Piepunine, A Novel Bis-diterpenoid Alkaloid from the Roots of *Aconitum piepunense*

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Further investigation on the roots of *Aconitum piepunense* led to the isolation of a novel bis-diterpenoid alkaloid designated as piepunine (**1**). Its structure was established by extensive interpretation of its 1D- and 2D-NMR data, high-resolution ESI-MS, and IR spectra. Piepunine represents the first example of an atisine–denudatine-type bis-diterpenoid alkaloids.

Introduction. – Diterpenoid alkaloids display a wide range of interesting bioactivities and chemical properties, as well as toxic and structural complexity. Most of these diterpenoid alkaloids were isolated from various species of *Aconitum* and *Delphinium*. Structurally, diterpenoid alkaloids can be classified as C_{18}-, C_{19}-, and C_{20}-diterpenoid alkaloids. An entire and update profile of each type of diterpenoid alkaloids was described by us in 'The Alkaloids' edited by *Cordell* [1–3]. The naturally rare bis-diterpenoid alkaloids could be regarded as a class of C_{20}-diterpenoid alkaloids. So far, only four types, namely the atisine–hetidine type, rearranged atisine–hetidine type, denudatine–denudatine type, and the heteratisine–hetidine type, of bis-diterpenoid alkaloids have been isolated from nature [1].

Aconitum piepunense HAND-MAZZ. Symb. Sin. belongs to the genus *Aconitum* in the Ranunculaceae, and is distributed mainly at an altitude of over 3000 m in the northwest of Yunnan Province in China [4]. Our earlier chemical investigation on this plant led to the discovery of some new C_{18}- and C_{19}-diterpenoid alkaloids [5][6]. Further investigation on the minor components of this plant now resulted in the isolation of a new bis-diterpenoid alkaloid, which we named piepunine (*Fig. 1*). This represents the first example of an atisine–denudatine-type bis-diterpenoid alkaloid. The isolation and structure determination of the new bis-diterpenoid alkaloid is described herein, and a plausible biogenetic pathway is proposed as well.

Results and Discussion. – Piepunine (**1**) was obtained as a white amorphous powder. Its molecular formula was deduced as $C_{44}H_{64}N_2O_4$ from a *quasi*-molecular-ion peak at m/z 685.4953 ($[M+H]^+$) in the HR-ESI-MS in conjunction with its ^{13}C-NMR data. The NMR spectra (*Table*) showed the presence of an EtN group (δ(H) 1.04 (t, J = 7.2 Hz), and 2.37–2.40 and 2.50–2.55 ($2m$); δ(C) 13.6 (q) and 50.8 (t)), two tertiary

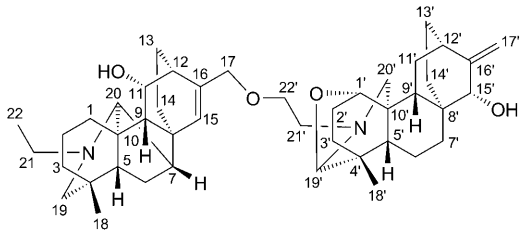


Fig. 1. *Piepunine* (**1**)*, isolated from* Aconitum piepunense

Me groups (δ(H) 0.70 and 0.96 (2*s*); δ(C) 26.0 and 26.1 (2*q*)), an exocyclic C=C bond (δ(H) 5.01 and 5.06 (2 br. *s*); δ(C) 157.2 (*s*) and 108.8 (*t*)), a trisubstituted C=C moiety (δ(H) 6.45 (*s*); δ(C) 141.2 (*s*) and 135.3 (*d*)), and an N,O-mixed acetal moiety (δ(H) 4.78 (*s*); δ(C) 94.2 (*d*)). All of the above-mentioned evidence, in combination with biogenetic considerations, suggested that piepunine is a bis-C_{20}-diterpenoid alkaloid [1]. The ^{13}C-NMR and DEPT data (*Table*) displayed the presence of four O-bearing CH groups (δ(C) 71.2, 71.3, 76.3, and 94.2) and two O-bearing CH_2 groups (δ(C) 72.6 and 68.0). The presence of six O-bearing C-atoms and four O-atoms, inferred from the molecular formula, indicated that this compound possesses two OH groups, one ether unit, and one acetal group. The δ(H) values of Me(18) (δ(H) 0.70) and Me(18′) (δ(H) 0.96) in the ^{1}H-NMR spectrum and the δ(C) values of two groups of quaternary C-atoms (C(4), C(8), and C(10) at δ(C) 35.8, 50.6, and 47.0; C(4′), C(8′), and C(10′) at

