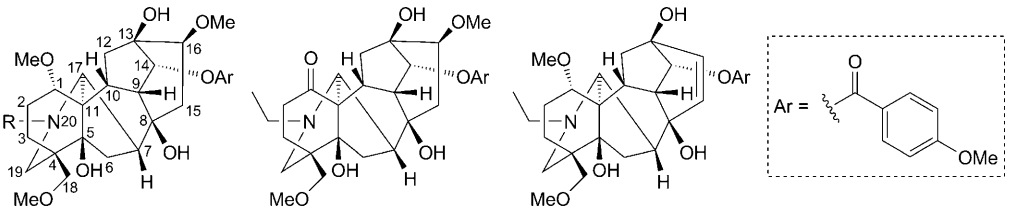
Three New C_{19}-Diterpenoid Alkaloids from *Aconitum hemsleyanum* var. *circinatum*

by **Feng Gao**[a])[b]), **Xiao-Yu Liu**[b]), and **Feng-Peng Wang***[b])

[a]) Agronomy College, Sichuan Agricultural University, No. 46 Xinkang Road, Yaan 625014, P. R. China
[b]) Department of Chemistry of Medicinal Natural Products, West China College of Pharmacy, Sichuan University, No. 17, Duan 3, Renmin Nan Road, Chengdu 610041, P. R. China
(phone/fax: +86-28-5501368; e-mail: wfp@scu.edu.cn)

Further phytochemical investigation of the roots of *Aconitum hemsleyanum* var. *circinatum* resulted in the isolation of three new aconitine-type C_{19}-diterpenoid alkaloids, hemsleyanines E–G (**1**–**3**, resp.). The structures of these new alkaloids were elucidated on the basis of spectral data including 2D-NMR.

Introduction. – There is a long and fascinating history of the utilization of the plants of *Aconitum* and *Delphinium* as a source of medicinal drugs by various civilizations. *Aconitum* preparations have been used as cardiotonics, febrifuges, sedatives, and anodynes, while *Delphinium* extracts have also been employed as sedatives and anthelmintics [1]. The diterpenoid alkaloids are considered to be the main bioactive components in these plants. In the course of our continuing investigation on the species of *Aconitum* and *Delphinium* [2–5], we studied the roots of *A. hemsleyanum* var. *circinatum* [6–8], which is endemic to the Emei Mountains of Sichuan Province in China and has been used as a folk remedy for the treatment of arthritic pain in Chinese traditional herbs [9]. Based on the isolation of 20 C_{19}-diterpenoid alkaloids from this plant [6–8], an additional phytochemical investigation was carried out, which led to the isolation of three new aconitine-type C_{19}-diterpenoid alkaloids, designated as hemsleyanines E–G (**1**–**3**, resp.). Herein, we report the isolation and structure elucidation of these new alkaloids.



1 Hemsleyanine E R = H
4 Hemsleyadine R = Et

2 Hemsleyanine F

3 Hemsleyanine G

Results and Discussion. – Hemsleyanine E (**1**) was isolated as an amorphous powder. The molecular formula $C_{30}H_{41}NO_9$ was deduced from the $[M+H]^+$ signal in

