Cycloartane Glycosides from *Cimicifuga dahurica*

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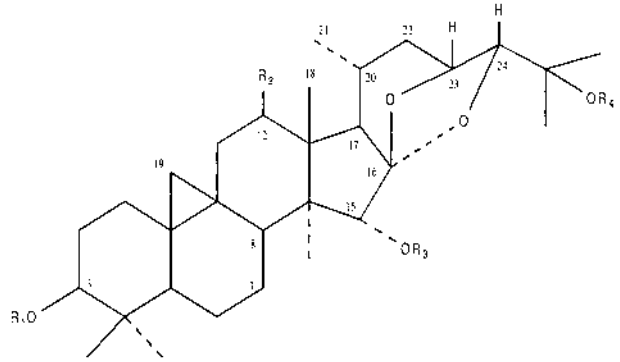
A new cycloartane bisdesmoside and two new trinorcycloartane glycosides, along with four known cycloartane compounds, were isolated from the rhizomes of *Cimicifuga dahurica* (Ranunculaceae). The structures of the new compounds were elucidated as 3-*O*-α-L-arabinopyranosyl cimigenol 15-*O*-β-D-glucopyranoside, 24-hydroxy-12β-acetoxy-25,26,27-trinorcycloartan-16,23-dione 3β-*O*-α-L-arabinopyranoside, and 16α,24α-dihydroxy-12β-acetoxy-25,26,27-trinor-16,24-cyclocycloartan-23-one 3β-*O*-α-L-arabinopyranoside by extensive NMR methods, FAB-MS, and hydrolysis.

Key words *Cimicifuga dahurica*; Ranunculaceae; cycloartane glycoside; trinorcycloartane glycoside

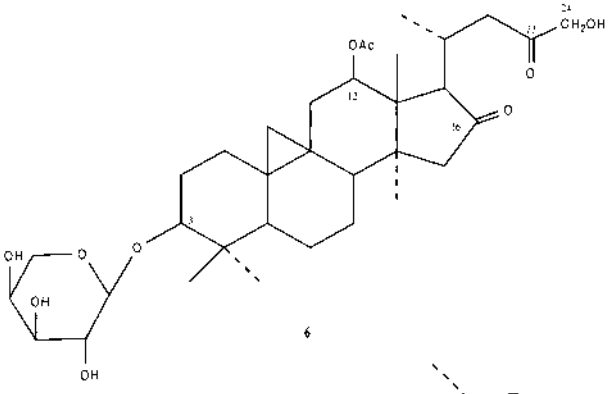
The rhizomes of *Cimicifuga dahurica* (TURCZ.) MAXIM., *C. heracleifolia* KOM., and *C. foetida* L. (Ranunculaceae) are used as antipyretic and analgesic remedies in Chinese medicine.[1] Another species, *C. racemosa* (black cohosh), is traditionally used to treat menopausal symptoms in Europe and North America. Chemical studies have resulted in the isolation of a series of 9,19-cycloartane triterpene glycosides from *Cimicifuga species*.[2—5] In a previous paper,[5] we reported on two cycloartane glycosides, cimigenol 3-*O*-α-L-arabinopyranoside and 25-*O*-acetyl-cimigenol 3-*O*-α-L-arabinopyranoside, from the rhizomes of *C. dahurica*. A reinvestigation of the ethanol extract of this plant has now led to the isolation of a new bisdesmoside (**5**) and two new trinorcycloartane glycosides (**6**, **7**), together with four known compounds (**1**—**4**).

