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Four New 9,19-Cyclolanostane Triterpenes from the Rhizomes of *Cimicifuga foetida* Collected in Yulong†

Wang, Haiyan[a,b](王海燕) Nian, Yin[a,b](年寅) Ma, Chengyou[a](马成友)
Liu, Jieqing[a](刘接卿) Song, Yanbo[a](宋彦波) Zhou, Lin[a](周琳)
Qiu, Minghua*[,a,b](邱明华)

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China*, *Kunming Institute of Botany*, *Chinese Academy of Sciences*, *Kunming 650204*, *China*
[b] *Graduate University of Chinese Academy of Sciences*, *Beijing 100049*, *China*

Four new 9,19-cyclolanostane-type triterpenes (**1**—**4**), were isolated from the rhizomes of *Cimicifuga foetida*. On the basis of spectroscopic analysis, their chemical structures were elucidated as 1,7-dien-cimigenol-3,12-dione (**1**), 1-en-cimigenol-3,11-dione (**2**), 11β-hydroxy-7-en-cimigenol-3-one (**3**), and (20*R*,24*R*)-24,25-epoxy-11β-hydroxy-7-en-9,19-cyclolanost-3,16,23-trione (**4**).

Keywords *Cimicifuga foetida*, cyclolanostane, cimigenolone

Introduction

The rhizomes of *Cimicifuga foetida* are an important constituent of a Traditional Chinese Medicine, namely "Shengma", and have been officially listed in the Chinese Pharmacopoeia as cooling and detoxification agents.[1] Previously, our research group studied on the chemical constituents of *C. foetida* collected from Yunnan and Guizhou provinces and successively reported a series of new cycloartane triterpene glycosides including a novel triterpene alkaloid, [2] as well as their antitumor and anticomplement activities.[3-6] In an attempt to fully explore the chemical constituents of this medicinal plant, we undertook phytochemical investigation on the rhizomes of *C. foetida*, collected from the high altitude area of Yulong country, Yunnan province. Four new compounds (**1**—**4**) (Figure 1), which were determined to be 1,7-dien-cimigenol-3,12-dione (**1**), 1-en-cimigenol-3,11-dione (**2**), 11β-hydroxy-7-en-cimigenol-3-one (**3**), and (20*R*,24*R*)-24,25-epoxy-11β-hydroxy-7-en-9,19-cyclolanost-3,16,23-trione (**4**), were characterised. This paper deals with the isolation and structural elucidation of these compounds.

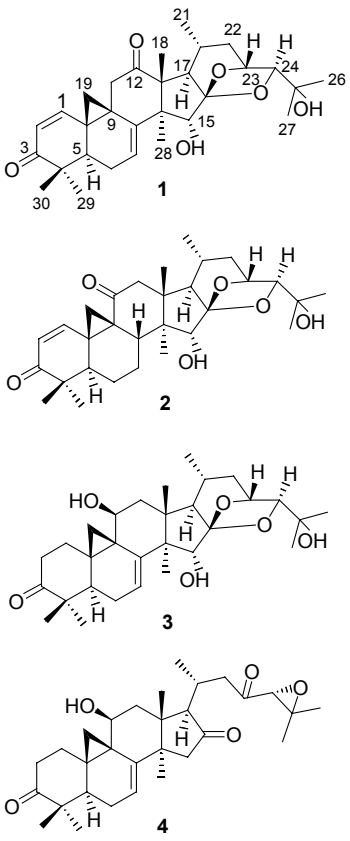

Figure 1 Structures of compounds **1**—**4**.

Experimental

General methods

Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in pyridine-d_5 on Bruker DRX-500 and

* E-mail: mhchiu@mail.kib.ac.cn
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Avance III-600 MHz spectrometers (Bruker, Zűrich, Switzerland), using chemical shift of TMS (0 ppm) as reference, whereas ESIMS and HR-ESI-TOF-MS data were obtained using a VG Autospec-3000 spectrometer. Infrared spectra were 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% H_2SO_4. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a YMC-Pack Pro C_{18} RS 10 mm×250 mm column. Silica gel (200—300 mesh, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40—63 μm, Merck), and Sephadex LH-20 (20—150 μm, Pharmacia) were used for column chromatography (cc).

