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New 9, 19-cycloartane triterpenoid from the root of *Cimicifuga foetida*

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[ABSTRACT]

AIM: To study the 9, 19-cycloartane triterpenes from the roots of *Cimicifuga foetida*.

METHOD: Chromatographic separations by silica gel, C_{18} reversed phase silica gel, and high-performance liquid chromatography (HPLC) were used. All of the structures were elucidated on the basis of spectroscopic analysis and chemical methods.

RESULTS: Five 9, 19-cycloartane triterpenes, (3*β*, 12*β*, 15*α*, 24*R*)-12, 2'-diacetoxy-24, 25-epoxy-15-hydroxy-16, 23-dione-3-*O*-*α*-L-arabinopyranoside (**1**), actein (**2**), 23-*epi*-26-deoxyactein (**3**), asiaticoside B (**4**), and 12*β*-hydroxycimigenol (**5**) were isolated from the roots of *Cimicifuga foetida*.

CONCLUSION: Compound **1** is a new triterpene with two acetoxy groups at C-2' and C-12.

[KEY WORDS] *Cimifuga foetida*; Ranuncluaceae; 9, 19-Cycloartane triterpene; Acetoxy groups

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Introduction

Cimicifuga species have a long history of being used as medicinal herbs [1]. In Europe and the United States, black cohosh (*C. racemosa*) is a well-known dietary supplement for women's health in alleviating menstrual pain and for menopausal disorders [2-3]. In China, the roots of *C. foetida* L., *C. dahurica* (Turcz.) Maxim., and *C. heracleifolia* Korn. are a source of a popular herbal medicine, "shengma", which has been used as an antipyretic and analgesic agent since ancient times [4-6]. Up to now, three main classes of compounds have been isolated from *Cimicifuga* spp: 9, 19-cycloartane triterpene glycosides, chromones, and cinnamic acid derivatives, of which the triterpene glycosides are considered to be the bioactive components [7]. Our research group has been studying the chemical constituents of *C. foetida*, *C. yunnanensis* P.K. Hsiao, *C. dahurica*, and *C. heracleifolia* [8-11] and reported a series of new cycloartane triterpenes, as well as their cytotoxic and anticomplement activities [12-15]. Further chemical investigations on the roots of *C. foetida* collected from Yulong County led to isolation of one new 9,19-cycloartane triterpene glycoside (**1**), together with four known compounds, actein (**2**) [16], 23-*epi*- 26-deoxyactein (**3**) [17], asiaticoside B (**4**) [18] and 12*β*-hydroxycimigenol (**5**) [19].

Results and Discussion

Compound **1** was obtained as a white powder. The IR spectrum showed absorptions of hydroxyl (3 442 cm^{-1}) and carbonyl groups (1 738 cm^{-1}), respectively. Its molecular formula ($C_{39}H_{58}O_{12}$) with eleven degrees of unsaturation was deduced from the analyses of ^{13}C-DEPT and HR-EI-MS data (*m/z* 718.393 7 $[M]^+$; calcd. 718.392 8).

The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of the characteristic cyclopropane methylene signals at δ_H 0.31 and 0.60 (each 1H, d, J = 4.2 Hz), one secondary methyl signal at δ_H 1.33 (d, J = 6.6 Hz), and six tertiary methyl groups at δ_H 0.95−1.58. Additionally, signals for an

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anomeric proton at δ_H 4.76 (1H, d, J = 7.8 Hz) and two acetyl methyl groups at δ_H 2.14 and 2.25 were observed. The ^{13}C DEPT spectrum of **1** exhibited 39 signals, of which 30 were attributed to the aglycon, five to a pentose residue, and four to two acetyl groups. The ^{13}C-DEPT spectrum of the aglycon of **1** also showed two ketone carbonyls at δ_C 219.59 and 205.63. The aforementioned data suggested that **1** was a 9,19-cycloartane triterpene glycoside with two acetoxy and two carbonyl groups. The NMR spectroscopic data of **1** (Table 1) closely resembled that of bugbanoside D, except for the presence of an additional acetoxy group and the absence of two olefinic carbons [20].

