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Phenylethanoid Glycosides from *Picria felterrae* Lour.

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Abstract: In our search for bioactive compounds from the whole plant of *Picria felterrae* Lour., three new phenylethanoid glycosides, picfeosides A–C (**1**–**3**), along with five known phenylethanoid glycosides, namely wiedemannioside (**4**), acteoside (**5**), acteoside isomer (**6**), *cis*-acteoside isomer (**7**), and *cis*-acteoside (**8**), were isolated using several chromatographic purification steps, including semipreparation HPLC on RP-18. The structures of the new compounds were elucidated on the basis of extensive spectroscopic analysis.
Key words: phenylethanoid glycoside; picfeosides A–C; *Picria felterrae* Lour.; Scrophulariaceae.

Picria felterrae Lour. (Scrophulariaceae) has been used as a traditional medicine in the south of China for the treatment of fever, herpes infection, cancer, and inflammation for more than 200 years (Zhong *et al.* 1979). Some triterpenoids have been isolated from the ethyl acetate (EtOAc) extract of this plant and have been regarded as the active components (Cheng *et al.* 1982; Hu *et al*. 1996). As part of our continuing chemical investigation of this plant (Zou *et al*. 2003a, 2003b; Wang *et al*. 2004a, 2004b), in the present study we report on the isolation and elucidation of the structure of three new phenylethanoid glycosides, picfeosides A–C (**1**–**3**), and five known phenylethanoid glycosides, namely wiedemannioside (**4**; Abougazar *et al*. 2003), acteoside (**5**; Sasaki *et al*. 1989), acteoside isomer (**6**; Miyase *et al.* 1982), *cis*-acteoside isomer (**7**; Wang *et al*. 1997), and *cis*-acteoside (**8**; Potterat *et al.* 1991), isolated from the aqueous-soluble fraction of the ethanolic extract (Fig. 1). The present paper describes the structure elucidation of the three new glycosides.

1 Results and Discussion

Picfeoside A (**1**) was obtained as an amorphous powder, $[\alpha]_D^{28}$ −65.2° (*c* 0.135, MeOH). Its molecular formula was determined as $C_{34}H_{44}O_{17}$ by HRFABMS and NMR data (Tables 1, 2). The ^{1}H-NMR spectrum in the aromatic region exhibited five aromatic protons, including two pairs of symmetrical ones, which appeared at δ 7.22 (2H, d, *J* = 7.5 Hz), 7.25 (2H, t, *J* = 7.5 Hz), and 7.19 (1H, t, *J* = 7.5 Hz), indicating that no substituted group was in the aglycon. The signals of H-7 and H-8 in the phenylethyl moiety appeared at δ 2.92 (2H, t, *J* = 7.4 Hz, H_2-7), 3.85 (1H, m, H-8a), and 4.25 (1H, m, H-8b). The ^{1}H-NMR signals of three aromeric protons at δ 6.26 (1H, d, *J* = 2.0 Hz), 6.21 (1H, d, *J* = 1.3 Hz), and 4.81 (1H, d, *J* = 7.9 Hz), as well as one methylene group of apiose at δ 4.09 and 4.17 (each 1H, d, *J* = 10.0 Hz) and one methyl group of rhamonose at δ 1.62 (3H, d, *J* = 5.7 Hz), were consistent with the configuration for a β-apiosyl unit, an α-rhamnosyl unit and a β-glucosyl unit. An HMBC experiment of **1** showed long-range correlations between the anomeric proton H-1‴ of the terminal apiose and the C-4″ of the internal rhamonose, H-1″ of the internal rhamonose and C-3′ of the glucose. The apiosyl and rhamnosyl groups were attached at C-3′ of the glucosyl moiety and C-4″ of the internal rhamnosyl moiety, respectively. In addition, the ^{1}H-NMR spectrum of **1** exhibited the

