Cimicifine A: A Novel Triterpene Alkaloid from the Rhizomes of *Cimicifuga foetida*[1])

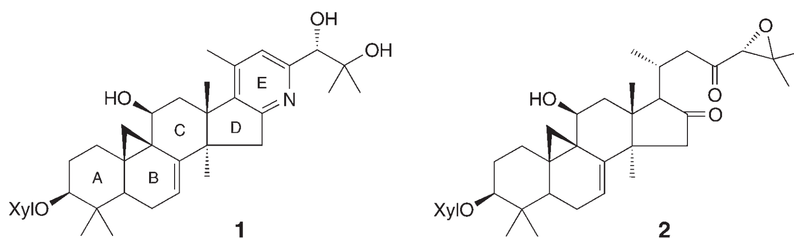
by **Li-Rong Sun**[a]), **Jian Yan**[a])[b]), **Lu Lu**[a])[b]), **Sheng-Ji Pei**[a]), **Zhong-Rong Li**[a]), **Lin Zhou**[a]), **Xian-Min Zhang**[a]), and **Ming-Hua Qiu***[a])[b])

[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, 650204, P. R. China
(phone: +86-871-5223327; fax: +86-871-5223255; e-mail: mhchiu@mail.kib.ac.cn)
[b]) Graduate School of the Chinese Academy of Sciences, Beijing, 100039, P. R. China

Cimicifine A (**1**), the first cycloartane triterpene alkaloid, was isolated from the rhizomes of *Cimicifuga foetida*, together with the known compound cimicifugoside H-1 (**2**). Their chemical structures were established by extensive NMR and MS analyses and by comparison with literature.

1. Introduction. – Throughout the world and for a long time the *Cimicifuga* species have been used as a medicinal herb [1]. In the United States and the European Union, *Cimicifuga racemosa* (L.) NUTT (black cohosh) [2][3] was applied as a herbal dietary supplement to relieve symptoms related to menopause [4][5]. A previous phytochemical investigation revealed several major constituents in *Cimicifuga* species: 9,19-cycloartane triterpenes and their glycosides, cinnamic acid derivatives, and chromones. Among them, triterpene glycosides were considered to be the main active components and were used as marker compounds to standardize its extract [6]. So far, more than 100 triterpenes and glycosides were found from the genus *Cimicifuga*, some of them showing pharmacological effects, such as antitumor and anti-HIV activities [7–10].

We now isolated a novel triterpene alkaloid **1**, called cimicifine A, besides the known alkaloid cimifugoside H-1 (**2**) [11], from *C. foetida* collected from Lijiang. The details of the isolation, structure determination, and proposed biogenetic pathway of **1** are discussed.



[1]) Part of the Ph.D. thesis of *L.-R. S.*, June, 2005 (http://www.kib.ac.cn/KIBEnglish/soft/95.htm).

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2. Result and Discussion. – The molecular formula of cimicifine A (**1**), $C_{35}H_{51}NO_8$, was established on the basis of negative-mode FAB-MS (*m*/*z* 612 ($[M-H]^-$)), and was further confirmed by a HR-FAB-MS (*m*/*z* 612.3531 ($[M-H]^-$)), corresponding to 11 degrees of unsaturation. The odd molecular mass showed an odd number of N atoms in **1**. Additionally, the alkaloid test with $BiI_3 \cdot 4\,KI$ on TLC was positive, indicating the presence of an N-atom. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of cimicifugoside H-1 (**2**) [11] revealed that **1** and **2** had a similar structure for rings A–C and a xylose moiety, which was further confirmed by analysis of 2D NMR spectra. Acid hydrolysis of **1** and trimethylsilylation [12] followed by gas chromatography (GC) of the product showed that the sugar of **1** was D-xylose [13], a common component of the triterpenoid glycosides isolated from *Cimicifuga* species [14][15]. The HSQC, HMBC, and ROESY data (*Fig. 1*) finally allowed to assign structure **1** to cimicifine A.