Table 1 ^1H NMR and ^{13}C-DEPT data of compound 1 (600 MHz and 150 MHz, in pyridine-d_5, J in Hz)

Position	δ_C	δ_H	Position	δ_C	δ_H	Position	δ_C	δ_H
1	32.45 (t)	1.53 (m) 1.10 (m)	**12**	77.85 (d)	5.54 (dd, 3.6, 10.2)	**27**	18.55 (q)	1.29 (s)
			13	45.14 (s)	----	**28**	12.29 (q)	1.15 (s)
2	30.22 (t)	2.28 (m) 1.86 (m)	**14**	47.16 (s)	----	**29**	25.81 (q)	1.09 (s)
			15	82.97 (d)	4.63 (s)	**30**	15.64 (q)	0.95 (s)
3	88.65 (d)	3.35 (dd, 4.2, 11.4)	**16**	219.59 (s)	----	**C3-Sugar**		
4	41.46 (s)	----	**17**	59.20 (d)	2.74 (d, 2.4)	**Ara-1'**	105.01 (d)	4.76 (d, 7.8)
5	47.31 (d)	1.29 (m)	**18**	14.09 (q)	1.58 (s)	**2'**	74.85 (d)	5.96 (t, 9.0)
6	20.97 (t)	1.54 (m) 0.73 (m)	**19**	31.43 (t)	0.60 (d, 4.2) 0.31 (d, 4.2)	**3'** **4'**	72.97 (d) 70.30 (d)	4.21 (dd, 3.6, 9.0) 4.31 (m)
7	26.74 (t)	2.13 (m) 1.17 (m)	**20** **21**	27.27 (d) 23.56 (q)	2.89 (m) 1.33 (d, 6.6)	**5'**	67.79 (t)	4.30 (m) 3.78 (d, 11.4)
8	47.34 (d)	1.93 (dd, 3.6, 10.2)	**22**	46.75 (t)	2.96 (m)	**12-COCH$_3$**	171.20 (s)	----
9	20.73 (s)	----	**23**	205.63 (s)	----	**12-COCH$_3$**	21.80 (q)	2.25 (s)
10	27.65 (s)	----	**24**	66.00 (d)	3.65 (s)	**2'-COCH$_3$**	170.54 (s)	----
11	36.63 (t)	2.80 (dd, 10.2, 15.6) 1.23 (dd, 3.6, 15.6)	**25** **26**	61.43 (s) 24.86 (q)	---- 1.31 (s)	**2'-COCH$_3$**	21.80 (q)	2.14 (s)

In the ^1H-^1H COSY spectrum (Fig. 1), a correlation of H-7 with H-8 was observed, which indicated that the double bond between C-7 and C-8 was reduced in **1**. In addition, the ^1H-^1H COSY correlations of a downfield resonance at δ_H 5.96 (t, J = 9.0 Hz) with H-3' (δ_H 4.21) and H-1' (δ_H 4.76), together with the HMBC correlation from a carbonyl group (δ_C 170.54) to the proton resonance (δ_H 5.96, t, J = 9.0 Hz) located the acetoxy group at C-2'.

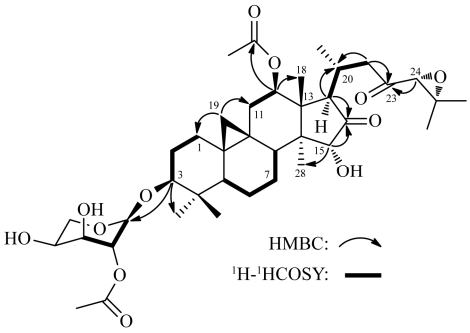

Fig. 1 Key HMBC and ^1H-^1H COSY correlations of compound 1

The relative configurations of H-3, H-5, H-8, H-12, and H-17 were established as in the α-orientation on the basis of the ROESY correlations of H-3/H-1'; H-3/H-5; H-8/Me-28; H-17/Me-28; and H-12/H-17 (Fig. 2). H-15 was elucidated as having a β-orientation on the basis of the ROESY correlation with Me-18. The absolute configuration of C-24 was assigned as *R*, by comparing the chemical shifts of H-24 and C-24 of **1** with those of known 9,19-cyclolanostane triterpene glycosides [20]. Therefore, the chemical structure of **1** was determined to be (3β, 12β, 15α, 24*R*)-12, 2'-diacetoxy-24, 25-epoxy-15-hydroxy-16, 23-dione-3-*O*-α-L-arabinopyranoside.

