HETEROCYCLES, Vol. 65, No. 2, 2005, pp. 365 - 370
Received, 27th September, 2004, Accepted, 8th December, 2004, Published online, 10th December, 2004

STRUCTURAL REVISION OF HEMSLEYADINE AND NEW ALKALOIDS HEMSLEYANINES A, B FROM *ACONITUM HEMSLEYANIUM* VAR. *CIRCINACUM*

Feng Gao and Feng-Peng Wang*

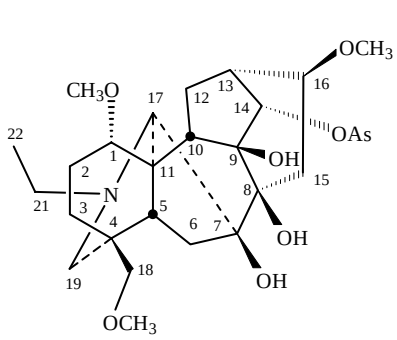
Department of Chemistry of Medicinal Natural Products, West China College of Pharmacy, Sichuan University, No. 17, Duan 3, Renmin Nan Road, Chengdu 610041, P. R. China (Email: wfp@wcums.edu.cn)

Abstract — Study on the phytochemistry of *Aconitum hemsleyanium* var. *circinacum* led to isolate two new C_{19}-diterpenoid alkaloids, hemsleyanines A (**6**) and B (**7**) together with six known alkaloids, yunaconitine, talatisamine, hemsleyadine, hemsleyasine, hemsleyanidine and isohemsleyanidine. The structures of these alkaloids including new alkaloids hemsleynines A and B were established on the basis of spectral data (^{1}H- and ^{13}C- NMR, 2D NMR, HRMS) and X-Ray analysis.

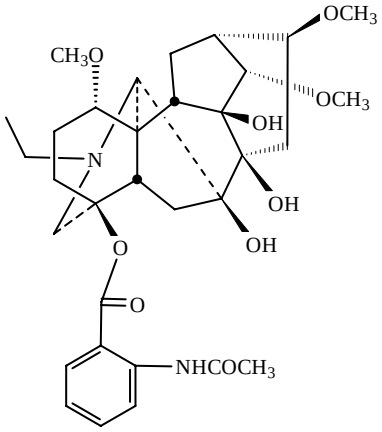
The literature search indicated that yunaconitine, talatisamine, hemsleyadine, hemsleyasine, hemsleyanidine, and isohemsleyanidine have been reported from the plant *Aconitum hemsleyanium* var. *circinacum*.[1~4] Further studies on the phytochemistry of the plant for chemotaxonomy of genus *Aconitum* L. from China have now led to the isolation of two new C_{19}-diterperoid alkaloids, hemsleyanines A (**6**) and B (**7**), together with six known alkaloids, yunaconitine, talatisamine, hemsleyadine, hemsleyasine, hemsleyanidine and isohemsleyanidine, from the roots of this plant. In addition, in this case, the structure of hemsleyadine was revised to be **3** by careful 2D NMR spectra interpretation and X-Ray analysis. The present paper deals with the isolation and structural determination of the new alkaloids including hemsleyadine.

Hemsleyadine (**3**), $C_{32}H_{45}NO_9$, was isolated as an amorphous powder substance and gave a positive Dragendorff's test. It is identical with hemsleyadine (**1**) previously reported by us[2] by comparison of mp, optical rotation, co-TLC (silica gel GF_{254}, $CHCl_3$-CH_3OH=97:3; ether-acetone=3:2), MS and NMR spectral data with the authentic sample. But, a newly carfull comparison of the ^{13}C- NMR spectrum of hemsleyadine with those of the analogues bearing the hydroxyl groups at C-7, C-8, and C-9, such as

