Cycloartane Triterpene Saponins from the Roots of *Cimicifuga foetida*

Nanqun Zhu,[†] Yi Jiang,[‡] Mingfu Wang,[†] and Chi-Tang Ho*[,†]

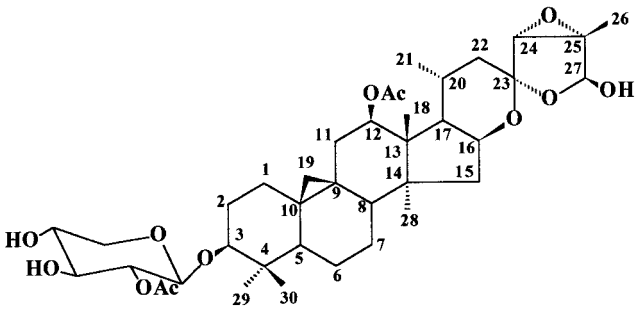
Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901, and Department of Drug Discovery, Shanghai Institute of Pharmaceutical Industry, Shanghai 200040, People's Republic of China

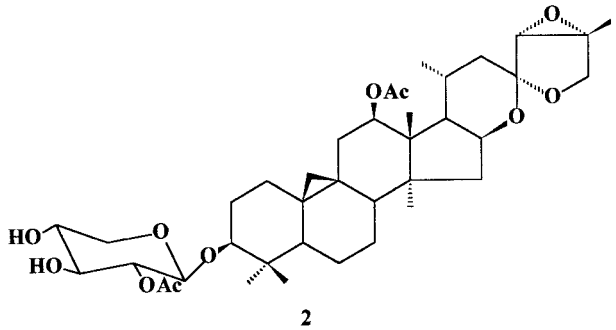
Received October 10, 2000

The structures of two new cycloartane-type saponin constituents of the roots of *Cimicifuga foetida* were established by the interpretation of spectroscopic data as 2′-*O*-acetylactein (**1**) and 2′-*O*-acetyl-27-deoxyactein (**2**), respectively. Also isolated were the known compounds actein, 27-deoxyactein, cimicifugoside H-1, and 15α-hydroxycimicidol 3-*O*-*β*-D-xyloside.

Cimicifuga racemosa (Ranunculaceae), commonly known as black cohosh, was used by Native Americans for the treatment of diarrhea, rheumatism, and sore throats.[1] Now, as a potential alternative to estrogen therapy in the treatment of menopausal symptoms, black cohosh has become a popular dietary supplement in the United States. In China and Japan, traditional medicine has long employed other *Cimicifuga* species, such as *C. simplex* (Ranunculaceae) and *C. foetida* (Ranunculaceae), for the alleviation of fever, pain, and inflammation.[2] In the market, Asian *Cimicifuga* species, such as *C. simplex*, *C. foetida*, or a combination of these species, have also been used for the same purpose as black cohosh extracts. Triterpene glycosides are considered to be the main active components and are used as marker compounds to standardize black cohosh extracts.[3] Until now, 16 triterpene glycosides have been isolated from black cohosh, more than 50 compounds of this type have been identified from *C. simplex*,[4] and more than 20 triterpene or triterpene glycosides have been reported from *C. foetida*.[5−10] Herein, we report the isolation and identification of two new cycloartane triterpenoids (**1** and **2**) along with four known triterpene glycosides, namely, actein, 27-deoxyactein, cimicifugoside H-1, and 15α-hydroxycimicidol 3-*O*-*β*-D-xyloside, from *C. foetida*.

