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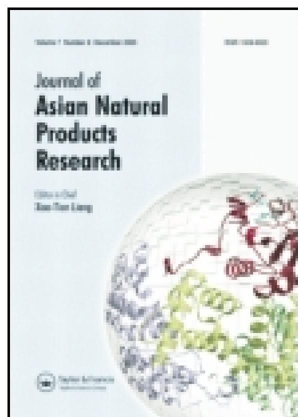

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Isoflavonoid glycosides from the flowers of *Pueraria lobata*

Ying-Lu Yu[ab], Yi-Ting Liao[c], Xian Li[a], Yang Ye[b], Chang-Qiang Ke[b], Xi-Qiang Li[b], Xin-Zhou Yang[b] and Mei-Cun Yao[c]*

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Pueraria lobata flower is a medicinal herb for treating intoxication, hepatic, and gastrointestinal tract lesions induced by alcohol. This study aims to investigate the isoflavonoid glycosides in *P. lobata* flowers. Two new isoflavone compounds were isolated from the extract of *P. lobata* flowers. Their structures were determined to be 5,6,7,4′-tetrahydroxyisoflavone-6,7-di-*O*-β-D-glucopyranoside and 5,6,7-trihydroxy-4′-methoxyisoflavone-6,7-di-*O*-β-D-glucopyranoside on the basis of spectroscopic means including HR-ESI-MS, UV, IR, ^{1}H, and ^{13}C NMR.

Keywords: *Pueraria lobata*; Leguminosae; isoflavonoid; 5,6,7,4′-tetrahydroxy isoflavone-6,7-di-*O*-β-D-glucopyranoside; 5,6,7-trihydroxy-4′-methoxyisoflavone-6,7-di-*O*-β-D-glucopyranoside

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1. Introduction

Pueraria lobata (Leguminosae) is a perennial leguminous vine endemic to eastern Asia. It is one of the oldest medicinal plants used in traditional Chinese medicine. Traditionally, this species has been used as Semen *Puerariae*, Radix *Puerariae*, and Flos *Puerariae* for more than 1000 years. *P. lobata* flowers have been widely employed to relieve fever and dysentery, promote the production of body fluid, lessen stiffness and pain, and treat alcohol abuse and rectal ulcers [1–4]. Modern pharmacological studies have demonstrated that *P. lobata* flowers can reduce ethanol absorption in the gastrointestinal tract [5,6], and have antidiabetic [7], antistress [8], antiviral [9], and antioxidant [10] properties. Most of pharmacological effects of *P. lobata* flower were attributed to its isoflavone components [5–9].

The chemical components of *P. lobata* have been widely investigated, and flavonoids and triterpenoids were the main secondary metabolites. Previous studies have led to the isolation of many isoflavone glycosides [11–13], flavonol glycosides and oleanane-type triterpene glycosides [11], as well as a tryptophan derivative [11]. The ingredients of *P. lobata* flowers included irisolidone, genistein, daidzein, glycitein, glycitin, 6″-*O*-xylosyl-tectoridin, 6″-*O*-xylosylglycitin, tectorigenin, tectoridin, kakkalide, kakkatin, kaikasaponon III, soyasaponin I, soyasaponin b, soyasaponin Ab, glycyrrhizin, rutin, biocanin A, ononin, β-sitosterol, robinin, nicotiflorin, and quercetin [14]. The present study reports the

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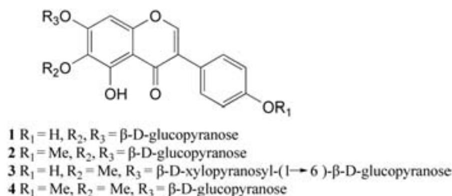

Figure 1. Structures of compounds **1**–**4**.

isolation and characterization of two new isoflavonoids from *P. lobata* (Figure 1).

