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金铁锁的新三萜皂甙*

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摘要：从金铁锁（*Psammosilene tunicoides* W. C. Wu et C. Y. Wu）根部分离得到 5 个齐墩果烷型五环三萜皂苷。它们的结构通过波谱和化学方法分别鉴定为：3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin **(1)**，3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin **(2)**，3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside（Lobatoside I，**3)**，3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-αL-rhamnopyranosyl（1→2）-β-D-fucopyranoside **(4)**，3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-6-O-acetylglucopyranosyl-(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside **(5)**。其中 5 为新化合物，1 和 2 为首次从自然界中分离得到。

关键词：金铁锁；石竹科；三萜皂甙
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A New Tritepenoid saponin from *Psammosilene tunicoides*

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Abstract：Five oleanane-type triterpenoid saponins were isolated from the roots of *Psammosilene tunicoides* W. C. Wu et C. Y. Wu. Their structures were elucidated by spectral and chemical methods as 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin **(1)**，3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin **(2)**，3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-(-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside（Lobatoside I，**3)**，3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-

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[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside (**4**), 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-6-O-acetyl-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside (**5**). Among them, compound 5 was a new triterpenoid saponin.

Key words: *Psammosilene tunicoides*; Caryophyllaceae; Triterpenoid saponin

Psammosilene tunicoides W. C. Wu et C. Y. Wu (Caryophyllaceae) is an only species in genus *Psammosilene* growing in southwest of China. It is a famous herb in Yunnan folk for stopping bleeding, relieving pain and promoting blood circulation (Lan, 1976). The crude saponins obtained from the plant have pain-relieving and anti-inflammatory activities (Song, 1981). As a part of our chemical studies on this plant, we report here the isolation and structure elucidation of five oleanane-type triterpenoid saponins.

Results and discussion

Compound **1** was obtained as a white amorphous powder. The negative ion FABMS spectrum of **1** showed a quasi molecular ion $[M-H]^-$ at m/z 807 compatible with the molecular formula $C_{42}H_{64}O_{15}$. Other significant peaks visible at m/z 645 $[M-H-162]^-$, 469 $[M-H-162-176]^-$ indicated the elimination of one hexosyl and one hexosyluronic acid unit. The ^1H and ^{13}C NMR spectra exhibited two anomeric proton and two anomeric carbon signals at δ 103.30 (4.88, d) and 106.33 (5.20, d).

Acid hydrolysis of **1** with 5% H_2SO_4-MeOH gave an aglycone which was identified as gypsogenin by comparison of its ^{13}C NMR spectrum with reported data (Murakami *et al*, 2001), and galactose and glucuronic acid (co-TLC with authentic samples). β-Configuration of the anomeric positions were inferred from the values of coupling constants in the ^1H NMR spectrum for both galactopyranosyl (J = 7.48 Hz) and glucuronopyranosyl (J = 6.8 Hz) moieties. The sequence of the sugars could be determined by the HMBC spectrum showing long range correlations between H－1 of glcUA (δ 4.88) and C－3 of the aglycone (δ 82.51), H－1 of gal (δ 5.20) and C－2 of glcUA (δ 83.58). Based on the above results, and the assumption that gal and glcUA are members of the commonly found D-series, the structure of **1** could be deduced to be 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin. After literature investigation, it was found that **1** was once obtained on acid hydrolysis of goyasaponin I from the fresh fruit of Japanese *Momordica charantia* L. (Murakami *et al*, 2001).

Compound **2** was also isolated as a white amorphous powder. Its molecular formula was assigned as $C_{47}H_{72}O_{19}$ by negative ion FABMS and ^{13}C NMR spectra. The ^{13}C and ^1H NMR spectra of **2** were very similar to those of **1** except that **2** had an additional xylose. In the HMBC spectrum, long range correlation was observed between H－1 of the additional xyl (δ 5.25) and C－3 (δ 86.13) of glcUA. The remaining spectral data were identical with those of **1**. So the structure of **2** was represented as 3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin. After literature investigation, it was found that Lacaille-Dubois *et al* (1993) once obtained **2** on acid hydrolysis of squarroside A from the roots of *Acanthophyllum squarrosum*.