Plant material

The rhizomes of *C. foetida* (82 kg) were collected in Yulong country, Yunnan province, China, in September 2010 and identified by Prof. Pei Shengji (Kunming Institute of Botandy, Chinese Academy of Sciences).

Extraction and isolation

The air-dried and powdered rhizomes of *C. foetida* (82 kg) were extracted three times with 95% EtOH at 70 ℃. After removal of the solvent by evaporation, the residue was extracted successively with EtOAc and *n*-BuOH. The EtOAc-soluble (5600 g) extract was subjected to silica gel chromatography and eluted with $CHCl_3$-MeOH (1∶0, 100∶1, 50∶1, 20∶1, 5∶1) to give five fractions (**Fr.A**—**Fr.E**). **Fr.C** (230 g) was fractionated into three sub-fractions (**Fr.C_1**—**Fr.C_3**) by performing silica gel chromatography, eluted with PE-Me_2CO (5∶1, 2∶1, 0∶1). **Fr.C_1** (60 g) was chromatographed repeatedly over RP-18 (45%, 60%, 80%, 100% MeOH-H_2O). The 60% fraction was divided into three fractions (**Fr.60%-1**—**Fr.60%-3**) after performing silica gel chromatography, eluted with PE-Me_2CO (1∶0, 10∶1, 5∶1). Compound **1** (2.5 mg), **3** (1.5 mg) and **4** (5.8 mg) were purified from fraction **Fr.60%-2** (2.2 g) by repeatly performing silica gel chromatography, eluted with $CHCl_3$-MeOH (50∶1) and then purified on sephadex LH-20 (eluting with MeOH) and HPLC (65% CH_2CN/H_2O). Compound **2** (1.8 mg) was obtained similarly from **Fr.60%-1** (1.2 g).

Compound **1**: A white powder, $[\alpha]_D^{20}$ −37.7 (*c* 0.09, MeOH); UV-vis (MeOH) λ_{max}: 204, 247 nm; ^{1}H NMR (600 MHz, Pyr) δ: 6.69 (d, J=10.0 Hz, 1H, H-1), 6.20 (d, J=9.9 Hz, 1H, H-2), 1.81—1.87 (m, 1H, H-5), 1.78—1.80 (m, 1H, H-6), 1.68—1.70 (m, 1H, H-6), 6.33—6.35 (m, 1H, H-7), 3.12 (s, 1H, H-11), 2.58 (s, 1H, H-11), 4.75 (s, 1H, H-15), 2.22 (overlapped, 1H, H-17), 1.33 (s, 3H, H-18), 1.21 (d, J=4.6 Hz, 1H, H-19), 0.85 (d, J=4.3 Hz, 1H, H-19), 1.64—1.66 (m, 1H, H-20), 1.17 (overlapped, 1H, H-21), 2.35—2.40 (m, 1H, H-22), 1.17 (overlapped, 1H, H-22), 4.84—4.85 (m, 1H, H-23), 3.83—3.85 (m, 1H, H-24), 1.49 (s, 3H, H-26), 1.51 (s, 3H, H-27), 1.31 (s, 3H, H-28), 1.17 (s, 3H, H-29), 1.02 (s, 3H, H-30); ^{13}C NMR see Table 1; IR (KBr) v: 3439, 2958, 2928, 2871, 2855, 1716, 1671, 1609, 1455, 1413, 1344, 1265, 1123, 1050 cm^{-1}; ESIMS *m/z*: 519 ([M+Na]$^+$); HR-ESI-TOF-MS calcd for $C_{30}H_{40}O_6Na$ 519.2722, found 519.2704.

