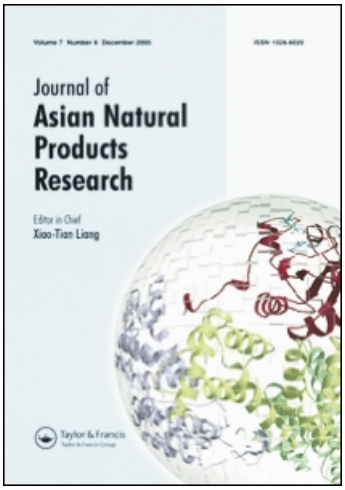


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Isolation of two new C-glucofuranosyl isoflavones from *Pueraria lobata* (Wild.) Ohwi with HPLC-MS guiding analysis

Hai-Jiang Zhang[a]; Xiao-Ping Yang[b]; Kui-Wu Wang[c]
[a] College of Life Science and Chemical Engineering, Huaiyin Institute of Technology, Huai'an, China [b] College of Pharmaceutical Science and Technology, Tianjin University, Tianjin, China [c] College of Food Science Biotechnology and Environmental Engineering, Zhejiang Gongshang University, Hangzhou, China

Online publication date: 21 April 2010

To cite this Article Zhang, Hai-Jiang , Yang, Xiao-Ping and Wang, Kui-Wu(2010) 'Isolation of two new C-glucofuranosyl isoflavones from *Pueraria lobata* (Wild.) Ohwi with HPLC-MS guiding analysis', Journal of Asian Natural Products Research, 12: 4, 293 — 299
To link to this Article: DOI: 10.1080/10286021003745445
URL: http://dx.doi.org/10.1080/10286021003745445

Journal of Asian Natural Products Research
Vol. 12, No. 4, April 2010, 293–299

ORIGINAL ARTICLE

Isolation of two new C-glucofuranosyl isoflavones from *Pueraria lobata* (Wild.) Ohwi with HPLC–MS guiding analysis

Hai-Jiang Zhang[a]*, Xiao-Ping Yang[b] and Kui-Wu Wang[c]

[a]*College of Life Science and Chemical Engineering, Huaiyin Institute of Technology, Huai'an 200023, China;* [b]*College of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China;* [c]*College of Food Science Biotechnology and Environmental Engineering, Zhejiang Gongshang University, Hangzhou 310035, China*

(*Received 8 December 2009; final version received 2 March 2010*)

A profiling analysis of the total isoflavone extract of the root of *Pueraria lobata* (Wild.) Ohwi was performed using HPLC coupled with ESI-ion trap mass spectrometry. A total of seven isoflavones were identified according to their retention times, UV, MS data, and comparing with the literature data. Among them, two proposed new compounds were isolated and their structures were determined to be 8-C-α-glucofuranosyl-7,4′-dihydroxyisoflavone and 8-C-β-glucofuranosyl-7,4′-dihydroxyisoflavone, named as neopuerarin A (**7**) and neopuerarin B (**6**), on the basis of chemical and spectral analyses.

Keywords: *Pueraria lobata*; kudzu; isoflavone; HPLC–MS; neopuerarin A; neopuerarin B

1. Introduction

Mass spectrometry (MS) is one of the most sensitive techniques for the rapid identification of compounds. HPLC–MS analysis for the natural product extract could provide very useful structural information on the constituents and be suitable for guiding the subsequent isolation for the target compounds. It is especially helpful for the isolation of the new constituents, particularly at minor or trace level in the plants so that much time-consuming and tedious useless work could be avoided.

Pueraria lobata (Wild.) Ohwi (kudzu) is a very important Chinese traditional medicine and has been used as antipyretic, diaphoretic, and antiemetic agents for many centuries in China. The main active compounds in *P. lobata* are believed to be the isoflavones. Up to now, many isoflavones have been isolated from this plant (Figure 1) [1,2]. In this paper, HPLC–MS analysis of the commercial total isoflavone extract of *P. lobata* was performed and two proposed new C-glucofuranosyl isoflavones were found. They were then isolated and their structures were elucidated by chemical and spectral analysis.

