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A flavonol tetraglycoside from *Sophora japonica* seeds

Jing-Hua Wang[a,*], Feng-Chang Lou[a], Ya-Lin Wang[b], Yu-Ping Tang[c]

[a]*Department of Phytochemistry, China Pharmaceutical University, Nanjing 210038, China*
[b]*Yunnan Baiyao Pharm. Ltd. Co., Kunming 650032, China*
[c]*Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*

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Abstract

A flavonol tetraglycoside, kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→2)- β-D-glucopyranoside-7-O-α-L rhamnopyranoside, together with nine known compounds were isolated from the seeds of *Sophora japonica* L. Their structures were elucidated on the basis of spectral and chemical evidence.

Keywords: *Sophora japonica*; Leguminosae; Seed; Flavonol glycoside

1. Introduction

Sophora japonica L. (Leguminosae) is widely cultivated in China, whose buds and fruits have been used as a hemostatic agent in traditional Chinese medicine. The flavones from its buds and pericarps were discovered as hemostatic constituents (Ishida et al., 1989; Tang et al., 2001). As chemical constituents of the seeds of this plants, triterpenes, phospholipids, alkaloids, amino acids, polysaccharides and fatty acids have been reported (Grishkovets and Gorbacheva, 1995; Mukhamedova and Glushenkova, 1997). To our knowledge, the structures of flavones in the seeds have not been reported. Our detailed study on the constituents of the seeds of *Sophora japonica* led to the discovery of a new flavonol tetraglycoside (**1**), together with nine known compounds, sophorabioside (**2**), sophoraflavonoloside (**3**), genistein 7,4′-di-*O*-β-D-glucopyranoside (**4**) (Watanabe et al., 1993), 1,6-di-*O*-galloyl-β-D-glucose (**5**) (Kashiwada et al., 1988), isoscutellarein (**6**) (Terashina et al., 1991), sissotrin (**7**), tectoridin (**8**) (Xu et al., 1999), kaempferol 7-*O*-α-L-rhamnopyranoside (**9**) (Liu et al., 1994) and sophororicoside (**10**). The known compounds were demonstrated by direct comparison with authentic samples (co-TLC, IR, MS, NMR) or with reported spectral and physical data. We describe here the isolation and elucidation of the new flavonol tetraglycoside (**1**).

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder and shown to be a kaempferol glycoside since acid hydrolysis afforded kaempferol, D-glucose and L-rhamnose. The NMR spectra of **1** (Table 1) showed the presence of two glucose and two rhamnose moieties from the four anomeric proton signals at δ 5.62 (1H, *d*, *J* = 7.7 Hz, H-1″), 4.58 (1H, *d*, *J* = 7.7 Hz, H-1‴), 4.37 (1H, *br s*, H-1⁗), 5.54 (1H, *br s*, H-1′′′′′) and two methyl groups at δ 1.05 (3H, d, *J* = 6.2 Hz, H-6⁗), 1.15 (3H, d, *J* = 6.1 Hz, H-6′′′′′), and the four anomeric carbon signals at δ 98.47 (C-1″), 104.06 (C-1‴), 99.83 (C-1⁗), 98.52 (C-1′′′′′) and two methyl groups at δ 17.78 (C-6⁗), 17.85 (C-6′′′′′). The molecular formula, $C_{39}H_{50}O_{24}$, was inferred from the HRFAB-MS ($[M–H]^-$ *m/z* 901.2592), and it was supported by the ESI-MS spectrum showing the 903 $[M+H]^+$, 925 $[M+Na]^+$ and 941 $[M+K]^+$ and the ^{13}C NMR spectrum (Table 1). In the ^1H NMR spectrum one rhamnosyl group exhibited an H-1′′′′′ signal at δ 5.54 typical of an attachment at the 7 position (Liu et al., 1994). Furthermore, the signal of H-1′′′′′ showed an HMBC correlation with the signal of C-7 (δ 161.54) in the HMBC spectrum (see Fig. 1). These showed the presence of 7-rhamnosyl residue. The other rhamnosyl group was linked to one glucosyl group at 6‴ position according to the observation of significantly

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* Corresponding author at: The Second Chemistry Examination Department, The Patent Office, State Intellectual Property Office of the People's Republic of China, No. 6 Xi Tu Cheng Road, Haidian District, Beijing 100088, PR China. Tel.: +86-10-62093849.
E-mail address: jh_wang202@hotmail.com (J.-H. Wang).