Table. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; $CDCl_3$) *of Piepunine* (**1**). δ in ppm, *J* in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
CH_2(1)	2.44–2.48 (*m*)	23.5 (*t*)	H–C(1′)	4.03 (*t*, *J* = 4.4)	71.3 (*d*)
CH_2(2)	1.47–1.52, 1.66–1.72 (2*m*)	23.0 (*t*)	CH_2(2′)	1.62–1.66, 1.90–1.95 (2*m*)	24.2 (*t*)
CH_2(3)	1.47–1.52, 1.68–1.72 (2*m*)	38.7 (*t*)	CH_2(3′)	1.22–1.26, 1.90–1.95 (2*m*)	47.5 (*t*)
C(4)	–	35.8 (*s*)	C(4′)	–	33.2 (*s*)
H–C(5)	1.15–1.19 (*m*)	54.0 (*d*)	H–C(5′)	1.62–1.66 (*m*)	49.1 (*d*)
CH_2(6)	1.54–1.59, 1.70–1.74 (2*m*)	25.7 (*t*)	CH_2(6′)	1.50–1.55, 1.50–1.55 (2*m*)	19.4 (*t*)
H–C(7)	2.03–2.08 (*m*)	34.0 (*d*)	CH_2(7′)	0.90–0.95, 1.18–1.22 (2*m*)	25.9 (*t*)
C(8)	–	50.6 (*s*)	C(8′)	–	45.5 (*s*)
H–C(9)	2.38–2.44 (*m*)	57.5 (*d*)	H–C(9′)	2.15–2.19 (*m*)	40.0 (*d*)
C(10)	–	47.0 (*s*)	C(10′)	–	36.9 (*s*)
H–C(11)	3.80 (*dd*, *J* = 10.8, 6.8)	71.2 (*d*)	CH_2(11′)	1.50–1.55, 1.66–1.72 (2*m*)	48.7 (*t*)
H–C(12)	1.90–1.94 (*m*)	34.4 (*d*)	H–C(12′)	2.32–2.36 (*m*)	36.0 (*d*)
CH_2(13)	1.22–1.26, 1.66–1.70 (2*m*)	31.3 (*t*)	CH_2(13′)	1.63–1.67 (*m*)	28.1 (*t*)
CH_2(14)	1.52–1.57, 1.92–1.97 (2*m*)	31.1 (*t*)	CH_2(14′)	1.13–1.17, 1.70–1.74 (2*m*)	31.0 (*t*)
H–C(15)	6.45 (*s*)	135.3 (*d*)	H–C(15′)	3.69 (br. *s*)	76.3 (*d*)
C(16)	–	141.2 (*s*)	C(16′)	–	157.2 (*s*)
CH_2(17)	3.89, 3.95 (*AB*, *J* = 12.0)	72.6 (*t*)	CH_2(17′)	5.01, 5.06 (2 br. *s*)	108.8 (*t*)
Me(18)	0.70 (*s*)	26.0 (*q*)	Me(18′)	0.96 (*s*)	26.1 (*q*)
CH_2(19)	2.15–2.19, 2.50–2.55 (2*m*)	56.9 (*t*)	H–C(19′)	4.78 (*s*)	94.2 (*d*)
H–C(20)	3.66 (br. *s*)	66.8 (*d*)	CH_2(20′)	2.33, 2.81 (*AB*, *J* = 10.0)	58.6 (*t*)
CH_2(21)	2.37–2.40, 2.50–2.55 (2*m*)	50.8 (*t*)	CH_2(21′)	2.83–2.88, 3.00–3.04 (2*m*)	55.9 (*t*)
Me(22)	1.04 (*t*, *J* = 7.2)	13.6 (*q*)	CH_2(22′)	3.43–3.47, 3.50–3.55 (2*m*)	68.0 (*t*)

