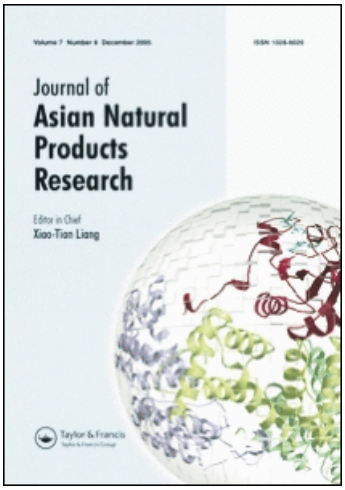
This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new isoflavone triglycosides from the small branches of *Sophora japonica*

YU-PING Tang[a]; HUA-XU Zhu[b]; JIN-AO Duan[a]
[a] Jiangsu Key Laboratory for TCM Formulae Research, Nanjing University of Traditional Chinese Medicine, Nanjing, China [b] Institute of Traditional Chinese Medicine, Nanjing University of Traditional Chinese Medicine, Nanjing, China

To cite this Article Tang, YU-PING , Zhu, HUA-XU and Duan, JIN-AO(2008) 'Two new isoflavone triglycosides from the small branches of *Sophora japonica*', Journal of Asian Natural Products Research, 10: 1, 65 — 70
To link to this Article: DOI: 10.1080/10286020701273858
URL: http://dx.doi.org/10.1080/10286020701273858

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research, Vol. 10, No. 1, January 2008, 65–70

Taylor & Francis
Taylor & Francis Group

Two new isoflavone triglycosides from the small branches of *Sophora japonica*

YU-PING TANG†*, HUA-XU ZHU‡ and JIN-AO DUAN†

†Jiangsu Key Laboratory for TCM Formulae Research, Nanjing University of Traditional Chinese Medicine, Nanjing 210046, China
‡Institute of Traditional Chinese Medicine, Nanjing University of Traditional Chinese Medicine, Nanjing 210029, China

Downloaded At: 19:04 22 January 2011

(Received 28 August 2006; revised 27 December 2006; in final form 14 January 2007)

Two new isoflavone triglycosides, genistein 4′-*O*-(6″-*O*-α-L-rhamnopyranosyl)-β-sophoroside (**1**), and genistein 4′-*O*-(6‴-*O*-α-L-rhamnopyranosyl)-β-sophoroside (**2**), together with five known compounds, namely, sophorabioside, genistin, rutin, quercetin 3-*O*-β-D-glucopyranoside, and kaempferol 3-*O*-β-D-glucopyranoside, were isolated from the small branches of *Sophora japonica* L. Their structures were elucidated on the basis of spectroscopic analyses and chemical evidence.

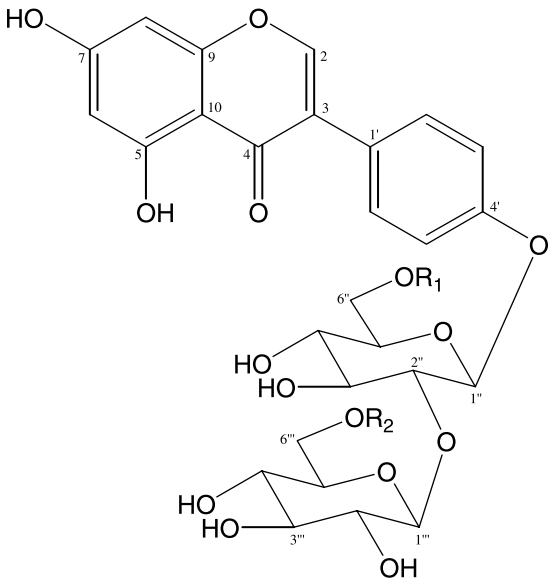
Keywords: *Sophora japonica*; Leguminosae; Branch; Isoflavone; Genistein; Triglycoside

