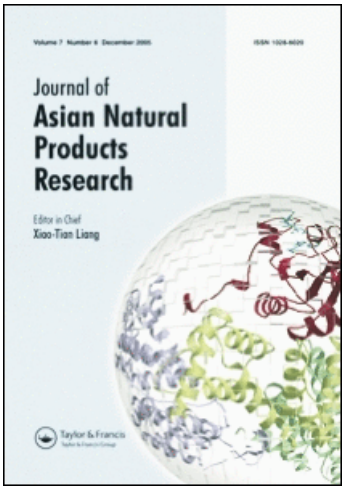

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Two new isoflavone glycosides from *Pueraria lobata*

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Two new isoflavone diglycosides, formononetin 8-*C*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (**1**) and formononetin 8-*C*-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranoside (**2**), were isolated from the roots of *Pueraria lobata*, together with four known compounds, 4′-methoxypuerarin (**3**), daidzin (**4**), genistin (**5**), and daidzein (**6**). The structures of these compounds were elucidated by the spectroscopic methods.

Keywords: *Pueraria lobata*; Leguminosae; isoflavone glycosides; formononetin 8-*C*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside; formononetin 8-*C*-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranoside

1. Introduction

Pueraria lobata (Willd.) ohwi (Leguminosae) is a perennial plant native to eastern Asia. Its root is an important Chinese traditional medicine as an antipyretic, antidiarrhetic, diaphoretic, and antiemetic agent [1–4]. Previous phytochemical investigations on this plant have led to the isolation of various isoflavones [5], aromatic glycosides [6], and saponins [7–9]. In our recent phytochemical research for this plant, two new isoflavone glycosides, formononetin 8-*C*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (**1**) and formononetin 8-*C*-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranoside (**2**), were isolated along with four known isoflavonoid glycosides, 4′-methoxypuerarin (**3**), daidzin (**4**), genistin (**5**), and daidzein (**6**). Their structures were determined by the MS and NMR spectroscopic analysis.

2. Results and discussion

The roots of *P. lobata* were extracted with boiling water, and the extract was precipitated with ethanol. The ethanol-soluble part was subjected to column chromatography (CC) with X-5 macroporous resin, industrial HPLC , and polyamide to furnish two new isoflavone diglycosides (**1** and **2**) and four known isoflavones (**3**–**6**).

Compound **1** was obtained as white amorphous powder. The molecular formula, $C_{27}H_{30}O_{13}$, was confirmed on the basis of the $[M - H]^-$ ion at *m/z* 561.1632 in the HR-ESI-MS spectra. The IR spectrum of **1** revealed the presence of hydroxyl group (3419 cm^{-1}), carbonyl group (1631 cm^{-1}), and aromatic ring (1608, 1588, and 1514 cm^{-1}). The absorption maxima at 250 and 304 nm in the UV spectrum and the proton singlet at δ 8.38 (H-2) in the ^1H NMR spectrum suggested **1** to be an isoflavone.

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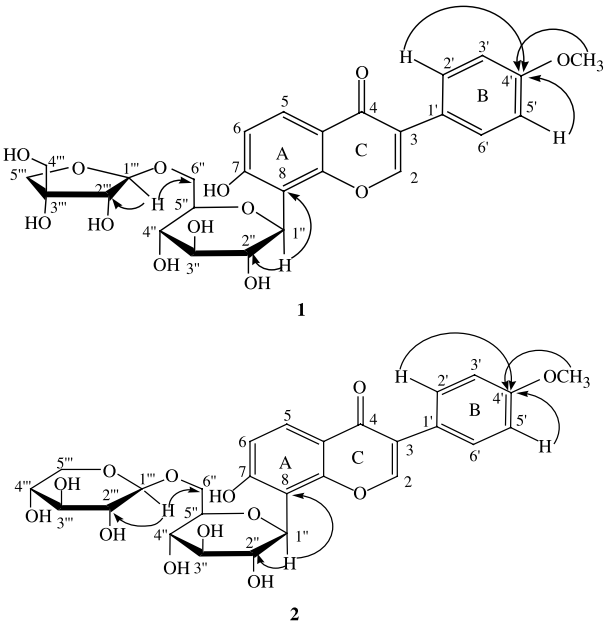

Figure 1. The structures and key HMBC correlations of compounds **1** and **2**.