Compound **1** was isolated as a white amorphous powder, and its molecular formula was determined from its ^{13}C NMR spectral and FABMS data (*m*/*z* 725 $[M + Na]^+$) as $C_{39}H_{58}O_{11}$. The ^1H NMR spectrum (Table 1) showed the presence of cyclopropane methylene groups at *δ* 0.29 and 0.62 (each 1H, d, $J = 4.0$ Hz), two acetyl methyl groups at *δ* 2.26 and 2.28, a secondary and five tertiary methyl groups at *δ* 0.88−1.88, protons of a five-membered sugar (*δ* 3.90, 4.25, 4.27, 4.42, 4.88, and 5.60), and five oxygen-substituted methine groups (*δ* 3.41, 4.04, 4.68, 5.17, and 5.83). The ^{13}C NMR spectrum of **l** (Table 1) showed the signal of one methylene carbon ascribable to a cyclopropane ring at *δ* 29.8, methine carbons at *δ* 63.7 (C-24), 73.7 (C-16), 77.4 (C-12), 88.6 (C-3), and 98.7 (C-27), two quaternary carbons at *δ* 65.9 (C-25) and 106.2 (C-23), and five carbons for the sugar, which were assigned with the help of 2D NMR spectra. This spectrum also exhibited two carbonyl groups at *δ* 171.1 and 170.5. All the spectral data of **1** showed a very close similarity to those of actein[11] and also suggested the configurations at C-23 C-24, C-25, and C-27 were the same as those of acetin, which were assigned as *R*, *R*, *S*, and *S*, respectively.[12] The sugar was identified as *β*-D-xylose by acid hydrolysis followed by TLC analysis with an authentic sample. Furthermore, compared with those of actein,[11] a significant difference in the ^1H NMR spectrum of the sugar moiety was the methine proton, which appeared at *δ* 5.60 instead of at *δ* 3.94. In addition, in the ^{13}C NMR spectrum, the signal due to C-1′ showed an upfield shift from *δ* 107.4 to 104.8, the signal for C-2′ shifted from *δ* 75.5 to 75.9, and the signal due to C-3′ exhibited an upfield shift from *δ* 78.5 to 76.4. These shifts could be explained by acetylation on carbon 2 of the xylose unit.[13] This result was also supported by the HMBC contour between the H-2′ signal and the carbonyl group signal at *δ* 170.5. On the basis of these data, the structure of **1** was assigned as 2′-*O*-acetylactein.





Compound **2** was also isolated as a white powder. The combination of its FABMS (*m*/*z* 709 $[M + Na]^+$) and ^{13}C NMR spectral data led to the elemental formula $C_{39}H_{58}O_{10}$ for **2**. On the basis of a comparison of the NMR spectral data with those reported in the literature for 27-deoxyactein,[11] **2** could be assigned as a close derivative of 27-deoxyactein. Following the same procedure as that employed for compound **1**, all the data, including correlations in the HMBC spectrum, proved compound **2** to be 2′-*O*-acetyl-27-deoxyactein.

* To whom correspondence should be addressed. Tel: 732-932-9611, ext 235. Fax: 732-932-6776. E-mail: ho@aesop.rutgers.edu.
[†] Rutgers University.
[‡] Shanghai Institute of Pharmaceutical Industry.

10.1021/np000483k CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 04/18/2001

Table 1. NMR Spectral Data of Compounds **1** and **2** (C_5D_5N, δ in ppm)

	1		**2**	
position	^{1}H	^{13}C	^{1}H	^{13}C
1	1.17 (m), 1.56 (m)	32.0	1.17 (m), 1.56 (m)	32.0
2	1.86 (m), 2.25(m)	30.0	1.86 (m), 2.24(m)	29.9
3	3.41 (dd, J = 11.0, 3.8 Hz)	88.6	3.45 (dd, J = 11.0, 4.0 Hz)	88.5
4		41.1		41.0
5		47.1		47.0
6	0.80 (m), 1.34 (m)	20.4	0.81 (m), 1.44 (m)	20.3
7	0.98 (m), 1.30(m)	25.7	1.00 (m), 1.29(m)	25.8
8	1.65 (m)	46.1	1.66 (m)	45.9
9		20.7		20.3
10		26.9		26.8
11	1.26 (m)	37.0	1.28 (m)	36.8
	2.70 (dd, J = 16.2, 9.0 Hz)		2.28 (dd, J = 16.0, 9.0 Hz)	
12	5.17(dd, J = 8.8, 4.0 Hz)	77.4	5.13(dd, J = 8.9, 3.4 Hz)	77.3
13		48.1		48.0
14		49.0		49.0
15	1.67 (m), 2.04 (m)	43.9	1.78 (m), 1.94 (m)	44.3
16	4.68 (m)	73.7	4.33 (m)	74.7
17	1.86 (m)	56.7	1.79 (m)	56.4
18	1.35 (s)	13.8	1.55 (s)	14.5
19	0.29 (d, J = 4.0 Hz)	29.8	0.24 (d, J = 4.2 Hz)	29.7
	0.62 (d, J = 4.0 Hz)		0.56 (d, J = 4.2 Hz)	
20	1.90 (1H, m)	26.3	2.30 (m)	23.5
21	1.05 (d, J = 6.1 Hz)	21.6	1.12 (d, J = 6.0 Hz)	21.5
22	1.75 (m), 2.30 (m)	37.8	1.57 (m), 1.70 (m)	37.7
23		106.2		106.1
24	4.04 (s)	63.7	3.77 (s)	62.5
25		65.9		62.7
26	1.88 (s)	13.4	1.50 (s)	13.7
27	5.83 (s)	98.7	3.72 (d, J = 10.3 Hz)	68.3
			4.15 (d, J = 10.3 Hz)	
28	0.88 (s)	19.8	0.93 (s)	19.8
29	1.20 (s)	26.0	1.16 (s)	25.6
30	1.00 (s)	15.4	0.99 (s)	15.3
CH_3CO-12		171.7		170.9
2.26 (s)	22.0	2.22 (s)	21.8	
1′	4.88 (d, J = 7.9 Hz)	104.8	4.87 (d, J = 7.9 Hz)	104.8
2′	5.60 (m)	75.9	5.61 (m)	75.8
3′	4.25 (m)	76.4	4.25 (m)	76.4
4′	4.27 (m)	71.6	4.27 (m)	71.5
5′	3.90 (dd, J = 10.5, 10.5 Hz)	67.3	3.80 (m)	67.3
	4.42 (dd, J = 10.4, 5.0 Hz)		4.42 (dd, J = 10.2, 5.0 Hz)	
CH_3CO-2′		170.5		170.3
2.28 (3H, s)	21.6	2.25 (3H, s)	21.5	