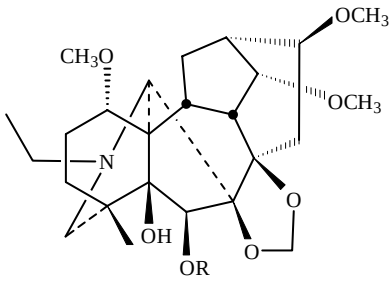
ranaconitne (**2**)[5] found that there are more differences, especially in C-7, C-8, and C-9, as well as that the signals for C-4 and C-11 are more deshielded (*ca*. 2 ppm) than those of ranaconitine (**2**).[5] Taking these findings into account, we decided to carry out supplemently the X-Ray diffraction analysis, resulting in the revision of the structure of hemsleyadine from **1** to **3** (Figure 1). The X-Ray analysis of hemsleyadine (**3**) showed that it is the new aconitine-type alkaloid containing the 5-hydroxyl group except for the lycoctonine-type alkaloids, such as bonvalol (**4**)[6] and bonvalone (**5**).[6] This also was supported by the observation of the HMBC correlations between the C-5 and H-3α, H-6 (α/β), H-7 (Figure 2) of hemsleyadine (**3**).



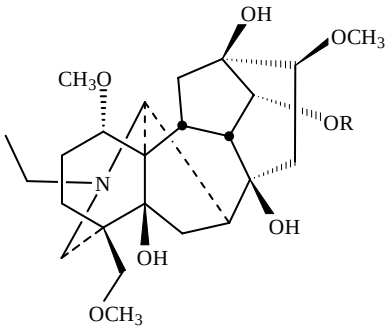
1



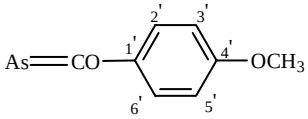
2



4 R= H
5 R= Ac



3 hemsleyadine R= As
6 hemsleyanine A
R= OC-C_6H_4-OH (*p*)
7 hemsleyanine B R= H



Hemsleyanine A (**6**), $C_{31}H_{43}NO_9$, mp 126-127.5℃, $[\alpha]_D^{20}$ +23.6° (c, 1.0, $CHCl_3$), was obtained as an amorphous powder. The ^{1}H- and ^{13}C- NMR spectra of hemsleyanine A showed one *N*- ethyl group (δ_H: 1.06, 3H, t, *J*=7.2 Hz; δ_C: 49.2 t, 13.5 q), three methoxyl groups(δ_H: 3.27, 3.31, 3.34, each 3H, s; δ_C: 56.6 q, 58.4 q, 59.6 q), and an *p*- hydroxylbenzoyl ester group (δ_H: 6.80, 7.84, each 2H, AA'BB' system, *J*=8.4 Hz; δ_C: see Table 1), The ^{1}H doublet (*J*=4.8 Hz) at δ_H: 5.19 in the ^{1}H NMR spectrum of hemsteyanine A was assigned to the H-14β based on the multiplicity and the coupling constant, resulting in location of the ester group to C-14. With the exception of the chemical shifts of the 14-ester group, the ^{1}H- and ^{13}C-NMR spectra of hemsleyanine A are almost identical with those of hemsleyadine(**3**)(Table 1), this thus leading

to the structure of hemsleyanine A to be assigned to **6**.

