New Triterpenoid Saponins from *Picria fel-tarrae*

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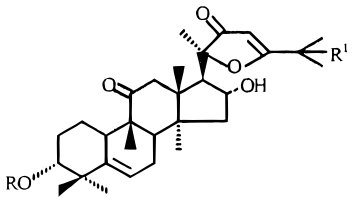
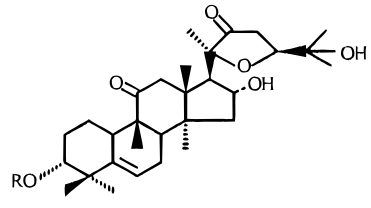
Three new triterpenoid saponins, named picfeltarraenins III (**1**), IV (**2**), and V (**3**), together with the known compounds picfeltarraenins IA, IB (**4**), and II, were isolated from whole plants of *Picria fel-tarrae*. The structures of **1**−**3** were elucidated on the basis of chemical and spectral methods.

Picria fel-tarrae Lour. is a plant belonging to the family Scrophulariacea. It grows in Guangdong, Guangsi, Guizhou, and Yunnan Provinces of the People's Republic of China and has been used as an herbal medicine by local peoples to treat inflammation. The 70% alcoholic extract of the plant was evaporated under vacuum and diluted with H_2O to yield fractions A−C of varying polarity, namely, an EtOAc extract (extract A), an EtOAc/95% EtOH (3:1) extract (extract B), and an aqueous extract (extract C). All three extracts demonstrated in vitro inhibitory effects against various bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Extract B was found to have significant inhibitory activity against carcinoma S180.[1] Previously, the isolation of picfeltarraegenins I−VI[2−5] obtained on the acid hydrolysis of extract B, and of picfeltarraenins IA, IB,[6] and II[7] from extract B, have been described. This paper deals with the isolation and structures of three new saponins named picfeltarraenins III (**1**), IV (**2**), and V (**3**).



1 R=α-L-Rha*p*(1→2)-β-D-Glc*p*; R^1=OH
2 R=α-L-Rha*p*(1→2)-β-D-Glc*p*(1→3)-β-D-Xyl*p*, R^1=H
4 R=α-L-Rha*p*(1→2)-β-D-Glc*p*; R^1=H



3 R=α-L-Rha*p*(1→2)-β-D-Glc*p*(1→3)-β-D-Xyl*p*

The crude saponins (30 g) from extract B were chromatographed over a Si gel column eluted with $CHCl_3$/MeOH/H_2O. The fractions obtained were further chromatographed on a RP-18 column eluted with MeOH/H_2O or CH_3CN/H_2O to yield picfeltarraenins IA (2.5 g), IB (1.52 g), II (0.43 g), III (**1**) (20 mg), IV (**2**) (220 mg), and V (**3**) (106 mg). The known compounds IA, IB (**4**), and II were identified by comparison of their spectral data with those described in the literature.[6,7]

Picfeltarraenin III (**1**) was obtained as an amorphous powder. The FABMS showed a quasi-molecular ion peak at *m*/*z* 831, corresponding to an $[M(C_{42}H_{64}O_{15}) + Na]^+$ ion. On the basis of its ^{1}H- and ^{13}C-NMR data, the aglycon of **1** was identified as picfeltarraeginin III [11,22-dioxo-3α,16α,25-trihydroxy-(20*S*,24)-epoxycucurbit-5,23-diene].[3] By comparison of its ^{1}H- and ^{13}C-NMR data (Tables 1 and 2), the sugar moiety of **1** was identified as α-L-rhamnopyranosyl(1→2)-*O*-β-D-glucopyranose, the same sugar moiety as that of picfeltarraenin IB (**4**). The interglycosidic linkages were deduced from an HMBC NMR experiment. As expected, correlations were observed between H-1 (δ 4.83) of glucose and C-3 (δ 83.6), H-1 (δ 6.54) of rhamnose and C-2 (δ 76.3) of glucose. Therefore, picfeltarraenin III (**1**) was identified as picfeltarraeginin III 3-*O*-α-L-rhamnopyranosyl(1→2)-*O*-β-D-glucopyranoside.