Negative ion ESI-MS of **1** showed a quasi-molecular ion $[M - H]^-$ at *m/z* 561, and fragment ions at *m/z* 429 $[M - H - 132]^-$, 339 $[M - H - 132 - 90]^-$, and 309 $[M - H - 132 - 120]^-$ were observed in the ESI-MS/MS spectrum. The loss of 132 units indicated the presence of one *O*-pentosyl substituent, and the strong loss of 90 and 120 units indicated the presence of one *C*-hexosyl substituent [10,11]. The mass difference of 268 units between compound **1** and the disaccharide unit (a hexose and a pentose) suggested that the aglycone moiety of compound **1** was substituted with one hydroxyl and one methoxyl group. In the ^{1}H NMR spectrum, the signals at δ 6.99 (2H, d, $J = 9.0$ Hz) and 7.53 (2H, d, $J = 9.0$ Hz) were assigned to H-3′,5′ and H-2′,6′ of the B-ring, respectively; the signals at δ 7.94 (1H, d, $J = 8.5$ Hz) and 7.00 (1H, d, $J = 8.5$ Hz) were assigned to H-5 and H-6 of the A-ring, respectively; and the singlet at δ 3.79 (3H, s) was ascribed to a methoxyl group. Therefore, it can be concluded that **1** was a 7,8,4′-trisubstituted isoflavone glycoside. The ^{13}C NMR spectrum of **1** exhibited 11 aliphatic carbon signals due to one pentosyl and one hexosyl moiety, and the DEPT-135 spectrum indicated the presence of three methylene moieties. These were in good agreement with the published data for the sugar moiety of dadzein 8-*C*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside, genistein 8-*C*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside [5], and acacetin 7-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside [12,13]. Thus, the sugar moiety of **1** was deduced to be β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside. In the HMBC spectrum of **1**, correlations of H-1″ (Glu H-1) at δ 4.81 with C-8 (δ 112.4) and C-2″ (δ 70.5) were observed (Figure 1), suggesting that the C-glucose moiety was attached to C-8. Meanwhile, the HMBC experiment showed the correlations of C-4′ at δ 158.8 with the protons at δ 3.79 (OCH_3) and 7.53 (H-2′,6′), and the proton at δ 4.78 (api H-1) with the carbons at δ 68.3 (Glu C-6), 75.5 (api C-2), and 78.6 (api C-3), which indicated the OCH_3 group was attached to C-4′ and the apiose moiety was linked to C-6″ of the glucose. The β-configuration of the glucose was confirmed by the *J* value ($J = 9.5$ Hz) of

Table 1. ^{1}H NMR and ^{13}C NMR spectral data for compounds **1** and **2** (DMSO-d_6)[a,b].

	1		**2**	
No.	^{1}H	^{13}C	^{1}H	^{13}C
2	8.38 s	152.8	8.40 s	152.9
3		124.2		124.1
4		174.7		174.7
5	7.94 d (8.5)	126.1	7.95 d (9.0)	126.2
6	7.00 d (8.5)	113.9	7.00 d (9.0)	114.0
7		161.5		161.0
8		112.4		112.5
4a		116.4		116.5
8a		156.1		156.2
1′		122.6		122.7
2′	7.53 d (9.0)	129.9	7.53 d (9.0)	129.9
3′	6.99 d (9.0)	113.5	6.99 d (9.0)	113.5
4′		158.8		158.8
5′	6.99 d (9.0)	113.5	6.99 d (9.0)	113.5
6′	7.53 d (9.0)	129.9	7.53 d (9.0)	129.9
1″	4.81 d (9.5)	73.3	4.79 d (9.0)	73.2
2″	4.04 dd (9.5, 9.0)	70.5	4.01 dd (9.0, 10.0)	69.8
3″	3.27 m	78.7	3.26 m	78.5
4″	3.19 m	70.4	3.20 m	70.3
5″	3.40 d (8.0)	79.9	3.40 m	80.0
6″	a: 3.94 m b: 3.39 d (8.0)	68.3	a: 4.02 m b: 3.26 m	69.2
1‴	4.78 d (3.0)	108.9	4.10 d (7.5)	103.8
2‴	3.74 d (3.0)	75.5	2.93 m	73.1
3‴		78.6	3.04 m	76.4
4‴	a: 3.84 m b: 3.57 d (9.5)	73.1	3.53 m	69.4
5″	3.31 (m)	62.8	a: 3.68 (m) b: 3.00 t (11.0)	65.5
4′-OCH_3	3.79 s	55.0	3.79 s	55.0

