Five New Triterpene Bisglycosides with Acyclic Side Chains from the Rhizomes of *Cimicifuga foetida* L.

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Five new triterpene bisglycosides, foetidinosides A (1), B (2), C (3), D (4) and E (5), including three of the cycloartane type, one of its derivative, and one of the lanostane type were isolated from the rhizomes of *Cimicifuga foetida*. Their structures were elucidated on the basis of spectroscopic data and chemical evidence. They were the first bisglycosides with acyclic side chains which were different from the typical triterpenes with side chains epoxidized with ring D in *Cimicifuga* species.

Key words *Cimicifuga foetida*; bisglycoside; lanostane; cycloartane; Ranunculaceae

Our group focuses on the chemical diversity and bioactivities of the genus *Cimicifuga* (Ranunculaceae) growing in the Yunnan-Guizhou Plateau.[1—4)] In our previous study, *Cimicifuga foetida* L. distributed at altitudes higher than 2000 m were found containing a series of cycloartane bisglycosides with disaccharide chains which were rarely reported before.[2,3)] Curiosity concerning the potential existence of cycloartane glycosides with more saccharides led to the unexpected isolation of one lanostane bisglycoside, three cycloartane bisglycosides, and one cycloartane derivative (**1**—**5**). These new compounds **1**—**5** obtained in low yields possessed highly oxidized acyclic side chains other than the typical triterpenes in *Cimicifuga* species with side chains epoxidized with ring D.[4)] This paper deals with the isolation and structrual elucidation of these compounds.

Results and Discussion

The roots of *C. foetida* collected in Lijiang county, Yunnan province, were extracted with 80% MeOH/H_2O, and the extract was partitioned between the $CHCl_3$-soluble and *n*-BuOH-soluble portions. The remaining water-soluble portion was separated by silica gel and octadecyl silica gel column chromatographies, and finally afforded five new triterpene bisglycosides (**1**—**5**).

The molecular formula of compound **1** was inferred as $C_{41}H_{70}O_{13}$ by negative high-resolution (HR)-electrospray ionization (ESI)-MS showing a $[C_{41}H_{69}O_{13}]^-$ ion peak at *m*/*z* 769.4742. In the ^{1}H-NMR spectrum (Table 1), there were seven tertiary methyl groups at δ_H 0.75, 1.04, 1.07, 1.12, 1.32, 1.33 and 1.42, and a secondary methyl group at δ_H 1.06 (partially overlaped). Additionally, two anomeric protons at δ_H 4.79 (d, J=7.6 Hz) and 5.07 (d, J=7.8 Hz) displayed downfield as well as an olefinic proton signal at δ_H 5.28 (d, J=5.3 Hz). The ^{13}C-NMR spectrum showed 30 resonances attributed to the aglycon part along with signals of a xylose and a glucose (Table 2). Then the planar structure of **1** was established as lanosta-9(11)-en-3,16,24,25-tetraol 3-*O*-xylosyl-24-*O*-glucoside (Fig. 1) by analysis of distortionless enhancement by poarization transfer (DEPT), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC) and ^{1}H–^{1}H correlation spectroscopy (COSY) experiments. HSQC-total correlation spectroscopy (TOCSY) was applied to determine the ambiguous assignments of the sugar moieties. The HMBC correlations of H-16 [δ_H 4.74 (dd, J=12.7, 7.4 Hz)] with C-13 (δ_C 44.8, C) and C-14 (δ_C 45.0, C); and H-15 (δ_H 1.78, 2.09) and H-17 (δ_H 1.80) with C-16 (δ_C 71.3, CH) positioned one hydroxyl group at C-16. The HMBC associations of H_3-26 (δ_H 1.33) and H_3-27 (δ_H 1.42) with C-25 (δ_C 72.1, C) indicated that the other hydroxyl group was at C-25. The olefinic bond was located at C-9 and C-11 due to the HMBC cross-peaks of H-11 [δ_H 5.28 (d, J=5.3 Hz)] with C-10 (δ_C 39.5, C) and C-13 (δ_C 44.8, C); H-12 (δ_H 1.99, 2.10) with C-9 (δ_C 149.0, C) and C-11 (δ_C 115.2, CH) while H_3-19 (δ_H 1.07) also showed a strong correlation with C-9. Hydrolysis of **1** afforded D-xylose and D-glucose, which were confirmed by GC analysis. The linkages of glycosides were determined by the clear HMBC associations of H-1′ [δ_H 4.79 (d, J=7.6 Hz)] with C-3 and H-1″ [δ_H 5.07 (d, J=7.8 Hz)] with C-24. The anomeric centre of both sugars are β oriented due to the J value of the anomeric protons. In rotating frame Overhauser enhancement spectroscopy (ROESY), the crosspeak between H-16 and H_3-28 indicated that the hydroxyl group at C-16 has a β configuration. The ^{13}C-NMR data of C-24 were comparable to analogue compounds having a 24*R* configuration.[5—7)] Consequently, the structure of **1** was elucidated as lanosta-9(11)-en-3β,16β,24*R*,25-tetraol 3-*O*-β-D-xylopranosyl-24-*O*-β-D-glucopyranoside.