2. Results and discussion

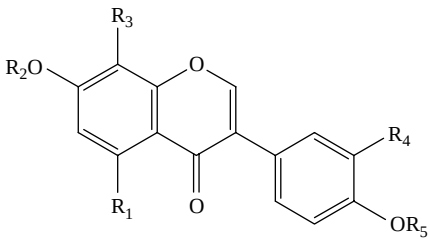
2.1 HPLC–MS analysis

The HPLC–MS conditions for the total isoflavone extract of *P. lobata* were optimized as described in Section 3.2 and the HPLC–MS chromatograms are shown in Figure 2. Based on mass spectral and UV spectral data (see Table 1), and comparing with the reference samples and the literature data [1–6], seven main compounds were identified as shown in Table 1.

*Corresponding author. Email: zhanghj3@hyit.edu.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286021003745445
http://www.informaworld.com



Glc: β-pyranoglucose

	R_1	R_2	R_3	R_4	R_5
Daidzein	H	H	H	H	H
Daidzin	H	Glc	H	H	H
Formononetin	H	H	H	H	CH_3
Genistein	OH	H	H	H	H
Genistin	OH	Glc	H	H	H
Genistein 8-C-glucoside	OH	H	Glc	H	H
Puerarin	H	H	Glc	H	H
PG-1	H	H	Glc	OH	H
PG-3	H	H	Glc	OCH_3	H

Figure 1. Structures of some isoflavones in the root of *P. lobata*.

As literatures reported [3–5], the neutral loss of 120 amu from the deprotonated ion in negative mode is believed to be the characteristic of C-glycoside and could be used for the differentiation from O-glycoside, the typically neutral loss of which is 162 amu. Therefore, all the peaks in Figure 2 were identified as the C-glycosides except for peak **4**, which showed a neutral loss of 162 amu.

Based on the retention time, MS and UV spectral data, and comparing with the reference samples, peaks **2** and **4** were unambiguously identified as puerarin and daidzin, respectively.

Peaks **1** and **5** were the isomeric compounds with the similar MS^n spectra, in which the consecutive neutral losses of 120 and 28 amu from $[M-H]^-$ of *m/z* 431 were observed. But they showed different UV spectra. Comparing with the literature data [1,2], peak **5** was identified as genistein 8-C-glycoside, while peak **1** was identified as PG-1.

From the MS^n spectra of peak **3**, the fragmentation of $[M-H]^-$ ion at *m/z* 445 gave product ions at *m/z* 415 $[M-H-CH_2O]^-$, 325 $[M-H-120]^-$, 310 $[M-H-120-CH_3]^-$, 297 $[M-H-120-CO]^-$, and 282 $[M-H-120-CH_3-CO]^-$. It indicated a $-OCH_3$ group in the aglycone skeleton. By comparing with the literature data [1,2], peak **3** was identified as PG-3.

Peaks **6** and **7** were the puerarin isomers of C-glycosyl isoflavone, which were identified as new compounds. The isolation and structural elucidation of them were then carried out, as reported in brief [7].

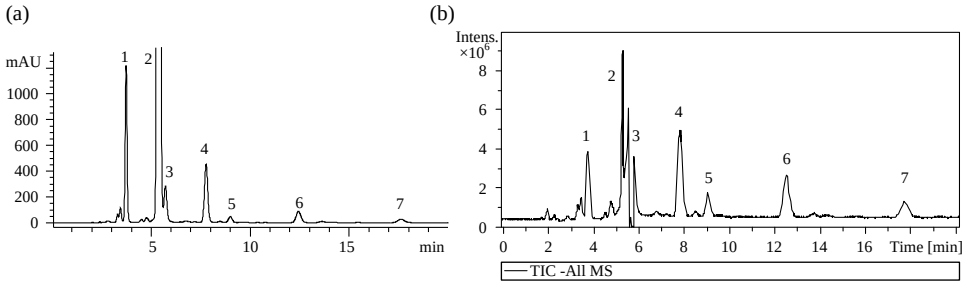


Figure 2. HPLC–MS analysis for the total isoflavone extract of *P. lobata*. (a) HPLC–UV chromatogram and (b) total ion current (TIC) chromatogram.

