New C_{19}-Diterpenoid Alkaloids from *Aconitum piepunense*

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Two new C_{19}-diterpenoid alkaloids, piepunensine A (1) and 18-acetylcammaconine (2), have been isolated from the roots of *Aconitum piepunense* together with five known alkaloids pengshenine B (3), talatisamine (4), aconosine (5), yunaconitine (6), and talatizidine (7). The structures of the new alkaloids were established on the basis of spectral data (1D- and 2D-NMR, HR-MS).

Key words *Aconitum piepunense*; C_{19}-diterpenoid alkaloid; piepunensine A; 18-acetylcammaconine

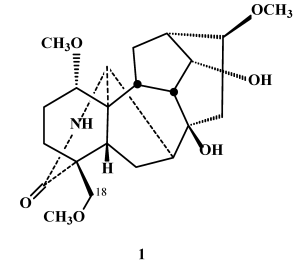
The plant *Aconitum piepunense* HAND-MAZZ. Symb. Sin. grows in Diqing county, Yunnan province, China at an elevation of 3000 m.[1)] To our knowledge, no phytochemical investigation of this plant has been undertaken. In the course of our continued studies of diterpenoid alkaloids from *Aconitum* and *Delphinium* plants, two new C_{19}-diterpenoid alkaloids, piepunensine A (**1**) and 18-acetylcammaconine (**2**), along with five known norditerpenoids pengshenine B (**3**), talatisamine (**4**), aconosine (**5**), yunaconitine (**6**), and talatizidine (**7**) were isolated from the roots of *Aconitum piepunense*. This paper reports the isolation and structural elucidation of these alkaloids.

Results and Discussion

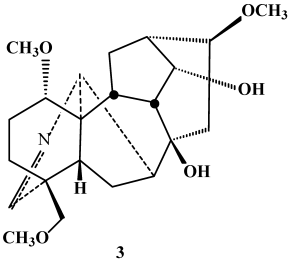
During the course of isolation, five known alkaloids pengshenine B (**3**),[6)] talatisamine (**4**),[7)] aconosine (**5**),[8)] yunaconitine (**6**),[9)] and talatizidine (**7**)[10)] were obtained and their structures were identified by comparison of the NMR data with reported values and co-TLC behavior with authentic samples. It is emphasised that piepunensine A (**1**) is the first known naturally occuring aconitine-type diterpenoid alkaloid with both the lactam and *N*-deethyl groups.

The molecular formula of piepunensine A (**1**) ($C_{22}H_{33}NO_6$) was determined by HR-ESI-MS $[M+Na]^+$ 430.2200. The NMR spectra strongly suggested an aconitine-type alkaloid for **1**.[2)] Its NMR and IR spectra displayed absence of an *N*-ethyl group and characteristic signals at δ_H 6.31 (1H, d, J=4.8 Hz), δ_C 174.5 (s) for an NH group and a lactam moiety (1648 cm^{-1}). The signal at δ 174.5 (s) was attributed to C-19 on the basis of the long-range ^1H–^{13}C HMBC correlations of 17-H (δ 3.63), 18-H (δ 3.57, 3.64) with C-19 (δ 174.5). Three methoxyl groups (δ_H 3.28, 3.35, 3.36, each 3H, s; δ_C 55.9 q, 56.5 q, 59.4 q) were present in the NMR spectra of **1**. These groups could be assigned at C-1, C-16, and C-18 due to the correlations between 1-OCH_3 (δ 3.28), C-1 (δ 84.3), 16-OCH_3 (δ 3.36), C-16 (δ 81.9), 18-OCH_3 (δ 3.35), and C-18 (δ 74.4), in HMBC spectrum. In the ^1H-NMR spectrum of **1**, one proton triplet (J=4.8 Hz) at δ 4.14 could be assigned to H-14β, suggesting the presence of a 14-hydroxyl group.[2,3)] The IR (3424 cm^{-1}) and ^{13}C-NMR (δ 71.5 s, 75.1 d) spectra also showed to have one each of secondary hydroxyl (14-OH) and tertiary hydroxyl group (8-OH). On the basis of these observations, the structure of piepunensine A was established as **1**.