Air-dried rhizomes of *C. dahurica* were extracted with 95% ethanol. The EtOAc-soluble fraction was subjected to silica gel column chromatography and further purified by octadecyl silica (ODS) chromatography to afford compounds **1**—**7**. Upon acid hydrolysis, compounds **2**—**4** afforded cimigenol (**1**), 7,8-didehydrocimigenol, and 12β-hydroxylcimigenol, respectively. On the other hand, alkaline hydrolysis of **3** in 1% Na_2CO_3 afforded **2** as the major product. The sugars obtained from the aqueous hydrolysates were determined to be D-xylose (in **2** and **3**), and L-arabinose (in **4**) by direct comparison with authentic samples using HPLC and optical rotation measurement. Compounds **1**—**4** were therefore elucidated to be cimigenol (**1**),[6] 7,8-didehydrocimigenol 3-*O*-β-D-xylopyranoside (**2**),[7] 25-*O*-acetyl-7,8-didehydrocimigenol 3-*O*-β-D-xylopyranoside (**3**),[7] and 12β-hydroxylcimigenol 3-*O*-α-L-arabinopyranoside (**4**)[8] by comparing their physical and spectral data [including one- and two-dimensional (1D, 2D)-NMR, FAB-MS] with the literature values.

Compound **5** was obtained as a white powder. The FAB-MS of **5** displayed a quasimolecular ion $[M+H]^+$ at *m*/*z* 777, consistent with a molecular formula of $C_{41}H_{60}O_{14}$. The NMR spectrum of **5** exhibited characteristic signals for cyclopropane methylene at δ_H 0.32 and 0.56 (each 1H, d, J=3.6 Hz) and δ_C 31.4. Acid hydrolysis of **5** yielded cimigenol (**1**) [high performance thin-layer chromatography (HPTLC), ^{1}H- and ^{13}C-NMR], D-glucose, and L-arabinose (identified by direct comparison of HPLC retention times and optical rotation values with authentic samples). A compari-



1. $R_1 = R_2 = R_3 = R_4 = H$
2. R_1 = Xyl, $R_2 = R_3 = R_4$ = H, 7,8-ene
3. R_1 = Xyl, $R_2 = R_3$ = H, R_4 = Ac, 7,8-ene
4. R_1 = Ara, R_2 = OH, $R_3 = R_4$ = H
5. R_1 = Ara, $R_2 = R_4$ = H, R_3 = Glc



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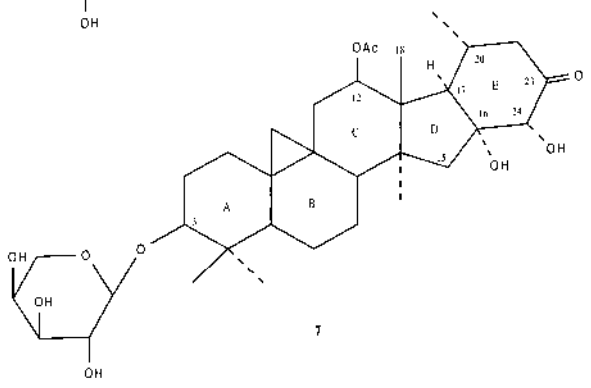

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Table 1. ^{1}H- and ^{13}C-NMR Data of **5**—**7** (Pyridine-d_5, δ in ppm)[a,b]