Picfeltarraenin IV (**2**) was isolated as colorless needles. The FABMS showed a quasi-molecular ion peak at *m*/*z* 949, corresponding to $[M(C_{47}H_{74}O_{18}) + Na]^+$. On the basis of its ^{1}H- and ^{13}C-NMR data, the aglycon of **2** was determined as picfeltarraeginin I.[2] The NMR and FABMS data indicated that **2** contained one pentose, one hexose, and one 6-deoxyhexose unit. Hydrolysis of **2** yielded glucose, rhamnose, and xylose. The β-configuration at the anomeric center of the glucopyranosyl moiety was suggested by the large coupling (J_{H1-H2} = 7.7 Hz) of the anomeric proton in the ^{1}H-NMR spectrum, and the configuration of the L-rhamnosyl group was determined as α on the basis of the C-5 signal at δ 69.6.[8] The xylosyl group was concluded to be in the β-configuration (J_{H1-H2}=7.5 Hz). ^{1}H- and ^{13}C-NMR chemical shifts were assigned (Tables 1 and 2) from a combination of 2D homonuclear ^{1}H-^{1}H (DQF-COSY, TOCSY) and heteronuclear ^{13}C-^{1}H (HMQC, HMBC) correlations that allowed unambiguous identifications of the aglycone and the various sugar moieties. The observation of cross-peaks in the HMBC spectrum arising from through-bond couplings over three bonds between the anomeric protons and carbons in adjacent systems allowed the determination of the sugar sequence and the aglycone linkage positions. Hence, cross-peaks between H-1 (δ 4.61) and C-1 (δ 101.2) of xylose and C-3 (δ 83.1) and H-3 (δ 3.44) of the aglycone, respectively, indicated that the xylose moiety was attached at C-3 of the aglycone. Cross-peaks between H-1 (δ 5.11) of glucose and C-3 (δ 90.0) of xylose, and H-1 (δ 6.57) of rhamnose and C-2 (δ 74.9) of glucose indicated that **2** consisted of a xylose

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Table 1. ^{1}H-NMR Spectral Data of Compounds **1**−**4**[a]