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shifted signals of C-6‴ (δ 64.75) and to HMBC correlations for the pairs H-1⁗/C-5⁗, H-1⁗/C-6‴, as well as H-6‴/C-1⁗ (see Fig. 1). Similarly, the glucosyl group substituted by the rhamnosyl group was attached to the other glucosyl group at 2″ position according to the observation of significantly shifted signals of C-2″ (δ 82.32) and to HMBC correlations for the pairs H-1‴/C-2″, H-1‴/C-5‴, H-5‴/C-6‴, H-6‴/C-5‴, as well as H-2″/C-1‴ and H-2″/C-1″ (see Fig. 1). Thus, the moiety comprising two glucoses and one rhamnose possesses rhamnosyl (1→6)-glucosyl (1→2)-glucosyl linkage. The interglycosidic linkage of this moiety at the C-3 position of the aglycone was determined from the C-3 signal at δ 133.08, which has been significantly shifted. In addition, the signal at δ 5.62 of H-1″ and the signal at δ 133.08 of C-3 were typical of kaempferol 3-*O*-β-D-glucoside. Thus, compound **1** was established as kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside. All anomeric carbon signals and anomeric proton signals of the four sugars were in accord with this structure.

Table 1
^{1}H and ^{13}C NMR spectral data for compound **5** (in DMSO-d$_6$)[a]

Position	^{1}HNMR ($J_{H,H}$ in Hz)	^{13}CNMR	HMBC (H→C)
2		156.33	
3		133.08	
4		177.53	
5		160.86	
6	6.44 (*d*; 2.0)	99.28	5,7,8,10
7		161.54	
8	6.81 (*d*; 2.0)	94.49	6,7,9,10
9		155.86	
10		105.46	
1′		120.67	
2′	8.11 (*d*; 8.8)	131.05	2,3′, 4′, 6′
3′	6.91 (*d*; 8.8)	115.18	1′, 2′, 4′, 5′
4′		160.03	
5′	6.91 (*d*; 8.8)	115.18	1′, 3′, 4′, 6′
6′	8.11 (*d*; 8.8)	131.05	2, 2′, 4′, 5′
5-OH	12.64 (*s*)		
4′-OH	10.20 (*s*)		
1″	5.62 (*d*; 7.7)	98.47	
2″	3.79 (*m*)	82.32	1″, 1‴, 3″
3″	3.65 (*m*)	76.52	2″, 4″
4″	3.31 (*m*)	68.03	5″
5″	3.37 (*m*)	76.89	4″
6″	3.49 (*m*) 3.64 (*m*)	60.83	4″, 5‴
1‴	4.58 (*d*; 7.7)	104.06	2″, 5‴
2‴	3.09 (*m*)	74.28	1‴, 3‴
3‴	3.17 (*m*)	76.39	2‴, 5‴
4‴	3.49 (*m*)	67.66	3‴
5‴	3.58 (*m*)	73.61	6‴
6‴	3.22 (*m*) 3.57 (*m*)	64.75	5‴, 1⁗
1⁗	4.37 (*br s*)	99.83	6‴, 3⁗, 5⁗
2⁗	3.31 (*m*)	71.59	3⁗, 4⁗
3⁗	3.47 (*m*)	70.21	2⁗, 4⁗
4⁗	3.08 (*m*)	71.91	3⁗, 5⁗, 6⁗
5⁗	3.34 (*m*)	68.14	3⁗, 6⁗
6⁗	1.05 (*d*; 6.2)	17.78	4⁗, 5⁗
1′′′′′	5.54 (*br* s)	98.52	7, 2′′′′′, 3′′′′′, 5′′′′′
2′′′′′	3.87 (*m*)	69.77	1′′′′′, 3′′′′′
3′′′′′	3.64 (*m*)	70.55	2′′′′′
4′′′′′	3.31 (*m*)	70.31	3′′′′′, 5′′′′′
5′′′′′	3.48 (*m*)	70.02	3′′′′′, 4′′′′′, 6′′′′′
6′′′′′	1.15 (*d*; 6.1)	17.85	4′′′′′, 5′′′′′

[a] All assignments were confirmed by HMQC, HMBC and ^{1}H–^{1}H COSY spectra.

3. Experimental

3.1. General

IR spectra were recorded on a Nicolet Impact-410 instrument. ESI-MS spectra were obtained on a VG Quattro mass spectrometer. FAB-MS was obtained on a Jeol JMS DX303HF mass spectrometer. The ^{1}H NMR spectra (400 MHz) and^{13}C NMR spectra (100 MHz) were recorded on a Brucker DRX-400 instrument.