Table 1. The HPLC–MS^n results of the total isoflavone extract of *P. lobata*.

Peak no.	t_R (min)	$[M-H]^-$	Fragment ion *m/z*	λ_{max} (nm)	Identity
1	3.73	431	MS^2[431]: 311, 283 MS^3[431 → 311]: 283	250, 290	PG-1
2	5.71	415	MS^2[415]: 325, 295, 267 MS^3[415 → 295]: 267	250, 306	Puerarin
3	5.33	445	MS^2[445]: 415, 325, 310, 297, 282 MS^3[445 → 325]: 310, 297, 282	250, 286, 308	PG-3
4	7.77	415	MS^2[461]: 415, 253	250, 298	Daidzin
5	8.99	431	MS^2[431]: 311, 283 MS^3[431 → 311]: 283	260	Genistein 8-C-glycoside
6	12.43	415	MS^2[415]: 295, 267 MS^3[415 → 295]: 267	250, 306	New compound
7	17.62	415	MS^2[415]: 295, 267 MS^3[415 → 295]: 267	250, 306	New compound

2.2 Structural characterization

Compound **7** (Figure 3) was obtained as a mild yellow amorphous powder with the UV absorption maxima at 250 and 306 nm. The IR spectrum showed the absorption bands at 3383 and 1633 cm^{-1} attributed to hydroxyl and carbonyl groups, respectively. The molecular formula was determined as $C_{21}H_{20}O_9$ by HR-ESI-MS at *m/z* 415.1033 $[M-H]^-$. Comparing the ^1H and ^{13}C NMR spectral data (see Table 2) of compound **7** with those of puerarin [1], they showed the similar pattern except for the difference in the sugar moiety region, which suggested that compound **7** is a typical isoflavone. The ^{13}C NMR and DEPT spectral data of the sugar moiety indicated that compound **7** is a C-glycosyl compound by the chemical shift of anomeric carbon at 79.9 ppm and the sugar moiety is an aldohexose unit which showed one CH_2 and five CH groups. By $^1H-^1H$COSY, HSQC, and HMBC spectra shown in Figure 3, the structure of the aglycone was elucidated and the site of the sugar linkage to aglycone in compound **7** was confirmed at the C-8

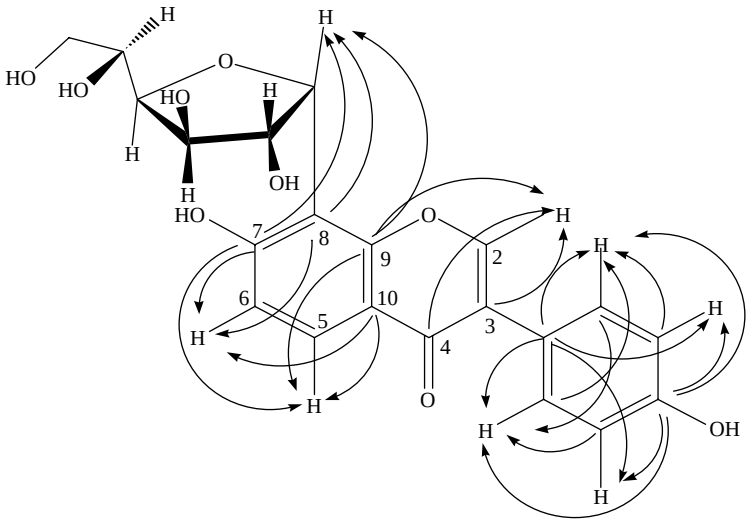


Figure 3. Key HMBC correlations of compound **7**.