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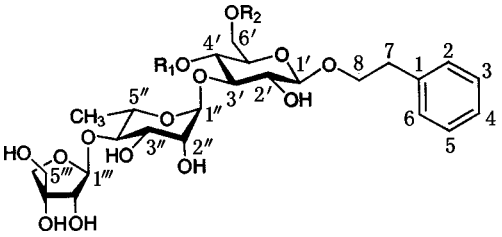
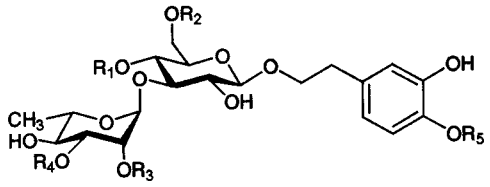


1 R_1 = *trans*-caffeoyl, R_2 = H
2 R_2 = *trans*-caffeoyl, R_1 = H
3 R_1 = R_2 = H



4 R_1 = *trans*-feruloyl $R_2 = R_3 = R_4$ = acetyl $R_5 = CH_3$
5 R_1 = *trans*-caffeoyl $R_2 = R_3 = R_4 = R_5$ = H
6 R_2 = *trans*-caffeoyl $R_1 = R_3 = R_4 = R_5$ = H
7 R_2 = *cis*-caffeoyl $R_1 = R_3 = R_4 = R_5$ = H
8 R_1 = *cis*-caffeoyl $R_2 = R_3 = R_4 = R_5$ = H

Fig. 1. Structures of compounds **1**–**8.**

characteristic three aromatic protons as an ABX system at δ 7.17 (1H, d, J = 8.0 Hz), 7.18 (1H, dd, J = 1.8, 8.0 Hz), and 7.58 (1H, d, J = 1.8 Hz), and two *trans*-olefinic protons at δ 6.69 (1H, d, J = 15.9 Hz) and 8.03 (1H, d, J = 15.9 Hz) for the *trans*-caffeoyl group. The chemical shift assignable to H-4′ of the glucosyl moiety was shifted downfield to δ 5.65 (1H, t, J = 8.1 Hz) and there was one more caffeoyl group in **1**. An HMBC experiment of **1** showed a ^3J interaction between the carbonyl carbon of the caffeoyl group and H-4′ of glucose. Accordingly, the structure of piefeoside A (**1**) was elucidated as β-phenylethoxy-3-O-[β-apiofuranosyl-(1→4)-α-rhamnopyranosyl]-4-O-caffeoyl-β-glucopyranoside.

Picfeoside B (**2**) has the composition $C_{34}H_{44}O_{17}$ as determined by HRFABMS, the same as that of **1**. The ^{1}H- and ^{13}C-NMR spectral data of **2** (Tables 1, 2) were strikingly similar to those of **1**. The only significant differences occurred at H-4′ and H_2-6′ of glucose. In the ^{1}H-NMR spectrum (Table 1), the signals of H-4′ (δ 5.65) of glucose for **1** were shifted upfield to δ 4.71 in **2**, whereas H_2-6′ (δ 4.32 and 4.20) was shifted downfield to δ 5.03 and 4.93, indicating that the caffeoyl moiety was located at C-6′ of glucose in **2**, instead of at C-4′ as in **1.** This assignment was in accordance with the observation of C-6′ of glucose being shifted downfield by Δ2.1 ppm (Table 2). The HMBC cross-peaks observed between H_2-6′ of glucose and the carbonyl carbon (δ 167.8) of the caffeoyl further confirmed the above elucidation. Therefore, the structure of **2** was established as β-phenylethoxy-3-O-[β-apiofuranosyl-(1→4)-α-rhamnopyranosyl]-6-O-caffeoyl-β-glucopyranoside.