The molecular formula of compound **2** was established as $C_{41}H_{62}O_{14}$ due to the molecular ion peak at *m*/*z* 777.4040 in the negative HR-ESI-MS. In the ^{1}H-NMR spectrum (Table 1), six quaternary methyls at δ_H 0.68, 0.91, 1.02,1.43, 1.59 and 1.72, and a secondary methyl at δ_H 1.04 (d, J=6.8 Hz) were observed. However, the conventional cyclopropane methylene upfield was not found. There were also two anomeric protons downfield at δ_H 4.79 (d, J=7.5 Hz) and 5.20 (d, J=7.8 Hz) which suggested the β-oriented anomeric centres of both sugars. Hydrolysis of **2** also afforded D-xylose and D-glucose, which were confirmed by GC analysis. Besides, three olefinic protons displayed at δ_H 5.37 (d, J=5.9 Hz), 5.40 (d, J=6.3 Hz) and 5.50 (s). The 41 signals in the ^{13}C-NMR spectrum were assigned to a xylose, a glucose while the rest 30 ones to the aglycone (Table 2). By analysis of DEPT, HSQC, HMBC and ^{1}H–^{1}H COSY, the planar struc-

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Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**5** (Pyridine d_5)

	1[b]	**2**[a]	**3**[a]	**4**[b]	**5**[a]
1	1.48, 1.81	5.50 s	1.23, 1.54	1.22, 1.54	1.19, 1.50
2	1.98, 2.34	2.33, 2.78 d (16.7)	1.94, 2.35 d (10.1)	1.93, 2.34 dd (12.4, 3.0)	1.92, 2.32 dd (12.5, 3.1)
3	3.37 dd (12.0, 4.0)	3.68 dd (8.2, 5.5)	3.50 dd (11.1, 3.7)	3.49 dd (11.6, 4.2)	3.48 dd (11.6, 4.3)
4					
5	0.96 d (10.8)	2.40	1.29	1.29	1.29
6	1.38, 1.63	2.32, 2.59 d (15.3)	0.50 q (11.9), 1.40	0.61 q (12.8), 1.44	0.70 dd (24.6, 12.3), 1.50
7	1.29, 1.62	5.40 d (6.3)	0.93 dd (23.6, 12.4), 1.14	0.92, 1.08	1.05, 1.29
8	2.32		1.34	1.43	1.68
9					
10					
11	5.28 d (5.3)	5.37 d (5.9)	1.04, 1.92	1.09, 1.94	1.05, 1.92
12	1.99, 2.10	2.14 dd (17.4, 5.8), 2.27	1.60, 1.65	1.69 t (7.25), 2H	1.68, 1.73
13					
14					
15	1.78, 2.09	2.07 d (17.8), 2.33	2.02 d (16.0), 2.19 dd (13.8, 7.7)	1.91, 2.17 dd (13.9, 8.0)	1.82 dd (13.1, 4.2), 2.21 dd (13.1, 7.9)
16	4.74 dd (12.7, 7.4)		4.32	4.43	5.07
17	1.80	2.36	1.89	2.70 dd (10.6, 8.2)	3.00 dd (10.7, 7.3)
18	1.12 s	0.68 s	1.23 s	1.29 s	1.44 s
19	1.07 s	3.11 d (13.5), 3.16 d (14.2)	0.22 s, 0.36 s	0.25 d (3.6), 0.44 d (3.1)	0.24 d (3.9), 0.50 d (3.4)
20	2.32	2.67 d (5.6)	2.73	2.46 dd (11.8, 4.8)	2.92 dd (10.6, 6.7)
21	1.06 d	1.04 d (6.8)	1.14 d (6.1)	1.22 d (6.8)	1.47 d (6.6)
22	1.67, 2.44	3.11 dd (19.6, 8.9), 4.02 dd (17.4, 9.2)	1.34, 2.65 t (12.7)	4.72 d (10.4)	4.91 s