Table 2. ^{1}H and ^{13}C NMR spectral data of neopuerarin B (**6**) and neopuerarin A (**7**) (in DMSO-d_6).

Position	Proton		Carbon	
	6	**7**	**6**	**7**
2	8.33 s	8.32 s	152.9	152.8
3	–	–	123.6	123.5
4	–	–	175.1	175.1
5	7.96 (d, $J = 8.5$ Hz)	7.90 (d, $J = 8.5$ Hz)	126.7	125.7
6	6.92 (d, $J = 8.5$ Hz)	6.88 (d, $J = 8.5$ Hz)	116.7	115.7
7	–	–	160.8	161.8
8	–	–	111.9	109.8
9	–	–	155.2	154.8
10	–	–	116.6	116.8
1′	–	–	122.7	122.6
2′	7.42 (d, $J = 7.5$ Hz)	7.38 (d, $J = 7.5$ Hz)	130.5	130.2
3′	6.83 (d, $J = 7.5$ Hz)	6.83 (d, $J = 7.5$ Hz)	115.4	115.2
4′	–	–	157.5	157.4
5′	6.83	6.83	115.3	115.2
6′	7.40	7.38	130.4	130.2
1″	5.30	5.72	80.4	79.9
2″	4.18	4.32	82.1	77.9
3″	3.80	4.20	80.8	75.2
4″	4.15	4.24	77.1	80.9
5″	3.80	3.80	69.0	68.5
6″	3.46–3.63	3.53–3.64	64.0	64.1

position by the appearance of the correlation between the anomeric proton of the sugar at δ_H 5.30 (H-1″) and the carbon atoms at δ_C 160.8 (C-7), 111.9 (C-8), and 155.2 (C-9) (Figure 3). Comparing the ^{13}C NMR spectral data with those of the puerarin [1,2], the carbon signals of C-1″, C-2″, C-4″ in the sugar moiety of compound **7** shifted 6.5–10.8 ppm to the downfield while the chemical shift of C-5″ moved more than 10 ppm to the upfield. Moreover, the coupling constant between the anomeric proton and H-2″ was not observed. It suggested that the sugar is in the furanose form. The ^{13}C NMR spectrum of the sugar moiety of compound **7** was in agreement with the reported data for the α-glucofuranosyl compound [8]. Therefore, compound **7** is determined to be 8-C-α-glucofuranosyl-7,4′-dihydroxyisoflavone, named neopuerarin A (Figure 4).

Compound **6** (Figure 3) was also obtained as a mild yellow amorphous powder with the UV absorption maxima at 250 and 306 nm. The IR spectrum showed the absorption bands at 3403 and 1628 cm^{-1} attributed to hydroxyl and carbonyl groups, respectively. The molecular formula was determined as $C_{21}H_{20}O_9$ by HR-ESI-MS at *m/z* 415.1107 $[M-H]^-$. The 1D and 2D NMR spectral data of compound **6** showed a similar pattern to compound **7**, which suggested that compound **6** is also an 8-C-aldohexofuranosyl-7,4′-dihydroxyisoflavone. Comparing the ^{13}C NMR spectral data of the sugar moiety of compound **6** with those of compound **7**, the chemical shifts of C-2″ and C-3″ moved about 5 ppm downfield while the signal of C-4″ shifted nearly 4 ppm upfield. Acid treatment of compound **6** using Besson's isomerization method [8] yielded puerarin and compound **7** (Figure 5). Therefore, compound **6** is determined to be 8-C-β-glucofuranosyl-7,4′-dihydroxyisoflavone,

