Hemsleyatine, a Novel C_{19}-Diterpenoid Alkaloid with 8-Amino Group from *Aconitum hemsleyanum*[1)]

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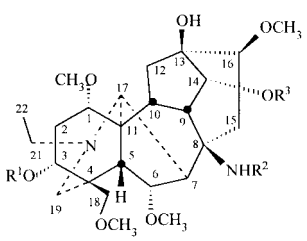
The new compound hemsleyatine (1) was isolated along with four known C_{19}-diterpenoid alkaloids: indaconitine (4), yunaconitine (5), chasmanine (6), and talatisamine (7) from the roots of *Aconitum hemsleyanum* Pritz. Structures were established by spectral analysis, including tow dimeusional (2D) NMR spectroscopy and a chemical method. Hemsleyatine (1) is the first C_{19}-diterpenoid alkaloid bearing the 8-amino group. In addition, the assignments of some ^{13}C signals for pseudaconine (3) were revised by comparison with those of hemsleyatine (1).

Key words *Aconitum hemsleyanum*; C_{19}-diterpenoid alkaloid; hemsleyatine

The plant *Aconitum hemsleyanum* Pritz belong to the Ranunculaceae and grows widely in the southwest part of China. It has been used as a folk remedy for treatment of arthritic pain.[2)] Apart from a preliminary study on the isolation of four known alkaloids (indaconitine, yunaconitine, chasmanine, and talatisamine) from this plant species,[3,4)] no further phytochemistry is reported. In this paper, we report the isolation and identification of hemsleyatine (**1**), a novel C_{19}-diterpenoid alkaloid having the 8-amino group together with four known alkaloids: indacontine (**4**), yunaconitine (**5**), chasmanine (**6**), and talatisamine (**7**).

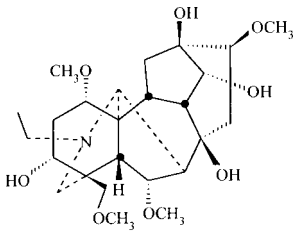
Results and Discussion

Hemsleyatine (**1**) was obtained as a white amorphous powder. High resolation electron impact (HR-EI)-MS of **1** indicated a molecular formula of $C_{25}H_{42}N_2O_7$ (M^+ at *m/z* 482.2989, Calcd 482.2992) with 6 degrees of unsaturation. The NMR data strongly suggested an aconitine-type alkaloid for hemsleyatine,[5)] bearing an *N*-ethyl group, and four methoxyl groups. In its ^1H-NMR spectrum, one proton doublet (J=4.8 Hz) signal at δ 3.90 could be assigned to H-14β,[5)] indicating the presence of a 14-hydroxyl group. The MS displayed a base peak at *m/z* 451 ($M^+ - OCH_3$), suggesting the presence of an 1α-OCH_3 group.[6)] The remaining three methoxyl groups could be located at C-6, C-16, and C-18 by a set of ^1H–^{13}C long-range correlations between the 6-OCH_3, 16-OCH_3, 18-OCH_3 and the related carbons C(6), C(16), and C(18), respectively (Table 1). In contrast, the chemical shift of the 16-OCH_3 was observed at the lower field (δ_H 3.42), implying it had a hydroxyl group at C-13.[7)] A double doublet (J=10.0, 4.8 Hz) signal at δ_H 3.70 was attributed to H-3 due to the presence of the multi-bond ^1H–^{13}C correlations between H-3 and (C-1, C-2, C-4, C-18) in the heteronuclear multiple bond connectivity (HMBC) spectrum of **1** (Table 1). Meanwhile, the stereochemistry of H-3 in **1** and **2** was deduced as the β-orientation, as in many cases, *e.g.*, 3-acetylaconitine (δ_H 4.70, dd, J=11.0, 7.0 Hz, H-3β),[8)] based on the coupling constants (J=10.0, 4.8 Hz for **1**; J=10.4, 7.6 Hz for **2**) in their ^1H-NMR spectra. The remaining amino group was placed at C-8 for the following reasons: 1) the MS of hemsleyatine showed that the molecular weight (*m/z* 482, M^+) is even; 2) the NMR spectra of acetyl derivative **2** (Table 2) displayed the presence of an secondary amide group (δ_H 5.87, s. 1H; 1.91, s, 3H; δ_C 168.5s, 24.2 q); and 3) the HMBC spectrum of **2** indicated the key ^1H–^{13}C long-range correlations between 8-N<u>H</u>Ac and C(8), C(9) (Table 2). The above findings led to the structural determination of hemsleyatine (**1**). Finally, unambiguous assignments of all of the ^1H and ^{13}C chemical shifts for hemsleyatine (**1**) and its acetylated derivative **2** were accomplished using two dimensional (2D) NMR techniques (^1H–^1H correlation spectroscopy (COSY), ^1H-detected heteronuclear multiple quantum coherence (HMQC), HMBC), together with revision of the assignments of the signals C(1), C(2), C(6), C(12), C(16), C(19), and C(21) for pseudaconine (**3**)[9)] by comparison with the ^{13}C-NMR data of hemsleyatine (**1**). It is worthy to note that hemsleyatine (**1**) is the first aconitine-type C_{19}-diter-penoid alkaloid bearing the amino group at C-8.