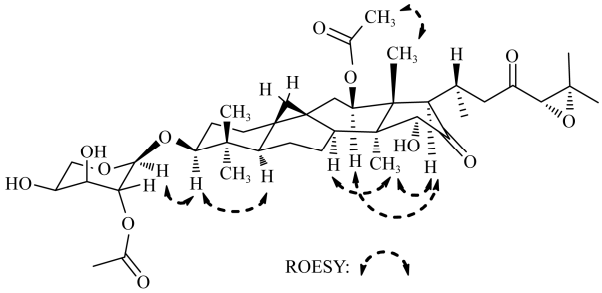

Fig. 2 Key ROESY correlations of compound 1

Compound **1** white powder (MeOH); UV (MeOH) λ_{max}: 203 and 240 nm; IR (KBr) ν_{max}: 3 442 (OH), 2 937, 1 738 (C=O), 1 635 (C=C), 1 456, 1 379, 1 240, 1 146, 1 055, 923, 605 cm^{-1}; positive HR-EI-MS *m/z* 718.3937 $[M]^+$, ($C_{39}H_{58}O_{12}$, Calcd. 718.3928), ^1H- and ^{13}C-DEPT data, see Table 1.

The known compounds actein (**2**), 23-*epi*-26-deoxyactein (**3**), asiaticoside B (**4**), and 12β-hydroxycimigenol (**5**) were identified by comparing their physical and spectroscopic data with reported data.

Experimental

General methods

Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. ^1H- and ^{13}C-DEPT spectra were recorded in pyridine-d_5 on Bruker DRX-500 and Avance III-600 MHz spectrometer (Bruker, Zűrich, Switzerland). ESI-MS and HR-EI-MS data were obtained using a Waters Autospec Premier-P776 spectrometer. Infrared spectrum was recorded on a Shimadzu IR-450 instrument with KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (200−250 μm thickness, silica gel 60 F_{254}, Qingdao

Marine Chemical, Inc.) and spots were visualized by heating after spraying with 10% aq. H_2SO_4 soln. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C-18 column (5 μm, 4.6 mm × 150 mm). Silica gel (200−300 mesh, Qingdao Marine Chemical, Inc.) and Lichroprep Rp-18 (40−63 μm, Merck) were used for column chromatography (cc).

Plant material

The roots of *C. foetida* (82 kg) were collected in 2010 from Yulong County, Yunnan Province and identified by Prof. PEI Sheng-Ji, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KUN No. 20100906) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

Extraction and isolation

The air-dried and powdered roots of *C. foetida* (82 kg) were refluedx with 95% MeOH (3 × 100 L× 5 h). The residue was extracted successively with EtOAc and *n*-BuOH. The EtOAc (5.6 kg) extract was subjected to silica gel cc, eluted with $CHCl_3$−MeOH ($CHCl_3$, 100 : 1, 50 : 1, 20 : 1, 5 : 1) to give five fractions (Fr. I−Fr. V). Fr. IV (350 g) was divided into five sub-fractions (Fr. IV.1−5) after performing silica gel cc, eluted with $CHCl_3$−acetone (10 : 1). Compounds **2** (50 g) and **3** (20 g) were recrystallized from Fr. IV.5. Fr. IV.3 (10 g) and Fr. IV.4 (20 g) were repeatedly chromatographed over Rp-18 (50%, 60%, 70%, 80%, and 90% MeOH−H_2O). Fr. IV.4.4 (80% MeOH−H_2O, 860 mg) of Fr. IV.4 was subjected to cc on silica gel (1.5 g) eluting with $CHCl_3$−acetone (5 : 1) to give fractions IV.4.4.1−7. Compounds **1** (1.2 mg) and **5** (20 mg) were purified from Fr. IV.4.4.6 (80 mg) by HPLC, eluted with CH_3CN−H_2O (45 : 55) (13.1 and 18.4 min). Fr. IV.3.4 (80% MeOH−H_2O, 1.3 g) of Fr. IV.3 was further purified by repeated CC of silica gel ($CHCl_3$−acetone, 25 : 1) and HPLC to obtain compound **4** (1.3 mg, CH_3CN−H_2O, 70 : 30, 15.5 min).

Hydrolysis and identification of the sugar moieties in compound 1

Compound **1** (1.2 mg) was dissolved in MeOH (5 mL), then 4% K_2CO_3 (5 mL) was added, and the solution was stirred at rt overnight. The solution was neutralized with 10% HOAc and extracted with EtOAc (3 × 15 mL). The EtOAc extract, after removal of solvent, was dissolved in MeOH (5 mL) and refluxed with 0.5 mol·L^{-1} HCl (3 mL) for 4 h. The aqueous layer was then neutralized with Ag_2CO_3, and the formed precipitate was filtered to give a monosaccharide, which had an R_f (EtOAc−$CHCl_3$−MeOH−H_2O, 3 : 2 : 2 : 1) and specific rotation $[\alpha]_D^{20}$ + 82.78 (*c* 0.05, MeOH) corresponding to the data for of L-arabinose.

References

[1] Nian Y, Wang HY, Su J, *et al*. A cytotoxic 4*α*-methyl steroid from the aerial parts of *Cimicifuga foetida* L. [J]. *Fitoterapia*, 2012, **83**(2): 293-297.