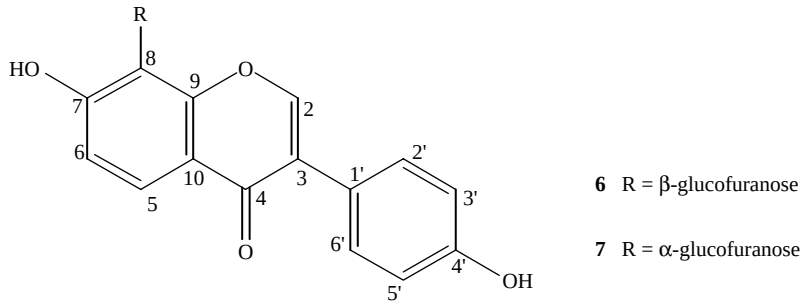


Figure 4. Structures of **6** (neopuerarin B) and **7** (neopuerarin A).

named neopuerarin B (Figure 4). To our knowledge, it is the second report of C-glucofuranosyl compounds in nature.

3. Experimental

3.1 Chemicals and materials

A commercial product of the total isoflavone extract of *P. lobata* was kindly donated by Zhongjing Modern TCM Company (Nanyang, China). The commercial reference compounds of puerarin and daidzin (purity > 98%, measured by HPLC with the area normalization method) were kindly gifted by Jinfeng Pharmaceutics Co. Ltd (Qingdao, China) and Dr Yang Xuedong, College of Pharmaceutical, Science and Technology, Tianjin University (Tianjin, China). Methanol for the HPLC analysis was of HPLC grade from Caledon (Ontario, Canada). Formic acid was of analytical grade from Yuanli Chemical Plant (Tianjin, China). Water for the sample preparation and the HPLC analysis was purified by a Milli-Q academic water purification system (Millipore, Bedford, MA, USA).

3.2 Instruments

HPLC–MS analysis for the total isoflavone extract of *P. lobata* was performed on an Agilent 1200-IT 6310 system equipped with an Agilent G1311A quaternary pump, a G1315D DAD detector, and a 6310 ion-trap mass spectrometer. The LC separation was performed using an Agela Venusil XBP-C18 column (4.6 × 250 mm, 5 μm), eluting with A (0.1% formic acid):B (MeOH) = 65:35 (v:v) at 1.0 ml/min. The column temperature was set at 30°C and the effluent was monitored at 250 nm. High-purity nitrogen

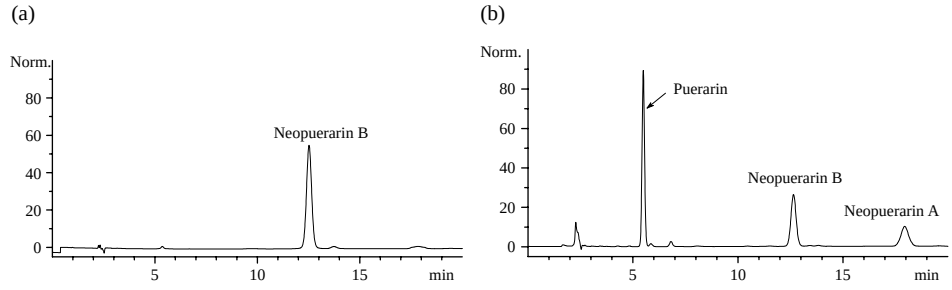


Figure 5. HPLC chromatogram of (a) neopuerarin B (**6**) and (b) acid-hydrolyzed neopuerarin B.

(N_2) was used as both drying gas with a flow rate of 12 l/min and as nebulizing gas with a pressure of 60 psi. Ultra-high pure helium (He) was used as the collision gas. The dry temperature was set at 350°C and the capillary voltage was set at 3500 V. The mass spectra were recorded in the scale from *m/z* 100 to 1000 in the negative mode. MS^n data were acquired in the Auto MS^n mode.

Melting points were measured on a YRT-3 micro-melting point apparatus (Tianjin, China). The UV spectra were recorded on a Shimadzu UV-2450 UV/Vis spectrophotometer. The IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrophotometer, with KBr pellets. The NMR spectra were measured with a Bruker DRX-500 spectrometer, at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. The HR-ESI-MS spectra were measured with a Varian IonSpec 7.0T high-resolution ESI-FT-ICR mass spectrometer.

