Hemsleyaconitines F and G, Two Novel C_{19}-Diterpenoid Alkaloids Possessing a Unique Skeleton from *Aconitum hemsleyanum*

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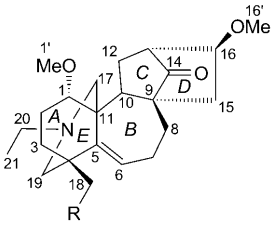
Hemsleyaconitines F and G (**1** and **2**, resp.) were isolated from the EtOH extract of *Aconitum hemsleyanum*. Their structures were elucidated by extensive analyses of the IR, 1D- and 2D-NMR, and MS data. The two C_{19}-diterpenoid alkaloids **1** and **2** possess a novel skeleton, featuring a five-membered *D*-ring between C(9), C(13), C(14), C(15), and C(16), which is quite different from the previously isolated six-membered *D*-ring analogs.

Introduction. – *Aconitum* L. (Ranunculaceae) is a large genus comprising *ca.* 300 species and widely distributed in the temperate regions of the Northern Hemisphere. Among them, 76 *Aconitum* species in China have found medicinal application, and some species possess analgesic, antirheumatic, and anti-arrhythmic activities. Recently, some novel diterpenoid alkaloids with unprecedented skeletons such as racemulosine [1] and vilmoraconitine [2] had been isolated from this genus. Therefore, from the phytochemical point of view, this genus is of interest for the identification of new natural compounds with interesting biological activities. The investigated natural compounds might possess potential as natural sources for the partial synthesis of high-value compounds. *Aconitum hemsleyanum* PRITZ., a herb used in folk medicine, is mainly distributed in Yunnan, Sichuan, and Guizhou Provinces in China, and has been used for the treatment of rheumatism and pains [3]. The aconitine-type C_{19}-diterpenoid alkaloids are the principal constituents of the above herb according to the literature [4–7]. Our previous study had led to the isolation of five new C_{19}-diterpenoid alkaloids from *A. hemsleyanum* [8]. During our further phytochemical investigation on this plant, two novel C_{19}-diterpenoid alkaloids, hemsleyaconitines F and G (**1** and **2**, resp.; see *Fig. 1*) were obtained from the 95% EtOH extract of its roots. To the best of our knowledge, both compounds are the first C_{19}-diterpenoid alkaloids with a unique five-membered ring formed by the C(9)–C(15) linkage, different from the common C_{19}-diterpenoid alkaloids featured with one six-membered ring through C(8)–C(15) linkage.

Results and Discussion. – Hemsleyaconitine F (**1**) was obtained as a colorless gum and showed positive reaction to *Dragendorff*'s reagent. The HR-ESI-MS (positive-ion



1 R = H
2 R = MeO

Fig. 1. *The structures of compunds* **1** *and* **2**

mode) exhibited a *quasi*-molecular-ion peak at *m/z* 374.2700 ($[M+H]^+$; calc. 374.2695) in accordance with the molecular formula $C_{23}H_{35}NO_3$ with an unsaturation degree of seven. The IR spectrum showed absorption for a cyclopentanone group at 1741 cm^{-1}. In the ^{1}H-NMR spectrum (*Table*), the signals of an olefinic H-atom at δ(H) 5.36 (br. *d*, $J=5.3$, H–C(6)), of two MeO groups at δ(H) 3.22 (*s*, MeO–C(1)) and 3.38 (*s*, MeO–C(16)), and of an EtN function at δ(H) 2.17 (*q*, $J=6.8$, 1 H of MeCH_2N), 2.39 (*q*, $J=6.8$, 1 H of MeCH_2N), and 1.02 (*t*, $J=7.1$, *Me*CH_2N) were observed. The ^{13}C-NMR (DEPT) spectrum displayed 23 C-atom signals including those of four Me groups, nine CH_2 groups, five CH groups, and five quaternary C-atoms. Detailed analyses of its NMR data suggested that compound **1** might be an aconitine type C_{19}-diterpenoid alkaloid possessing rings *A*, *B*, *C*, and *E* similar to those of 14-dehydrogenicunin B [9], but a trisubstituted C=C bond was indicated according to the H-atom signal at δ(H) 5.36 (br. *d*, $J=5.3$) in the ^{1}H-NMR, and the C-atom signals at δ(C) 119.1 (*d*) and 137.2 (*s*) in the ^{13}C-NMR. Compared with 14-dehydrogenicunin B, there were two more CH_2 units in compound **1** as the DEPT spectrum suggested. The CH_2 group (δ(C) 49.7, *t*) was assigned to C(17) according to the HMBC spectrum (*Fig. 2*), indicating there was no connection between C(7) and C(17). The long-range correlations between the non-O-bearing quaternary C-atom with the signal at δ(C) 48.3 (C(9)) and H_a–C(15), H_b–C(15), and H–C(13), and C-atom with the signal at δ(C) 79.9 (C(16)) and H–C(13), H_a–C(15), H_b–C(15), and MeO–C(16), and C-atom with the signal at δ(C) 218.5 (C(14)) and H–C(13) and H–C(16) suggested a rearranged five-membered ring *D* comprising C(9), C(13), C(14), C(15), and C(16) in compound **1**. The cross-peak of C(5) with H–C(6) and H–C(7), of C(6) with H–C(7), of C(11) with H–C(6) in the HMBC spectrum allowed us to place the C=C bond between C(5) and C(6). Furthermore, the cross-peaks between H–C(7) and H–C(6)/H–C(8), H–C(12) and H–C(10)/H–C(13), H–C(16) and H–C(13)/H–C(15) observed in the ^{1}H,^{1}H-COSY spectrum (*Fig. 2*) supported the above conclusions.