3.2. Plant material

The fruits of *Sophora japonica* L. were collected from mature trees, growing in Nanjing, People's Republic of China, in November 1998, and were identified by Professor Luoshan Xu, China Pharmaceutical University. The seeds were obtained after getting rid of pericarps of the fruits. Their voucher specimen were deposited in the herbarium of China Pharmaceutical University.

3.3. Extraction and isolation

Dried and powdered seeds of *S. japonica* (2.5 kg) were defatted with petroleum ether followed by extracting sequentially with 60 and 95% EtOH in a percolator. The combined EtOH extracts were concd under vacuum until only H_2O remained and the soln partitioned with EtOAc and *n*-BuOH. The *n*-BuOH extract (25 g) was subjected to silica gel CC eluting with $CHCl_3$–MeOH (20:1) followed by stepwise addition of MeOH to give three Frs. (A_1–A_3). Fr. A_1 was applied to a silica gel column which was eluted with $CHCl_3$–MeOH (10:1) to give compounds **2** (105 mg), **3** (76 mg) and **9** (12 mg) after crystallizing from their respective Frs. and recrystallization from MeOH. Fr. A_2 was subjected to silica gel CC using a $CHCl_3$–MeOH (8:1) gradient followed by CC on Sephadex LH-20 with MeOH to yield **5** (5 mg). Fr. A_3 was purified on polyamide CC using a H_2O–EtOH gradient followed by repeated CC on Sephadex LH-20 with MeOH–H_2O–Me_2CO (1:1:2) to afford **1** (15 mg). The EtOAc extract (15 g) was applied to silica gel CC with $CHCl_3$–MeOH (50:1) followed by stepwise addition of MeOH. Compounds **6** (6 mg), **7** (9 mg), **8** (6 mg), **9** (5 mg) and **10** (260 mg) were obtained after crystallizing from their corresponding Frs. and recrystallization from MeOH. TLC was conducted on

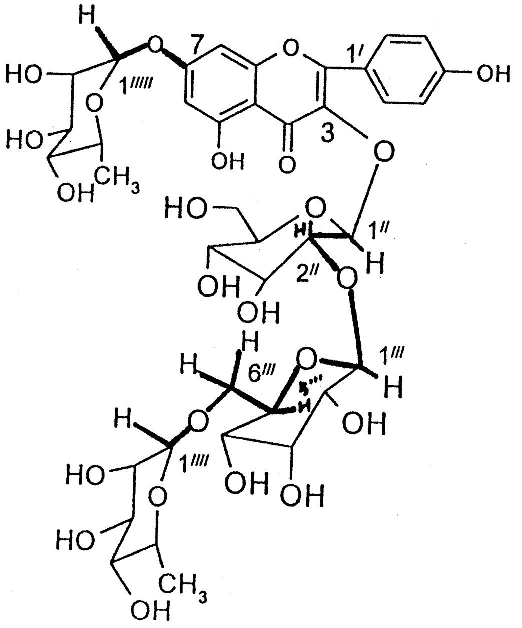

Fig. 1. Structure of **1** with bold lines indicating selected, diagnostic ^1H–^{13}C long-range correlations observed in the HMBC spectrum.

precoated silica gel $60GF_{254}$ plates (Merck) using $CHCl_3$–MeOH–HCOOH (8:1:0.5) and EtOAc–HCOOH–HOAc–H_2O (10:1:1:3), the flavonoid spots were visualized under UV light (365 nm) and by spraying the plates with 2% $AlCl_3$ in EtOH.

3.4. Acid hydrolysis

A solution of each of the flavonol glycosides in 5 ml of 5% HCl was heated for 3 h. Each reaction mixture was extracted with EtOAc. The EtOAc Fr. (aglycone) and the aqueous Fr. (sugar) were concentrated to dryness for identification.

3.5. Kaempferol 3-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside (5)

Yellow amorphous powder. Soluble in H_2O and MeOH, insoluble in $CHCl_3$. UV colour on a silica gel TLC: purple, changing to yellow after spraying with $AlCl_3$. IR (KBr) υ_{max} 3442, 2931, 1653, 1601, 1501, 1490, 1448, 1178, 1074 cm^{-1}. HRFAB-MS *m/z*: 901.2592 $[M-H]^-$ (calc. for $C_{39}H_{49}O_{24}$ 901.2598). ESI-MS *m/z*: 903 $[M+H]^+$(30), 925 $[M+Na]^+$(100), 941 $[M+K]^+$(20). ^1H and ^{13}C NMR spectral data see Table 1.

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