Actein, 27-deoxyactein, cimicifugoside H-1, and 15α-hydroxycimicidol 3-*O*-*β*-D-xyloside were confirmed by comparison of their physical and spectral data with those published in the literature.[5,11]

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Optical rotations were obtained on a JASCO DIP-181 polarimeter. Elemental composition was analyzed on a Carlo Erba 1106 instrument. IR spectra were recorded on a Nicolet 5000 infrared spectrometer. FABMS were measured with a MAT-711 mass spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AM-400 instrument, and chemical shifts are reported in ppm with the solvent (pyridine-d_5) as the reference. TLC was carried out on silica gel $HSGF_{254}$ plates (Yantai Institute of Chemical Technology, Yantai, People's Republic of China). Spots were visualized by 10% H_2SO_4 in ethanol solution followed by heating. Silica gel H (60 μm, Qingdao, People's Republic of China) was used for column chromatography.

Plant Material. Roots of *C. foetida* were collected in the area of Dabieshan, Anhui Province, People's Republic of China, in October 1997 and were identified by Professor Siqi Luo, of Shanghai Institute of Pharmaceutical Industry, Shanghai, People's Republic of China**.** The material was air-dried, and a voucher specimen (No. 09710) was deposited at Shanghai Institute of Pharmaceutical Industry, Shanghai, People's Republic of China.

Extraction and Isolation. The air-dried roots (4.5 kg) were extracted with 90% EtOH (5 L × 3) below 65 °C. The alcohol was evaporated, the remaining residue was mixed with water (500 mL) at around 65 °C, and the resulting mixture was filtered. The hot water-insoluble material (50 g) was dissolved in CH_2Cl_2 (100 mL), and the solution was subjected to Si gel column chromatography using CH_2Cl_2 containing increasing amounts of MeOH as solvent (up to CH_2Cl_2−MeOH 5:1), which yielded seven fractions.

Fraction 3, eluted with CH_2Cl_2−MeOH (15:1), was subjected to chromatography on a Si gel column with cyclohexane−acetone (1.2:1), giving two additional fractions (I and II). Repeated chromatography on a Si gel column by $CHCl_3$−MeOH (10:1) on subfraction I gave compound **1** (10 mg). Subfraction II was purified by Si gel column chromatography with $CHCl_3$−MeOH (10:1) as eluent, to afford **2** (12 mg). Fraction 4 was subjected to passage over a Si gel column (cyclohexane−acetone, 1:1) to yield actein (1.2 g) and 27-deoxyactein (1.5 g). Fraction 5 was rechromatographed on a Si gel column, eluted by $CHCl_3$−MeOH (8:1) to give cimicifugoside (1.0 g) and 15α-hydroxycimicidol 3-*O*-*β*-D-xyloside (160 mg).