Picfeoside (**3**) was isolated as an amorphous powder, with a molecular formula of $C_{25}H_{38}O_{14}$, as determined by HRFABMS and NMR data (Tables 1, 2). The negative FABMS of **3** exhibited a pseudomolecular ion at *m/z* 561 $[M–H]^+$, which was 162 mass units lower than that of **2**, suggesting the absence of a caffeoyl group. The upfield shift of H_2-6′ (δ 4.49 and 4.41 in **3**, δ 5.03 and 4.93 in **2**) of glucose indicated the presence of a free OH group in this position (Table 1). This assumption was supported by the ^{1}H-NMR spectrum of **3**, revealing the resonances of three anomeric protons: δ 4.75 (d, J = 7.8 Hz), 6.30 (brs), and 6.44 (d, J = 1.6 Hz), consistent with the presence of a β-glucose, an α-rhamnose, and a β-apiose moiety, respectively. A ^3J correlation in the HMBC spectrum between H-4″ of rhamnose and the C-1‴ of apiose, between H-3′ of glucose and C-1″ of rhamnose, and between H-8 of the aglycon and C-1′ of glucose, showed that **3** was a decaffeoyl derivative of **2**. Accordingly, **3** was assigned as β-phenylethoxy-3-O-[β-apiofuranosyl-(1→4)-α-rhamnopyranosyl]-β-glucopyranoside, which was given the trivial name picfeoside C.

This is the first report on the isolation of phenylethanoid glycosides from *P. felterrae*. It is of note that there are very few examples of phenyethanoid

Table 1 ^{1}H-NMR (400 MHz, δ in ppm, *J* in Hz, pyridine-d_5) data for compounds **1**–**3**

Position	Compound **1**	Compound **2**	Compound **3**
Aglycon			
2	7.22 d (7.5)	7.18 d (7.5)	7.20 d (7.5)
3	7.25 t (7.5)	7.23 t (7.5)	7.25 t (7.5)
4	7.19 t (7.5)	7.15 t (7.5)	7.19 t (7.5)
5	7.25 t (7.5)	7.23 t (7.5)	7.25 t (7.5)
6	7.22 d (7.5)	7.18 d (7.5)	7.20 d (7.5)
7	2.92 t (7.4)	2.94 t (7.4)	2.91 t (7.4)
8	4.25 m	4.10 m	4.20 m
	3.85 m	3.85 m	3.78 m
Glucose			
1′	4.81 d (7.8)	4.75 d (7.8)	4.75 d (7.8)
2′	4.01 t (8.1)	4.08 t (8.5)	3.97 t (8.8)
3′	4.45 t (8.1)	4.34 t (8.5)	4.37 t (8.8)
4′	5.65 t (8.1)	4.71 t (8.5)	4.22 t (8.8)
5′	4.04 m	4.00 m	3.85 m
6′	4.32 m	5.03 m	4.49 m
	4.20 m	4.93 m	4.41dd (4.4, 11.7)
Rhamnose			
1″	6.21 d (1.3)	6.28 d (1.3)	6.30 br s
2″	4.70 br s	4.65 br s	4.70 br s
3″	4.62 dd (3.0, 9.0)	4.63 dd (3.0,9.0)	4.66 dd (3.0, 9.3)
4″	4.38 t (9.0)	4.45 t (9.0)	4.48 overlap
5″	5.11 m	4.98 m	5.07 m
6″	1.62 d (3H, 5.7)	1.68 d (3H, 6.1)	1.70 d (3H, 6.4)
Apiose			
1‴	6.26 d (2.0)	6.38 d (1.8)	6.44 d (1.6)
2‴	4.75 d (2.0)	4.75 d (1.8)	4.81 d (1.6)
4‴	4.50 d (12.0)	4.55 d (12.0)	4.58 d (12.0)
	4.28 d (12.0)	4.30 d (12.0)	4.32 d (12.0)
5‴	4.17 d (10.0)	4.15 d (10.0)	4.17 d (10.7)
	4.09 d (10.0)	4.10 d (10.0)	4.09 d (10.7)
Caffeoyl			
2	7.58 d (1.8)	7.57 d (1.8)	
5	7.17 d (8.0)	7.18 d (8.2)	
6	7.18 dd (1.8, 8.0)	7.06 dd (1.8, 8.2)	
7	8.03 d (15.9)	7.95 d (15.8)	
8	6.69 d (15.9)	6.64 d (15.8)	

