*-*β*-D-glucopyranoside (**2**) and quercetin-3-*O*-rutinoside (**3**)] from the root barks of this hardwood species.

Compound **1** was obtained as a yellow amorphous powder with optical rotation $[\alpha]_D^{20}$ –93.8° (*c* 0.5; MeOH) and mp 169–171°C. The molecular formula was determined to be $C_{28}H_{24}O_{15}$ from its positive FAB-MS, which exhibited a quasi-molecular ion peak at *m/z* 601 for $[M + H]^+$ and *m/z* 623 for $[M + Na]^+$. The presence of the phenolic hydroxyl group in compound **1** was recognized in the TLC chromogenic reaction by the gray-green color when spraying with 1% ethanolic $FeCl_3$ (R_f values 0.66 and 0.25 in solvents A and B, respectively) [7–9]. The IR spectrum of **1** also showed the absorption of hydroxyl (3410 cm^{-1}), conjugated ketone (C=O, 1680 cm^{-1}), and aromatic double bond (C=C, 1615 cm^{-1}). Its UV spectrum exhibited absorption maxima at 265, 276, 299, and 330 nm.

The ^{13}C NMR spectrum of compound **1** displayed 28 carbon resonances, while the DEPT experiment sorted these signals into one methyl, 12 methines, and 15 quaternary carbons, as shown in Table 1. The ^1H NMR spectrum of **1** (Table 1) revealed a pair of ABX type phenyl protons at δ 7.65 (1H, d, J = 2.2 Hz), 6.92 (1H, d, J = 8.3 Hz), and 7.52 (1H, dd, J = 8.3, 2.2 Hz), which were assignable to H-2′, H-5′, and H-6′, respectively. A set of AB style proton doublets with coupling constant J = 1.9 Hz was recognized at δ 6.23 and 6.41, which were ascribed to H-6 and H-8, respectively. In the ^{13}C NMR spectrum, the flavonol nucleus of **1** was confirmed by signals resonating at δ 159.6 (C-2), 135.3 (C-3), and 179.5 (C-4) [10]. Thus, the aglycone part was identified as quercetin [11].

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TABLE 1. NMR Spectroscopic Data for Compound **1** (MeOH-d_4, δ, ppm, J/Hz)

C atom	δ_H	δ_C	DEPT	C atom	δ_H	δ_C	DEPT
2	–	159.6	qC	Rha			
3	–	135.3	qC	1″	5.31 (1H, br.s)	103.5	CH
4	–	179.5	qC	2″	4.15 (1H, m)	72.2	CH
5	–	163.1	qC	3″	3.53 (1H, m)	70.9	CH
6	6.23 (1H, d, J = 1.9)	100.2	CH	4″	5.08 (1H, dd, J = 10.1, 9.8)	75.2	CH
7	–	166.3	qC	5″	4.36 (1H, m)	69.5	CH
8	6.41 (1H, d, J = 1.9)	95.2	CH	6″	1.02 (3H, d, J = 6.3)	17.8	CH_3
9	–	158.5	qC	Galloyl			
10	–	105.8	qC	1‴	–	121.8	qC
1′	–	123.0	qC	2‴	7.10 (1H, s)	109.9	CH
2′	7.65 (1H, d, J = 2.2)	117.7	CH	3‴	–	146.3	qC
3′	–	145.9	qC	4‴	–	139.8	qC
4′	–	149.5	qC	5‴	–	146.3	qC
5′	6.92 (1H, d, J = 8.3)	116.2	CH	6‴	7.10 (1H, s)	109.9	CH
6′	7.52 (1H, dd, J = 8.3, 2.2)	123.1	CH	7‴	–	168.4	qC

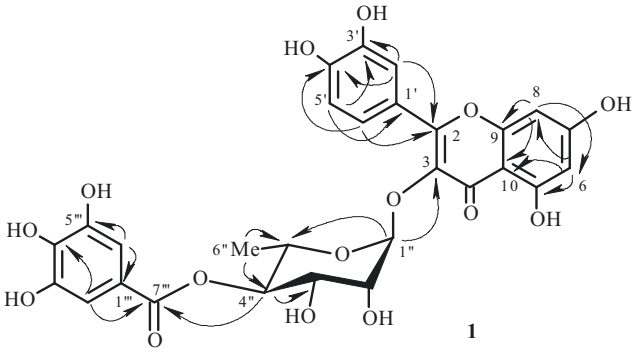

Fig. 1. Key HMBC correlations of compound **1**.

In the ^{1}H NMR spectrum of **1**, the rhamnosyl sugar of α-L-configuration was characterized by its anomeric proton resonating at δ 5.31 (H-1″) as a broad singlet, and its secondary methyl protons distinctively appeared at δ 1.02 (3H, J = 6.3 Hz, H-6″) as a doublet [12]. As shown in Table 1 and Fig. 1, the HMBC spectroscopic correlations of **1** showed interlinks between the rhamnosyl anomeric proton δ 5.31 (1H, br.s, H-1′) and the quaternary carbon δ 135.3 (C-3), which confirmed that the rhamnosyl moiety was attached at C-3 of the quercetin aglycone. From the information above, the presence of quercitrin (quercetin-3-*O*-α-L-rhamnopyranoside) residue was confirmed [13].