23	1.25, 1.83		4.66 d (9.4)	1.86, 2.51 d (12.3)	4.33
24	3.92	4.65 s	3.56 s	4.37	4.33
25					
26	1.33 s	1.72 s	1.62 s	1.46 s	1.70 s
27	1.42 s	1.59 s	1.59 s	1.50 s	1.68 s
28	0.75 s	1.02 s	0.87 s	0.87	1.11 s
29	1.32 s	1.43 s	1.34 s	1.33 s	1.33 s
30	1.04 s	0.91 s	1.06 s	1.06 s	1.05 s
Xyl-[c]					
1′	4.79 d (7.6)	4.79 d (7.5)	4.88 d (7.5)	4.86 d (7.5)	4.87 d (7.6)
2′	4.03 t (8.2)	4.01	4.04	4.02 t (8.2)	4.03
3′	4.17 t (8.8)	4.16 t (8.7)	4.17 t (8.7)	4.17 t (8.7)	4.15
4′	4.23	4.22	4.20	4.22	4.22
5′	3.79 t (10.7), 4.41 dd (11.2, 5.2)	3.73 t (10.6), 4.34 dd (11.2, 5.6)	3.79 t (10.7), 4.41 dd (11.2, 5.2)	3.73 t (10.7), 4.35	3.75 t (10.6), 4.35
Glc-[c]					
1″	5.07 d (7.8)	5.20 d (7.8)	4.76 d (7.5)	4.80 d (7.9)	5.03 d (7.9)
2″	4.07 t (8.3)	4.07	4.04	3.99 t (7.7)	4.05
3″	4.21	4.26	4.20	4.21	4.13
4″	4.20	4.23	4.20	4.22	3.95
5″	3.93	3.96	3.90	3.75	3.93
6″	4.30 dd (11.6, 5.6), 4.52 dd (11.6, 2.1)	4.36 dd (11.4, 5.5), 4.51 d (10.1)	4.34, 4.52 d (11.2)	4.34, 4.43	4.12, 4.59 d (10.5)

a) Recorded at 400 Hz. *b*) Recorded at 500 Hz. *c*) Assigned by HSQC-TOCSY.

ture was determined as 9,10-*seco*-cycloarta-1(10),7,9-trien-3,24,25-triol-16,23-dione 3-*O*-xylosyl-25-*O*-glucoside (Fig. 2). HSQC-TOCSY was used to clarify the ambiguous part of the assignments in the sugar moieties. The HMBC correlations of H-1 [δ_H 5.50 (s)] with C-3 (δ_C 83.9, CH) and C-19 (δ_C 43.6, CH_2); H-2 [δ_H 2.78 (d J=16.7 Hz)] with C-1 (δ_C 120.6, CH), C-3, C-4 (δ_C 39.0, C) and C-10 (δ_C 138.7, C); H-7 [δ_H 5.40 (d, J=6.3 Hz)] with C-5 (δ_C 51.3, CH), C-6 (δ_C 25.1, CH_2), C-9 (δ_C 142.6, C) and C-14 (δ_C 45.9, C); H-6 [δ_H 2.32, 2.59 (d, J=6.3 Hz)] with C-5, C-7 (δ_C 125.3, CH), C-8 (δ_C 137.9, C) and C-10; H-11 [δ_H 5.37 (d, J=5.9 Hz)] with C-8, C-12 (δ_C 36.7, CH_2) and C-13 (δ_C 43.4, C) and H-12 with C-9, C-11 (δ_C 121.3, CH), C-13, C-14 and C-18 (δ_C 16.7, CH_3) located the three olefinic bonds at C-1–C-10, C-7–C-8 and C-9–C-11 respectively. The distribution of olefinic bonds were confirmed by UV. The experiment value of 248 (3.8) nm of the UV (MeOH) λ_{max} (log ε) matched the calculated value of λ_{max} of 244 nm derived from the Woodward–Fieser rules.[8] Two carbonyl groups were positioned at C-16 and C-23 due to the HMBC associations of H-15 [δ_H 2.07 (d, J=17.8 Hz), 2.33] and H-17 (δ_H 2.36) with C-16 (δ_C 218.5, C) and H-22 [δ_H 3.11 (dd, J=19.6, 8.9 Hz), 4.02 (dd,