Table 1 ^{13}C NMR spectral data for compounds **1**—**4** (150 MHz, C_5D_5N)

No.	1	2	3	4
1	151.7 (d)	150.2 (d)	22.0 (t)	22.6 (t)
2	128.0 (d)	128.0 (d)	37.2 (t)	37.6 (t)
3	204.1 (s)	204.1 (s)	215.7 (s)	216.0 (s)
4	45.5 (s)	46.8 (s)	49.0 (s)	49.5 (s)
5	40.4 (d)	41.9 (d)	45.5 (d)	45.8 (d)
6	22.2 (t)	24.7 (t)	29.0 (t)	29.4 (t)
7	117.6 (d)	18.9 (t)	114.8 (d)	115.8 (d)
8	145.4 (s)	46.6 (d)	147.2 (s)	147.5 (s)
9	26.4 (s)	35.9 (s)	28.7 (s)	28.7 (s)
10	33.9 (s)	38.8 (s)	28.6 (s)	29.5 (s)
11	45.0 (t)	210.0 (s)	63.2 (d)	63.4 (d)
12	209.7 (s)	53.1 (t)	49.2 (t)	47.6 (t)
13	51.4 (s)	42.2 (s)	41.5 (s)	44.9 (s)
14	56.3 (s)	47.5 (s)	50.5 (s)	46.5 (s)
15	77.5 (d)	78.7 (d)	78.2 (d)	50.2 (t)
16	112.1 (s)	111.9 (s)	112.2 (s)	219.0 (s)
17	51.6 (d)	57.8 (d)	58.9 (d)	61.5 (d)
18	14.1 (q)	18.8 (q)	21.1 (q)	20.8 (q)
19	30.6 (t)	30.1 (t)	18.0 (t)	18.6 (t)
20	24.9 (d)	24.5 (d)	24.0 (d)	28.0 (d)
21	20.6 (q)	19.9 (q)	19.5 (q)	20.8 (q)
22	38.7 (t)	38.3 (t)	38.0 (t)	47.9 (t)
23	72.3 (d)	72.3 (d)	72.0 (d)	206.1 (s)
24	90.8 (d)	90.7 (d)	90.3 (d)	66.3 (d)
25	71.4 (s)	71.4 (s)	71.0 (s)	61.3 (s)
26	27.6 (q)	27.7 (q)	26.9 (q)	18.8 (q)
27	25.7 (q)	25.8 (q)	25.3 (q)	25.0 (q)
28	20.1 (q)	12.3 (q)	19.4 (q)	28.1 (q)
29	22.0 (q)	22.3 (q)	22.7 (q)	23.3 (q)
30	19.3 (q)	20.1 (q)	20.3 (q)	20.7 (q)

Compound **2**: A white powder $[\alpha]_D^{20}$ +277.9 (*c* 0.03, MeOH); UV-vis (MeOH) λ_{max}: 202, 256, 375 nm; ^{1}H NMR (600 MHz, Pyr) δ: 7.43 (d, J=10.3 Hz, 1H, H-1), 6.13 (d, J=10.3 Hz, 1H, H-2), 2.44—2.47 (m, 1H, H-5), 2.09—2.11 (m, 1H, H-6), 1.57—1.59 (m, 1H, H-6), 1.37—1.38 (m, 1H, H-7), 1.04 (overlapped, 1H, H-7), 2.07—2.09 (m, 1H, H-8), 2.80—2.82 (m, 1H, H-12), 2.61 (overlapped, 1H, H-12), 4.38 (s, 1H, H-15), 1.66 (overlapped, 1H, H-17), 0.97 (s, 3H, H-18), 1.91 (d, J=4.0 Hz, 1H, H-19), 1.56 (d, J=4.0 Hz, 1H, H-19), 1.67 (overlapped, 1H, H-20), 0.80 (d, J=6.4 Hz, 3H, H-21), 2.62 (overlapped, 1H, H-22), 1.02 (overlapped, 1H, H-22), 4.76—4.84 (m, 1H, H-23), 3.80—3.81 (m, 1H, H-24), 1.51 (s, 3H, H-26), 1.50 (s, 3H, H-27), 1.30 (s, 3H, H-28), 1.17 (s, 3H, H-29), 0.97 (s, 3H, H-30); ^{13}C NMR: see Table 1; IR (KBr) v: 3441, 2967, 2933, 2872, 1709, 1676, 1631 cm^{-1}; ESIMS *m/z*: 521 ([M+Na]$^+$);

HR-ESI-TOF-MS calcd for $C_{30}H_{42}O_6Na$ 521.2879, found 521.2869.