[a] Signals were assigned by means of the HMQC and HMBC spectra (*J* Hz).
[b] Experiments were done on 500 MHz for the ^{1}H and 125 MHz for ^{13}C NMR spectra, respectively, and the *J* values (parentheses) are in Hertz.

its anomeric proton due to axial interaction. According to the above evidence, **1** was characterized as formononetin 8-*C*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (Figure 1). The ^{1}H NMR and ^{13}C NMR spectral data were assigned by the HMBC and HMQC spectra and listed in Table 1.

Compound **2** was obtained as white amorphous powder. The molecular formula, $C_{27}H_{30}O_{13}$, was established by negative HR-ESI-MS at *m/z* 561.1624 $[M - H]^{-}$. Its UV absorption maxima, the negative ESI-MS, and ESI-MS/MS spectra were completely identical to those of compound **1**, suggesting the similar aglycone and sugar moieties in **1** and **2**. The ^{1}H NMR, ^{13}C NMR spectra (Table 1) and the DEPT-135 spectral data of **2** showed close resemblance with those of **1**, except for the signals due to terminal sugar unit. The nature of the terminal sugar units of **2** was evident from the ^{13}C NMR spectrum that showed the presence of one *O*-xylopyranosyl unit, and the terminal sugar unit was confirmed to be linked to the C-6″ of the glucose on the basis of the correlations between the proton at δ 4.10 (xyl H-1) and the carbons at δ 69.2 (Glu C-6), 73.1 (xyl C-2), and 76.4 (xyl C-3) in the HMBC spectrum. These data are consistent with those of daidzein 8-*C*-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranoside [5]. The β-configuration of the two sugars was confirmed by the *J* values ($J = 9.0$ and 7.5 Hz) of their anomeric protons. Thus, **2** was identified as formononetin

8-*C*-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranoside (Figure 1).

Compound **3** was determined as 4′-methoxypuerarin by comparison of the ^{1}H NMR, ^{13}C NMR, IR, UV, and MS spectral data with those of compounds **1** and **2**. The structures of the other three known compounds were identified as daidzin (**4**), genistin (**5**), and daidzein (**6**) by comparison of the ^{1}H NMR, ^{13}C NMR, IR, UV, and MS spectral data with the values in literature [5].

3. Experimental

3.1 General experimental procedures

Melting points were obtained using an XT-4 melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 polarimeter. The UV and IR spectra were recorded on a Waters 2996 photodiode array detector (PDA) and a Perkin-Elmer 683 infrared spectrophotometer in KBr disks, respectively. The high-resolution mass spectra were recorded on the Macromass Q-TOF mass spectrometer fitted with a Lockspray interface. The MS/MS experiments were performed on a Finnigan TSQ/SSQ triple quadrupole mass spectrometer equipped with an ESI interface. Argon was used as the collision gas and the pressure was set at 3.0 mTorr. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) and Bruker AV500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer. Analytical HPLC experiments used to monitor each fraction were carried out on Waters 2695 chromatograph with a PDA detector and a Tigerkin ODS-2 column (5 μm, 4.6 × 250 mm I.D., Dalian Sipore Co. Ltd, Dalian, Liaoning, China). CC was performed on X-5 macroporous resin and polyamide with a pump. Preparative HPLC was carried out on self-made industrial chromatography with a single wavelength ultraviolet detector (310 nm) and a Tigerkin ODS-2 column (10–20 μm, 80 × 360 mm, I.D.), and the flow was set at 200 ml/min.