Table 2. ^{13}C-NMR Spectral Data with DEPT of Compounds **1**—**5** (Pyridine d_5)

	1[b]	**2**[a]	**3**[a]	**4**[b]	**5**[a]
1	36.6 t	120.6 d	32.2 t	32.2 t	32.2 t
2	27.3 t	32.4 t	30.0 t	30.1 t	30.1 t
3	88.8 d	83.9 d	88.5 d	88.5 d	88.6 d
4	39.9 s	39.0 s	41.3 s	41.3 s	41.4 s
5	53.2 d	51.3 d	47.5 d	47.6 d	47.6 d
6	21.5 t	25.1 t	21.0 t	21.1 t	21.1 t
7	28.5 t	125.3 d	26.1 t	26.2 t	26.6 t
8	42.1 d	137.9 s	47.8 d	48.1 d	48.2 d
9	149.0 s	142.6 s	19.9 s	20.0 s	20.1 s
10	39.5 s	138.7 s	26.3 s	26.3 s	26.4 s
11	115.2 d	121.3 d	26.4 t	26.5 t	26.4 t
12	37.6 t	36.7 t	32.9 t	32.9 t	33.2 t
13	44.8 s	43.4 s	45.7 s	45.4 s	45.9 s
14	45.0 s	45.9 s	47.0 s	47.2 s	47.3 s
15	47.6 t	47.9 t	48.1 t	48.4 t	49.1 t
16	71.3 d	218.5 s	83.2 d	83.1 d	72.3 d
17	56.2 d	59.9 d	57.5 d	53.1 d	52.4 d
18	15.8 q	16.7 q	19.5 q	19.6 q	19.4 q
19	22.6 q	43.6 t	30.1 t	30.1 t	30.0 t
20	30.1 d	27.0 d	26.5 d	37.1 d	36.4 d
21	17.9 q	20.4 q	17.6 q	11.7 q	14.6 q
22	32.5 t	47.2 t	42.0 t	68.7 d	85.8 d
23	29.4 t	212.4 s	68.5 d	38.6 t	75.6 d
24	90.6 d	81.4 d	80.0 d	76.5 d	76.3 d
25	72.1 s	79.6 s	73.7 s	72.7 s	74.4 s
26	27.2 q	23.2 q	26.8 q	25.7 q	24.7 q
27	25.0 q	23.7 q	28.3 q	26.4 q	28.7 q
28	19.4 q	25.1 q	20.3 q	20.4 q	20.6 q
29	28.4 q	24.9 q	25.8 q	25.7 q	25.8 q
30	17.1 q	15.4 q	15.5 q	15.5 q	15.5 q
Xyl-[c]					
1′	107.8 d	107.3 d	107.6 d	107.5 d	107.6 d
2′	75.6 d	75.5 d	75.6 d	75.6 d	75.1 d
3′	78.7 d	78.6 d	78.7 d	78.6 d	78.7 d
4′	71.1 d	71.2 d	71.3 d	71.2 d	71.3 d
5′	67.2 t	67.2 t	67.2 t	67.1 t	67.2 t
Glc-[c]					
1″	105.7 d	97.8 d	107.0 d	106.5 d	106.5 d
2″	75.6 d	75.4 d	76.0 d	75.8 d	75.1 d
3″	78.5 d	78.8 d	78.4 d	78.8 d	78.7 d
4″	71.7 d	71.5 d	71.8 d	71.8 d	72.0 d
5″	78.5 d	78.6 d	78.4 d	78.2 d	78.8 d
6″	62.6 t	62.6 t	62.6 t	62.8 t	63.2 t

a) Recorded at 100 Hz. *b*) Recorded at 125 Hz. *c*) Assigned by HSQC-TOCSY.