Compound **3**: A white powder, $[\alpha]_D^{20}$ −26.7 (*c* 0.19, MeOH); UV-vis (MeOH) λ_{max}: 202, 386 nm; ^{1}H NMR (600 MHz, Pyr) δ: 1.76—1.79 (m, 1H, H-1), 1.66 (overlapped, 1H, H-1), 2.82—2.87 (m, 1H, H-2), 2.34—2.37 (m, 1H, H-2), 1.61—1.63 (m, 1H, H-5), 3.01—3.05 (m, 1H, H-6), 1.86—1.88 (m, 1H, H-6), 6.20—6.21 (m, 1H, H-7), 4.58—4.60 (m, 1H, H-11), 2.74—2.78 (m, 1H, H-12), 2.04—2.06 (m, 1H, H-12), 4.65 (s, 1H, H-15), 1.51 (d, overlapped, 1H, H-17), 1.30 (s, 3H, H-18), 2.12 (overlapped, 1H, H-19), 1.14 (overlapped, 1H, H-19), 1.66 (overlapped, 1H, H-20), 0.86 (d, *J*=6.5 Hz, 3H, H-21), 1.04—1.07 (m, 1H, H-22), 2.26—2.31 (m, 1H, H-22), 4.79—4.80 (m, 1H, H-23), 3.84 (d, *J*=9.0 Hz, 1H, H-24), 1.52 (s, 3H, H-26), 1.51 (s, 3H, H-27), 1.38 (s, 3H, H-28), 1.13 (s, 3H, H-29), 1.11 (s, 3H, H-30); ^{13}C NMR see Table 1; IR (KBr) *v*: 3440, 2966, 2929, 2870, 1706, 1633 cm^{-1} ; ESIMS *m*/*z*: 523 ([M+Na]$^+$); HR-ESI-TOF-MS calcd for $C_{30}H_{44}O_6Na$ 523.3035, found 523.3027.

Compound **4**: A white powder, $[\alpha]_D^{20}$ −18.7 (*c* 0.16, MeOH); UV-vis (MeOH) λ_{max}: 201 nm; ^{1}H NMR (600 MHz, Pyr) δ: 1.79 — 1.81 (m, 1H, H-1), 1.64 (overlapped, 1H, H-1), 2.85 (overlapped, 1H, H-2), 2.36 (overlapped, 1H, H-2), 1.63—1.66 (m, 1H, H-5), 3.02—3.05 (m, 1H, H-6), 1.83—1.85 (m, 1H, H-6), 5.14 (overlapped, 1H, H-7), 4.55—4.58 (m, 1H, H-11), 2.86 (overlapped, 1H, H-12), 2.22—2.25 (m, 1H, H-12), 2.49—2.51 (m, 1H, H-15), 2.35 (overlapped, 1H, H-15), 2.41—2.42 (m, 1H, H-17), 1.14 (s, 3H, H-18), 2.11 (d, *J*=3.9 Hz, 1H, H-19), 1.08 (d, *J*=3.9 Hz, 1H, H-19), 2.64—2.66 (m, 1H, H-20), 1.06 (d, *J*=6.1 Hz, 3H, H-21), 3.69—3.73 (m, 1H, H-22), 2.61—2.65 (m, 1H, H-22), 3.79 (s, 1H, H-24), 1.37 (s, 3H, H-26), 1.38 (s, 3H, H-27), 1.21 (s, 3H, H-28), 1.20 (s, 3H, H-29), 1.24 (s, 3H, H-30); ^{13}C NMR see Table 1; IR (KBr) *v*: 3444, 2965, 2933, 1730, 1633, 1454, 1384, 1115 cm^{-1}; ESIMS *m*/*z*: 505 ([M+Na]$^+$); HR-ESI-TOF-MS calcd for $C_{30}H_{42}O_5Na$ 505.2929, found 505.2925.