3.2 Plant material

The dried roots of *P. lobata* were collected in Yuexi, Anhui Province of China, in November 2004 and authenticated by Xirong He, Institute of Medication, Xiyuan Hospital of China Academy of Traditional Chinese Medicine. A voucher specimen (0471) is deposited in the Dalian Institute of Chemical Physics, Chinese Academy of Science, China.

3.3 Extraction and isolation

The dried roots of *P. lobata* (10 kg) were extracted with boiling water (100 L × 2) under reflux for 2 h, and the combined extracts were concentrated. The residue (2.0 kg) was dissolved in H_2O and then precipitated with 65 and 80% ethanol, respectively. After removal of the deposition, the soluble part was concentrated *in vacuo* to yield 968 g of the residue. The residue was subjected to CC on the X-5 macroporous resin (30 L), eluted with water, and then 10, 20, 40, 70, and 95% EtOH to get six fractions (A–F). In each eluting condition, 120 L was used. Fraction F (15.9 g) was subjected to preparative HPLC with MeOH (A)–H_2O (B) as mobile phase at a flow of 200 ml/min. The gradient profile was as follows: 0–45 min linearly from 30% A to 55% A, 45–50 min linearly from 55% A to 70% A, and then held for 10 min. Compounds **4** (78 mg) and **6** (40 mg), as well as 9 fractions (F-1–F-9), were also obtained. Fraction F-4 (0.8 g, t_R = 30.4–36.0 min) was subjected to CC on the polyamide (950 ml) and eluted with water, and then 15, 30, 50, and 95% EtOH. In each eluting condition, 3800 ml was used and the samples collected by each 950 ml. Compounds **1** (85 mg), **2** (21 mg), **3** (16 mg), and **5** (45 mg), as well as other 13 fractions, were obtained.

*3.3.1 Formononetin 8-C-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (**1**)*

White amorphous powder (H_2O), mp 166–168°C; $[\alpha]_D^{25}$: 27.3 (*c* 0.82, DMSO); UV λ_{max} (ACN/H_2O) nm: 250, 304; IR (KBr) ν_{max}:

3419 (OH), 2931 (CH), 1631 (C═O), 1610 (aromatic), 1588, 1514, 1445, 1249, 1180, and 1082 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS *m/z* 561.1632 $[M - H]^-$ (calcd for $C_{27}H_{30}O_{13}$, 561.1608); positive ion ESI-MS *m/z*: 563.1 $[M + H]^+$, 585.1 $[M + Na]^+$; negative ion ESI-MS *m/z*: 561.1 $[M - H]^-$; negative ion ESI-MS/MS *m/z*: 429.3 $[M - H - Api]^-$, 375.4 $[M - H - Api - 3H_2O]^-$, 339.4 $[M - H - Api - C_3H_6O_3]^-$, 309.4 $[M - H - Api - C_4H_8O_4]^-$, 281.3 $[M - H - Api - C_4H_8O_4\text{-}CO]^-$.

3.3.2 *Formononetin 8-C-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranoside (**2**)*

White amorphous powder (H_2O), mp 143–145°C; $[\alpha]_D^{25}$: −9.2 (*c* 0.55, DMSO); UV λ_{max} (ACN/H_2O) nm: 250, 307; IR (KBr) ν_{max}: 3427 (OH), 2918 (CH), 1633 (C═O), 1608 (aromatic), 1589, 1514, 1331, 1249, 1211, and 1113 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS *m/z*: 561.1624 $[M - H]^-$ (calcd for $C_{27}H_{30}O_{13}$, 561.1608); positive ion ESI-MS *m/z*: 563.1 $[M + H]^+$, 585.1 $[M + Na]^+$; negative ion ESI-MS *m/z*: 561.1 $[M - H]^-$; negative ion ESI-MS/MS *m/z*: 429.3 $[M–H\text{-}Xyl]^-$, 375.4 $[M–H–Api\text{-}3H_2O]^-$, 339.4 $[M–H–Xyl\text{-}C_3H_6O_3]^-$, 309.4 $[M–H–Api\text{-}C_4H_8O_4]^-$, $[M - H–Api\text{-}C_4H_8O_4\text{-}CO]^-$.