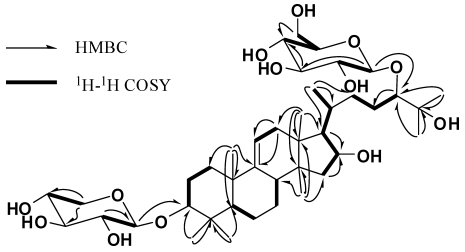


Fig. 1. HMBC and ^{1}H–^{1}H COSY Correlations of **1**

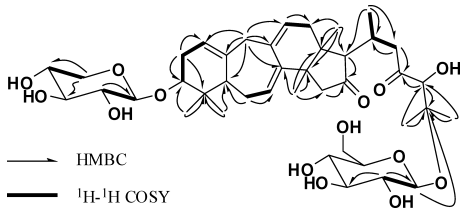


Fig. 2. HMBC and ^{1}H–^{1}H COSY Correlations of **2**

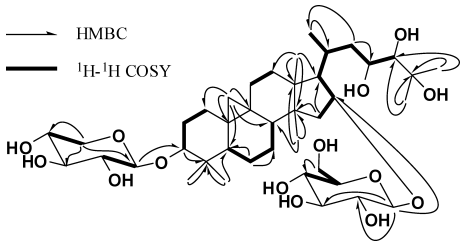
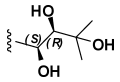
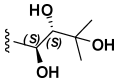
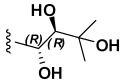
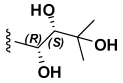


Fig. 3. HMBC and ^{1}H–^{1}H COSY Correlations of **3**

J=17.4, 9.2 Hz)] and H-24 [δ_H 4.65, (s)] with C-23 (δ_C 212.4, C). The HMBC cross-peaks of H-24 with C-23, C-25 (δ_C 79.6, C), C-26 (δ_C 23.2, CH_3) and C-27 (δ_C 23.7, CH_3) resulted in the hydroxyl group at C-24. The linkages of glycosides were deduced by the HMBC associations of H-1′ [δ_H 4.79 (d, J=7.5 Hz)] with C-3 and H-1″ [δ_H 5.20 (d, J=7.8 Hz)] with C-25. The ROESY experiment displayed normal cross-peaks of H-3 with H-5, and H_3-28 with H-17 indicating that there was no configuration change in the cycloartane skeleton except for the cleavage of the C-9–C-10 bond. The configuration of C-24 was not determined. Therefore, compound **2** was characterized as 9,10-*seco*-cycloarta-1(10),7,9-trien-3β,24ζ,25-triol-16,23-dione 3-*O*-β-D-xylopranosyl-25-*O*-β-D-glucopyranoside.

The molecular formula of compound **3** was $C_{41}H_{70}O_{14}$ deduced by the molecular ion peak appearing at *m*/*z* 785.4665 in the negative HR-ESI-MS. The typical cycloproprane methylene at δ_H 0.22 (s) and 0.36 (s); six quaternary methyls at δ_H 0.87, 1.06, 1.23, 1.34, 1.59 and 1.62 and a secondary methyl at δ_H 1.14 (d, J=6.1 Hz) were observed in the ^{1}H-NMR spectrum (Table 1). Two anomeric protons downfield at δ_H 4.88 (d, J=7.5 Hz) and 4.76 (d, J=7.5 Hz) revealed the existence of two sugars with the β configuration. Hydrolysis experiment with GC detection also proved that **3** possessed the same type of sugar moieties (D-xylose and D-glucose) as the compounds above. However, in the ^{13}C-NMR spectrum, there were no characteristc signals of hemiketal or acetal carbons.[9—12] The planar structure was elucidated to be cycloarta-3,16,23,24,25-pentaol 3-*O*-xylosyl-24-*O*-glucoside by comprehending the information of DEPT, HSQC, HMBC and ^{1}H–^{1}H COSY experiments (Fig. 3). The HMBC correlations of H-22 [δ_H 1.34, 2.65 (d, J=12.7 Hz)] with C-23 (δ_C 68.5, CH); H-24 [δ_H 3.56 (s)] with C-22 (δ_C 42.0, CH_2), C-25 (δ_C 73.7, C), C-26 (δ_C 26.8, CH_3) and C-27 (δ_C 28.3, CH_3) and H_3-26 [δ_H 1.62 (s)] and H_3-27 [δ_H 1.59 (s)] with C-24 and C-25 indicated the location of the three hydroxyl groups at C-23, C-24 and C-25. H-20 (δ_H 2.73), H-21 [δ_H 1.14 (d, J=6.1 Hz)], H-22, H-23 [δ_H 4.66 (d, J=9.4 Hz)] and H-24 also correlated with neighbouring protons in ^{1}H–^{1}H COSY. The glycosidation positions were proved at C-3 and C-16 considering the HMBC cross-peaks btween H-1′ [δ_H 4.88 (d, J=7.5 Hz)] and C-3 (δ_C 88.5, CH) as well as between H-1″ [δ_H 4.76 (d, J=7.5 Hz)] and C-16 (δ_C 83.2, C). The ROESY associations of H-15α, which concluded by the cross-peaks of H-15α with H_3-28, and H-17 with H-16 suggested that the glycosidated hydroxyl group at C-16 has the β form. Comparing the NMR data of C-22—C-25 with refer-