the HR-FAB-MS (*m/z* 560.2875; calc. 560.2860) and the ^{13}C-NMR data (*Table*). The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited characteristic NMR features of an aconitine-type C_{19}-diterpenoid alkaloid [10], bearing three MeO groups (δ(H) 3.26, 3.32, 3.40 (3*s*); δ(C) 55.7, 58.1, 59.4 (3*q*)), and an anisoyl ester (δ(H) 6.90, 7.97 (2*d*, J = 8.4, each 2 H), δ(H) 3.84 (*s*, 3 H); δ(C) see *Table*), but lacking the typical *N*-ethyl group. The *doublet* (J = 8.4 Hz) at δ(H) 5.16 in the ^{1}H-NMR spectrum could be assigned to the H_β–C(14) based on the multiplicity and the coupling constant, resulting in the location of the anisoyl ester group at C(14) [10]. Three MeO groups could be located at C(1), C(16), and C(18), respectively, according to the HMBCs (*Fig. 1*) between 1-MeO (δ(H) 3.26) and C(1) (δ(C) 82.1), 16-MeO (δ(H) 3.40) and C(16) (δ(C) 83.0), as well as 18-MeO (δ(H) 3.32) and C(18) (δ(C) 78.0). Comparison of the NNR spectra of **1** with those of hemsleyadine (**4**) [6], whose structure was confirmed by single crystal X-ray analysis, showed that the latter had an additional *N*-ethyl group. The ^{13}C-NMR data of **1** and **4** are very similar except for C(7), C(17), and C(19) (*Table*), which could be contributed to the effect of *N*-deethylation. The structure of hemsleyanine E, therefore, was established as **1** by careful analysis of the ^{1}H-, ^{13}C-NMR, and 2D-NMR (^{1}H,^{1}H-COSY, HMQC, and HMBC) spectra. Hemsleyanine E (**1**) is a rare C_{19}-diterpenoid alkaloid containing a 5β-OH group, and without *N*-ethyl group.

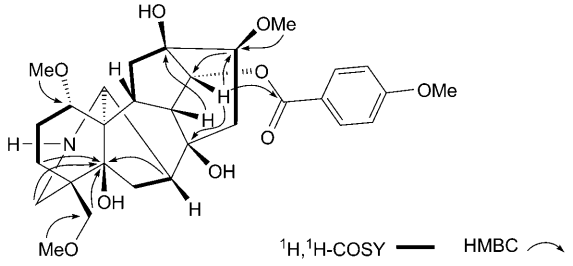


Fig. 1. *Selected ^{1}H,^{1}H-COSY and HMBC correlations of hemsleyanine E* (**1**)

Hemsleyanine F (**2**), a white amorphous compound, had a molecular formula of $C_{31}H_{41}NO_9$ (*m/z* 571.2662; calc. 571.2781) derived from the HR-EI-MS. The NMR spectra of **2** showed an *N*-ethyl group (δ(H) 1.09 (*t*, J = 7.2, 3 H), 2.40–2.44, 2.46–2.49 (2*m*, 1 H each); δ(C) 13.5 (*q*), 48.5 (*t*)), two MeO groups (δ(H) 3.34, 3.43 (2*s*, each 3 H); δ(C) 58.3, 59.5 (2*q*)), and an anisoyl ester (δ(H) 6.89, 7.92 (2*d*, J = 8.8, each 2 H), δ(H) 3.83 (*s*, 3 H); δ(C) see *Table*), which strongly suggested an aconitine-type C_{19}-diterpenoid alkaloid for **2** [10]. The anisoyl ester group could also be located at C(14) due to the one-H-atom *doublet* signal (J = 4.8 Hz) at δ(H) 5.19 in the ^{1}H-NMR spectrum. Comparison of the NMR data of **2** with those of the known hemsleyadine (**4**) revealed that the latter had an additional MeO group but the former had a quaternary C-atom at δ(C) 212.2, whose HMBCs (*Fig. 2*) between H–C(2) (δ(H) 2.40–2.44, 3.24–3.28) and H–C(3) (δ(H) 1.70–1.74, 2.43–2.47) indicated that the two compounds are very similar only except for the substituents at C(1): hemsleyanine F contains an oxo group at C(1) instead of MeO. Therefore, the structure of hemsleyanine F was assigned as **2**. This structure was also confirmed by extensive analysis of its 2D-

Table. *^{1}H- and ^{13}C-NMR Data of* **1**–**3**[a]), *and ^{13}C-NMR Data of Compound* **4**. δ in ppm, J in Hz.