1. Introduction

Sophora japonica L. (leguminosae) is widely distributed throughout China. Its buds and fruits have been used as a hemostatic agent in traditional Chinese medicine, and flavonoids were discovered as hemostatic constituents from the buds of *S. japonica* [1]. Triterpenes, phospholipids, alkaloids, amino acids, polysaccharides, and fatty acids have been reported from its seeds [2,3]. Recently we systematically investigated the chemical constituents in the pericarps [4,5] and seeds [6] of *S. japonica*, and isolated several compounds, including flavonol, isoflavone, and their glycosides. To our knowledge, no phytochemical investigation on the branches of this species has been reported. In the present study, the 95% aqueous ethanolic extract of the small branches of *S. japonica* was separated by repeated column chromatography to give seven flavonoid glycosides, namely, genistein 4′-*O*-(6″-*O*-α-L-rhamnopyranosyl)-β-sophoroside (**1**), genistein 4′-*O*-(6‴-*O*-α-L-rhamnopyranosyl)-β-sophoroside (**2**), sophorabioside, genistin, rutin, quercetin 3-*O*-β-D-glucopyranoside and kaempferol 3-*O*-β-D-glucopyranoside. Compounds **1** and **2** (figure 1) are new isoflavone triglycosides. Here, we report the isolation and structure elucidation of **1** and **2**.

*Corresponding author. Email: yupingtang@njutcm.edu.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2008 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020701273858



1 R_1 = rha R_2 = H
2 R_1 = H R_2 = rha

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

Downloaded At: 19:04 22 January 2011

2. Results and discussion

Compounds **1** and **2** were obtained from the *n*-BuOH-soluble part of a 95% aqueous ethanolic extract of the small branches of *S. japonica*. Compound **1** obtained as a colourless amorphous powder, whose molecular formula, $C_{33}H_{39}O_{19}$, was inferred from HRFAB-MS at *m/z* 739.2098 $[M - H]^-$, and it was supported by ^{13}C NMR and DEPT spectra, which showed 31 resonance signals including one methyl, two methylenes, 20 methines, and eight quaternary carbons. The IR spectrum of compound **1** showed strong absorption bands at 3418 (OH), 1652 (α,β-unsaturated C═O), 1612, 1582, 1496 (C═O, aromatic), and a broad band at 1160–1000 cm^{-1}, indicating its glycosidic nature. The presence of a singlet at δ 8.38, the typical proton signal of H-2, in the ^1H NMR spectrum and UV absorption maximum at 261 nm suggested that it was an isoflavonoid [5,7]. Upon acid hydrolysis of **1**, genistein, glucose, and rhamnose were identified by comparing with the authentic samples on the TLC. Genistein was also identified by comparison of the UV and ^1H NMR spectral data with those in the literature [8]. ^1H NMR and ^{13}C NMR spectra of **1** showed the presence of a genistein moiety and three sugar residues [5,7,9].

A methyl doublet, which appeared at δ 1.20 in the ^1H NMR spectrum of **1**, was assigned to the C-6 methyl protons of a rhamnose residue. All ^1H NMR and ^{13}C NMR signals of the rhamnosyl moiety could be assigned on the basis of 2D NMR spectral data (table 1). A TOCSY experiment showed a correlation between the C-6 methyl protons of rhamnose residue and the anomeric proton at δ 4.34, demonstrating that they belonged to the same spin system. The anomeric proton of the rhamnosyl residue showed a HMBC correlation with a carbon signal at δ 66.0, which was assigned to C-6″ of the first glucosyl moiety. Therefore,

Table 1. ^{1}H NMR and ^{13}C NMR spectroscopic assignments (δ/ppm) for compounds **1** and **2** in DMSO-d_6[†].