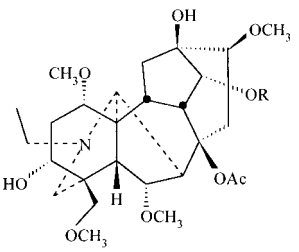


1 R^1=R^2=R^3=H

2 R^1=R^2=R^3=Ac



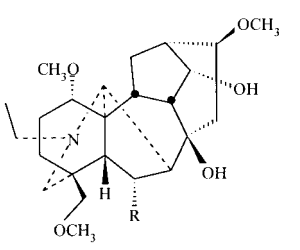
3



4 R=Bz

5 R=As

As = CO–C$_6$H$_4$–OCH$_3$



6 $R=OCH_3$

7 R=H

Experimental

General Experimental Procedures Melting points are uncorrected. Optical rotations were measured with a PE-341 polarimeter at 20±1 °C; ^1H-

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Table 1. NMR Data of Compounds **1** and **3**

No.	**1**				**3**
	δ_H (J=Hz)	δ_C	^{1}H COSY	HMBC (H→C)	δ_C
1	3.06 dd (9.0, 6.2)	82.8 d	H-2α, H-2β	C-2, C-3, C-10, C-11, C-17, C-1′	82.4[a] (84.5)[b]
2	2.08 m (α)	33.6 t	H-1, H-2β	C-1, C-3, C-4, C-11	33.8[a] (35.9)[a]
	2.32 m (β)		H-1, H-2α	C-1, C-3, C-11	
3	3.70 dd (10.0, 4.8)	71.6 d	H-2α, H-2β	C-1, C-2, C-4, C-18	72.2
4	—	43.4 s	—	—	43.5
5	2.06 d (6.8)	47.7 d	H-6	C-4, C-7, C-11, C-19	49.3
6	4.04 d (6.4)	83.2 d	H-5, H-7	C-4, C-5, C-7, C-8, C-11, C-6′	83.2[a] (82.4)[b]
7	1.82 m	54.5 d	H-6, H-17	C-5, C-6, C-8, C-11, C-15, C-17	52.2
8	—	53.7 s	—	—	72.9
9	2.10 m	49.1 d	H-10, H-14	C-8, C-10, C-12, C-13, C-14, C-15	50.4
10	1.86 m	42.4 d	H-9, H-12β	C-8, C-9, C-12	42.0
11	—	50.2 s	—	—	50.2
12	1.90 m (α)	35.6 t	H-12β	C-9, C-10, C-13, C-16	35.9[a] (33.8)[b]
	2.34 m (β)		H-12α, H-10	C-10, C-11, C-13, C-14, C-16	
13	—	76.7 s	—	—	76.9
14	3.90 d (4.8)	79.3 d	H-9	C-8, C-9, C-13, C-16	79.6
15	2.02 m (β)	42.0 t	H-15β, H-16	C-8, C-16	40.1
	2.46 m (α)		H-15α, H-16	C-7, C-8, C-9, C-13, C-16	
16	3.39 m	84.7 d	H-15α, H-15β	C-8, C-12, C-13, C-16′	84.5 (83.2)[b]
17	3.12 s	61.9 d	H-7	C-5, C-6, C-8, C-10, C-11	62.4
18	3.60 d (9.2)	77.0 t	H-18 (3.74)	C-3, C-4, C-5, C-19, C-18′	77.5
	3.74 d (9.2)		H-18 (3.60)	C-3, C-4, C-5, C-19, C-18′	
19	2.44 (hidden)	47.4 t	(hidden)	C-3, C-4, C-5, C-21	47.4[a] (48.7)[b]
	2.90 (hidden)		(hidden)	C-3, C-4, C-5, C-17	
21	2.52 m	48.9 t	H_2-22	C-17, C-19, C-22	48.7[a] (47.4)[b]
22	1.09 t (7.2)	13.5 q	H_3-21	C-21	13.7
1′	3.24 s	55.9 q	—	C-1	56.3
6′	3.34 s	57.6 q	—	C-6	57.4
16′	3.42 s	58.1 q	—	C-16	57.9
18′	3.32 s	59.1 q	—	C-18	59.4
NH_2	4.77 br s	—	—		—