Negative FABMS and ^{13}C NMR spectra of compound **3** suggested the molecular formula $C_{65}H_{102}O_{32}$. There were six anomeric carbon and six anomeric proton signals in the ^{1}H and ^{13}C NMR spectra. Complete acid hydrolysis of **3** afforded gypsogenin as an aglycone and glucose, glucuronic acid, galactose, fucose, rhamnose and xylose by co-TLC with authentic sugar samples. Alkaline hydrolysis of **3** with 5% aqueous KOH gave a prosaponin which was identified as compound **1**. These data indicated that two sugars (galactose, glucuronic acid) must be bound by a glycosidic linkage to the aglycone at C－3, whilst the four remaining sugar moieties must be bound to the aglycone by a glycosidic ester linkage at C－28. Sugar proton signals in the ^{1}H NMR spectra were assigned by $^{1}H-^{1}H$ cosy experiments. Using this technique, the spin-systems staring with the aromeric proton signals could be determined. Thereafter the ^{13}C signals was assigned by the C－H connectivities observed as cross-peaks in the HMQC spectra. The linkage site of C－28 sugar moieties could be determined by the HMBC spectrum showing correlations between H－1 of glc (δ 5.37) and C－3 of rha (δ 82.14), H－1 of xyl (δ 5.43) and C－4 of rha (δ 78.58), H－1 of rha (δ 5.94) and C－2 of fuc (δ 74.87), H－1 of fuc (δ 5.91) and C－28 of the aglycone (δ 176.58). Thus, the structure of **3** was elucidated to be 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (Lobatoside I). It had been isolated from the seed of *Actinostemma lobatum* Maxim. (Fujioka *et al*, 1992).

Compound **4** possessed the molecular formula $C_{70}H_{110}O_{36}$ which was determined by negative ion FABMS and ^{13}C NMR spectra. The ^{13}C and ^{1}H NMR spectra of **4** were similar to those of **3** except that **4** had an additional xylose. Alkaline hydrolysis of **4** gave compound **2** as a prosaponin which indicated that the additional xylose must be bound to C－3 of glc-UA. So the structure of **4** was determined to be 3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-glucuronopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside. Frechet *et al* (1991) had isolated it from the roots of *Gypsophila paniculata* and *G. arrostii*.

Compound **5** was obtained as a white powder. Its molecular formula was assigned as $C_{67}H_{104}O_{33}$ by negative ion FABMS showing a quasi molecular ion peak at m/z 1436 $[M]^-$. The molecular weight of **5** was 42 amu more than that of **3** which suggested **5** possessed an additional acetyl group. Further comparison of the ^{1}H and ^{13}C NMR spectra of the two compounds also revealed some differences in the tetrasaccharide linked to C－28 of the aglycone. The signal of C－6 of glucose appeared at δ 62.94 in **3** was shifted 2 ppm to the lower field, and C－5 signal was shifted upfield for 3.39ppm (δ 75.60), which implied the acetylation of C－6 of glucose. This was supported by the presence of $[M-162-42]^-$ ion peak at m/z 1232 in the FABMS spectrum. Furthermore, the HMBC spectrum exhibited long range correlations between H－6 of glucose and the ketonic carbon of the acetyl confirming the attachment of the acetyl group to the C－6 position of glucose. Thus, the structure of **5** was elucidated to be 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→4)-[β-D-6-O-acetylglucopyra-nosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside.

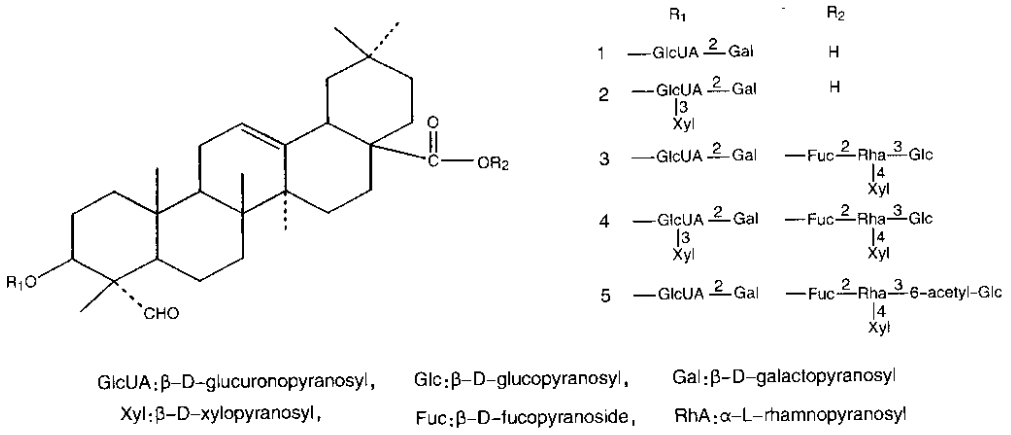

Fig. 1 Structures of compounds **1 – 5**

Experimental

General MPs: uncorrected; ^{1}H NMR, ^{13}C NMR and 2D – NMR spectra were recorded on Bruker AM – 400MHz or DRX – 500 spectrometers with TMS as internal standard and C_5D_5N as solvent; FABMS data were recorded on a VG Autospec – 3000 spectrometer.