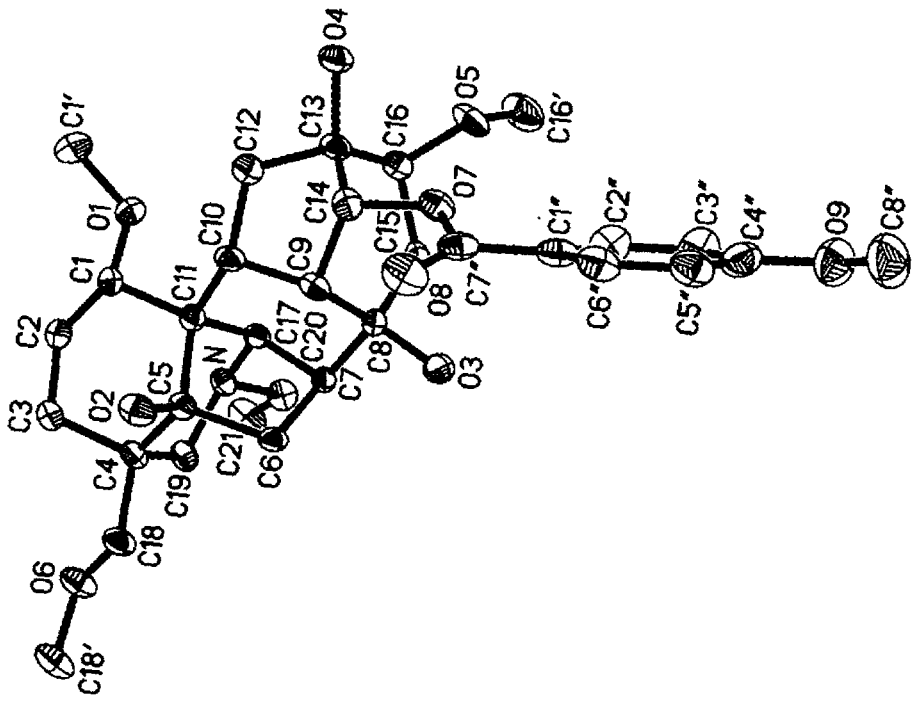


Figure 1. Single-crystal X-Ray structure of hemsleyadine (**3**)

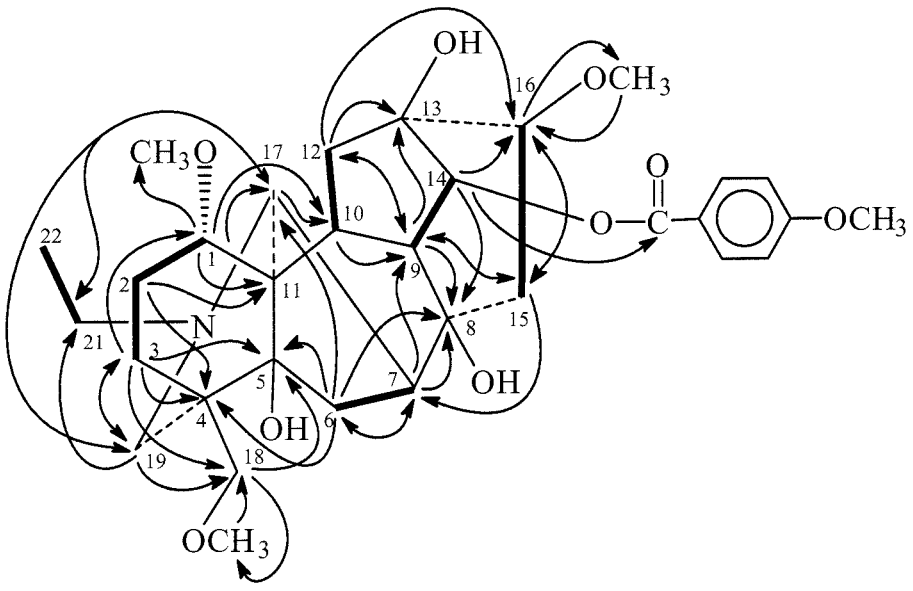


Figure 2. (—) key ^{1}H-^{1}H COSY of hemsleyadine (**3**);(↷) key HMBC correlations of hemsleyadine (**3**)

Hemsleyanine B (**7**), mp 92-93℃, $[\alpha]_D^{20}$-35.2° (c, 1.0, $CHCl_3$), was isolated as an white amorplcous powder and assigned to the molecular formula $C_{24}H_{39}NO_7$ by HR-EI-MS spectrum. ^{1}H NMR spectrum of hemsleyanine B exhibited the presence of an *N*- ethyl group (*δ*: 0.98, 3H, t, *J*=7.2 Hz) and three methoxyl

groups (δ: 3.18, 3.27, 3.35, each 3H, s). Its ^{13}C NMR spectrum is very similar to those of hemsleyanine A (**6**) (Table 1). As expected, there are only minor differences mainly restricted to the vicinity of the C-14 function. Coupling with comparison of the co-TLC (silica gel GF_{254}, $CHCl_3$-CH_3OH= 97:3, cyclohexane-acetone=3:2) and MS spectrum of hemsleyanine B with those of the hydrolytic product of **6**, therefore, the structure of hemsleyanine B was established as **7**.