	1		**2**		**3**		**4**
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(C)
H–C(1)	3.24–3.28 (*m*, H_β)	82.1 (*d*)	–	212.2 (*s*)	3.22–3.26 (*m*, H_β)	83.6 (*d*)	83.3
CH_2(2)	1.75–1.78 (*m*, H_α), 2.15–2.21 (*m*, H_β)	25.0 (*t*)	2.40–2.44 (*m*, H_α), 3.24–3.28 (*m*, H_β)	40.9 (*t*)	2.00–2.04 (*m*, H_α), 2.05–2.09 (*m*, H_β)	26.2 (*t*)	25.7
CH_2(3)	1.38–1.42 (*m*, H_α), 2.10–2.15 (*m*, H_β)	26.4 (*t*)	1.70–1.74 (*m*, H_α), 2.43–2.47 (*m*, H_β)	31.7 (*t*)	1.35–1.38 (*m*, H_α), 1.70–1.74 (*m*, H_β)	28.4 (*t*)	27.9
C(4)	–	41.3 (*s*)	–	41.2 (*s*)	–	41.1 (*s*)	40.8
C(5)	–	82.8 (*s*)	–	88.8 (*s*)	–	83.8 (*s*)	83.7
CH_2(6)	1.78–1.82 (*m*, H_α), 2.17–2.20 (*m*, H_β)	34.8 (*t*)	1.80–1.85 (*m*, H_α), 2.13–2.16 (*m*, H_β)	34.2 (*t*)	1.85–1.89 (*m*, H_α), 2.20–2.24 (*m*, H_β)	33.4 (*t*)	34.2
H–C(7)	1.90–1.93 (*m*, H_β)	51.7 (*d*)	2.10 (br. *s*, H_β)	47.1 (*d*)	2.50–2.54 (*m*, H_β)	41.2 (*d*)	45.4
C(8)	–	74.2 (*s*)	–	74.6 (*s*)	–	74.3 (*s*)	73.4
H–C(9)	2.75 (*dd*, J = 9.6, 4.4, H_β)	45.7 (*d*)	2.76 (*t*, J = 5.2, H_β)	45.9 (*d*)	2.84 (*dd*, J = 9.6, 4.8, H_β)	46.1 (*d*)	46.8
H–C(10)	2.57–2.61 (*m*, H_β)	35.8 (*d*)	2.17–2.20 (*m*, H_β)	31.6 (*d*)	2.40–2.45 (*m*, H_β)	36.7 (*d*)	36.3
C(11)	–	50.7 (*s*)	–	63.5 (*s*)	–	50.5 (*s*)	50.2
CH_2(12)	1.99–2.02 (*m*, H_β), 2.08–2.12 (*m*, H_α)	34.9 (*t*)	1.33–1.38 (*m*, H_β), 2.60–2.64 (*m*, H_α)	39.3 (*t*)	2.00–2.04 (*m*, H_β), 2.05–2.09 (*m*, H_α)	41.0 (*t*)	35.7
C(13)	–	76.1 (*s*)	–	75.9 (*s*)	–	77.3 (*s*)	76.3
H–C(14)	5.16 (*d*, J = 4.8, H_β)	79.8 (*d*)	5.19 (*d*, J = 4.8, H_β)	80.0 (*d*)	5.24 (*d*, J = 4.8, H_β)	81.2 (*d*)	80.1