a) The revised data. *b*) The data reported in ref. 9.

Table 2. NMR Data of Compound **2**

No.	δ_H (J=Hz)	δ_C	^{1}H COSY	HMBC (H→C)
1	3.02 m	82.0 d	H-2α, H-2β	C-2, C-10, C-11, C-17
2	2.30 m (α)	31.8 t	H-1, H-2β, H-3	C-1, C-3, C-4, C-11
	2.38 m (β)		H-1, H-2α, H-3	C-1, C-3, C-4, C-11
3	4.87 dd (10.4, 7.6)	71.6 d	H-2α, H-2β	C-2, C-4, C-19, 3-C<u>O</u>-CH_3
4	—	42.4 s	—	—
5	3.06 br s	46.3 d	H-6	C-4, C-6, C-11, C-17
6	3.85 d (6.8)	84.0 d	H-5, H-7	C-4, C-7, C-6′
7	3.04 m	48.5 d	H-6, H-17	C-6, C-8, C-9, C-11, C-17
8	—	57.6 s	—	—
9	2.27 d (6.8)	46.5 d	H-14	C-7, C-10
10	1.97 m	41.4 d	H-12α, H-12β	C-8, C-9, C-11, C-12, C-13
11	—	49.7 s	—	—
12	1.94 m (α)	35.8 t	H-10, H-12β	C-9, C-10, C-11, C-13, C-16
	2.34 m (β)		H-10, H-12α	C-11, C-13
13	—	75.5 s	—	—
14	4.66 d (4.8)	80.3 d	H-9	C-8, C-13, C-16
15	2.44 m (β)	37.6 t	H-15α, H-16	C-8, C-16
	2.78 m (α)		H-15β, H-16	C-7, C-8, C-9, C-13, C-16
16	3.31 m	84.0 d	H-15α, H-15β	C-13, C-16′
17	3.08 s	60.8 d	H-7	C-8, C-11
18	2.97 d (8.8)	71.9 t	H-18 (3.93)	C-3, C-4, C-5, C-18′
	3.93 d (8.8)		H-18 (2.97)	C-3, C-4
19	1.99 (hidden)	47.6 t	H-19 (2.70)	C-4, C-17, C-21
	2.70 (hidden)		H-19 (1.99)	C-3, C-5
21	2.60 m	49.0 t	H-22	C-22
22	1.10 t (7.2)	13.5 q	H-21	C-21
3-OAc	2.06 s	170.2 s	—	—
		21.2 q	—	3-C<u>O</u>CH_3
14-OAc	2.08 s	172.3 s	—	—
		21.2 q	—	14-C<u>O</u>CH_3
8-NHAc	1.91 s	168.5 s	—	—
		24.2 q	—	8-NHC<u>O</u>CH_3
1′	3.22 s	56.2 q	—	C-1
6′	3.23 s	57.5 q	—	C-6
16′	3.37 s	58.6 q	—	C-16
18′	3.20 s	58.8 q	—	C-18
NH	5.87 s	—	—	C-8, C-9, 8-NHC<u>O</u>CH_3
13-OH	3.97 s	—	—	C-12, C-13, C-16