The relative configuration of compound **1** was established by the ROESY spectrum. As shown in *Fig. 3*, the correlations between H–C(10), assumed to be β-oriented with reference to the aconitine type C_{19}-diterpenoid alkaloid, and H–C(13), H–C(1), and H–C(16′) were observed, indicating the β-orientation of H–C(1), H–C(13), and H–C(16′). Thus, the structure of compound **1** was determined as displayed in *Fig. 1*, named as hemsleyaconitine F (**1**).

Hemsleyaconitine G (**2**) was also obtained as a colorless gum and reacted positively to the *Dragendorff*'s reagent. The molecular formula was determined as $C_{24}H_{37}NO_4$

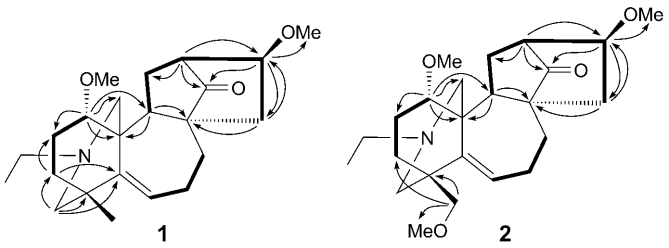


Fig. 2. *Selected* $^1H,^1H$*-COSY* (—) *and HMBC* (→) *correlations of compounds* **1** *and* **2**

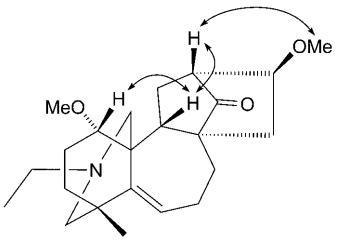


Fig. 3. *Selected ROESY correlations of compound* **1**

based on EI-MS (*m*/*z* 403 (M^+)) and HR-ESI-MS (positive-ion mode; *m*/*z* 404.2795 ($[M+H]^+$; calc. 404.2800)). The ^{1}H- and ^{13}C-NMR spectra of compound **2** were almost identical to those of compound **1**, except that signals of an additional MeO (δ(H) 3.27 (*s*, MeO–C(18)); δ(C) 59.3 (*q*)) and of a CH_2 group (δ(H) 2.88 (*d*, $J = 8.9$, H_a–C(18)), 3.06 (*d*, $J = 8.9$, H_b–C(18)); δ(C) 78.4 (*t*)) appeared. Considering that compound **1** contained one more quarternary Me group than compound **2**, it could be concluded that compound **2** might be a derivative of compound **1** with an additional MeO group at C(18). This assumption was supported by the HMBC spectrum in which the cross-peaks between *Me*O–C(18) (δ(C) 59.3, *q*) and H–C(18), C(3) (δ(C) 33.6, *t*), C(4) (δ(C) 37.4, *s*), and H–C(18) (δ(H) 2.88, 3.06) emerged. The full NMR data assignments of compound **2** were achieved according to the ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY spectral analyses. Consequently, the structure of compound **2** was elucidated as shown in *Fig. 1* and as hemsleyaconitine G (**2**).