Table 2 ^{13}C-NMR (100 MHz, δ in ppm, pyridine-d_5) data for compounds **1**–**3**

Position	Compound **1**	Compound **2**	Compound **3**
Aglycon			
1	139.2 s	139.3 s	139.3 s
2	129.4 d	129.5 d	129.5 d
3	128.8 d	128.8 d	128.8 d
4	126.6 d	126.6 d	126.6 d
5	128.8 d	128.8 d	128.8 d
6	129.4 d	129.5 d	129.5 d
7	36.6 t	36.5 t	36.6 t
8	70.7 t	70.8 t	70.6 t
Glucose			
1′	104.2 d	104.5 d	104.4 d
2′	76.4 d	75.6 d	75.7 d
3′	80.7 d	82.7 d	83.2 d
4′	70.2 d	69.7 d	69.5 d
5′	75.8 d	75.4 d	78.5 d
6′	62.2 t	64.3 t	62.5 t
Rhamnose			
1″	102.9 d	102.6 d	102.6 d
2″	72.7 d	72.8 d	72.9 d
3″	72.8 d	73.0 d	73.0 d
4″	79.8 d	79.9 d	80.0 d
5″	68.5 d	68.2 d	68.1 d
6″	19.3 q	18.8 q	18.9 q
Apiose			
1‴	111.5 d	111.5 q	111.6 d
2‴	78.2 d	78.3 d	78.2 d
3‴	80.4 s	80.5 s	80.6 s
4‴	75.1 t	75.1 t	75.1 t
5‴	66.0 t	65.6 t	65.7 t
Caffeoyl			
1	126.8 s	126.8 s	
2	115.8 d	115.8 d	
3	147.7 s	147.8 s	
4	150.8 s	150.2 s	
5	116.9 d	116.8 d	
6	123.4 d	122.3 d	
7	146.9 d	146.3 d	
8	114.7 d	114.8 d	
O–C = O	167.1 s	167.8 s	

glycosides with a trisaccharide moiety containing apiose (Kyriakopoulou *et al*. 2001) and, in such glycosides, the phenylethol moiety without substitution on its aromatic ring is infrequent (Jimenez and Riguera 1994). Therefore, compounds **1**–**3** may be considered, to some extent, as the characteristic aqueous-soluble constituents of *P. felterrae*.

2 Experimental

2.1 General experimental procedures

Optical rotations were determined with a Perkin-Elmer model 2401 polarimeter (Perkin Elmer, Norwalk, CT, USA). UV spectra were taken on a Shimadizu UV

210A spectrometer (Shimadizu, Japan). IR spectra were run on a Bio-Rad FTS-135 grating infrared spectrophotometer (Bio-Rad, Hercules, CA, USA). 1D- and 2D-NMR spectra were recorded with a Brucker AM-400 (Brüker, CH-6304 Zug, Switzerland) spectrometer. Chemical shifts (δ) were obtained using TMS as an internal standard. FABMS and HRFABMS were obtained using a VG Auto Spec-3000 (VG, Manchester, England) or a Finnigan MAT 90 (Finnigan, USA) instrument. MPLC was performed using a Büchi model 688 (Brüker, CH-6304 Zug, Switzerland) apparatus on columns containing RP-18 Si gel (40–63 μm; Merck, Darmstadt, Germany) and HPLC was performed using an Agilent 1100 series (Agilent Technologies Deutschland GmbH, 76337 Waldbronn, Germany) on a ZORBAX (Agilent Technologies Deutschland GmbH, 76337 Waldbronn, Germany) SB-C_{18} (9.4×250 mm) column. Silica gel-precoated plates (Qingdao Marine Chemical, Qingdao, China) were used for TLC and detections were performed by spraying with 10% H_2SO_4 solution followed by heating to 200 ºC for 30 s.