CH_2(15) or H–C(15)	2.27–2.31 (*m*, H_α), 2.63–2.66 (*m*, H_β)	41.8 (*t*)	2.32–2.35 (*m*, H_α), 2.50–2.54 (*m*, H_β)	43.0 (*t*)	5.56 (*d*, J = 9.6)	130.1 (*d*)	41.0
H–C(16)	3.32–3.36 (*m*, H_α)	83.0 (*d*)	3.28–3.32 (*m*, H_α)	83.2 (*d*)	5.90 (*d*, J = 9.6)	135.0 (*d*)	83.5
H–C(17)	3.12 (br. *s*)	58.3 (*d*)	2.90 (br. *s*)	64.6 (*d*)	3.01 (br. *s*)	63.5 (*d*)	63.0
CH_2(18)	2.98 (*AB*, J = 9.2), 3.58 (*AB*, J = 9.2)	78.0 (*t*)	3.12 (*AB*, J = 9.6), 3.66 (*AB*, J = 9.6)	77.5 (*t*)	2.98 (*AB*, J = 11.2), 3.68 (*AB*, J = 11.2)	79.1 (*t*)	78.3
CH_2(19)	2.48 (*d*, J = 10.2), 2.60 (hidden)	50.4 (*t*)	2.18 (hidden), 2.73 (hidden)	56.5 (*t*)	2.42 (hidden), 2.86 (hidden)	55.7 (*t*)	55.1
CH_2(21)	–	–	2.40–2.44 (*m*), 2.46–2.49 (*m*)	48.5 (*t*)	2.30–2.34 (*m*), 2.63–2.68 (*m*)	49.0 (*t*)	48.7
Me(22)	–	–	1.09 (*t*, J = 7.2)	13.5 (*q*)	1.00 (*t*, J = 7.2)	13.4 (*q*)	13.2
1-MeO	3.26 (*s*)	55.7 (*q*)	–	–	3.24 (*s*)	56.4 (*q*)	56.0
16-MeO	3.40 (*s*)	58.1 (*q*)	3.43 (*s*)	58.3 (*q*)	–	–	57.8
18-MeO	3.32 (*s*)	59.4 (*q*)	3.34 (*s*)	59.5 (*q*)	3.33 (*s*)	59.5 (*q*)	59.1
ArC=O	–	166.4 (*s*)	–	166.2 (*s*)	–	167.2 (*s*)	166.5
C(1′)	–	122.4 (*s*)	–	122.5 (*s*)	–	121.8 (*s*)	122.3
H–C(2′,6′)	7.97 (*d*, J = 8.4)	131.7 (*d*)	7.92 (*d*, J = 8.8)	131.8 (*d*)	7.80 (*d*, J = 8.8)	131.7 (*d*)	131.3
H–C(3′,5′)	6.90 (*d*, J = 8.4)	113.5 (*d*)	6.89 (*d*, J = 8.8)	113.6 (*d*)	6.90 (*d*, J = 8.8)	113.8 (*d*)	113.2
C(4′)	–	163.2 (*s*)	–	163.4 (*s*)	–	163.8 (*s*)	162.9
4′-MeO	3.84 (*s*)	55.2 (*q*)	3.83 (*s*)	55.3 (*q*)	3.84 (*s*)	55.4 (*q*)	54.9