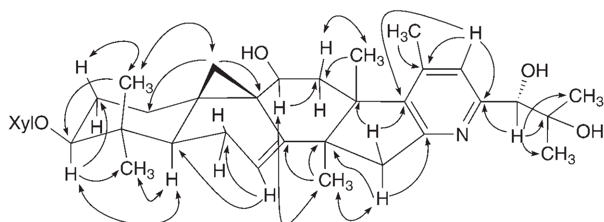


Fig. 1. *Key HMBC* (→) *and ROESY* (↔) *correlations of* **1**

The ^{1}H-NMR spectrum of **1** (see *Exper. Part*) exhibited 7 tertiary Me signals at δ(H) 1.06, 1.16, 1.36, 1.41, 1.51, 1.64, and 2.20, the characteristic cyclopropane CH_2 signals at δ(H) 1.02 and 2.01 (2*d*, $J = 3.2$ Hz, CH_2(19)), and the anomeric-proton signal of a xylose unit at δ(H) 4.89 (*d*, $J = 7.5$ Hz, H–C(1′)). The ^{13}C-NMR spectrum (including DEPT) displayed 35 C-atoms accounting for five C-atoms of a glycosidic moiety (δ(C) 107.6, 75.6, 78.7, 71.3, and 67.2), 11 quaternary C-atoms, and 6 CH, 6 CH_2, and 7 Me groups. Firstly, all δ(H) and δ(C) of **1** were assigned by a detailed analysis of the HSQC plot. Then, in the HMBC spectrum, an informative correlation was observed between the anomeric H–C(1′) signal at δ(H) 4.89 and a CH signal at δ(C) 88.5 (C(3)), indicating that the sugar moiety was linked to C(3). This was further supported by a correlation between H–C(3) (δ(H) 3.60) of the aglycon and H–C(1′) (δ(H) 4.89) of the xylose unit in the ROESY plot. In the ^{1}H-NMR spectrum, the coupling constant of H–C(1′) ($J = 7.5$ Hz) [13] indicated that the xylose unit was present as the β-D-anomer in the $^4C_1$ configuration. HMBC Correlations of δ(H) 1.02 and 2.01 (CH_2(19)) with δ(C) 29.5 (C(1)), 146.4 (C(8)), 27.4 (C(9)), and 63.1 (C(11)) and of δ(H) 4.60 (*dd*, J = 3.5, 9.0 Hz, H–C(11)) with δ(C) 27.4 (C(9)) and 44.5 (C(12)) were present. Further pertinent correlations (see *Fig. 1*) allowed us to establish the partial structure **1a** for **1** (see *Fig. 2*). However, the remaining NMR data of **1** were different from those of cimicifugoside H-1 (**2**) and other known 9,19-cycloartane-type triterpenes, suggesting a novel structure for **1**. There were still five unassigned signals of olefinic C-atoms in the ^{13}C-NMR. The lower-field δ(C) of two sp^2 C-atoms (δ(C) 162.5 (C(16)) and 160.3 (C(23))) suggested the presence of a C=N group. The remaining 5 unsaturation degrees and the ^{13}C NMR data (δ(C) 162.5, 141.9, 142.6, 122.9, and 160.3) were compatible with a pyridine ring (C(16)–C(17)–C(20)–C(22)–C(23)) in **1**. This was confirmed by a UV maximum at 271 nm and HMBC correlations from δ(H) 7.56 (*s*, H–C(22)) to δ(C) 142.6 (C(20)), 141.9 (C(17)), and 160.3 (C(23)) (*Fig. 1*), thus establishing the fragment **1b** (*Fig. 2*). A strong correlation between δ(H) 2.20 (*s*, Me(21)) and δ(C) 142.6 (C(20)) suggested that C(21) was connected to C(20). HMBC Correlations of δ(H) 2.83 and 3.24 (2*d*, J = 15.2 Hz, CH_2(15)) with δ(C) 162.5 (C(16)), 141.9 (C(17)), 51.4 (C(14)), and 48.7 (C(13)) and correlations of δ(H) 1.36 (*s*, Me(18)) with δ(C) 44.5 (C(12)), 48.7 (C(13)), and 141.9 (C(17)) suggested that the fragments **1a** and **1b** were connected to form **1c** *via* C(15)

and C(13) (*Fig. 2*). Long-range correlations between the signals of a pair of geminal Me groups at δ(H) 1.64 (*s*, Me(26)) and 1.51 (*s*, Me(27)) and δ(C) 73.8 (C(25)) and 79.6 (C(24)) indicated the presence of **1d**, and the significant correlation δ(H) 5.04 (*s*, H−C(24))/δ(C) 160.3 (C(23)) revealed the connection of **1c** with **1d** (*Fig. 2*) resulting in structure **1**. The configuration of **1** was elucidated on the basis of a ROESY experiment (*Fig. 1*) and coupling constants. In the ROESY plot, the correlation H−C(11) (J = 3.5, 9.0 Hz)/Me(28) suggested that HO−C(11) was β-oriented. The obvious correlations H_α−C(3) (J = 3.6, 11.2 Hz) [16]/H_α−C(5), H_α−C(3)/H−C(1′), CH_2(19)/Me(30), and CH_2(19)/Me(18) suggested that the configurations of rings A, B, C, and D were the same as in compound **2**. These key findings, together with other pertinent peaks, determined the configuration of **1** as (3*S*,5*R*,9*S*,11*S*,13*S*,14*R*). The configuration (*S*) at C(24) was deduced tentatively by means of the proposed biogenetic pathway (see *Scheme*, below), involving an acid or alkaline-catalyzed opening of the 24,25-epoxy moiety of **2** [17–20].