	1		***2***	
Position	^{13}C	^{1}H	^{13}C	^{1}H
2	154.7 d	8.38 (s)	154.6 d	8.39 (s)
3	124.5 s		124.4 s	
4	180.3 s		180.3 s	
5	162.2 s		162.2 s	
6	99.3 d	6.23 (d, 1.8)	99.3 d	6.24 (d, 2.0)
7	164.6 s		164.5 s	
8	94.1 d	6.40 (d, 1.8)	94.0 d	6.41 (d, 2.0)
9	157.9 s		157.8 s	
10	104.9 s		104.8 s	
1′	122.3 s		122.2 s	
2′,6′	130.4 d	7.49 (d, 8.2)	130.3 d	7.49 (d, 8.4)
3′,5′	116.0 d	7.06 (d, 8.2)	115.9 d	7.08 (d, 8.4)
4′	157.3 s		157.2 s	
5-OH		12.91 (s)		12.91 (s)
7-OH		10.90 (s)		10.89 (s)
1″	98.2 d	5.54 (d, 7.0)	98.3 d	5.54 (d, 7.0)
2″	82.2 d	3.53 (m)	82.2 d	3.56 (m)
3″	76.4 d	3.56 (m)	76.5 d	3.57 (m)
4″	69.8 d	3.18 (m)	69.7 d	3.25 (m)
5″	75.6 d	3.20 (m)	76.9 d	3.26 (m)
6″	66.0 t	3.34 (m), 3.19 (m)	60.9 t	3.49 (m), 3.62 (m)
1‴	104.0 d	4.65 (d, 7.6)	104.0 d	4.58 (d, 7.7)
2‴	74.5 d	3.14 (m)	74.3 d	3.08 (m)
3‴	76.6 d	3.24 (m)	76.4 d	3.18 (m)
4‴	70.1 d	3.20 (m)	69.5 d	3.30 (m)
5‴	76.9 d	3.17 (m)	75.3 d	3.39 (m)
6‴	60.8 t	3.55 (m), 3.64 (m)	66.1 t	3.22 (m), 3.59 (m)
1⁗	100.3 d	4.34 (brs)	100.3 d	4.35 (brs)
2⁗	70.2 d	3.37 (m)	70.6 d	3.27 (m)
3⁗	69.9 d	3.69 (m)	70.2 d	3.42 (m)
4⁗	71.7 d	3.21 (m)	71.8 d	3.08 (m)
5⁗	68.2 d	3.82 (m)	68.1 d	3.28 (m)
6⁗	17.8 q	1.20 (d, 6.2)	17.7 q	1.01 (d, 6.2)

[†] 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR.
Carbon multiplicities were determined by DEPT experiments. S = quaternary, d = methane, t = methylene, q = methyl carbons.

Downloaded At: 19:04 22 January 2011

glycosylation of the first glucose at the C-6″ position became evident. A TOCSY experiment showed a correlation between the two C-6 protons (δ 3.19, 3.34) of the first glucosyl moiety and the anomeric proton at δ 5.54, demonstrating that they belonged to the same spin system. A carbon signal at δ 157.3 was assigned to C-4′, on the basis of its long-range ^{13}C—^{1}H correlations observed in the HMBC experiment with the two proton signals at δ 7.06 (H-3′,5′) and δ 7.49 (H-2′,6′). And the C-4′ showed a three-bond correlation with the anomeric proton of a first glucosyl unit at δ 5.54. The anomeric proton of the second glucosyl residue at δ 4.65 showed a long-range correlation with a carbon signal at δ 82.2, corresponding to a proton at δ 3.53 in the HMQC spectrum. The latter showed a ^{1}H—^{1}H correlation, observed in the DQF-COSY experiment, with the anomeric proton of the first glucosyl moiety at δ 5.54. Therefore, glycosylation of the first glucosyl moiety at the C-2″ position was confirmed. 2D NMR allowed the assignment of all ^{1}H NMR and ^{13}C NMR signals of the first and second glucosyl moieties (table 1). The β-configuration of two anomeric carbons was deduced from the coupling constant of H-1″ ($J = 7.0$ Hz) and H-1‴ ($J = 7.6$ Hz) observed in the ^{1}H NMR spectrum [10]. These showed the presence of flavonoid 4′-*O*-(6″-*O*-α-L-rhamnopyranosyl)-

β-sophorosyl residue in compound **1**. Therefore, compound **1** was characterised as genistein 4′-*O*-(6″-*O*-α-L-rhamnopyranosyl)-β-sophoroside.