proton(s)	1	2	3	4
1	1.80 m	2.30 dd (17.2, 3.1)	2.27 m	2.03 m
	1.72 m	1.85 m	1.82 m	1.78 m
2	2.01 m	2.02 m	2.00 m	1.99 m
3	3.52 t (7.8)[b]	3.44 t (7.2)	3.42 t (7.8)	3.49 t (7.8)
6	5.61 m	5.69 brs	5.66 brs	5.61 m
7	1.79 m	1.85 m	1.81 m	1.80 m
8	2.40 br d (11.8)	2.41 br d (11.1)	2.42 br d (12.3)	2.41 m
10	1.79 m	1.85 m	1.81 m	1.79 m
12	3.04 d (13.4)	3.05 d (14.5)	3.18 d (11.4)	3.08 d (14.5)
	2.65 d (13.4)	2.58 m	2.78 d (11.4)	2.58 d (14.6)
15	2.24 m	1.85 m	1.81 m	1.82 m
	1.72 m	1.67 m	1.63 dd (19.2, 6.5)	1.66 m
16	4.89 m	4.74 m	5.66 m	4.75 m
17	2.98 d (6.6)	2.94 d (6.55)	2.84 d (7.0)	2.95 d (6.5)
18	0.95 s	0.96 s	1.02 s	0.96 s
19	1.11 s	1.17 s	1.14 s	1.12 s
21	1.68 s	1.62 s	1.45 s	1.63 s
23	6.13 s	5.58 s	3.21 dd (18.1, 12.4)	5.58 s
			2.56 dd (18.1, 6.4)	
24			4.17 m	
25		2.60 m		2.60 q (6.8)
26	1.67 s	1.08 d (6.60)	1.54 s	1.08 d (6.9)
27	1.71 s	1.09 d (6.6)	1.34 s	1.09 d (6.8)
28	1.43 s	1.41 s	1.41 s	1.43 s
29	1.34 s	1.32 s	1.30 s	1.34 s
30	1.36 s	1.37 s	1.34 s	1.35 s
Xyl				
X_1		4.61 d (7.5)	4.58 m	
X_2		4.20 m	4.02 m	
X_3		4.08 m	4.06 m	
X_4		4.08 m	4.05 m	
X_5		4.22 m	4.22 m	
		3.55 br t	3.55 br t	
Glc				
G_1	4.83 d (7.3)	5.11 d (7.7)	5.12 d (7.8)	4.83 d (7.3)
G_2	4.31 m	4.10 m	4.01 m	4.27 m
G_3	4.30 m	4.19 m	4.19 m	4.25 m
G_4	4.15 t (8.5)	4.03 m	4.13 m	4.14 t (8.6)
G_5	3.89 m	4.03 m	4.13 m	3.86 m
G_6	4.52 dd (11.4, 1.9)	4.58 m	4.58 m	4.52 d (8.0)
	4.34 m	4.22 m	4.21 m	4.31 m
Rha				
R_1	6.54 s	6.57 s	6.51 s	6.53 s
R_2	4.75 m	4.79 m	4.79 m	4.75 m
R_3	4.57 dd (9.3, 3.0)	4.51 dd (9.5, 3.4)	4.52 dd (9.2, 3.3)	4.58 dd (9.3, 3.1)
R_4	4.32 m	4.22 m	4.22 m	4.29 m
R_5	4.89 m	4.74 m	4.52 m	4.84 m
R_6	1.74 d (6.0)	1.71 d (6.9)	1.71 d (5.7)	1.75 d (6.0)

[a] Measured at 400 MHz, with reference to δ 7.56 in C_5D_5N. Assignments were based on ^{1}H−^{1}H DQFCOSY, TOCSY, HMQC, and HMBC experiments for compounds **1**−**4**. [b] Coupling constants (J in Hz) are given in parentheses.

unit bearing glucose at C-3 and rhamnose at C-2. Consequently, the structure of **2** was elucidated as picfeltarraeginin I 3-*O*-α-L-rhamnopyranosyl(1→2)-*O*-*β*-D-glucopyranosyl(1→3)-*O*-*β*-D-xylopyranoside.

Picfeltarraenin V (**3**), an amorphous powder, exhibited a quasimolecular ion peak at m/z 967 in the FABMS, corresponding to $[M(C_{47}H_{70}O_{19}) + Na]^+$. By comparison of its ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) with those of **2**, it was apparent that both compounds had the same sugar moieties. On the basis of its ^{1}H- and ^{13}C-NMR data (Tables 1and 2), the aglycon of **3** was identified as picfeltarraeginin II.[3] Thus, picfeltarraenin V (**3**) was elucidated as picfeltarraeginin II 3-*O*-α-L-rhamnopyranosyl(1→2)-*O*-*β*-D-glucopyranosyl (1→3)-*O*-*β*-D-glucopyranoside.

All ^{1}H- and ^{13}C-NMR signals of the known compound IB (**4**) were also assigned (Tables 1 and 2) by means of