2′-*O*-Acetylactein (1): white amorphous powder; mp 143−146 °C; $[\alpha]_D$ −56.6° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3450 (OH), 1735 (CO) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1;

FABMS *m*/*z* 725 $[M + Na]^+$; *anal*. calcd for $C_{39}H_{58}O_{11}$, C 66.64, H 8.32, found, C 66.47, H 8.54.

2′-*O*-Acetyl-27-deoxyactein (2): white amorphous powder; mp 147−149 °C; $[\alpha]_D$ −34.1° (*c* 0.74, MeOH); IR (KBr) ν_{max} 3430 (OH), 1730 (CO) cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 709 $[M + Na]^+$; *anal*. calcd for $C_{39}H_{58}O_{10}$, C 68.20, H 8.51; found, C 68.11, H 8.78.

Actein: colorless plates (acetone); mp 223−225 °C (lit. mp 232−233 °C);[11] $[\alpha]_D$ −64.1° (*c* 0.90, MeOH) {lit. $[\alpha]_D$ −66.0° [*c* 1.6, MeOH−$CHCl_3$ (1:1)]};[11] and exhibited spectral data consistent with literature values.[11]

27-Deoxyactein: colorless needles (acetone); mp 258−260 °C (lit. mp 252−254 °C);[11] $[\alpha]_D$ −43.2° (*c* 1.15, MeOH) {lit. $[\alpha]_D$ −44.3° [*c* 1.4, MeOH−$CHCl_3$ (1:1)]};[11] and exhibited spectral data consistent with literature values.[11]

Cimicifugoside H-1: colorless needles (MeOH); mp 260−262 °C (lit. mp 260−262 °C);[11] $[\alpha]_D$ −41.7° (*c* 0.68, MeOH) [lit. $[\alpha]_D$ −43.5° [*c* 0.50, MeOH)];[11] and exhibited spectral data consistent with literature values.[11]

15α-Hydroxycimicidol 3-*O*-*β*-D-xyloside: white powder; mp 270−274 °C; $[\alpha]_D$ −45.0° (*c* 0.55, MeOH) {lit. $[\alpha]_D$ −45.7° [*c* 0.38, MeOH−$CHCl_3$ (1:1)]};[5] and exhibited spectral data consistent with literature values.[5]

References and Notes

(1) Liberman, S. *J. Women's Health* **1998**, *7*, 525−529.
(2) Jiangsu New Medical College. *A Dictionary of Traditional Chinese Materia Medica*; Shanghai Scientific and Technological Publishing Co.: Shanghai, 1977; p 451.
(3) Liske, E. *Adv. Ther*. **1998**, *15*, 45−53.
(4) Shao, Y.; Harris, A.; Wang, M.; Zhang, H.; Cordell, G. A.; Bowman, M.; Lemmo, E. *J. Nat. Prod*. **2000**, *63*, 905−910.
(5) Kadota, S.; Li, J. X.; Tanaka, K.; Namba, T. *Tetrahedron* **1995**, *51*, 1143−1166.
(6) Li, J. X.; Kadota, S.; Pu, X. F.; Namba, T. *Tetrahedron Lett*. **1994**, *35*, 4575−4576.
(7) Li, C. J.; Li, Y. H.; Xiao, P. G.; Mabry, T. J.; Watson, W. H.; Krawiec, M. *Phytochemistry* **1996**, *42*, 489−494.
(8) Li, C. J.; Li, Y. H.; Xiao, P. G. *Yaoxue Xuebao* **1994**, *29*, 934−936.
(9) Li, C. J.; Li, Y. H.; Chen, S. F.; Xiao, P. G. *Yaoxue Xuebao* **1994**, *29*, 449−453.
(10) Li, C. J.; Li, Y. H.; Chen, D. H.; Xiao, P. G. *Chin. Chem. Lett*. **1993**, *4*, 957−958.
(11) Koeda, M.; Aoki, Y.; Sakurai, N.; Nagai, M. *Chem. Pharm. Bull*. **1995**, *43*, 771−776.
(12) Kusano, A.; Takahira, M.; Shibano, M.; In, Y.; Ishida, T.; Miyase, T.; Kusano, G. *Chem. Pharm. Bull*. **1998**, *46*, 467−472.
(13) Yoshimoto, K.; Itatani, Y.; Tsuda, Y. *Chem. Pharm. Bull*. **1980**, *28*, 2065−2076.

NP000483K