Compound **2** was obtained as a colourless amorphous powder. The molecular formula, $C_{33}H_{40}O_{19}$, was established from HRFAB-MS at *m/z* 739.2105 $[M - H]^-$, consistent with ^{13}C NMR and DEPT spectra. The UV, IR, and NMR spectra of **2** suggested the existence of a genistein 4′-*O*-β-sophoroside unit, as found in **1** (table 1). The difference between compounds **1** and **2** was the linkage position of the rhamnose residue. The anomeric proton of the rhamnosyl residue at δ 4.35 showed a HMBC correlation with a carbon signal at δ 66.1, corresponding to two protons at δ 3.22 and δ 3.59 in the HMQC spectrum, and the carbon signal at δ 66.1 showed a triplet in the DEPT spectrum, which indicated the ^{13}C NMR signal at δ 66.1 was 6-position carbon signal of a glucosyl residue, and the rhamnosyl residue was linked to 6-position of the glucose unit. A TOCSY experiment showed a correlation between the H-6 signal of the glucose and the anomeric proton of the outer glucosyl moiety at δ 4.58, demonstrating that the rhamnosyl moiety was linked to 6-position of the outer glucose unit. In this way, a 4′-*O*-(6‴-*O*-α-L-rhamnopyranosyl)-β-sophoroside could be characterised unambiguously. Thus, compound **2** was identified as genistein 4′-*O*-(6‴-*O*-α-L-rhamnopyranosyl)-β-sophoroside.

Two of the known compounds were identified by comparing their physical and spectral data with literature values [7,9], namely, sophorabioside, and genistin. In addition, the other three known substances were identified by comparison of spectral data with those of authentic samples: rutin, quercetin 3-*O*-β-D-glucopyranoside, and kaempferol 3-*O*-β-D-glucopyranoside. And all of five compounds were previously isolated from fruits of *S. japonica* [5].

Downloaded At: 19:04 22 January 2011

3. Experimental

3.1 General experimental procedures

Melting points were determined on an Electrothermal 9200 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer model 241 polarimeter. UV and IR spectra were measured on a Shimadzu UV-1601 instrument and on a Perkin–Elmer 983 spectrometer, respectively. All NMR spectra were run on a Bruker DRX-500 instrument operating at 500 MHz for ^1H and 125 MHz for ^{13}C, using standard pulse sequences. Chemical shifts are reported on the δ scale in parts per million, downfield from TMS. Carbon multiplicities were determined from DEPT-135 and DEPT-90 experiments. All 2D NMR spectra were recorded using pulsed field gradients. $^1H—^1H$ correlations were observed in double quantum filtered (DQF) COSY and TOCSY experiments. One-bond $^{13}C—^1H$ correlations were observed in a HMQC experiment. Long-range $^{13}C—^1H$ correlations were observed in a HMBC experiment. FAB-MS were obtained on a JEOL JMS DX-303HF mass spectrometer.

Column chromatography was performed on Si gel (Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia), and RP-18 (Shimadzu). TLC was carried out on precoated Si gel 60 F_{254} plates (Merck), developed with EtOAc/HOAc/HCOOH/H_2O (30:0.9:1.1:8, upper phase, and 10:1:1:2), *n*-BuOH/HOAc/H_2O (4:1:5, upper phase), and for sugars EtOAc/HOAc/CH_3OH/H_2O (13:4:3:3). For visualisation, 1% methanolic $AlCl_3$ was

used for the isoflavonoids, and thymol solution (0.5 g thymol in 95 ml of ethanol and 5 ml of H_2SO_4) was used for the sugars (plates were heated to 120°C for 15–20 min).

3.2 Plant material

Small branches of *S. japonica* L. were collected from mature trees, growing in Nanjing, China, in 2000, and were identified by Professor Luoshan Xu (China Pharmaceutical University). A voucher specimen (No. CPUT-001128) is deposited in the herbarium of China Pharmaceutical University.

3.3 Extraction and isolation

Downloaded At: 19:04 22 January 2011

Dried and powdered small branches of *S. japonica* (about 20.0 kg) were extracted three times with 95% ethanol using an ultrasonic apparatus for 3 h each time, with the solvent removed under reduced pressure, and the residue (about 530 g) dissolved in hot water. This residue was left in the refrigerator overnight and filtered. The filtrate was partitioned with CH_2Cl_2, EtOAc, and *n*-BuOH, successively. The *n*-BuOH-soluble fraction (96 g) was concentrated and subjected to Si gel column chromatography eluting with CH_2Cl_2/CH_3OH (20:1) followed by stepwise addition of CH_3OH to yield 14 fractions. Fraction 9 (16.5 g) was subjected to Si gel (CH_2Cl_2/CH_3OH, 10:3) and Sephadex LH-20 (CH_3OH) chromatography and purified by HPLC (RP_{18}, 4 μm, 260 nm, CH_3OH/H_2O (0.5% acetic acid), 18:82; **1**, $t_R = 10.75$ min; **2**, $t_R = 11.76$ min) to give compounds **1** (11 mg) and **2** (10 mg), respectively. Fraction 7 (23.1 g) was subjected to Si gel column chromatography (CH_2Cl_2/CH_3OH, 10:2) to give sophorabioside (5.1 g) and rutin (6.2 g). Fraction 6 (5.6 g) was subjected to Si gel (CH_2Cl_2/CH_3OH, 10:2) column chromatography, to give genistin (54 mg), quercetin 3-*O*-β-D-glucopyranoside (14 mg), and kaempferol 3-*O*-β-D-glucopyranoside (18 mg).