	5		**6**		**7**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	33.0	1.22	32.2	1.10	32.1	1.10
		1.56		1.49		1.49
2	30.5	1.95	30.1	1.88	30.0	1.87
		2.36		2.33		2.28
3	88.9	3.48 dd (11.6, 4.4)	88.2	3.46 dd (11.6, 4.4)	88.2	3.44 dd (11.6, 4.0)
4	41.4	—	41.5	—	41.3	—
5	47.9	1.30	47.2	1.23	47.4	1.23
6	21.9	0.76	20.7	0.66	20.8	0.72
		1.64		1.47		1.46
7	26.8	1.22	26.3	0.94	26.1	0.96
		2.46		1.13		1.24
8	49.1	1.73	45.8	1.59	46.9	1.48
9	20.7	—	20.0	—	19.5	—
10	27.0	—	27.6	—	26.7	—
11	26.3	1.04	36.3	1.21	38.0	1.16
		2.03		2.66		2.95
12	34.2	1.56	76.4	5.49 dd (9.2, 3.2)	77.4	5.37 dd (9.0, 3.2)
		1.64				
13	41.5	—	48.8	—	51.1	—
14	47.8	—	43.8	—	48.8	—
15	88.3	4.37 s	50.9	2.08 d (18.0)	49.8	2.02
				2.29 d (18.0)		2.25
16	111.8	—	217.5	—	82.7	—
17	59.7	1.50	61.5	2.76	63.9	2.45
18	20.1	1.14 s	13.8	1.35 s	13.5	1.33
19	31.4	0.32 d (3.6)	30.7	0.27 d (4.0)	30.4	0.30 d (4.0)
		0.56 d (3.6)		0.58 d (4.0)		0.57 d (4.0)
20	24.3	1.62	26.8	2.80	25.7	2.24
21	20.2	0.86 d (6.4)	23.3	1.26 d (6.4)	21.3	0.99 d (6.8)
22	38.3	0.93	43.6	2.69	45.6	2.46
		2.20		3.02 dd (17.6, 7.2)		2.56
23	71.6	4.71 br d (8.8)	211.0	—	210.8	—
24	89.8	3.53 s	69.6	4.46 d (18.4)	82.3	4.52 s
				4.53 d (18.4)		
25	71.0	—		—		—
26	26.1	1.23 s		—		—
27	27.8	1.40 s		—		—
28	13.1	1.28 s	19.5	0.97 s	20.4	1.39 s
29	26.1	1.23 s	26.0	1.29 s	25.8	1.28 s
30	15.8	1.05 s	15.8	0.97 s	15.6	1.00 s
Ac			21.6	2.23 s	21.8	2.12 s
			170.6	—	170.4	—
ara 1′	107.6	4.80 d (7.6)	107.6	4.79 d (7.2)	107.5	4.77 d (7.2)
2′	73.1	4.47	73.1	4.44 t (7.2)	73.0	4.44 dd (7.2, 7.2)
3′	74.9	4.18	74.9	4.17 dd (7.2, 2.8)	74.7	4.15 dd (7.2, 3.2)
4′	69.7	4.35	69.8	4.32 br s	69.6	4.30 br s
5′	66.9	4.28	67.0	4.30 dd (10.8, 2.4)	66.9	3.78 br d (10.8)
		3.78		3.80 d (10.8)		4.29 br d (10.8)
glc 1″	105.3	4.99 d (7.2)				
2″	75.9	4.11				
3″	78.7	4.28				
4″	72.7	4.15				
5″	78.1	4.06				
6″	62.3	4.33				
		4.47				

a) Assignments were established by interpretation of the distortionless enchancement by polarization transfer (DEPT), DQF-COSY, HMQC, and HMBC spectra. *b*) *J* values (in Hz) are given in parentheses. Overlapped signals are reported without designating multiplicity.

son of the ^{13}C-NMR data of **5** with those of **1** indicated glycosylation shifts at C-3 (+11.1 ppm) and C-15 (+8.2 ppm). Long-range heteronuclear maltiple bond connectivity (HMBC) correlations were observed between 3α-H (δ 3.48) of the aglycon and C-1′ (δ 107.6) of arabinose, as well as between 15-H (δ 4.37) of the aglycon and C-1″ (δ 105.3) of glucose. Hence, compound **5** was elucidated to be 3-*O*-α-L-arabinopyranosyl cimigenol 15-*O*-β-D-glucopyranoside. Examination of the double quantum filtered correlation spectroscopy (DQF-COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), and HMBC spectra led to the assignments of all proton and carbon signals as shown in Table 1.