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a]

carbon	1	2	3	4
1	25.5	25.4	25.1	25.4
2	27.3	26.9	26.5	27.2
3	83.6	83.1	82.8	83.5
4	42.0	41.8	41.5	41.9
5	142.7	142.5	142.1	142.7
6	118.6	118.7	118.3	118.6
7	24.3	24.1	23.7	24.1
8	36.1	35.9	35.4	36.0
9	48.2	48.1	47.8	48.1
10	43.6	43.3	43.1	43.4
11	212.8	212.9	213.0	212.8
12	48.7	48.6	48.6	48.6
13	49.2	49.0	48.7	49.1
14	50.9	50.6	50.1	50.7
15	46.6	46.4	46.0	46.5
16	69.9	69.6	69.3	69.8
17	59.4	58.9	58.9	59.1
18	20.2	20.0	19.6	20.0
19	20.5	20.4	20.1	20.5
20	91.0	90.0	83.9	91.0
21	23.6	23.1	20.3	23.3
22	206.9	206.8	217.0	206.9
23	100.9	101.0	37.6	101.1
24	195.2	195.0	79.6	195.2
25	69.9	30.3	69.9	30.4
26	28.3	19.3	25.9	19.4
27	28.6	19.6	27.0	19.6
28	25.3	25.1	24.7	25.2
29	22.3	22.2	21.8	22.2
30	19.2	19.0	19.0	18.9
Xyl				
X_1		101.2	100.8	
X_2		74.7	74.4	
X_3		90.0	89.6	
X_4		69.3	69.5	
X_5		66.3	65.9	
Glc				
G_1	100.7	104.4	104.4	100.7
G_2	76.3	74.9	74.6	76.2
G_3	80.1	78.5	78.3	80.0
G_4	72.0	71.6	71.2	72.3
G_5	77.9	78.6	78.2	77.8
G_6	63.3	62.5	62.2	63.1
Rha				
R_1	101.3	101.2	100.8	101.1
R_2	72.6	72.4	71.9	72.5
R_3	72.6	72.3	72.1	72.5
R_4	74.3	74.1	73.8	74.2
R_5	69.3	69.6	69.1	69.2
R_6	19.0	19.1	18.8	19.0

[a] Measured at 100 MHz, with reference to δ 135.5 in C_5D_5N. Assignments were based on ^{1}H−^{1}H DQF COSY, TOCSY, HMQC, and HMBC experiments for compounds **1**−**4**.

^{1}H−^{1}H DQF−COSY, TOCSY, HMQC, and HMBC NMR experiments. For all of the compounds **1**−**4**, we made an unusual observation in that the glycosylation shifts of C-2 of the glucosyl residues were almost zero. Methanolysis of the permethylates of **4** provided methyl 3,4,6-tri-*O*-methylglucopyranoside and methyl 2,3,4-tri-*O*-methylrhamnopyranoside, which supported the structural proposals being made.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. UV spectra were recorded on a Shimadzu UV-250 instrument using MeOH as solvent. IR spectra were measured with a Perkin-Elmer 559B apparatus. NMR spectra were obtained on Bruker AMX-400 NMR spectrometer with chemical shifts reported in ppm. MS were determined on a MAT 95 double-focusing mass

spectrometer using glycerin plus NaCl as matrix. GLC was carried out on a 1 m × 0.4 cm column containing 10% PEGS on Chromosorb W; column temperature 170 °C; carrier gas N_2 (40 mL/min).

Plant Material. *Picria fel-tarrae* Lour. was collected at Wuzhou, Guangsi Province, People's Republic of China, in June 1992. A voucher sample of the plant is deposited at the herbarium of the Department of Phytochemistry, Shanghai Institute of Materia Medica, Academia Sinica, Shanghai, People's Republic of China.

Extraction and Isolation. The whole plants of *P. fel-tarrae* (1.0 kg) were powdered and extracted three times at room temperature with 70% EtOH (each time for 4 days). A total of 70 g of extract was obtained after concentration *in vacuo*. The extract was diluted with H_2O to yield extracts A, B, and C successively, with an EtOAc extract (extract A, 11.1 g), an EtOAc/95% EtOH (3:1) extract (extract B, 32.0 g), and a H_2O extract (extract C, 28.0 g) being produced. The crude saponins (30 g) produced in extract B were subjected to column chromatography on Si gel, eluted with $CHCl_3$/MeOH/H_2O (4:1:0.1). The fractions obtained were purified by repeated RP-18 column chromatography (Lobar), with elution by MeOH/H_2O (3:2) or CH_3CN/H_2O (2:3) to yield picfeltarraenins IA (2.5 g), IB (1.52 g), II (0.43 g), III (**1**) (20 mg), IV (**2**) (220 mg), and V (**3**) (106 mg).