[a]) Recorded in $CDCl_3$, ^{1}H-NMR at 400 MHz, and ^{13}C-NMR at 100 MHz.

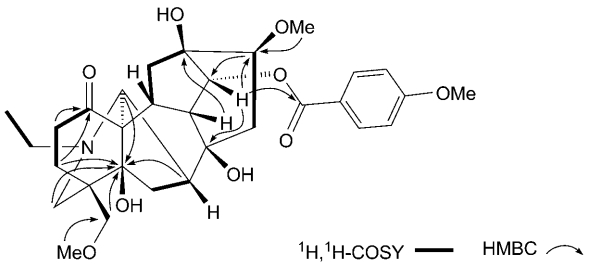


Fig. 2. *Selected ^{1}H,^{1}H-COSY and HMBC correlations of hemsleyanine F* (**2**)

NMR (^{1}H,^{1}H-COSY, HMQC, and HMBC) spectra (*Table*). Hemsleyanine F (**2**) is an unusual natural C_{19}-diterpenoid alkaloid with an oxo group at C(1).

Hemsleyanine G (**3**), with a molecular formula of $C_{31}H_{41}NO_8$ (according to the HR-EI-MS), also exhibited characteristic NMR spectral features of a C_{19}-diterpenoid alkaloid containing an *N*-Et group (δ(H) 1.00 (*t*, $J = 7.2$, 3 H), 2.30–2.34, 2.63–2.68 (2*m*, 1 H each); δ(C) 13.4 (*q*), 49.0 (*t*)), two MeO groups (δ(H) 3.24, 3.33 (2*s*, each 3 H); δ(C) 56.4 (*q*), 59.5 (*q*)), an anisoyl ester group (δ(H) 6.90, 7.80 (2*d*, $J = 8.8$, each 2 H), δ(H) 3.84 (*s*, 3 H); δ(C) see *Table*), and a disubstituted (*Z*)-C=C bond (δ(H) 5.56, 5.90 (2*d*, $J = 9.6$, each 1 H); δ(C) 130.1, 135.0 (2*d*)). Two MeO groups could be located at C(1) and C(18) due to the correlations between 1-MeO (δ(H) 3.24) and C(1) (δ(C) 83.6) and between 18-MeO (δ(H) 3.33) and C(18) (δ(C) 79.1) in the HMBC experiment (*Fig. 3*). On the other hand, a *doublet* at δ(H) 5.24 ($J = 4.8$ Hz) could be attributed to H_β–C(14), implying the presence of the anisoyl group at C(14). The disubstituted C=C bond was located between C(15) and C(16) mainly based on the presence of critical HMBCs from H–C(15) (δ(H) 5.56 (*d*, $J = 9.6$)) to C(7) (δ(C) 41.2), C(8) (δ(C) 74.3), C(9) (δ(C) 46.1), and C(13) (δ(C) 77.3), as well as H–C(16) (δ(H) 5.90 (*d*, $J = 9.6$)) to C(8) (δ(C) 74.3), C(13) (δ(C) 77.3), and C(14) (δ(C) 81.2). Finally, unambiguous assignments of ^{1}H- and ^{13}C-chemical shifts for hemsleyanine G (**3**) (*Table*) were accomplished using the 2D-NMR techniques (^{1}H,^{1}H-COSY, HMQC, and HMBC). All available evidence suggested the structure of hemsleyanine G as **3**.

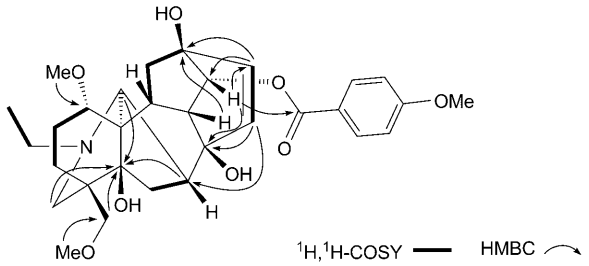


Fig. 3. *Selected ^{1}H,^{1}H-COSY and HMBC correlations of hemsleyanine G* (**3**)

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Experimental Part

General. Silica gel GF_{254} and *H* (*Qindao Sea Chemical Factory*, P. R. China) were used for TLC and column chromatography (CC), resp.; spots on TLC were detected with modified *Dragendorff*'s reagent. A polyvinyl sulfonic ion exchange resin (H-form, cross linking 1×1, *Chemical Factory of Nankai University*, P. R. China) was used for the extraction of total alkaloids. M.p.: thermal values analysis with microscope; uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet FT-IR 200SXY* spectrophotomer. ^{1}H- and ^{13}C-NMR spectra: *Varian Unity INOVA 400/54* NMR spectrometer in $CDCl_3$ with TMS as the internal standard. ESI- and HR-MS: *VG Auto spec 3000* or *Finnigan MAT 90* instrument.

Plant Material. The sample of *A. hemsleyanum* var. *circinatum* W. T. WANG was collected in the Emei Mountains, Sichuan Province, China, and authenticated by Prof. *W. T. Wang* from the Institute of Botany, Chinese Academy of Sciences, where a voucher specimen has been deposited.