The FAB-MS of **6** displayed a quasimolecular ion

$[M+Na]^+$ at *m/z* 643, consistent with the molecular formula of $C_{34}H_{52}O_{10}$. Acid hydrolysis of **6** yielded L-arabinose. Similar to **5**, the ^{1}H-NMR spectrum of **6** (Table 1) exhibited signals for cyclopropane methylene at δ 0.27 and 0.58 (each 1H, d, J=4.0 Hz). A comparison of the NMR data between **6** and a known trinorcycloartane glycoside, cimicifugenoside H-3,[9] suggested they possess an identical side chain structure. The proton signals at δ 1.26, 2.76, 2.80, 2.69, and 3.02 could be assigned to 21-H, 17-H, 20-H, 22-H_a, and 22-H_b, respectively, by DQF-COSY experiments. Following the above assignments, the locations of two ketonic carbons could be deduced based on HMBC data. Thus long-range correlations between signals at δ_C 217.5 and δ_H 2.76 (17-H), as well as between δ_C 211.0 and δ_H 2.69 (22-H_a)/3.02 (22-H_b) were observed, indicating that both 16-C and 23-C belong to ketonic carbons. Moreover, HMBC correlations could be demonstrated between δ_C 211.0 (C-23) and δ_H 4.53 (24-H_a)/4.46 (24-H_b), between the acetyl carboxyl (δ_C 170.6) and 12α-H (δ_H 5.49), as well as between the anomeric carbon (δ_C 107.6) of arabinose and 3-H (δ_H 3.46) of the aglycone. All available evidence led to the conclusion that compound **6** is 24-hydroxy-12β-acetoxy-25,26,27-trinorcycloartan-16,23-dione 3β-*O*-α-L-arabinopyranoside.

The FAB-MS of compound **7** displayed quasimolecular ions $[M+H]^+$ and $[M+Na]^+$ at *m/z* 621 and 643, respectively, suggesting the same molecular formula as that of **6**. Analysis of the NMR data of **7** and a comparison with those of **6** indicated that the former was different from the latter by the formation of an E ring, as well as the loss of the CH_2OH group in **7**. The HMBC results supported the above observation. Thus the HMBC spectrum of **7** exhibited long-range correlation signals between H-15/C-24 and H-17/C-24, suggesting that a bond is formed between C-16 and C-24.[9] The relative stereochemistry at these two carbons was then deduced from an nuclear Overhauser effect (NOE) experiment. Upon irradiation of 18-H_3 (δ 1.33), enhancement of 24-H (δ 4.52) was observed. Such a result could only arise in the case of a D/E *cis*-ring junction and 24α-H. It followed that both the 16- and 24-hydroxyl groups must locate in an α-configuration. Thus the structure of compound **7** was established to be 16α,24α-dihydroxy-12β-acetoxy-25,26,27-trinor-16,24-cyclocycloartan-23-one 3β-*O*-α-L-arabinopyranoside.

It should be noted that for cimigenol derivatives such as **1**—**5**, the biogenetic precursors are generally accepted to be hydroshengmanol and shengmanol,[2] whereas for compounds **6** and **7**, the parental structures may be the genins of cimicifugosides H-1 and H-2.[9] These parental structures have yet to be found in *C. dahurica*.

Experimental

Melting points were measured on a Leica Galen III micro melting point apparatus and were uncorrected. Optical rotations were measured on a Pekin-Elmer 241 polarimeter. ^{1}H-, ^{13}C- and 2D-NMR spectra were recorded on a JEOL JNM-EX400 spectrometer. FAB-MS spectra were determined in positive ion mode on a Finnigan MAT TSQ7000 spectrometer. Column chromatography was carried on silica gel (100—200 mesh) and ODS (10—40 μm). TLC was conducted on Silica gel 60 F_{254} and RP-18 F_{254} S plates (Merck). HPLC was performed using an ODS column (Waters, NOVA-Pak C_{18}, 3.9×300 mm).

Plant Materials The rhizomes of *C. dahurica* (TURCZ.) MAXIM. were collected in Yianbian, Jilin Province, China. The plant was authenticated by Dr. Zhe-Bin Zheng, and a voucher specimen (No. 930812) has been deposited in the herbarium of the China Pharmaceutical University.