3.3.3 *4′-Methoxypuerarin (**3**)*

White amorphous powder (MeOH–H_2O), mp 151–153°C; $[\alpha]_D^{25}$: + 10.2 (*c* 0.54, DMSO); UV λ_{max} (ACN/H_2O) nm: 250, 304; ^1H NMR (400 MHz, DMSO-d_6): δ 8.38 (1H, s, H-2), 7.97 (1H, d, *J* = 8.8 Hz, H-5), 7.02 (1H, d, *J* = 8.8 Hz, H-6), 7.52 (2H, d, *J* = 8.8 Hz, H-2′,6′), 7.00 (2H, d, *J* = 8.8 Hz, H-3′,5′), 4.84 (1H, d, *J* = 8.8 Hz, Glu H-1), 3.79 (1H, s, 4′-OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 153.2 (C-2), 124.4 (C-3), 175.2 (C-4), 126.6 (C-5), 113.9 (C-6), 161.3 (C-7), 112.8 (C-8), 117.0 (C-4a), 156.3 (C-8a), 123.0 (C-1′), 130.3 (C-2′,6′), 115.0 (C-3′,5′), 159.2 (C-4′), 73.6 (Glu C-1), 70.9 (Glu C-2), 78.7 (Glu C-3), 70.6 (Glu C-4), 81.9 (Glu C-5), 61.5 (Glu C-6), 55.4 (4′-OCH_3); positive ion ESI-MS *m/z* 431.0 $[M + H]^+$; negative ion ESI-MS *m/z* 429.0 $[M - H]^-$.

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References

[1] W.M. Keung and B.L. Vallee, *Phytochemistry* **47**, 499 (1998).
[2] W.M. Keung and B.L. Vallee, *Proc. Natl Acad. Sci.* **90**, 10008 (1993).
[3] S.M. Boue, T.E. Wiese, S. Nehls, S. Elliott, C.H. Carter-Wientjes, B.Y. Shih, J.A. McLachlan, and T.E. Cleveland, *J. Agric. Food Chem.* **51**, 2193 (2003).
[4] J.S. Lee, *Clin. Chim. Acta* **347**, 121 (2004).
[5] K. Junei, F. Junich and C. Junko, *Chem. Pharm. Bull.* **35**, 4846 (1987).
[6] N. Toshihiro, K. Junei, F. Junichi, S. Yusuke, I. Mutsumi, S. Yoshiaki, I. Yayoi, Y. Ichiro, and K. Manki, *Phytochemistry* **33**, 1207 (1993).
[7] T. Arao, J. Kinjo, T. Nohara, and R. Isobe, *Chem. Pharm. Bull.* **45**, 362 (1997).
[8] T. Arao, T. Idzu, J. Kinjo, T. Nohara, and R. Isobe, *Chem. Pharm. Bull.* **44**, 1970 (1996).
[9] T. Arao, J. Kinjo, T. Nohara, and R. Isobe, *Chem. Pharm. Bull.* **43**, 1176 (1995).
[10] G. Rath, A. Touré, M. Nianga, J.L. Wolfender, and K. Hostettmann, *Chromatographia* **41**, 332 (1995).
[11] P. Waridel, J.L. Wolfender, K. Ndjoko, K.R. Hobby, H.J. Major, and K. Hostettmann, *J. Chromatogr. A* **926**, 29 (2001).
[12] C.C.W. Wanjala and R.R.T. Majinda, *Phytochemistry* **51**, 705 (1999).
[13] J. Cheng, Y.Y. Zhao, B. Wang, L. Qiao, and H. Liang, *Chem. Pharm. Bull.* **53**, 419 (2005).