2. Results and discussion

Compound **1** was isolated as yellow needle crystals with the molecular formula $C_{27}H_{30}O_{16}$, as determined by a quasi-molecular ion $[M + Na]^+$ at *m/z* 633.1456 generated from HR-ESI-MS. The UV spectrum showed absorption maxima at 219, 271, and 333 nm. The IR spectrum displayed strong absorption bands at 3407, 1658, 1614, 1515, and 1457 cm^{-1}, suggesting the presence of an isoflavonoid moiety [15]. The ^1H NMR spectrum displayed signals of a downfield proton at δ_H 13.51 assigned to a hydrogen-bonded hydroxyl group at C-5, an isolated aromatic proton at δ_H 8.44 assignable to H-2, two pairs of aromatic protons at δ_H 7.39 (1H, d, $J = 8.4$ Hz) and 6.83 (1H, d, $J = 8.4$ Hz) due to H-2′,6′ and H-3′,5′ as an $AA'XX'$ system, almost identical with those of 6-hydroxygenistein [16]. Complete acid hydrolysis with HCl yielded D-glucose by the GC analysis of its leucine derivative, which was compared with the standard compound [17]. The signals of two anomeric protons appeared at δ_H 5.01 (d, $J = 7.3$ Hz) and 4.87 (d, $J = 7.4$ Hz), indicating β-D-configuration for the glucosyl units. Their positions were determined by the HMBC long-range correlations (Figure 2). The HMBC correlation between an anomeric proton at δ_H 4.87 (d, $J = 7.4$ Hz) and C-6 indicated that one β-D-glucose unit was attached to C-6. Furthermore, the HMBC correlation of another anomeric proton

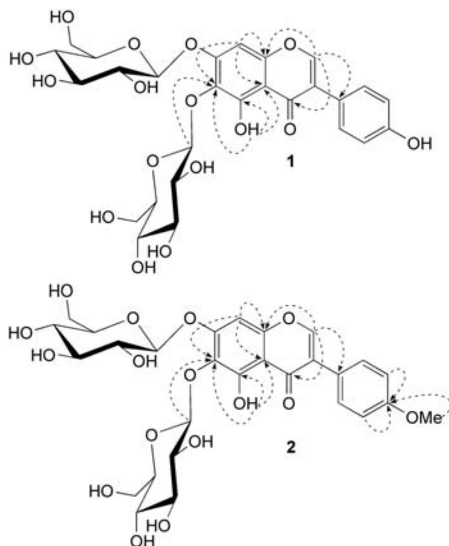

Figure 2. Key HMBC correlations of compounds **1** and **2**.

at δ_H 5.01 (d, $J = 7.3$ Hz) and C-7 indicated that the other β-D-glucose unit was attached to C-7. Therefore, the structure of **1** was elucidated as 5,6,7,4′-tetrahydroxyisoflavone-6,7-di-*O*-β-D-glucopyranoside.

Compound **2** was also isolated as yellow needle crystals with the molecular formula $C_{28}H_{32}O_{16}$, as determined by a quasi-molecular ion $[M + Na]^+$ at *m/z* 647.1603 in the HR-ESI-MS spectrum. Comparison of its ^1H and ^{13}C NMR spectral data with those obtained for **1** (Table 1) revealed the structural analogy of these two compounds. The only difference observed was the presence of an additional methoxyl group at C-4′ in **2** instead of a free hydroxyl group in **1**, which was further confirmed by the HMBC correlations of H-3′, H-5′ and the methoxyl group at δ_H 3.79 with the oxygenated carbon signal at δ_C 159.3 (Figure 2). Thus, the structure of **2** was elucidated as 5,6,7-trihydroxy-4′-methoxyisoflavone-6,7-di-*O*-β-D-glucopyranoside.

By comparison with the previously published data, the known compounds **3** and **4** were identified as 4′,5,7-trihydroxy-

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Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** in DMSO-d_6 (δ in ppm, J in Hz).