18-Acetylcammaconine (**2**) was isolated as white amorphous powder with mp 123—125 °C. The HR-ESI-MS at *m/z* 450.2862 corresponded to the protonated molecular ion $[M+H]^+$ ($C_{25}H_{40}NO_6$). The NMR spectrum of **2** exhibited characteristic features of the aconitine-type C_{19}-diterpenoid alkaloids,[2)] bearing an *N*-ethyl group (δ_H 1.06, 3H, t, J=7.2 Hz; δ_C 13.5 q, 49.3 t), two methoxyl groups (δ_H 3.28, 3.34, each 3H, s; δ_C 56.2 q, 56.4 q), and an acetyl group (δ_H 2.06, s; δ_C 20.8 q, 171.0 s). Comparison of the MS and NMR spectra of **2** with those of cammaconine (**8**)[4)] showed that it had an additional acetyl group instead of a hydroxyl group. The ^{13}C-NMR spectra of **2** and **8** are very similar except for C-4 and C-18 due to the substituted effect (OH→OAc)[5)]



1



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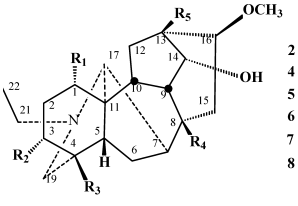

2 R_1=OCH_3, R_2=R_5=H, R_3=CH_2OAc, R_4=OH
4 R_1=OCH_3, R_2=R_5=H, R_3=CH_2OCH_3, R_4=OH
5 R_1=OCH_3, R_2=R_3=R_5=H, R_4=OH
6 R_1=OCH_3, R_2=R_5=OH, R_3=CH_2OCH_3, R_4=OAc
7 R_1=R_4=OH, R_2=R_5=H, R_3=CH_2OCH_3
8 R_1=OCH_3, R_2=R_5=H, R_3=CH_2OH, R_4=OH

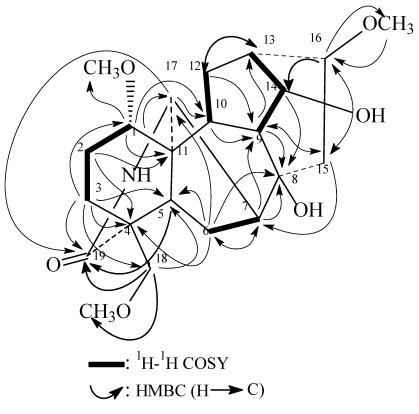

Fig. 1. The Key ^1H–^1H COSY and HMBC Correlations of Piepunensine A (**1**)

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(Table 1). Herein, this extra acetyl group could be located at C-18 according to the correlations between H_2-18 (δ 3.77, 3.81) and 18-O<u>CO</u>CH_3 (δ 171.0). Therefore, the structure of 18-acetylcammaconine (**2**) was determined.

Experimental

General Experimental Procedures Melting points were ascertained by thermal values analysis using a microscope and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectra were obtained on a Nicolet FT-IR 200SXY spectrophotomer. ^{1}H- and ^{13}C-NMR were measured in $CDCl_3$, with TMS as internal standard, on a Varian Unity INOVA 400/54 NMR spectrometer. MS spectra were measured by Finnigan LCQ and Micromass Auto Ultima-Tof spectrometer. Silica gel H (Qindao Sea Chemical Factory, China) was used for TLC and column chromatography.

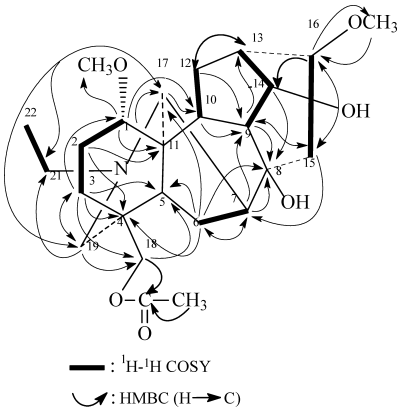

Fig. 2. The Key ^{1}H–^{1}H COSY and HMBC Correlations of 18-Acetylcammaconine (**2**)

Plant Material *Aconitum piepunense* Hand-Mazz. Symb. Sin. was collected in Diqing County of Yunnan Province, China in August 2004, and authenticated by Professor Qin-Er Yang, Institute of Botany, Chinese Academy of Sciences, China. A voucher specimen has been deposited in West China College of Pharmacy, Sichuan University.