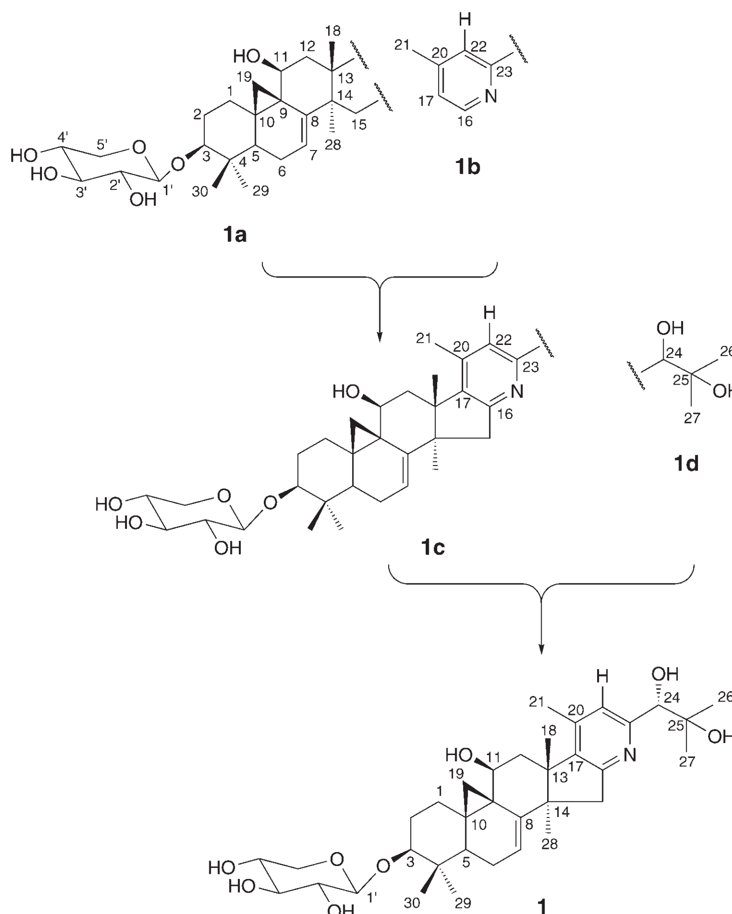
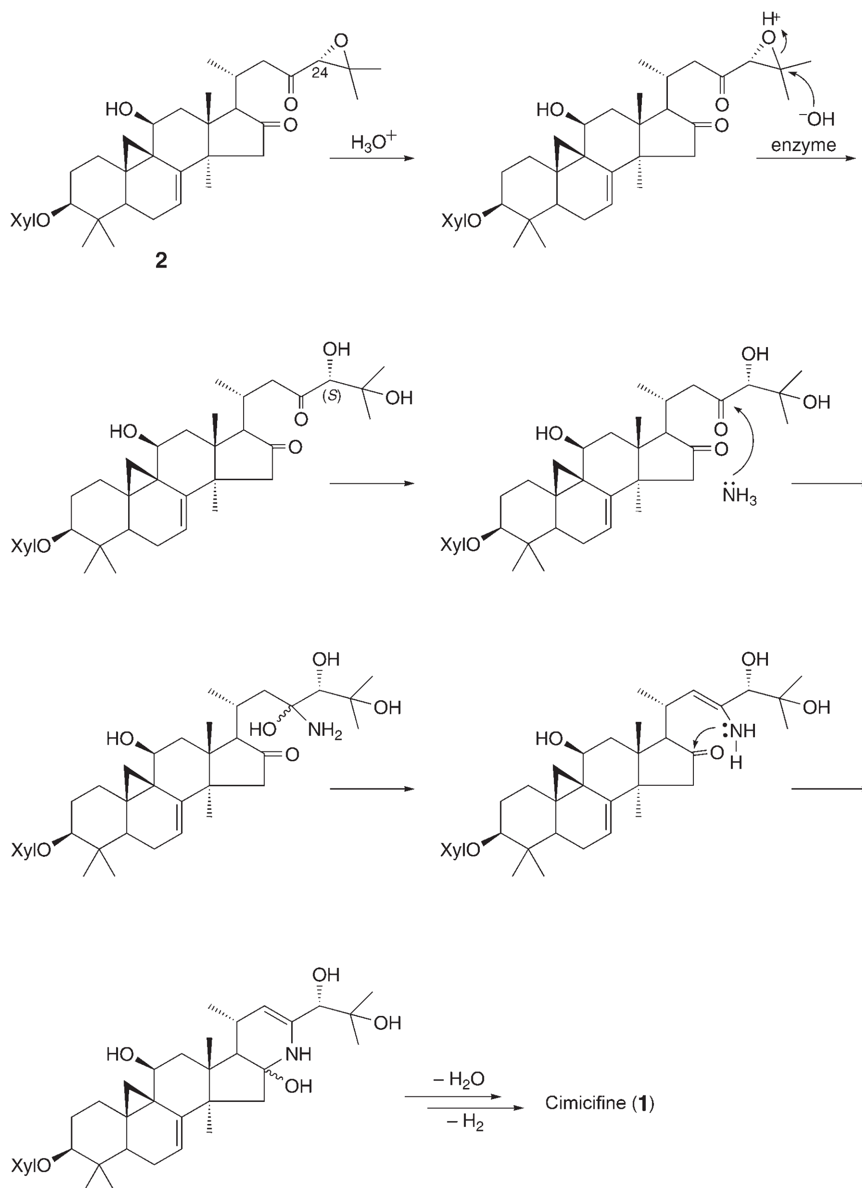