Results and Discussion

Compound 1: isolated as a white powder, giving a pseudo-molecular ion at *m*/*z* 519 [M+Na]$^+$ in the positive ion ESIMS. Analysis of the ^{13}C NMR and HR-TOF-MS (*m*/*z* 519.2704 [M+Na]$^+$) determined its molecular formula as $C_{30}H_{40}O_6$. The IR spectrum showed absorptions for hydroxyl groups (3439 cm^{-1}), carbonyl groups (1716 cm^{-1}), and α,β-unsaturated ketone unit (1671 and 1609 cm^{-1}). In the ^{1}H NMR spectrum, there were seven methyl groups at δ_H 1.49 (3H, s), 1.33 (s, 3H), 1.31 (s, 3H), 1.17 (s, 3H), 1.02 (3H, s), 1.17 (overlapped, 3H) and the characteristic cyclopropane methylene signals at δ_H 1.21 (d, *J*=4.6 Hz, 1H), δ_H 0.85 (d, *J*=4.3 Hz, 1H). In the ^{13}C NMR spectrum (Table 1), a characteristic ketal signal was observed at δ_C 112.1 (s)[7]. These evidence suggested **1** with a cimigenol skeleton. A comparison of the NMR data of **1** with the known compound 7-en-cimigenol[7] showed that, structurally, **1** resembled 7-en-cimigenol, with the major differences being that the signals due to C-1, C-2 and C-12, and a hydroxyl methine for C-3 were replaced by the signals of an α,β-unsaturated ketone unit at δ_C 128.0 (d), 151.7 (d) and 204.1 (s), and a carbonyl signal at δ_C 209.7 (s). Significant HMBC correlations of coupling carbonyl signal (δ_C 204.1) with H_3-29 (δ_H 1.17), H_3-30 (δ_H 1.02) and H-1 (δ_H 1.76—1.79, m) suggested the α,β-unsaturated ketone unit at C-1, C-2, and C-3 (Figure **2**). In addition, the carbonyl group at δ_C 209.7 showed correlations with H-11 (δ_H 3.12, 1H; 2.58, 1H) and H_3-18 (δ_H 1.33, 3H), indicating that the carbonyl group instead of the hydroxyl group was at C-12. This deduction was further supported by the downfield shift of C-11 and C-13 by about δ 19.6 and 10.2 in ^{13}C spectrum, respectively. Unambiguous ROESY correlations (Figure 3) of H-15 with H_3-18 revealed an α-orientation of 15-OH. The relative configurations of C-23 and C-24 were assigned as (23*R*, 24*S*), respectively, by comparing the coupling constants of the C-23 and C-24 of **1** with those of known compounds.[4,8] Therefore, compound **1** was identified as 1,7-dien-cimigenol-3,12-dione.

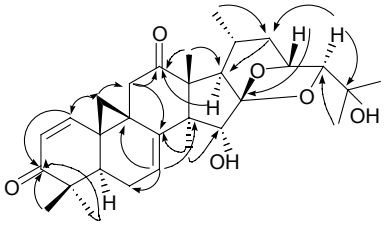

Figure 2 The key HMBC correlations of compound **1**.

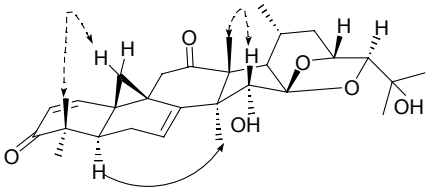

Figure 3 The key ROSEY correlations of compound **1**.