	1		**2**	
	δ(H)[a]	δ(C)[b]	δ(H)[a]	δ(C)[b]
1				
2	8.44 (1H, s)	154.8	8.48 (1H, s)	155.1
3		122.4		122.8
4		180.8		180.7
4a		106.7		106.7
5		152.9		152.9
6		129.1		129.2
7		156.3		156.3
8	6.93 (1H, s)	94.6	6.95 (1H, s)	94.6
8a		152.9		152.9
1′		121.2		122.1
2′	7.39 (1H, d, $J = 8.4$)	130.3	7.52 (1H, d, $J = 7.8$)	130.3
3′	6.83 (1H, d, $J = 8.4$)	115.1	7.02 (1H, d, $J = 7.8$)	113.9
4′		157.4		159.3
5′	6.83 (1H, d, $J = 8.4$)	115.1	7.02 (1H, d, $J = 7.8$)	113.9
6′	7.39 (1H, d, $J = 8.4$)	130.3	7.52 (1H, d, $J = 7.8$)	130.3
4′-OMe			3.79 (3H, s)	55.3
5-OH	13.51 (1H, s)			
Glc (C-6)				
1	4.87 (1H, d, $J = 7.4$)	101.1	4.88 (1H, d, $J = 7.4$)	101.1
2[c]	–	73.3	–	73.3
3	3.05–3.45 (m)	75.8	3.02–3.45 (m)	75.8
4	3.05–3.45 (m)	69.7	3.02–3.45 (m)	69.7
5	3.05–3.45 (m)	77.2	3.02–3.45 (m)	77.2
6[c]	3.71 (1H, br d, $J = 12.0$) –	60.8	3.59 (1H, br d, $J = 11.6$)	60.8
Glc (C-7)				
1	5.01 (1H, d, $J = 7.3$)	103.4	5.02 (1H, d, $J = 7.4$)	103.4
2[c]	–	74.1	–	74.1
3	3.05–3.45 (m)	76.2	3.02–3.45 (m)	76.3
4	3.05–3.45 (m)	69.7	3.02–3.45 (m)	69.7
5	3.05–3.45 (m)	77.4	3.02–3.45 (m)	77.4
6[c]	3.76 (1H, br d, $J = 11.6$) –	60.8	3.74 (1H, br d, $J = 12.1$) –	60.8

Notes: [a]At 400 MHz.
[b]At 100 MHz.
[c]Hidden under water peak.

6-methoxyisoflavone 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside [18] and tectoridine [13], respectively.

The present study reports the isolation of two new isoflavone glycosides, 5,6,7,4′-tetrahydroxyisoflavone-6,7-di-*O*-β-D-glucopyranoside (**1**) and 5,6,7-trihydroxy-4′-methoxyisoflavone-6,7-di-*O*-β-D-glucopyranoside (**2**), together with two known ones, 4′,5,7-trihydroxy-6-methoxyisoflavone-7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**3**) and tectoridine (**4**), from the flowers of *P. lobata*. All of these compounds, except for tectoridine (**4**), are reported from this plant for the first time.

The genus *Pueraria* comprises about seven species in China, in which *P. lobata*, *P. thomsonii* Benth., and *P. thunbergiana* have been widely investigated on the chemical constituents. It was reported that HPLC profiles for flowers extract of *P. lobata* and *P. thomsonii* Benth. had no

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significant differences [9]. The main isoflavones of *P. thomsonii* Benth. flowers are tectorigenin-7-*O*-xylosylglucoside and tectoridin, but that of *P. lobata* is kakkalide [10]. The substances of these two kinds of flowers undergo a certain degree of change depending on the storage period [11]. Other previous studies showed that *P. lobata* and *P. thunbergiana* comprised very similar chemical constituents: puerarin, formononetin, daidzin, daidzein, coumestrol, genistein-8-C-apiosyl (1 → 6) glucoside and daidzein-4,7-diglucoside in the roots [19]; irisolidone, kakkalidone, kakkalide, genistein, tectoridine, irisolidone 7-*O*-β-D-glucopyranoside, and 4′,5,7-trihydroxy-6-methoxyisoflavone-7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside in the flowers [18]. The close analog in the chemical constituents of *P. lobata* and *P. thunbergiana* suggested that these two species may be related chemosystematically. Moreover, the occurrence of 4′,5,6,7-tetrasubstituted isoflavone glycoside rich in the flowers of *P. lobata* and *P. thunbergiana* is more interesting, since isoflavonoids with such a substituted pattern are less common in *Pueraria* and may be useful as chemotaxonomic markers in the genus.