δ(C) 33.2, 45.5, and 36.9) in the ^{13}C-NMR spectrum suggested that the two moieties of this bis-C_{20}-diterpenoid alkaloid consist of a denudatine-type C_{20}-diterpenoid-alkaloid moiety and an atisine-type C_{20}-diterpenoid-alkaloid moiety [1]. Comparison of the NMR data of the denudatine moiety of piepunine with those of denudatine [7], a representative denudatine-type C_{20}-diterpenoid alkaloid, revealed that they shared a similar NMR spectral pattern. The presence of the denudatine moiety was confirmed by the correlations Me(18)/C(19), CH_2(19)/C(20), H–C(20)/C(8), and H–C(7)/C(10) in the HMBC spectrum (*Fig. 2*). A typical exocyclic C=C bond was isomerized to the endocyclic C(15)=C(16) bond in the denudatine section, which was confirmed by the correlations from H–C(7) to the olefinic C-atom C(15), and from the olefinic H–C(15) to C(17) in the HMBC spectrum (*Fig. 2*). An OH group was positioned at C(11) due to the evident correlations between H–C(11) (δ(H) 3.80 (*dd*)) to C(8), C(10), and C(16) in the HMBC spectrum. Similarly, the NMR data of the atisine moiety of piepunine and those of isoatisine, a typical example of the atisine-type C_{20}-diterpenoid alkaloids, are very close to each other [8]. The N,O-mixed acetal moiety was attributed to C(19′) according to the long-range correlations from Me(18′) to C(19′), and from H–C(19′) to C(20′) (*Fig. 2*). An O-ether linkage was assigned to C(19′) and C(1′) based on an HMBC from H–C(19′) to C(1′). Another OH group was located at C(15′) according to the cross peaks between H–C(15′) (δ(H) 3.69 (br. *s*)) and C(12′), and between H–C(15′) and C(17′) in the HMBC spectrum. The typical EtN group was replaced by an CH_2CH_2N moiety, which was supported by the ^{1}H,^{1}H-COSY correlation between CH_2(21′) and CH_2(22′), and the HMBCs from H–C(19′) to C(21′), and from H–C(20′) to C(21′). The connection of the denudatine moiety and the atisine moiety was accomplished through an O-ether linkage between C(17) and C(22′), which was established by the HMBCs CH_2(17)/C(22′) and CH_2(22′)/C(17).

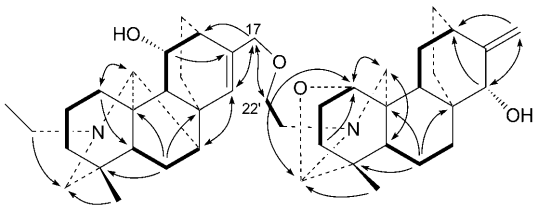


Fig. 2. *Key ^{1}H,^{1}H-COSY* (—) *and key HMBC* (H → C) *correlations of piepunine* (**1**)

The relative configuration at the stereogenic centers of piepunine was deduced from corresponding correlations in the NOEDS (nuclear *Overhauser* difference spectrum). As shown in *Fig. 3*, a correlation between H–C(15′) and H–C(9′) in the selective NOE experiment indicated that the OH group at C(15′) was α-oriented. The signal of H–C(15) was significantly increased when the signal of H–C(11) (δ(H) 3.80 (*dd*, J = 10.8, 6.8 Hz)) was irradiated, indicating the OH group at C(11) to be in α-orientation. Thus, the structure of piepunine was established as (11α)-17-[(15α)-20,22-deepoxy-1,19-epoxyatisin-22-yl)oxy]-15,16-didehydro-16,17-dihydrodenudatine.

A plausible biogenetic pathway for piepunine is proposed in the *Scheme*. The C(16)=C(17) bond in a denudatine-type C_{20}-diterpenoid alkaloid **A** could be oxidized

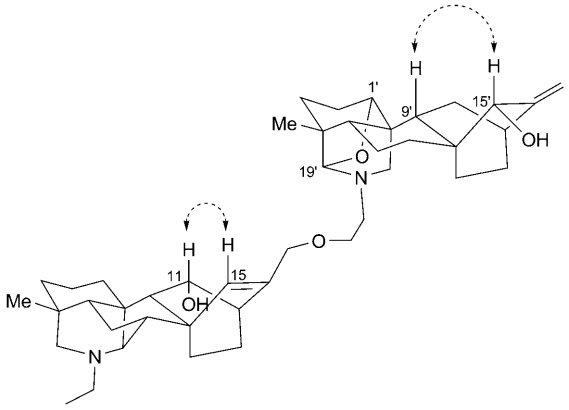
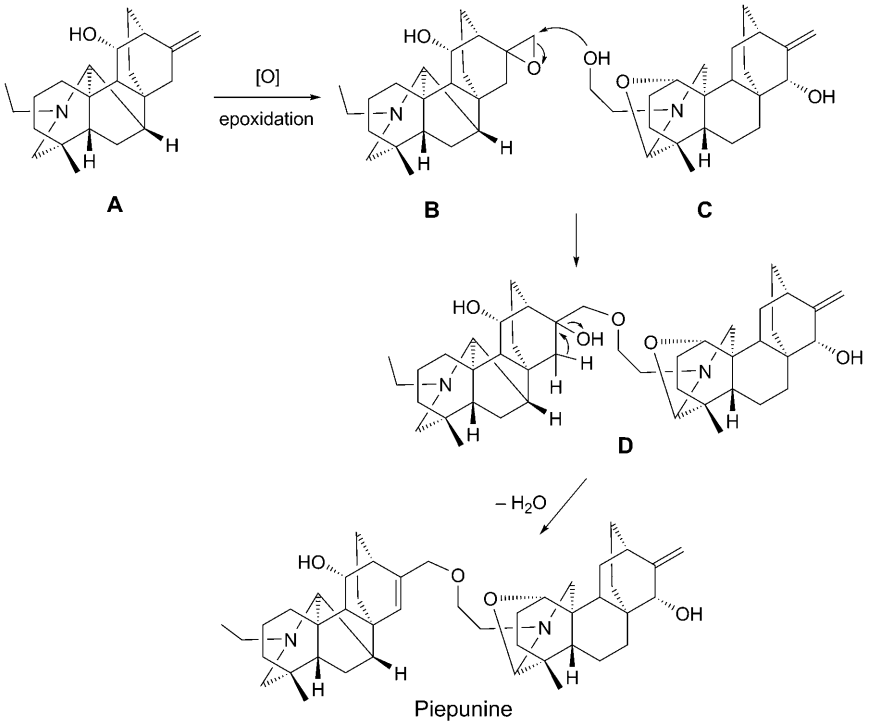