Table 3. Comparison of NMR Data of Total Four Configurations of C-23 and C-24

	3	 Alisol A[13]	Alisol P[14]	 Alisol E[15]	 Orbicoside[16,17]	 Cumingianoside Q[18]	23,24,25-Trihydroxy cycloartan-3-one[19]
C-22	42.0	40.0	39.9	38.5	43.0	42.0	40.0
C-23	68.5	69.5	69.6	73.2	73.2	69.4	69.7
H-23	4.66 d (br 9.4)	3.77 dd (9.6, 3.6)	3.77 dd (9.3, 3.5)	3.47 (t-like)	4.32 td (8.4, 3)	4.52 br t (7)	4.13 dd (9.4, 4.8)
C-24	80.0	77.6	77.4	79.1	79.1	77.0	75.0
H-24	3.56 s (br)	3.00 s (br)	3.00 s (br)	3.27 d (6)	3.78 d (8.4)	3.57 br s	3.18 s (br)
C-25	73.7	74.1	74.4	71.6	74.3	73.8	74.3

ence data[13—19] (Table 3), the configurations of C-23 and C-24 in **3** were 23*R* and 24*S*, respectively. In sum, **3** was established as cycloarta-3β,16β,23*R*,24*S*,25-pentaol 3-*O*-β-D-xylopyranosyl-16-*O*-β-D-glucopyranoside.

Compound **4** is an isomer of **3** (molecular ion peak at *m*/*z* 785.4696 in the negative HR-FAB-MS). The major changes in their NMR data were the hydroxylation of C-22 and C-24 in **4** instead of C-23 and C-24 in **3** (Tables 1, 2). The HMBC experiment showed correlations of H-23 [δ_H 1.86, 2.51 (d, J=12.3 Hz)] with C-22 (δ_C 68.7, CH); H_3-26 [δ_H 1.46 (s)] and H_3-27 [δ_H 1.50 (s)] with C-24 (δ_C 76.5, CH) and C-25 (δ_C 72.7, C). Furthermore, their cross-peaks of H-22 [δ_H 4.72 (d, J=10.4 Hz)], H-23 and H-24 (δ_H 4.37) also can be observed in ^{1}H–^{1}H COSY spectrum (Fig. 4). Consequently, the structure of **4** was concluded as cycloarta-3β,16β,22ζ,24ζ,25-pentaol 3-*O*-β-D-xylopyranosyl-16-*O*-β-D-glucopyranoside.