2.2 Plant materials

The whole plant of *Picria felterrae* Lour. was collected in Wuzhou, China, in October 2001. A voucher specimen (PF-0101) is deposited in the herbarium of the test center of Guilin Sanjin Pharmaceutical Co., China.

2.3 Extraction and isolation

The air-dried plant (10 kg) was pulverized and extracted successively with ethanol (EtOH; 2×100 L) under reflux. The combined filtrate was concentrated under reduced pressure and adsorbed subsequently on a Diaion HP-20 column (Mitsubishi, Chemical Corporation, Tokyo, Japan), which was eluted with H_2O and methanol (MeOH), respectively. The fraction eluted with MeOH was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (increasing polarity from 19:1 to 1:1) to give 10 fractions (I–X). Part (25 g) of fraction X (225 g) was rechromatographed on a silica gel column using $CHCl_3$-MeOH mixtures (increasing polarity) as eluents to give five subfractions (I–V). Subfraction II (2.3 g) was subjected to MPLC on an RP-18 and afforded compounds **5** (40 mg), **6** (25 mg), and **7** (200 mg) with elution of MeOH-H_2O (1:1). Compounds **1** (40 mg), **2** (15 mg), **3** (70 mg), and **4** (20 mg) were separated from subfraction III (2.5 g) by semipreparation HPLC on an RP-18 with MeOH-H_2O in a gradient elution mode. Subfraction IV was chromatographed on an MCI (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), gel column, and then on an RP-18 using a mixture of MeOH and H_2O (1:1) as the eluent by semipreparation HPLC to gave compound **8** (60 mg).

2.4 Identification

Compound 1 $C_{34}H_{44}O_{17}$, yellowish amorphous powder, $[\alpha]_D^{28}$ −65.2º (*c* 0.135, MeOH). UV λ_{max} (MeOH, log ε) nm: 331 (4.14). IR ν_{max} cm^{-1}: 3 440 (OH), 1 693 (C═O), 1 631 (C═C), 1 516 (aromatic rings). Negative HRFABMS *m/z*: 723.253 8 $[M–H]^+$ (calculated for $C_{34}H_{43}O_{17}$ 723.250 0). Negative FAB-MS (glycerol) *m/z*: 723 $[M–H]^+$. For ^1H- and ^{13}C-NMR spectral data, see Tables 1 and 2.

Compound 2 $C_{34}H_{44}O_{17}$, yellowish amorphous powder, $[\alpha]_D^{24}$ −55.0º (*c* 0.100, MeOH). UV λ_{max} (MeOH, log ε) nm: 331 (4.11), 245 (3.90). IR ν_{max} cm^{-1}: 3 442 (OH), 1 690 (C═O), 1 632 (C═C), 1 517. Negative HRFABMS *m/z*: 723.252 6 $[M–H]^+$ (calculated for $C_{34}H_{43}O_{17}$ 723.250 0). Negative FAB-MS (glycerol) *m/z*: 723 $[M–H]^+$, 591 $[M–133]^+$, 560 $[M–caffeoyl–H]^+$. For ^1H- and ^{13}C-NMR spectral data, see Tables 1 and 2.

Compound 3 $C_{25}H_{38}O_{14}$, amorphous powder, $[\alpha]_D^{25}$ −76.8º (*c* 0.254, MeOH). UV λ_{max} (MeOH, log ε) nm: 207 (3.98). IR ν_{max} cm^{-1}: 3 421 (OH), 1 695 (C═O), 1632 (C═C). Negative HRFABMS *m/z*: 561.214 7 $[M–H]^+$ (calculated for $C_{25}H_{37}O_{14}$ 561.218 3). Negative FAB-MS (glycerol) *m/z*: 561 $[M–H]^+$, 429 $[M–133]^+$. For ^1H- and ^{13}C-NMR spectral data, see Tables 1 and 2.

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