Hemsleyaconitines F and G (**1** and **2**, resp.) are the first two new aconitine-type C_{19}-diterpenoid alkaloids with a rearranged five-membered *D* ring comprising C(9), C(13), C(14), C(15), and C(16), which is different from the six-membered *D* ring of the roported aconitine alkaloids and could not be well explained biogenetically.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China), Al_2O_3 (*Shanghai Wusi Chemical Reagents Company, Ltd.*), and *Sephadex LH-20* (*Pharmacia Fine Chemical Co. Ltd.*, Germany). M.p.: *XRC-1* micro-melting-point apparatus; uncorrected. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrophotometer. IR Spectra: *Bio-Rad FTS-135* spectrometer. 1D- and 2D-NMR spectra: *Bruker AM-*

Table. *^{1}H- and ^{13}C-NMR Data and HMBCs of* **1** *and* **2**. At 400 and 100 MHz, respectively, in $CDCl_3$; δ in ppm, J in Hz.

Position	**1**			**2**		
	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)	HMBC (H → C)
1	3.22–3.27[a])	85.0 (*d*)	1′, 2, 11, 17	3.21–3.26[a])	84.8 (*d*)	1′, 2, 11, 17
2a	1.99–2.05 (*m*)	26.2 (*t*)	1, 3, 4, 5, 6	2.04–2.11 (*m*)	25.9 (*t*)	1, 3, 4, 5
2b	2.86–2.93 (*m*)		1, 4, 5, 6	2.81–2.87 (*m*)		1, 3, 4
3a	1.37–1.43 (*m*)	39.0 (*t*)	2, 4, 5, 19	1.64–1.69 (*m*)	33.6 (*t*)	1, 2, 4
3b	1.66 (*dd*, *J* = 13.5, 5.5)		1, 2, 4, 11, 19	1.68–1.75 (*m*)		1, 2, 4
4		33.4 (*s*)			37.4 (*s*)	
5		137.2 (*s*)			137.2 (*s*)	
6	5.36 (*d*, *J* = 5.3)	119.1 (*d*)	7, 9, 11	5.35 (*d*, *J* = 5.9)	118.9 (*d*)	7, 9, 11
7a	1.95–2.03 (*m*)	22.7 (*t*)	5, 6, 11	1.86–1.93 (*m*)	22.1 (*t*)	5, 6,10, 11
7b	2.15–2.21 (*m*)		5, 6, 9	2.16–2.22 (*m*)		5, 6, 10
8a	2.04–2.09 (*m*)	46.7 (*t*)	9	2.04–2.10 (*m*)	46.8 (*t*)	9
8b	2.41–2.48 (*m*)		9	2.42–2.49 (*m*)		9
9		48.3 (*s*)			48.3 (*s*)	
10	1.15 (*dd*, *J* = 10.7, 6.3)	41.3 (*d*)	1, 4, 7, 9, 11	1.38 (*dd*, *J* = 10.5, 6.1)	37.1 (*d*)	1, 6, 11,12, 17
11		41.1 (*s*)			40.6 (*s*)	
12a	1.32 (*dd*, *J* = 11.8, 4.4)	36.2 (*t*)	1, 4, 7, 9, 11	1.31 (*dd*, *J* = 11.9, 2.9)	36.2 (*t*)	5, 13, 14, 16
12b	2.87–2.93 (*m*)		9	2.85–2.92 (*m*)		5, 6, 10, 13,16
13	2.72 (br. *s*)	48.7 (*d*)	9, 12, 14, 16	2.71 (br. *s*)	48.8 (*d*)	9, 12, 14, 16
14		218.5 (*s*)			218.5 (*s*)	
15a	2.16–2.22 (*m*)	37.3 (*t*)	9, 16	2.61 (*dd*, *J* = 15.3, 6.1)	37.3 (*t*)	16
15b	2.62 (*dd*, *J* = 14.9, 8.6)		9, 16	2.16–2.22 (*m*)		9, 16
16	3.35–3.43[a])	79.9 (*d*)	13, 14, 15, 16′	3.35–3.43[a])	79.9 (*d*)	13, 14, 15, 16′
17a	2.11 (*d*, *J* = 11.0)	49.7 (*t*)	1, 11	2.15–2.21[a])	50.4 (*t*)	1, 10, 11, 19
17b	2.62 (*d*, *J* = 11.2)		1, 11	2.99 (*d*, *J* = 11.1)		1, 10, 11, 19
18	0.73 (*s*)	25.9 (*q*)	3, 4, 17	2.88 (*d*, *J* = 8.9), 3.06 (*d*, *J* = 8.9)	78.4 (*t*)	3, 4, 18, 18′
19a	2.17–2.23[a])	57.9 (*t*)	5, 17	2.12–2.19[a])	54.3 (*t*)	3, 4, 11, 19
19b	2.45 (*d*, *J* = 14.0)		3, 4, 5, 17	2.47 (*d*, *J* = 11.2)		3, 4, 19
Me*CH*$_2$N	2.17 (*q*, *J* = 6.8), 2.39 (*q*, *J* = 6.8)	52.4 (*t*)	17, 19, 21	2.09 (*q*, *J* = 6.8), 2.40 (*q*, *J* = 6.8)	52.6 (*t*)	17, 19, 21
*Me*CH$_2$N	1.02 (*t*, *J* = 7.1)	12.4 (*q*)	20	1.02 (*t*, *J* = 7.1)	12.4 (*q*)	20
MeO–C(1)	3.22 (*s*)	54.6 (*q*)	1	3.21 (*s*)	54.7 (*q*)	1
MeO–C(16)	3.38 (*s*)	55.9 (*q*)	16	3.37 (*s*)	56.0 (*q*)	16
MeO–C(18)				3.27 (*s*)	59.3 (*q*)	18