Picfeltarraenin III (1): amorphous powder; $[\alpha]^{20}_D$ + 25.7° (*c* 0.090, MeOH); UV (MeOH) λ max (log ϵ) 262 (4.05) nm; IR υ max (KBr) 3400, 1685, 1580, 1160−950 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; FABMS *m/z* 853 $[M + 2 \times Na - H]^+$, 831 $[M + Na]^+$; *Anal.* calcd for $C_{42}H_{64}O_{15}\cdot 2H_2O$: C, 59.70; H, 8.11; Found: C, 59.34; H, 7.93.

Picfeltarraenin IV (2): colorless needles; $[\alpha]^{20}_D$ + 24.0° (*c* 0.083, MeOH); UV (MeOH) λ max (log ϵ) 261 (3.92) nm; IR υ max (KBr) 3410, 1685, 1580, 1160−950 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; FABMS *m/z* 971 $[M + 2 \times Na - H]^+$, 949 $[M + Na]^+$; *Anal.*calcd for $C_{47}H_{74}O_{18}\cdot 4H_2O$: C, 56.50; H, 8.27; Found: C, 56.13; H, 7.94.

Picfeltarraenin V (3): amorphous powder; $[\alpha]^{20}_D$ + 11.9° (c 0.097, MeOH); UV (MeOH) λ max (log ϵ) 210 (3.35) nm; IR υ max (KBr) 3420, 1750 1685, 1580, 1160−950 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; FABMS *m/z* 989 $[M + 2 \times Na - H]^+$, 967 $[M + Na]^+$; *Anal.* calcd for $C_{47}H_{76}O_{19}\cdot 4H_2O$: C, 55.50; H, 8.32; Found: C, 55.84; H, 8.73.

Acidic Hydrolysis of 1−3. A MeOH solution of each glycoside (**1**−**3**), together with standard sugar samples, was applied at points about 1 cm from the bottom of a HPTLC Si gel plate and hydrolyzed with HCl vapor for 2 h at 50 °C. Each plate was then heated at 60 °C for 2 h to remove residual HCl, and developed using $CHCl_3$/CH_3OH/H_2O (8:2:0.1) as eluent. Each plate was sprayed with 10% H_2SO_4 (in EtOH), and then heated at 110 °C.

Methylanolysis of 4. Compound **4** (100 mg) was methylated according to Hakomori's method[9] using NaH (500 mg) and CH_3I (15 mL) in DMSO. The crude permethylated product was purified by preparative TLC [Si gel, petroleum ether/EtOAc/Me_2CO (3:1:0.5)] to afford a permethylate, which showed no hydroxy absorption in the IR spectrum. The permethylated saponin was methanolyzed for 2.5 h with 2 N HCl (5 mL) at 80 °C. The methylated methyl monosaccharides were examined and identified (by comparison with the corresponding authentic samples)[10] by TLC and GLC. TLC: solvent, C_6H_6/Me_2CO (3:1); detection with ceric sulfate. GLC: t_R 1.25 and 2.06 min (α- and β-methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranose); 9.30 and 11.42 min (α- and β-methyl 3,4,6-tri-*O*-methyl-D-glucopyranose).

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(10) Authentic sugar samples were obtained by methanolysis of the permethylates of Gyp XXXXVI[11] and L-rhamnose.
(11) Compound Gyp XXXXVI, 2α,3β,12β,20(*S*)-tetrahydroxydammar-24-ene-3-*O*-[β-D-glucopyranosyl(2→1)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranoside, was previously isolated from *Gynostemma pentaphyllum* by my group.

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