and ^{13}C-NMR spectra were acquired on a Varian INOVA-400/54 spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard; IR spectrum was recorded on a Nicolet FT-IR 200SXV spectrophotometer; High-resolution mass spectra were obtained from the VG 70 A mass spectrometer; TLC was performed on silica gel GF_{254} percolated plates, sprayed with a Dragendorff's reagent for detection; Column chromatography was carried out on silica gel H (10—40 μm). A polyvinyl sulfonic ion exchange resin (H-form, cross linking 1×1, Chemical Factory of Nankai University, China) was used in the extraction of total alkaloids.

Plant Material The plants *Aconitum hemsleyanum* Pritz were collected from Emei mountain in Sichuan province, China, in November, 1997. The voucher specimen was deposited at the herbarium of the West China College of Pharmacy, Sichuan University.

Extraction and Isolation According to the literature method,[10] 5.0 kg of dried powdered roots of *Aconitum homsleyanum* Pritz was percolated with 0.05 mol/l HCl until 50 l of percolation was collected. A column of 5.0 kg wet resin (dry weight 0.5 kg) was used to treat the percolates. After exchange, the resin was washed repeated in a section filter with deionized water. Then, the resin column was basified with 10% NH_4OH, and eluted with 95% EtOH (5000 ml). Evaporation of the eluting liquid under reduced pressure gave the syrupy total alkaloid (38 g), which was fractionated by a pH gradient method into four portions I (pH 3, 7.0 g); II (pH 7, 20.0 g); III (pH 9, 1.5 g); IV (pH 11, 0.5 g). Part I was chromatographed successively over Chromatotron (silica gel H) eluting with petroleum ether–acetone (4 : 1→3 : 1) to give indaconitine (**4**) (75 mg) and yunaconitine (**5**) (115 mg). Part II was chromatographed on silica gel H column eluting with petroleum ether–acetone–diethylamine (90 : 10 : 1→50 : 50 : 1) to afford hemsleyatine (**1**) (290 mg), chasmanine (**6**) (84 mg), and talatisamine (**7**) (200 mg). The known alkaloids were identified on the basis of comparison of the ^{1}H-NMR data with reported values[11—14] and co-TLC behavior with the authentic samples.

Hemsleyatine (**1**): White amorphous powder, mp 89—90 °C (chloroform–acetone–diethylamine) $[\alpha]_D$ +36.5°(c=0.55, $CHCl_3$); ^{1}H- and ^{13}C-NMR ($CDCl_3$) δ: see Table 1; IR (KBr) cm^{-1}: 3456, 3446, 3428, 3410, 2929, 1096; HR-EI-MS *m/z*: 482.2989 (Calcd for $C_{25}H_{42}N_2O_7$: 482.2992); EI-MS *m/z* (%): 482 (M^+, 4), 467 (7), 451 (100), 96 (17), 58 (23).

Acetylation of Hemsleyatine (1) To a mixed solution of acetic anhydride (2 ml) and pyridine (5 ml) hemsleyatine (**1**) (100 mg) was added and the solution was allowed to stand at room temperature overnight. Evaporation under reduced pressure gave a residue, which was chromatographed on a silica gel H column (3.5 g) eluting with petroleum ether–acetone (2 : 1) to give compound **2** (23 mg) as a white amorphous powder, mp 267—268 °C (petroleum ether–acetone); ^{1}H- and ^{13}C-NMR ($CDCl_3$) δ: see in Table 2; HR-EI-MS *m/z*: 608.3301 (Calcd for $C_{31}H_{48}N_2O_{10}$ 608.3308). EI-MS *m/z* (%): 608 (M^+, 4), 593 (7), 577 (100), 549 (51), 517 (37), 96 (9), 60 (20).

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References and Notes

1) Dedicated to Prof. Xiao-Tian Liang on the the occasion of his 80th birthday.
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