[a]) Overlapped signals.

400 and *DRX-500* spectrometers; chemical shifts δ in ppm with reference to the solvent signals. MS: *VG Autospec-3000* spectrometer at 70 eV; in *m/z*. HR-ESI-MS: *API Qstar-Pulsar-1* spectrometer.

Plant Material. The roots of *Aconitum hemsleyanum* PRITZ. were collected in Wuding County, Yunnan Province, P. R. China, in October, 2006, and authenticated by Prof. Dr. *Li-Gong Lei* from Kunming Institute of Botany. A voucher specimen (No. KIB 2006-10-01) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The roots of *A. hemsleyanum* (54 kg) were powdered and extracted three times with 95% EtOH for 2 h under reflux. After removing solvent, the crude extract was dissolved in 15 l of 2% HCl soln. and filtered. The acidic soln. was basified to pH 9.0 with NH_3 (25%), and then extracted with $CHCl_3$ to obtain a crude alkaloid extract (460 g) after removal of $CHCl_3$ in vacuum. The extract was chromatographed over SiO_2 column (4.6 kg, 200–300 mesh) and eluted with petroleum ether (PE)/acetone/Et_2NH 10:1:1 → 5:1:1 to provide six fractions, *Frs. 1–6*. *Fr. 1* (11.3 g) was repeatedly chromatographed (SiO_2: PE/Et_2NH 30:1; Al_2O_3: PE/AcOEt 8:1; *Sephadex LH20:* $CHCl_3$/MeOH 1:1) to yield **1** (10 mg) and **2** (8 mg).

Hemsleyaconitine F (= rel-(*2*R,*3*S,*4a*R,*8*R,*11*S)*-13-Ethyl-1,3,4,5,6,8,9,10,11,11b-decahydro-3,11-dimethoxy-8-methyl-2*H*-2,4a-methano-8,11a-(methanoiminomethano)dibenzo[*a,c*]*[7]annulen-15-one*; **1**). Colorless gum. $[\alpha]_D^{25.7} = +24.65$ ($c = 0.43$, $CHCl_3$). IR (KBr): 2969, 2850, 2819, 1741, 1631, 1106, 1093. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 373 (32, M^+), 342 (86, $[M - MeO]^+$), 300 (95), 270 (100), 211 (67), 141 (84), 91 (63), 71 (100). HR-ESI-MS (pos.): 374.2695 ($[M + H]^+$, $C_{23}H_{35}NO_3^+$; calc. 374.2700).

Hemsleyaconitine G (= rel-(*2*R,*3*S,*4a*R,*8*S,*11*S)*-13-Ethyl-1,3,4,5,6,8,9,10,11,11b-decahydro-3,11-dimethoxy-8-(methoxymethyl)-2*H*-2,4a-methano-8,11a-(methanoiminomethano)dibenzo[*a,c*]*[7]annulen-15-one*; **2**). Colorless gum. $[\alpha]_D^{21.5} = +11.90$ ($c = 0.21$, $CHCl_3$). IR (KBr): 2971, 2858, 2822, 1741, 1633, 1106, 1093. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 403 (32, $M]^+$), 372 (100, $[M - MeO]^+$), 358 (71), 330 (34), 298 (34), 72 (33). HR-ESI-MS (pos.): 404.2795 ($[M + H]^+$, $C_{24}H_{37}NO_4^+$; calc. 404.2800).

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