The NMR data showed that **5** was also a cycloartane bisglycoside (Tables 1, 2). Hydrolysis reaction with GC detection confirmed that the sugars were D-xylose and D-glucose as in the other compounds. The negative HR-ESI-MS inferred a molecular formula of $C_{41}H_{70}O_{15}$ (molecular ion peak *m*/*z* 801.4625) which suggested that **5** was an analogue of **4** with an extra hydroxyl group. The HMBC correlations of H-15 [δ_H 1.82 (dd, J=13.1, 7.9 Hz), 2.51 (dd, J=13.1, 4.2 Hz)] and H-17 [δ_H 3.00 (dd, J=10.7, 7.3 Hz)] with C-16 (δ_C 72.3, CH) and H_3-26 [δ_H 1.70 (s)] and H_3-27 [δ_H 1.68 (s)] with C-25 (δ_C 74.4, C) and C-24 (δ_C 76.3, CH) implied hydroxylated positions at C-16, C-24 and C-25. The ^{1}H–^{1}H COSY associations of H-20 [δ_H 2.92 (dd, J=10.6, 6.7 Hz)], H-22 [δ_H 4.91 (s)] and H-23 (δ_H 4.33) exhibited an other hydroxyl group at C-23. The glycosidated hydroxyls were located at C-3 and C-22 due to the HMBC correlations of H-1′ [δ_H 4.87 (d, J=7.6 Hz)] with C-3 (δ_C 88.6, CH) and H-1″ [δ_H 5.03 (d, J=7.9 Hz)] with C-22 (δ_C 85.8, CH). The anomeric centres of both sugars were β oriented due to the J value of the anomeric protons mentioned above. The structure of **5** was determined accordingly as cycloarta-3β,16β,22ζ,23ζ,24ζ,25-hexaol 3-*O*-β-D-xylopyranosyl-22-*O*-β-D-glucopyranoside.

Compounds **1**—**3** and **5** were tested for cytotoxicity against human promyelocytic leukemia (HL-60) cells, human hepatocellular carcinoma (SMMC-7721) cells, carcinomic human alveolar basal epithelial (A-549) cells, human breast cancer (SK-BR-3) cells and human pancreatic adenocarcinoma (PANC-1) cells by the 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide (MTT) method with *cis*-diamminedichloroplatinum (DDP) as a positive control. Compound **1** exerted moderate inhibition against HL-60 and SMMC-7721 cell growth with IC_{50} values of 12.64 and 30.59 μM respectively. The rest compounds were inactive with IC_{50} values >40 μM.

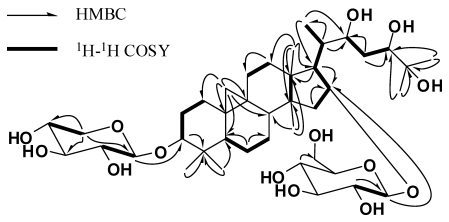


Fig. 4. HMBC and ^{1}H–^{1}H COSY Correlations of **4**

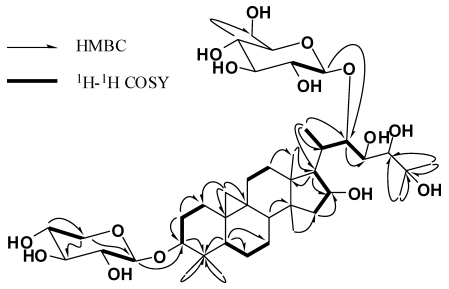


Fig. 5. HMBC and ^{1}H–^{1}H COSY Correlations of **5**

Compounds **1**—**5** widened our outlook of triterpenoids in *Cimcifuga*. The large quantity of cycloartane type triterpenoids have their side chains epoxidized with ring D in *Cimicifuga*. However, these five trace triterpenoid bisglycosides seem to have C-16 or side chain glycosylated so as to keep from epoxidation. Their roles in the biosynthesis process are interesting to seek after as well as their possible bioactivities.

Experimental

General Procedure Optical rotations were recorded on a Horiba SEPA-300 polarimeter; UV spectra on a Shimadzu UV-2401A spectrophotometer; and IR spectra on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H-, ^{13}C-NMR and 2D-NMR spectra were recorded on a Bruker AV-400 MHz or a DRX-500 spectrometer with TMS as an internal standard. MS were recorded using VG Autospec-300, Finnigan MAT-90 and API Qstar-Plusar-1 spectrometers. Column chromatography was carried out on silica gel (200—300 mesh, Qingdao Haiyang Chemical Ltd.) and Lichroprep RP-18 (40—63 μm, Merck, 4.5 cm×20 cm). TLC was performed on GF_{254} pre-coated plates (0.20—0.25 mm, Qingdao Haiyang Chemical Co., Ltd.) and detected by dipping with 10% H_2SO_4 followed by heating. GC experiments were achieved by using a GC-17A with an H_2 flame ionization detector (FID)

(Shimadzu). The GC column was a DB-1 capillary column (15 m× 0.25 mm). Sample D-glucose and D-xylose were purchased from Sinopharm Chemical Reagent Co., Ltd. as well as hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS). Sample L-xylose and L-glucose were commercially obtained from Acros Organics along with L-cysteine methyl easter hydrochloride.