Fig. 2. *Structural fragments of compound* **1**

To discard the possibility that **1** is an artifact formed during the separation procedures, the freshly made MeOH extract from *C. foetida* was analyzed by LC/MS and compared by TLC. We found that **1** was indeed present in the fresh sample. Besides, no ammonia was used in the isolation procedure. Therefore, **1** is a natural

product and represents a novel type of a natural cycloartane triterpene alkaloid. Tentatively, we propose a biogenetic pathway in which the pyridine ring originates from a coupling reaction between NH_3 and the 16,26-diketone moiety, such as present in **2**, followed by elimination of H_2O (*Scheme*).

Scheme. *Proposed Biogenetic Pathway*



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Experimental Part

General. TLC: visualization by spraying with 10% H_2SO_4 soln. followed by heating. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical*, China); *Lichroprep RP-18* (40–63 μm; *Merck*, Darmstadt, Germany); and *Sephadex LH-20* (*Pharmacia Fine Chemical Co. Ltd.*). Melting points: *X-4* apparatus; uncorrected. Optical rotations: *Horiba SEAP-300* spectropolarimeter. UV Spectra: λ_{max} (log ε) in nm. IR Spectra: *Shimadzu IR-450* instrument; in cm^{-1}; KBr pellets. NMR Spectra: *Bruker AV-400* or *DRX-500* instruments; chemical shifts δ in ppm relative to $SiMe_4$ as the internal standard; *J* in Hz. FAB-MS and HR-EI-MS: *VG Autospec-3000* spectrometer; in *m/z* (rel. int. in % of the base peak).

Plant Material. The rhizomes of *Cimicifuga foetida* were collected in Lijiang, Yunnan Province, China, in July, 2003, and identified by Prof. *Zong-yu Wang*, Kunming Institute of Botany, CAS.

Extraction and Isolation. The powdered and dried rhizomes *C. foetida* (23.2 kg) were extracted with 90% MeOH under reflux and concentrated to give a crude extract (3.9 kg) which was suspended in H_2O and partitioned successively with petroleum ether, AcOEt, and BuOH. The AcOEt fraction (1.72 kg) was repeatedly subjected to CC (silica gel), and the alkaloid fractions were further purified with prep. HPLC (*Agilent 1100* HPLC system, 9.4 cm × 25 cm, MeOH/H_2O 70:30): cimicifine A (**1**; 27.1 mg) and cimicifugoside H-1 (**2**; 35.4 mg).