Extraction and Isolation. The powdered roots (4.0 kg) of *A. hemsleyanum* var. *circinatum* were percolated with 0.05M HCl (40 l). Wet resin (dry weight 40 kg) was added to the percolate, followed by repeated washing on a suction filter with deionized H_2O. The air-dried resin was then alkalized with 10% aq. NH_4OH (1.8 l) and continuously extracted with Et_2O (5.0 l), and evaporated to give the total crude alkaloids (68.0 g) as yellowish amorphous powder. The crude alkaloids (38.2 g) were chromatographed on a SiO_2 column eluting with $CHCl_3$/MeOH 200 : 1 → 7 : 1 gradient system to give *hemsleyadine* (**4**; 2.6 g) and fractions *A* (3.2 g), *B* (10.8 g), *C* (9.6 g), and *D* (6.2 g). *Fr. B* (10.8 g) was chromatographed on a SiO_2 column eluting with $CHCl_3$/MeOH 97 : 3 to afford fractions *B-1* (420 mg), *B-2* (1.2 g), *B-3* (4.2 g), and *B-4* (3.8 g). *Fr. B-2* was separated on a SiO_2 column eluting with petroleum ether (PE)/Me_2CO 3 : 1 to give three subfractions *B-2-1* (120 mg), *B-2-2* (400 mg), and *B-2-3* (700 mg). Further SiO_2 chromatography of fraction *B-2-1* eluting with cyclohexane/Me_2CO (3 : 1) produced *hemsleyanine G* (**3**, 82 mg). *Fr. B-4* was chromatographed over a SiO_2 column with PE/Me_2CO 2 : 1 to give fractions *B-4-1* (1.2 g) and *B-4-2* (1.6 g). CC of *Fr. B-4-2* with cyclohexane/Me_2CO/Et_2NH 80 : 20 : 1 as eluent gave fractions *B-4-2-1* (76 mg), *B-4-2-2* (180 mg), and *B-4-2-3* (560 mg). In addition, *Fr. B-4-2-3* was chromatographed on a SiO_2 column (PE/Me_2CO/Et_2NH 80 : 20 : 1) to provide *hemsleyanine F* (**2**; 77 mg). Further SiO_2 chromatography of *Fr. D* eluting with $CHCl_3$/MeOH 96 : 4 gave *hemsleyanine E* (**1**; 103 mg).

Hemsleyanine E (*=(1α,7β,14α,16β)-5,8,13-Trihydroxy-1,16-dimethoxy-4-(methoxymethyl)aconitan-14-yl 4-Methoxybenzoate*; **1**). White amorphous powder. M.p. 81 – 82°. $[\alpha]_D^{20} = +60.2$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3447, 2928, 1701, 1606, 1512, 1459, 1102. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): *Table.* ESI-MS: 560 (100, $[M+H]^+$). HR-FAB-MS: 560.2875 ($[M+H]^+$, $C_{30}H_{42}NO_9^+$; calc. 560.2860).

Hemsleyanine F (*=(7β,14α,16β)-20-Ethyl-5,8,13-trihydroxy-16-methoxy-4-(methoxymethyl)-1-oxo-aconitan-14-yl 4-Methoxybenzoate*; **2**). White amorphous powder. M.p. 83 – 84°. $[\alpha]_D^{20} = -44.3$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3443, 2938, 1699, 1615, 1503. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): *Table.* ESI-MS: 518 (100, $[M+H]^+$). HR-EI-MS: 571.2662 (M^+, $C_{31}H_{41}NO_9^+$; calc. 571.2781).

Hemsleyanine G (*=(1α,7β,14α)-20-Ethyl-5,8,13-trihydroxy-1-methoxy-4-(methoxymethyl)aconit-15-en-14-yl 4-Methoxybenzoate*; **3**). White amorphous powder. M.p. 87 – 88°. $[\alpha]_D^{20} = +63.3$ ($c = 1.0$, $CHCl_3$). ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): *Table.* ESI-MS: 556 (100, $[M+H]^+$). HR-EI-MS: 555.2695 (M^+, $C_{31}H_{41}NO_8^+$; calc. 555.2832).

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