Extraction and Isolation Powdered roots (3.6 kg) of *Aconitum piepunense* were percolated with 0.1 mol/l HCl (40 l). The filtrate was then alkalized with 28% aqueous NH_4OH (1.2 l) to pH>9, extracted with ethyl acetate (each 20 l) for 5 cycles, and evaporated to give the total crude alkaloids (36.6 g). The crude alkaloids (36 g) were chromatographed over silica gel column eluting with petroleum ether–acetone (6 : 1→3 : 1) gradient system to give fractions A (1.3 g), B (4.4 g), C (6.1 g), D (11.4 g), and E (11.5 g). Fraction B (4.4 g) was chromatographed on a silica gel column eluting with cyclohexane–acetone (8 : 1) to give talatisamine (**4**) (0.4 g), aconosine (**5**) (0.6 g), and the fraction B-1 (1.1 g) which was chromatographed on a silica gel column eluting with $CHCl_3$–CH_3OH (98 : 2) to afford piepunensine A (**1**) (58 mg) and pengshenine B (**3**) (120 mg). Silica gel column chromatography of fraction D (11 g) eluting with ether–ethyl acetate (6 : 1) gave 18-acetylcammaconine (**2**) (120 mg), yunaconitine (**6**) (60 mg), and talatizidine (**7**) (45 mg).

Piepunensine A (**1**): White amorphous powder, mp 94—96 °C; $[\alpha]_D^{20}$ −16.9° (c=0.5, $CHCl_3$); IR (KBr) cm^{-1}: 3424, 2932, 1647, 1458, 1094; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1; HR-ESI-MS: $[M+Na]^+$ 430.2200, Calcd for $C_{22}H_{33}NO_6Na$, 430.2205.

18-Acetylcammaconine (**2**): White amorphous powder, mp 123—125 °C; $[\alpha]_D^{20}$ −1.7° (c=0.5, $CHCl_3$); IR (KBr) cm^{-1}: 3434, 2945, 1735, 1224, 1085;

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds (**1**, **2**, **8**) (^{1}H: 400 MHz, ^{13}C: 100 MHz; $CDCl_3$)

No.	**1**		**2**		**8**
	δ_C	δ_H Mult (J=Hz)	δ_C	δ_H Mult (J=Hz)	δ_C
1	84.3 d	3.25 m	86.0 d	3.10 m	86.3
2	26.3 t	1.80 m (α)	25.6 t	1.82 m (α)	25.8
		2.22 m (β)		2.09 m (β)	
3	30.3 t	1.70 m (α)	32.4 t	1.63 m (α)	33.2
		2.04 m (β)		1.82 m (hidden) (β)	
4	51.1 s	—	37.8 s	—	39.1
5	36.9 d	2.32 m	37.5 d	2.38 m (hidden)	37.6
6	26.1 t	1.67 m (hidden) (α)	24.8 t	1.28 m (α)	24.6
		2.09 m (β)		1.44 m (β)	
7	54.8 d	2.14 m	45.9 d	1.69 m	45.9
8	71.5 s	—	72.7 s	—	73.7
9	45.7 d	2.34 m	46.9 d	2.30 m	47.0
10	43.0 d	2.06 m	45.7 d	1.82 m	46.0
11	47.1 s	—	48.7 s	—	48.8
12	27.0 t	1.85 m (hidden) (β)	27.6 t	1.72 m (hidden) (β)	27.7
		1.89 m (α)		1.92 m (α)	
13	45.7 d	1.86 m (hidden)	46.0 d	2.20 m	45.6
14	75.1 d	4.14 t (4.8)	75.5 d	4.14 t (4.4)	75.6
15	36.7 t	2.18 m (α)	38.3 t	2.12 m (α)	38.3
		2.38 m (β)		2.56 m (β)	
16	81.9 d	3.47 m	82.2 d	3.43 m	82.3
17	56.2 d	3.63 br s	62.6 d	3.18 br s	63.0
18	74.4 t	3.57 ABq (10.0)	70.0 t	3.77 ABq (11.2)	68.8
		3.64 ABq (10.0)		3.81 ABq (11.2)	
19	174.5 s	—	52.7 t	2.07 ABq (11.2)	53.1
				2.53 ABq (11.2)	
21	—	—	49.3 t	2.36 m	49.2
				2.46 m	
22	—	—	13.5 q	1.06 t (7.2)	13.7
1-OCH_3	55.9 q	3.28 s	56.2 q	3.28 s	56.3
16-OCH_3	56.5 q	3.36 s	56.4 q	3.34 s	56.5
18-OCH_3	59.4 q	3.35 s	—	—	—
OAc	—	—	171.0 s	—	—
			20.8 q	2.06 s	—

^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1; HR-ESI-MS: $[M+H]^+$ 450.2862, Calcd for $C_{25}H_{40}NO_6$, 450.2856.

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