Cimicifine A (=*(3β,11β,24*S*)-11,24,25-Trihydroxy-16,23-nitrilo-9,19-cyclolanosta-7,17(20),22-trien-3-yl β-*D*-Xylopyranoside*; **1**): Amorphous solid. M.p. 275–277°. $[\alpha]_D^{24} = -12.7$ ($c = 0.2$, MeOH). UV (MeOH): 271 (0.28). IR: 3442 (OH), 2956, 1631, 1451, 1420, 1373, 1075, 1043, 995, 975, 590. ^{1}H-NMR: 7.56 (*s*, H–C(22)); 5.34 (*d*, *J* = 7.0, H–C(7)); 5.04 (*s*, H–C(24)); 4.89 (*d*, *J* = 7.5, H–C(1′)); 4.60 (*dd*, *J* = 9.0, 3.5, H–C(11)); 4.33 (*dd*, *J* = 11.1, 6.0, 1 H, CH_2(5′)); 4.22 (*dd*, *J* = 9.2, 4.8, H–C(4′)); 4.17 (*t*, *J* = 8.7, H–C(3′)); 4.04 (*t*, *J* = 7.8, H–C(2′)); 3.74 (*t*, *J* = 10.8, 1 H, CH_2(5′)); 3.60 (*dd*, *J* = 11.2, 3.6, H–C(3)); 3.24 (*d*, *J* = 15.2, H_β–C(15)); 3.07 (*dd*, *J* = 13.6, 9.8, H_α–C(12)); 2.83 (*d*, *J* = 15.2, H_α–C(15)); 2.75 (*d*, *J* = 10.7, 2.8, H_β–C(2)); 2.45 (*d*, *J* = 13.2, H_β–C(12)); 2.43 (*m*, H_β–C(1)); 2.20 (*s*, Me(21)); 2.17 (*m*, H_α–C(1)); 2.01 (*d*, *J* = 3.2, 1 H, CH_2(19)); 1.97–1.93 (*m*, H_α–C(6)); 1.79–1.72 (*m*, H_β–C(6)); 1.74 (*d*, *J* = 2.8, H_α–C(2)); 1.64 (*s*, Me(26)); 1.51 (*s*, Me(27)); 1.41 (*s*, Me(29)); 1.39 (*d*, *J* = 5.2, H–C(5)); 1.36 (*s*, Me(18)); 1.16 (*s*, Me(30)); 1.06 (*s*, Me(28)); 1.02 (*d*, *J* = 3.2, 1 H, CH_2(19)). ^{13}C-NMR: 162.5 (C(16)); 160.3 (C(23)); 146.4 (C(8)); 142.6 (C(20)); 141.9 (C(17)); 122.9 (C(22)); 115.0 (C(7)); 107.6 (C(1′)); 88.5 (C(3)); 79.6 (C(24)); 78.7 (C(3′)); 75.6 (C(2′)); 73.8 (C(25)); 71.3 (C(4′)); 67.2 (C(5′)); 63.1 (C(11)); 51.4 (C(14)); 48.7 (C(13)); 44.5 (C(12)); 44.2 (C(15)); 44.0 (C(5)); 40.8 (C(4)); 29.9 (C(10)); 29.5 (C(1)); 28.7 (C(28)); 27.8 (C(2)); 27.8 (C(26)); 27.4 (C(9)); 26.0 (C(29)); 25.9 (C(27)); 24.4 (C(18)); 22.1 (C(6)); 18.9 (C(19)); 18.4 (C(21)); 14.6 (C(30)). FAB-MS (neg.): 612 (75, $[M-H]^-$). HR-FAB-MS: 612.3531 ($C_{35}H_{50}NO_8^-$; calc. 612.3536).

Acid Hydrolysis of **1**. To a soln. of **1** (7 mg) in MeOH (1 ml), 1N HCl (2 ml) was added, and the mixture was refluxed in a water bath for 2 h. After cooling to r.t., the soln. was extracted with $CHCl_3$ (3 × 3 ml) and the aq. layer lyophilized *in vacuo* to give a sugar residue.

Identification of the Sugar Component of **1** *and Determination of its Absolute Configuration.* As described in [19], pyridine solns. (100 μl each) of the sugar residue (0.04M) and of L-cysteine methyl ester hydrochloride (0.06M) were mixed and heated at 60° for 1 h. The trimethylsilylation reagent HMDS/TMCS (1,1,1,3,3,3-hexamethyldisilazane/chlorotrimethylsilane; 150 μl) was added, and heating at 60° was continued for another 30 min. The precipitate was centrifuged off, and the supernatant (1 μl) was subjected to GC (*DB-5* column (15 m × 0.25 mm i.d., film thickness 0.25 μm), oven temp. 180°, injection-port temp. 250°, carrier gas helium, 10 psi, injection volume 1 μl, split ratio 1:50): t_R 3.50 min.

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