3. Experimental

3.1 General experimental procedures

All melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer polarimeter 341. CD spectra were recorded on a JASCO J-720W spectrophotometer. UV spectra were detected on a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were determined on a Nicolet Magna FT-IR 750 spectrometer (ν_{max} in cm^{-1}). NMR spectra were recorded on a Bruker AM 400 MHz NMR spectrometer. Samples were dissolved in DMSO-d_6 (residual ^1H 2.50 and ^{13}C 39.5 ppm). The chemical shifts were given on δ (ppm) scale with tetramethylsilane as an internal standard, and coupling constants (J) were in Hz. The ESI-MS and HR-ESI-MS data were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer. Preparative and semi-preparative HPLC system: two PrepStar SD-1 solvent delivery modules, a ProStar UV–vis 320 detector and a ProStar 701 Fraction Collector (Varian, Walnut Creek, CA, USA); a LiChrospher 100 RP-18 (Merck, Darmstadt, Germany) column (250 × 22 mm i.d., 5 μm) was used for preparative isolation. Gas chromatography: Shimadzu GC 14-BPF apparatus equipped with a 5% OV225/AW-DMCS-Chromosorb W (80–100 mesh) column (2.5 m × 3 mm) and a hydrogen-flame ionization detector.

3.2 Plant material

P. lobata flowers in the present research were purchased from Qingping Herbal Market in Guangzhou, Guangdong Province, China, in September 2006, and identified by Prof. Jin-Gui Shen of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (20060013B) has been deposited in the Herbarium of the institute.

3.3 Extraction and isolation

The air-dried *P. lobata* flowers (50 g) were extracted with 75% EtOH (200 ml ×3) at room temperature. After the evaporation of the solvent under reduced pressure, the residue was dissolved in water (10 ml) and then subjected to column chromatography (CC) (10.0 cm × 40 cm, D101 resin, 150 g). First, 500 ml of water was used to remove hydrosoluble pigments and saccharides, and then 1500 ml of 60% ethanol to elute the resin. The ethanol elute was collected and evaporated to dryness to obtain an extract. The extract was subjected to CC (5.0 cm × 30 cm, MCI gel, 100 ml) and eluted in turn with 20, 40, 60, 80, and 100% EtOH aqueous solution

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(each 500 ml) to afford subfractions 1.1–1.5. Subfraction 1.1 (225 mg) was evaporated to dryness and then resolved in methanol. Compound **1** (100 mg) was crystallized from the methanol solution. Subfraction 1.2 (261 mg) was also evaporated to dryness and then dissolved in methanol. Compound **2** (87 mg) was obtained also by crystallization. The subfraction 1.4 (325 mg) was subjected to preparative HPLC (H_2O: CH_3CN 95: 5 → 65: 35, 150 min, linear gradient) to yield compounds **3** (56 mg) and **4** (78 mg).

*3.3.1 5,6,7,4′-Tetrahydroxyisoflavone-6,7-di-O-β-D-glucopyranoside (**1**)*

Yellow needle crystals, mp 232–233°C, $[\alpha]_D^{20}$ −48.0 ($c = 0.5$, methanol); UV (MeOH) λ_{max} (log ε): 219 (4.21), 271 (3.81), 333 (1.72) nm; IR (KBr) ν_{max} (cm^{-1}): 3407 (OH), 1658 (C=O), 1515 and 1457 (aromatic C=C), 1269 (C—O), 1100–1000 (glycoside); ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) spectral data see Table 1. HR-ESI-MS *m/z*: 633.1456 $[M + Na]^+$ (calcd for $C_{27}H_{30}O_{16}Na$, 633.1432).

*3.3.2 5,6,7-Trihydroxy-4′-methoxy isoflavone-6,7-di-O-β-D-glucopyranoside (**2**)*

Yellow needle crystals, 87 mg, mp 205–207°C, $[\alpha]_D^{20}$ −38.0 ($c = 0.5$, methanol); UV (MeOH) λ_{max} (log ε): 221 (4.19), 277 (3.90), 329 (1.71) nm; IR (KBr) ν_{max} (cm^{-1}): 3398 (OH), 1656 (C=O), 1515 and 1459 (aromatic C=C), 1292 and 1272 (C—O), 1100–1000 (glycoside); ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) spectral data, see Table 1; HR-ESI-MS *m/z*: 647.1603 $[M + Na]^+$ (calcd for $C_{28}H_{32}O_{16}Na$, 647.1588).

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