The ^{1}H NMR spectrum of **1** also displayed one singlet at δ 7.10 integrated to two protons due to a pair of symmetric galloyl protons (H-2‴ and H-6‴). The ^{13}C NMR spectrum of **1** showed a carboxylic carbon at δ 168.4 (C-7‴). The protonated aromatic carbons C-2‴/C-6‴ and the hydroxy-bearing carbons C-3‴/C-5‴ gave two strong singlets at δ 109.9 and 146.3, respectively, because of the symmetrical skeleton of the galloyl moiety. The linkage of the galloyl moiety to C-4″ of the quercitrin was confirmed by the significant downfield shift of H-4″ to δ 5.08 (approximately Δ+1.96, from δ_H 3.12) and C-4″ to δ 75.2 (approximately Δ+4.0, from δ_C 71.2) when compared with those in quercitrin [13, 14]. In addition, the HMBC correlations of **1** (Fig. 1) showed cross peaks between δ_H 5.08 (H-4″) of the quercitrin and the carboxylic carbon C-7‴ (δ 168.4) of the galloyl group, which further evidenced that the galloyl residue was connected at C-4″ of the quercitrin moiety. According to the above-discussed properties and spectral data, compound **1** was elucidated as quercetin-3-*O*-(4″-galloyl)-α-L-rhamnopyranoside.

The chemical structures of the known flavonol glycosides **2** and 3 were identified by comparison of their spectral data with the literature values as kaempferol-3-*O*-β-D-glucopyranoside [15] and quercetin-3-*O*-rutinoside [16], respectively. Though **2** and **3** have been found in other parts of *S. japonica* previously, this is the first report of compounds **2** and **3** from the roots of this tree.

EXPERIMENTAL

General. FAB-MS (positive mode) spectra were recorded on a Micromass Autospec M363 spectrometer. NMR spectra were recorded in MeOH-d_4 on a Bruker Avance DPX 400 spectrometer with tetramethylsilane as an internal standard (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Melting points (uncorrected) were determined on an Electro Thermal 9100 apparatus. Optical rotations were measured with a Jasco DIP-1000 digital polarimeter. IR spectra were obtained with a PerkinElmer BX FT-IR spectrometer, following the KBr disk method. A Jenway 6405 spectrophotometer was used to record the UV spectra. Fraction eluents were collected with a fraction collector (SBS-160). TLC analysis was carried out on DC-Plastikfolien Cellulose F (Merck) plates and developed with *t*-BuOH–AcOH–H_2O (3:1:1, v/v/v, solvent A) and AcOH–H_2O (3:47, v/v, solvent B). TLC spots were detected by UV light (254 and 365 nm) and by spraying with 1% $FeCl_3$ in EtOH solution followed by heating. All reagents were of analytical grade.

Plant Material. Root barks of *S. japonica* were collected in October 2013 from the campus forest of Tianjin University of Science and Technology, China. Taxonomic identification was done by Prof. Dan Wang, Institute of Chemical Industry of Forest Products, CAF, China. A voucher specimen (No. 131002) has been deposited at the Herbarium of Tianjin Key Laboratory of Pulp and Paper, College of Material Science and Chemical Engineering, Tianjin University of Science and Technology.

Extraction and Isolation. The air-dried and finely powdered root bark of *S. japonica* (6.85 kg) was extracted four times with 70% acetone solution (v/v) at room temperature and filtered. The filtrate was concentrated under reduced pressure and fractionated sequentially using solvents of increasing polarity (*n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH); then each fraction was freeze-dried to obtain dried powders. A portion of the CH_2Cl_2 (13.25 g) fraction was subjected to silica gel column chromatography and eluted with a step gradient solvent systems using CH_2Cl_2–EtOAc (20:1→1:1→1:9, v/v) to give fractions SJC_1, SJC_2, SJC_3, and SJC_4. Fraction SJC_2 (8.02 g) was purified by Sephadex LH-20 column chromatography and eluted with MeOH–H_2O (4:1, 1:2 and 1:5, v/v) and EtOH–hexane (3:1 and 1:2, v/v) repeatedly to give compounds **1** (23 mg) and **2** (40 mg). Fraction SJC_3 (3.16 g) was also separated by Sephadex LH-20 column chromatography with MeOH–H_2O (2:1, 1:3, and 1:6, v/v) to give a yellow amorphous compound **3** (36 mg). The whole purification process was guided and monitored by TLC experiments.

Quercetin-3-*O*-(4″-galloyl)-α-L-rhamnopyranoside (1). Yellow amorphous powder; R_f 0.66 (solvent A) and 0.25 (solvent B); mp 169–171°C; $[\alpha]_D^{20}$ –93.8° (*c* 0.5; MeOH). UV (MeOH, λ_{max}, nm): 265, 276, 299, 330. IR (KBr, ν_{max}, cm^{-1}): 3410 (OH), 1680 (conjugated ketone C=O), 1615 (aromatic C=C). Positive FAB-MS *m/z:* 601 $[M + H]^+$, 623 $[M + Na]^+$ for suggested molecular weight 600 and calculated for $C_{28}H_{24}O_{15}$; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data are summarized in Table 1.

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