3.3 Sample preparation

The extract (51.8 mg) was dissolved with 10 ml mobile phase and filtered through 0.45 μm nylon membrane. The filtered solution was subjected to HPLC–MS analysis with an injection volume of 40 μl.

3.4 Isolation

The extract of total isoflavone (30 g) was chromatographed on RP-C18 silica gel (40–60 μm, self-synthesized), eluting with 25% MeOH, 40% MeOH, and 60% MeOH, successively. The 40% elutes were concentrated by Rota-vapor and then purified by semi-preparative HPLC (Agela Venusil XBP-C18 column, 10 × 250 mm, 5 μm), eluting with H_2O–MeOH (60:40 v/v) at 2.0 ml/min with a Shimadzu LC-10AT pump and the effluents were monitored at 250 nm using a Shimadzu SPD-10A UV detector, to afford compound **6** (26.33 mg) and compound **7** (14.88 mg).

*3.4.1 Compound **6***

A mild yellow amorphous powder; mp 157–160°C. UV (CH_3OH) λ_{max} (log ε): 250 (3.33), 306 (2.87) nm; IR (KBr) ν_{max}: 3403, 1628, 1515, 1257, 1028 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectral data are given in Table 2; HR-ESI-MS *m/z* 415.1029 $[M-H]^-$ (calcd for $C_{21}H_{19}O_9$, 415.1028).

*3.4.2 Compound **7***

A mild yellow amorphous powder; mp 149–152°C. UV (CH_3OH) λ_{max} (log ε): 250 (3.32), 306 (2.83) nm; IR (KBr) ν_{max}: 3383, 1633, 1515, 1256, 1037 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectral data are given in Table 2; HR-ESI-MS *m/z* 415.1033 $[M-H]^-$ (calcd for $C_{21}H_{19}O_9$, 415.1028).

3.5 Acid hydrolysis of compound 6

Compound **6** (1 mg) was dissolved in 4 ml of MeOH–4 M HCl (1:1) and was refluxed for 1 h. The solution was neutralized with 2 M NaOH and then extracted with 2 ml of *n*-BuOH three times. The *n*-BuOH layer was combined and concentrated *in vacuo*. The residue was dissolved with 2 ml of MeOH and then subjected to HPLC–MS analysis (Figure 5).

References

[1] J.E. Kinjo, J.I. Furusawa, J. Baba, T. Takeshita, M. Yamasaki, and T. Nohara, *Chem. Pharm. Bull.* **35**, 4846 (1987).

[2] Y. Ohshima, T. Okuyama, K. Takahashi, T. Takizawa, and S. Shibata, *Planta Med.* **54**, 250 (1988).

[3] J.K. Prasain, K. Jones, M. Kirk, L. Wilson, M. Smith-Johnson, C. Weaver, and

S. Barnes, *J. Agric. Food Chem.* **51**, 4213 (2003).
[4] H.J. Rong, J.F. Stevens, M.L. Deinzer, D.L. Cooman, and D.D. Keukeleire, *Planta Med.* **64**, 620 (1998).
[5] Y. Zhang, Q. Xu, X.Z. Zhang, J.P. Chen, X.M. Liang, and A. Kettrup, *Anal. Bioanal. Chem.* **383**, 787 (2005).
[6] H.J. Zhang and X.P. Yang, *J. Pharm. Biomed. Anal.* **49**, 843 (2009).
[7] H.J. Zhang, X.P. Yang, and K.W. Wang, *Chin. Chem. Lett.* **20**, 720 (2009).
[8] M.K. Park, J.H. Park, Y.G. Shin, W.Y. Kim, J.H. Lee, and K.H. Kim, *Planta Med.* **4**, 363 (1996).