[2] Lieberman SJ. A review of the effectiveness of *Cimicifuga racemosa* (black cohosh) for the symptoms of menopause [J]. *J Women's Health*, 1998, **7**(5): 525-529.

[3] Tatsuji H, Hiroshi O, Shiya S, *et al*. Inhibitory effect of ferulic acid and isoferulic acid on murine interleukin-8 production in response to influenza virus *in vitro* and *in vivo* [J]. *Planta Med*, 1995, **61**(3): 221-226.

[4] Liske E, Wustenberg P. Therapy of climacteric complaints with *Cimicifuga racemosa*: herbal medicine with clinically proven evidence [J]. *Menopause*, 1998, **5**(4): 250-255.

[5] *Pharmacopoeia of the Peoples Republic of China* (2010) [S]. Vol. 1. Beijing, 2010: 68-69.

[6] McKenna DJ, Jones K, Humphrey S, *et al*. Black cohosh: efficacy, safety, and use in clinical and preclinical applications [J]. *Altern Ther*, 2001, **7**(3): 93-100.

[7] Liske E. Therapeutic efficacy and safety of *Cimicifuga race- mosa* for gynecologic disorders [J]. *Adv Ther*, 1998, **15**(1): 45- 53.

[8] Nian Y, Zhang MX, Li Y, *et al*. Cycloartane triterpenoids from the aerial parts of *Cimicifuga foetida* Linnaeus [J]. *Phytochemistry*, 2011, **72**(11-12): 1473-1481.

[9] Fang ZZ, Nian Y, Li W, *et al*. Cycloartane triterpenoids from *Cimicifuga yunnanensis* induce apoptosis of breast cancer cells (MCF7) *via* p53-dependent mitochondrial signaling pathway [J]. *Phytother Res*, 2011, **25**(1): 17-24.

[10] Nian Y, Wang HY, Zhou L, *et al*. Cytotoxic cycloartane triterpenes of the traditional Chinese medicine "Shengma" (*Cimicifuga dahurica*) [J]. *Planta Med*, 2013, **79**: 60-69.

[11] Nian Y, Wang HY, Su J, *et al*. Cytotoxic cycloartane triterpenes from the roots of *Cimicifuga heracleifolia* [J]. *Tetrahedron*, 2012, **68**(32): 6521-6527.

[12] Lu L, Chen JC, Li Y, *et al*. Studies on the constituents of *Cimicifuga foetida* collected in Guizhou Province and their cytotoxic activities [J]. *Chem Pharm Bull*, 2012, **60**(5): 571-571.

[13] Li DS, Nian Y, Sun Y, *et al*. Three new cycloartane (9, 19-cyclolanostane) glycosides from *Cimicifuga foetida* [J]. *Helv Chim Acta*, 2011, **94**(4): 632-638.

[14] Sun LR, Yan J, Lu L, *et al*. Cimicifine A: a novel triterpene alkaloid from the rhizomes of *Cimicifuga foetida* [J]. *Helv Chim Acta*, 2007, **90**(7): 1313-1318.

[15] Qiu M, Kim JH, Lee HK, *et al*. Anticomplement activity of cycloartane glycosides from the rhizome of *Cimicifuga foetida* [J]. *Phytother Res*, 2006, **20** (11): 945-948.

[16] Li CJ, Li YH, Chen SF, *et al*. Triterpenoids from *Cimicifuga foetida* L. [J]. *Acta Pharm sin*, 1994, **29** (6): 449-453.

[17] Chen SN, Li W, Fabricant DS, *et al*. Isolation, structure elucidation, and absolute configuration of 26-deoxyactein from *Cimicifuga racemosa* and clarification of nomenclature associated with 27-deoxyactein [J]. *J Nat Prod*, 2002, **65** (4): 601-605.

[18] Gao J, Huang F, Zhang J, *et al*. Cytotoxic cyclartane triterpene saponins from *Actaea asiatica* [J]. *J Nat Prod*, 2006, **69**(10): 1500-1502.

[19] Kusano A, Shibano M, Kusano G. Four new glycosides from the aerial parts of *Cimicifuga simplex* Wormsk. [J]. *Chem Pharm Bull*, 1995, **43**(7): 1167-1170.

[20] Kusano A, Shibano M, Tsujamoto D, *et al*. Four new cycloart-7-enol glycosides from the underground parts of *Cimicifuga simplex* Wormsk. [J]. *Chem Pharm Bull*, 2001, **49** (4): 437-441.

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