Compound 2: isolated as a white powder. The combination of the HR-TOF-MS (*m*/*z* 521.2869 [M+Na]$^+$) and ^{13}C NMR data led to the determination of its formula as $C_{30}H_{42}O_6$. The IR and NMR spectra of **2** resembled those of compound **1**. In the NMR of **2,** the signals due to the olefinic carbons (C-7/C-8) were replaced by those of a methylene and a methine. In the HMBC, the correlation between the carbonyl signal at δ_C 209.9 and H-19 (δ_H 1.91, 1H; 1.56, 1H), and H-12 (δ_H 2.81, 1H; 2.61, 1H), suggested that the carbonyl group shifted from C-12 to C-11 in **2**, which was confirmed by the upfield shift of C-9 by about δ 5.4 and the downfield shifts of H-19 by about δ 0.70 and 0.71. The configurations of C-23 and C-24 were determined as *R* and *S*, by the same way as that of compound **1**. Thus,

compound **2** was elucidated as 1-en-cimigenol-3,11-dione.

Compound 3: a white powder. Its molecular formula $C_{30}H_{44}O_6$ was deduced from HR-TOF-MS (*m*/*z* 523.3027 $[M+Na]^+$). The IR spectrum showed absorption of OH groups at 3440 cm^{-1}, C=O groups at 1706 cm^{-1} and olefinic bonds at 1633 cm^{-1}. The ^1H and ^{13}C NMR spectroscopic data of **3** resembled those of 7-en-cimigenol.[7] The signals of a hydroxyl methine (δ_C 63.2) and a carbonyl (δ_C 215.7) were observed, while the signals of a methylene and a methine due to C-11 and C-3 disappeared. The hydroxy group located at C-11 was based on the HMBC correlation between δ_C 63.2 with H-12 (δ_H 2.76, 1H; 2.05, 1H), H-19 (δ_H 2.12), and significant COSY correlations of H-11 (δ_H 4.58, 1H) and H-12 (δ_H 2.76, 1H; 2.05, 1H). In the HMBC spectrum, the correlation of δ_C 215.7 with H-2 (δ_H 2.84, 1H; 2.35, 1H), H_3-29 (δ_H 1.13, 3H) and H_3-30 (δ_H 1.11, 3H) revealed that the carbonyl group was located at C-3. The ROESY correlations of H-11 with H_3-28 suggested that the 11-OH was β-orientation. Accordingly, compound **3** was characterized as 11β-hydroxy-7-en-cimigenol-3-one.

Compound 4: isolated as a white powder. The molecular formula $C_{30}H_{42}O_5$, was established on the basis of ^{13}C NMR, and HR-TOF-MS (*m*/*z* 505.2925 $[M+Na]^+$). Its IR spectrum exhibited absorption at 3444 and 1730 cm^{-1}, owing to hydroxyl and carbony groups, respectively. The NMR spectroscopic data of **4** was identical to the aglycone of cimicifugosides H-1,[9] except that the CH signal at δ_C 78.0 due to C-3 was instead of a carbonyl signal at δ_C 216.0, which was supported by the HMBC associations of the carbonyl carbon at δ_C 216.0 with H_3-29 (δ_H 1.20) and H_3-30 (δ_H 1.24). In the ROESY spectrum, H-11 showed correlations with H_3-28, which indicated that the hydroxyl group at C-11 was β configuration. The configuration of C-24 was assigned to *R* by comparision of the chemical shifts with those of known compounds having a 24*R* configuration.[10,11] Ultimately, compound **4** was elucidated as (20*R*,24*R*)-24,25-epoxy-11β-hydroxy-7-en-9,19-cyclolanost-3,16,23-trione.

Conclusions

All the four new compounds (**1**—**4**) were the non-glycosides triterpenes with 3-one from the genus of *Cimicifuga*. Compound **1** and **2** were respectively 12-one and 11-one, which were rarely reported before. Moreover, compound **4** was a triterpenoid aglycone with acyclic sides chains which was different from the typical triterpenes with side chains epoxidized with ring D in *Cimicifuga* species.

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(Zhao, X.)