Plant Material The roots of *C. foetida* were collected in Lijiang county, Yunnan province in July 2004 and identified by Prof. Pei Shengji (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (KIB 04072601) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The air-dried and powdered roots of *C. foetida* (10 kg) were extracted three times with MeOH under reflux. After removal of the solvent by evaporation, the residue (950 g) was suspended in H_2O and partitioned sequentially with $CHCl_3$ and *n*-BuOH. The remaining water-soluble portion (105 g) was subjected to silica gel chromatography and eluted with $CHCl_3$–MeOH (10 : 1, 8 : 1, 5 : 1, 1 : 1) to give four fractions (fr. 1—4). Fr. 2. (0.5 g) was chromatographed repeatedly over RP-18 (60—70% MeOH–H_2O) to yield **2** (13 mg), **1** (25 mg) and **3** (14 mg) successively, while fr. 3. (0.3 g) afforded **5** (13 mg) and **4** (17 mg) in the same polarity of elution as above.

Compound **1**: A white powder, $[\alpha]_D^{19}$ +16.3° (c=0.12, C_5H_5N); HR-ESI-MS (769.4742; Calcd for $C_{41}H_{69}O_{13}$, 769.4738). IR (KBr) cm^{-1}: 3425, 2942, 2859, 1729, 1447, 1087. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

Compound **2**: A white powder; $[\alpha]_D^{22}$ +77.8° (c=0.11, MeOH); HR-ESI-MS (777.4040; Calcd for $C_{41}H_{61}O_{14}$, 777.4061). UV (MeOH) λ_{max} (log ε) nm: 202 (3.7), 248 (3.8); IR (KBr) cm^{-1}: 3416, 2885, 1729, 1073, 1044. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

Compound **3**: A white powder; $[\alpha]_D^{19}$ −4.8° (c=0.10, C_5H_5N); HR-ESI-MS (785.4665; Calcd for $C_{41}H_{69}O_{14}$, 785.4687). IR (KBr) cm^{-1}: 3400, 2933, 1387, 1077, 1051. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

Compound **4**: A white powder; $[\alpha]_D^{19}$ +6.8° (c=0.07, MeOH); HR-FAB-MS (785.4696; Calcd for $C_{41}H_{69}O_{14}$, 785.4687). IR (KBr) cm^{-1}: 3407, 2933, 1088, 1052. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

Compound **5**: A white powder; $[\alpha]_D^{19}$ −6.3° (c=0.16, MeOH); HR-ESI-MS (801.4625; Calcd for $C_{41}H_{69}O_{15}$, 801.4636). IR (KBr) cm^{-1}: 3417, 2940, 2870, 1075, 1044. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

Sugar Analysis Compounds **1**—**5** (each 3 mg) were separately refluxed with 2 N HCl/1,4-dioxane 1 : 1 (2 ml) at 100°C for 2 h. After neutralizing with Ag_2CO_3 (300 mg), $CHCl_3$ (2 ml×3) was used for extraction. The filtrate of the aqueous layer was concentrated to dryness under reduced pressure and then dissolved in pyridine (100 μl). After that, L-cysteine methyl ester hydrochloride (1.1 mg) was added, and the mixture was kept at 60 °C for 1 h. Then the trimethylsilylation reagents HMDS (100 μl) and TMCS (50 μl) were added successively, and the mixture was kept at 4—8 °C for 8 h. The filtrate was subjected to GC analysis under the following conditions: column temperature, 180 °C→250 °C, 8 °C/min; column pressure, 80 kPa; injector and detector temperature, 250 °C and 280 °C respectively; injection volume, 8 μl; and split ratio, 1/30. GC analysis showed the presence of D-xylose and D-glucose while compared with the authentic D- and L-xylose (t_R 7.414 and 7.759 min respectively) and glucose (t_R 9.821 and 10.023 min respectively).

Acknowledgments This work was supported by the Natural Science Foundation of Yunnan (No. 2005C0010Z) and Natural Science Foundation of China (No. 30772636), as well as Foundation of Key State Lab. of Phytochemistry and Plant Resources in West China (P2008ZZ05), Innovative Key Projects of Chinese Academy of Sciences (CAS) (KSCX2-YW-R-194) and CAS action-plan for West Development (29KZCX2-XB2-15-03).

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