Fig. 3. *Key NOE correlations* (←- - -→) *of piepunine* (**1**)

to the corresponding epoxide **B**. A critical nucleophilic attack at the oxirane moiety of **B** by a primary OH group of an atisine-type C_{20}-diterpenoid alkaloid **C** may generate the corresponding bis-diterpenoid alkaloid **D**, which could be converted to piepunine by elimination of water.

Scheme. *Plausible Biogenetic Pathway of Piepunine* (**1**)



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Experimental Part

General. TLC and column chromatography (CC): silica gel GF_{254} and *H* (*Qindao Sea Chemical Factory*, P. R. China) resp.; detection (TLC) with modified *Dragendorff*'s reagent. Melting points: thermal-values analysis with microscope; uncorrected. Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectrum: *Nicolet-FI-IR-200SXY* spectrophotomer. ^{1}H- and ^{13}C-NMR Spectra: *Varian-Unity-INOVA-400/54* NMR spectrometer; in $CDCl_3$ with Me_4Si as the internal standard. ESI-MS and HR-MS: *VG-Auto-spec-3000* or *Finnigan-MAT-90* instrument; in *m*/*z*.

Plant Material. The sample of *Aconitum piepunense* was collected from Diqing County of Yunnan Province in China in August 2004, and authenticated by Prof. *Qin-Er Yang* at the Institute of Botany, Chinese Academy of Sciences. A voucher specimen has been deposited with West China College of Pharmacy, Sichuan University.

Extraction and Isolation. The powdered roots (3.6 kg) of *Aconitum piepunense* were percolated with 0.1M HCl (40 l). The filtrate was then alkalinized to pH > 9 with 28% aq. NH_4OH soln. (1.2 l), and extracted with AcOEt (5 × 20 l). The solvent was evaporated to give a crude alkaloid extract (36.6 g), most of which (36.0 g) was subjected to CC (SiO_2, petroleum ether/Me_2CO 6:1 → 3:1): *Fractions A* (1.3 g), *B* (4.4 g), *C* (6.1 g), *D* (11.4 g), and *E* (11.5 g). *Fr. C* (6.1 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 98:2): *Frs. C-1* (2.9 g), *C-2* (1.93 g), and *C-3* (420 mg). Further CC of *Fr. C-2* (SiO_2, petroleum ether/AcOEt/Et_2NH 86:14:1) provided piepunine (**1**; 90 mg).

Piepunine (=*(11α)-17-[(15α)-20,22-deepoxy-1,19-epoxyatisin-22-yl)oxy]-15,16-didehydro-16,17-dihydrodenudatine* = rel-*(2*R*,3*R*,6*S*,6a*R*,6b*R*,8*S*,10*S*,10a*S*,11a*R*)-1-{(3*S*,6a*S*,6b*R*,8*R*,10a*S*,11*S*,11a*S*,13*R*)-2-[(1-Ethyl-3-methyl-1,2,3,4,5,6,6b,7,11,11a-decahydro-7-hydroxy-8,10a-ethano-11,3,6a-ethanylylidene-8*H*-*indeno[2,1*-b*]azocin-9-yl)methoxy]ethyl}dodecahydro-3-methyl-9-methylene-2,6-epoxy-8,10a-ethano-11,3,6a-ethanylylidene-8*H*-indeno[2,1*-b*]azocin-10-ol*; **1**): White amorphous powder. M.p. 83–85°. $[\alpha]_D^{20} = -51.0$ ($c = 0.5$, $CHCl_3$). IR (KBr): 3424, 2927, 2867, 1653, 1456, 1373, 1185, 1064, 947, 893, 830. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 685.4953 ($[M + H]^+$, $C_{44}H_{65}N_2O_4^+$; calc. 685.4944).

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