Extraction and Isolation Air-dried rhizomes of *C. dahurica* (4.0 kg) were extracted three times with 95% EtOH for 3 h each under reflux. The EtOH extract was concentrated and fractionated by successive extraction using *n*-hexane (1000 ml×3), EtOAc (1000 ml×3), and *n*-BuOH (1000 ml×3) to afford an *n*-hexane fraction (30 g), an EtOAc fraction (110 g), and an *n*-BuOH fraction (100 g). The EtOAc fraction (70 g) was subjected to silica gel column chromatography (700 g, $CHCl_3$–MeOH, 99 : 1→75 : 25), followed by ODS column chromatography (100 g, MeOH–H_2O, 4 : 6→9 : 1), to afford **1** (50 mg), **2** (80 mg), **3** (230 mg), **4** (70 mg), **5** (210 mg), **6** (20 mg), and **7** (80 mg).

Compound **5**: White powder (MeOH), mp 224—225 °C, $[\alpha]_D$ +15.9° (c=0.32, MeOH). IR (KBr) cm^{-1}: 3450 (OH), 3034, 1634, 1450, 1065 (C–O–C), 987. FAB-MS *m/z* 777 $[M+H]^+$. *Anal.* Calcd for $C_{41}H_{60}O_{14}$: C, 61.19; H, 7.46. Found: C, 61.25; H, 7.41. ^{1}H- and ^{13}C-NMR data: see Table 1.

Compound **6**: White powder (MeOH), mp 233—235 °C. Alkaline blue tetrazolium reaction on TLC was positive. IR (KBr) cm^{-1}: 3500—3300 (OH), 1735, 1731, 1710 (C=O), 1450, 1040, 992. FAB-MS *m/z* 621 $[M+H]^+$, 643 $[M+Na]^+$. *Anal.* Calcd for $C_{34}H_{52}O_{10}$: C, 65.81; H, 8.39. Found: C, 65.87; H, 8.34. ^{1}H- and ^{13}C-NMR data: see Table 1.

Compound **7**: White powder (MeOH), mp 257—259 °C, $[\alpha]_D$ −102.1° (c=0.18, MeOH). Alkaline blue tetrazolium reaction on TLC was positive. IR (KBr) cm^{-1}: 3500—3350 (OH), 1734, 1720 (C=O), 1632, 1450, 1040, 991. FAB-MS *m/z* 621 $[M+H]^+$, 643 $[M+Na]^+$. *Anal.* Calcd for $C_{34}H_{52}O_{10}$: C, 65.81; H, 8.39. Found: C, 65.86; H, 8.31. ^{1}H- and ^{13}C-NMR data: see Table 1.

Acid Hydrolysis and Identification of Sugars in 5—7 A solution of the compound (20 mg) in 50% MeOH containing HCl (0.5 N) was heated under reflux for 3 h. The reaction mixture was neutralized with NaOH (0.5 N), diluted with H_2O, and then partitioned with EtOAc. The EtOAc solution was chromatographed on a Sephadex LH-20 column (*ca.* 40 g) to afford the aglycon, which was identified by comparison with NMR data. The water layer was concentrated, filtered, and passed through a NOVA-Pak C_{18} cartridge (Waters), followed by repeatedly separation on HPLC [chromatographic conditions: mobile phase: MeCN–H_2O (3 : 1); flow rate: 0.6 ml/min; detection: refractive index (RI)] to afford D-glucose (3.1 mg in **5**, 16.8 min) and L-arabinose (2.8 mg in **5**, 3.3 mg in **6**, and 3.5 mg in **7**, 13.7 min). The optical rotation values of the monosaccharides were as follows: D-glucose, $[\alpha]_D^{25}$ +53.3° in **5** (lit.[10] +52.7°); L-arabinose, $[\alpha]_D^{25}$ +102.1° in **5**, +103.8° in **6**, and +103.6° in **7** (lit.[10] +103.0°).

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