3.3.1 Genistein 4′-O-(6″-O-α-L-rhamnopyranosyl)-β-sophoroside (1). Colourless amorphous powder; mp 248–249°C; $[\alpha]_D^{25}$ −45 (*c* 0.006, DMSO); UV (CH_3OH) λ_{max} 261, 325 nm; IR ν_{max}^{KBr} 3418, 2977, 2929, 1652, 1612, 1582, 1512, 1496, 1444, 1383, 1366, 1243, 1182, 1160–1000 cm^{-1}; ^1H NMR and ^{13}C NMR (DMSO-d_6) spectral data: see table 1; HRFAB-MS *m/z* 739.2098 $[M - H]^-$ (calcd for $C_{33}H_{39}O_{19}$, 739.2086).

3.3.2 Genistein 4′-O-(6‴-O-α-L-rhamnopyranosyl)-β-sophoroside (2). Colourless amorphous powder; mp 245–246°C; $[\alpha]_D^{25}$ −67 (*c* 0.004, DMSO); UV (CH_3OH) λ_{max} 261, 324 nm; IR ν_{max}^{KBr} 3419, 2976, 2929, 1652, 1612, 1582, 1512, 1496, 1443, 1382, 1367, 1246, 1180, 1160–1000 cm^{-1}; ^1H NMR and ^{13}C NMR (DMSO-d_6) spectral data: see table 1; HRFAB-MS *m/z* 739.2105 $[M - H]^-$(calcd for $C_{33}H_{39}O_{19}$, 739.2086).

3.4 Acid hydrolysis

For acid hydrolysis, a solution of each compound (**1** and **2**) in 5 ml of 6% HCl was heated for 3 h. Each reaction mixture was extracted with EtOAc. The EtOAc fraction (aglycon) and the aqueous fraction (sugars) were concentrated to dryness for identification.

Acknowledgements

The NMR and FAB-MS spectra were provided by Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. We are also pleased to thank Prof. Luoshan Xu (Division of Pharmacognosy, China Pharmaceutical University) for the plant identification.

References

[1] H. Ishida, T. Umino, K. Tsuji, T. Kosuge. *Chem. Pharm. Bull.*, **37**, 1616 (1989).
[2] K.H.S. Mukhamedova, A.I. Glushenkova. *Chem. Nat. Compd.*, **33**, 445 (1997).
[3] V.I. Grishkovets, L.A. Gorbacheva. *Chem. Nat. Compd.*, **31**, 596 (1995).
[4] Y. Tang, J. Hu, J. Wang, F. Lou. *J. Asian Nat. Prod. Res.*, **4**, 1 (2002).
[5] Y. Tang, F. Lou, J. Wang, S. Zhuang. *J. Nat. Prod.*, **64**, 1107 (2001).
[6] J. Wang, F. Lou, Y. Wang, Y. Tang. *Phytochemistry*, **63**, 463 (2003).
[7] K. Watanabe, J. Kinjo, T. Nohara. *Chem. Pharm. Bull.*, **41**, 394 (1993).
[8] W.Z. Huang, J.A. Duan, Z.L. Li. *Zhongguo yaoke daxue xuebao*, **31**, 8 (2000).
[9] K.R. Markham, B. Terlai, R. Stanley, H. Geiger, T.L. Mabry. *Tetrahedron*, **34**, 1389 (1978).
[10] K.R. Markham, H. Geiger. *The Flavonoids: Advances in Research Since 1986*, J.B. Harborne (Ed.), pp. 441–497, Chapman & Hall, London (1994).

Downloaded At: 19:04 22 January 2011