Table 1 ^{13}C NMR chemical shifts of aglycone moieties of compounds **1 – 5** (in C_5D_5N)

C	1	2	3	4	5
1	38.15	38.20	38.27	38.34	38.50
2	28.36	28.12	25.00	25.51	25.10
3	82.51	82.63	83.71	82.52	83.42
4	55.11	55.25	54.20	55.18	54.68
5	48.44	48.20	48.56	49.00	48.66
6	20.49	20.56	20.70	21.00	20.56
7	32.62	32.81	32.54	32.73	32.51
8	40.11	40.25	40.34	40.43	40.52
9	47.96	47.92	47.95	48.03	47.89
10	36.37	36.20	36.34	36.44	36.45
11	23.88	23.78	23.92	23.68	23.88
12	122.34	122.15	122.56	122.60	122.65
13	145.00	144.68	144.24	144.22	144.19
14	42.30	42.56	42.28	42.35	42.31
15	28.36	28.45	28.25	28.29	28.32
16	23.68	23.75	23.60	23.68	23.65
17	46.75	46.50	47.08	47.14	47.10
18	42.11	42.08	42.01	42.11	42.05
19	46.58	46.42	46.50	46.57	46.55
20	31.05	30.86	30.89	30.91	30.90
21	34.34	34.50	34.04	34.12	34.15
22	33.36	33.45	32.54	32.53	32.55
23	209.57	210.15	210.12	210.21	210.32
24	11.00	11.12	11.16	11.15	11.20
25	15.72	15.86	15.95	15.93	16.02
26	17.44	17.49	17.46	17.53	17.41
27	25.02	25.62	26.17	26.15	26.08
28	180.25	180.50	176.58	176.58	176.61
29	33.36	33.28	33.30	33.30	33.25
30	23.88	23.75	23.92	23.91	23.88

Plant material　The dried roots of *Psammosilene tunicoides* were purchased from the Yunnan Baiyao Drug Factory in Kunming, Yunnan.

Extraction and isolation　The dried and powdered roots of *Psammosilene tunicoides* were extracted with EtOH (90%) under reflux, and the solution was evaporated *in vacuo*. The residue was suspended in acetone to afford crude saponin as a precipitate, which was subjected to silica gel column chromatography, eluting with $CHCl_3$-MeOH-H_2O (8:2:0.2–6.5:3.5:0.8) to give two main fractions. The two fractions were further purified on silica gel and Rp-18 column chromatography to yield compounds **1 – 5**.

Table 2　^{13}C NMR chemical shifts of sugar moieties of compounds **1 – 5** (in C_5D_5N)

	C	1	2	3	4	5
3-O-						
glcUA	1	103.30	103.85	103.12	104.02	103.25
	2	83.58	75.69	82.51	75.36	82.79
	3	77.16	86.13	77.01	86.30	76.85
	4	73.05	71.50	72.96	71.37	72.95
	5	77.16	78.62	76.37	78.69	77.28
	6	171.46	172.15	172.00	172.04	171.56
gal	1	106.33	104.50	106.14	104.33	106.44
	2	74.49	73.89	74.30	74.54	74.38
	3	74.99	74.98	74.42	75.36	74.65
	4	70.22	70.55	70.19	70.28	70.31
	5	77.77	77.19	77.25	77.14	77.62
	6	62.26	62.09	62.16	62.00	62.18
xyl	1		105.18		105.46	
	2		75.36		75.54	
	3		78.52		78.38	
	4		70.88		70.92	
	5		67.42		67.37	
28-O-						
fuc	1			95.13	95.16	95.35
	2			74.87	74.97	75.06
	3			75.49	75.54	75.53
	4			73.24	72.98	73.14
	5			72.36	72.35	72.55
	6			17.03	16.99	17.12
rha	1			102.16	102.16	101.15
	2			71.00	71.37	71.52
	3			82.14	82.52	82.56
	4			78.58	78.69	78.40
	5			69.05	69.13	69.12
	6			19.05	19.06	18.29
glc	1			105.45	106.37	105.75
	2			75.94	75.95	75.31
	3			78.58	78.94	77.69
	4			71.95	72.11	71.62
	5			78.89	78.69	75.60
	6			62.94	62.30	64.96
	CH_3					21.25
	CO					172.89
xyl	1			105.23	105.29	104.95
	2			75.94	75.95	75.85
	3			79.40	79.44	79.32
	4			71.31	72.98	71.52
	5			67.15	67.37	67.30

Compound 1 $C_{42}H_{64}O_{15}$, white amorphous powder, mp 283 – 290℃, $[\alpha]_D^{21}$ + 470.52 (c 0.29, MeOH); FABMS m/z: 807 $[M-H]^-$, 645 $[M-H-162]^-$, 469 $[M-H-162-176]^-$; ^1H NMR (C_5D_5N, 400 MHz): δ 5.20 (1H, d, J = 7.48 Hz, H-1_{gal}), δ 4.88 (1H, d, J = 6.80 Hz, H-1_{glcUA}); ^{13}C NMR data, see Table 1 and 2.

Compound 2 $C_{47}H_{72}O_{19}$, white amorphous powder, mp 235 – 238℃; FABMS m/z: 940 $[M]^-$, 808 $[M-132]^-$, 778 $[M-162]^-$, 646 $[M-132-162]^-$, 470 $[M-132-162-176]^-$; ^1H NMR (C_5D_5N, 400 MHz): 5.19 (1H, d, J = 7.2 Hz, H-1_{glcUA}), 5.25 (1H, d, J = 7.2 Hz, H-1_{xyl}), 4.92 (1H, d, J = 7.5 Hz, H-1_{gal}); ^{13}C NMR data, see Table 1 and 2.

Compound 3 $C_{65}H_{102}O_{32}$, white powder, mp 235 – 240℃, $[\alpha]_D^{21}$ – 1.57 (c 0.635, C_5H_5N); FABMS m/z: 1394 $[M]^-$, 1232 $[M-162]^-$, 1055 $[M-H-162-176]^-$, 807 $[M-162-132-146\times2]^-$, 761 $[M-H-176-162\times2-132]^-$, 469 $[M-H-176-162\times2-146\times2-132]^-$; ^1H NMR (C_5D_5N, 400 MHz): δ 5.94 (1H, H-1_{rha}), 5.91 (1H, H-1_{fuc}), 5.43 (1H, H-1_{xyl}), 5.37 (1H, H-1_{glc}), 5.13 (1H, H-1_{gal}), 4.72 (1H, H-1_{glcUA}); ^{13}C NMR data, see Table 1 and 2.

Compound 4 $C_{70}H_{110}O_{36}$, white powder, mp 223 – 224℃, $[\alpha]_D^{26}$ – 4.14 (c 0.3, MeOH); FABMS m/z: 1525 $[M-H]^-$, 1393 $[M-H-132]^-$, 1363 $[M-H-162]^-$, 1231 $[M-H-162-132]^-$, 1055 $[M-H-162-132-176]^-$, 807 $[M-H-132\times2-162-146\times2]^-$; ^1H NMR (C_5D_5N, 400 MHz): δ 5.94 (1H, d, J = 7.8 Hz, H-1_{fuc}), 5.91 (1H, s, H-1_{rha}), 5.42 (1H, d, J = 7.2 Hz, H-1_{xyl}), 5.36 (1H, d, J = 7.8 Hz, H-1_{glc}), 5.29 (1H, d, J = 7.2 Hz, H-1_{glcUA}), 5.17 (1H, d, J = 7.2 Hz, H-1_{xyl}), 4.85 (1H, m, H-1_{gal}); ^{13}C NMR data, see Table 1 and 2.

Compound 5 $C_{67}H_{104}O_{33}$, white powder, mp 228 – 230℃, $[\alpha]_D^{26}$ + 10.15 (c 0.012, MeOH); FABMS m/z: 1436 $[M]^-$, 1274 $[M-162]^-$, 1232 $[M-162-42]^-$, 1098 $[M-162-176]^-$, 808 $[M-162-42-132-146\times2]^-$; ^1H NMR (C_5H_5N, 400 MHz): δ 5.92 (1H, H-1_{rha}), 5.95 (1H, H-1_{fuc}), 5.45 (1H, H-1_{xyl}), 5.31 (1H, H-1_{glc}), 5.08 (1H, H-1_{gal}), 5.19 (1H, H-1_{glcUA}); ^{13}C NMR data, see Table 1 and 2.

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