EXPENIMENTAL

General Experimental Procedures. Optical rotations were recorded on a PerKin-Elmer 341 polarimenter. IR spectra were obtained on a Nicolet FT-IR SXY spectrophotomer. ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity INOVA 400/54 NMR spectrometer in $CDCl_3$ with TMS as the internal standard. MS spetrum were measured on Finnigan LCQ and Micromass Auto Ultima-Tof spectrometer. Silica gel GF_{254} and H (Qindao Sea Chemical Factory, China) were used for TLC, and column chromatography, respectively. A polyvinyl sulfonic ion exchange resin (H-form), cross linking 1 $\times$1, Chemical Factory of Nankai University, China) was used for the extraction of total alkaloids.

Plant Material The *Aconitum hemsleyanium* var. *circinacum* was collected in Emei mountant, Sichuan province, China and authenticated by Professor W. T. Wang of the Institute of Botany, Chinese Academy of Sciences, where a voucher specimen has been deposited.

Extraction and Isolation According to method reported in the literature,[7] powdered roots (4.0 kg) of *Aconitum hemsleyanium* var. *circinacum* were percolated with 3% 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% aqueous NH_4OH (1.8 L) and continuously extracted with ether (5.0 L), and evaporated to give the total crude alkaloids (68.0 g) as yellowship amorphous powder substance. The crude alkaloids (38.2 g) was chromatographed over silica gel column eluting with $CHCl_3$-CH_3OH (200:1→7:1) gradient system to give hemsleyadine (**3**) (2.6 g), fractions A (3.2 g), B (10.8), C (9.6 g), and D (6.2 g). Fraction C (9.6 g) was chromatographed on a silica gel column eluting with $CHCl_3$-CH_3OH (97:3) to afford hemsleyanine B (**7**) (320 mg) and hemsleyasine (4.2 g). Fraction D was chromatographed using silica gel column eluting with cyclohexane-acetone (3:1) to give hemsleyanine A (**6**) (12 mg), as well as isohemsleyanidine (20 mg). Silica gel column chromatograplry of fraction A (3.2 g) eluting with ether-acetone (3:1) gave yunaconitine (26 mg), talatisamine (900 mg), and hemsleyanidine (18 mg). The known alkaloids were determined by comparison of co-TLC (silica gel GF_{254}, $CHCl_3$-CH_3OH=96:4; ether-acetone=3:2) and ^{1}H- and ^{13}C- NMR spectral data with those of the authentic samples.

Table 1. ^{1}H- and ^{13}C- NMR spectral data of compounds (**3**, **6**, and **7**) (^{1}H: 400 MHz, ^{13}C: 100 MHz; $CDCl_3$)

No.	**3**		**6**	**7**
	δ_C	δ_H Mult (*J*= Hz)	δ_C	δ_C
1	83.3 d	3.90 m	83.6 d	84.8 d
2	25.7 t	1.92 m (α)	25.9 t	25.7 t
		2.00 m (β)		
3	27.9 t	1.21 m (α)	28.3 t	28.2 t
		2.18 m (β)		
4	40.8 s	-	41.0 s	41.0 s
5	83.7 s	-	84.3 s	84.3 s
6	34.2 t	1.67 m (hidden) (α)	34.6 t	34.4 t
		1.86 m (hidden) (β)		
7	45.4 d	1.86 m (hidden)	45.2 d	44.5 d
8	73.4 s	-	74.8 s	73.2 s
9	46.8 d	2.67 t (5.6)	47.5 d	49.0 d
10	36.3 d	2.39 m	36.1 d	36.5 d
11	50.2 s	-	50.5 s	50.3 s
12	35.7 t	2.20 m (β)	36.2 t	35.3 t
		2.36 m (*a*)		
13	76.3 s	-	77.0 s	77.0 s
14	80.1 d	5.03 d (4.8)	80.8 d	79.4 d
15	41.0 t	2.28 m (hidden) (α)	41.0 t	39.5 t
		2.38 m (hidden) (β)		
16	83.5 d	3.18 m	83.6 d	83.8 d
17	63.0 d	3.02 br s	63.6 d	63.7 d
18	78.3 t	2.87 ABq (9.2)	78.6 t	78.6 t
		3.47 ABq (9.2)		
19	55.1 t	1.73 ABq (12.0)	55.3 t	55.3 t
		2.43 ABq (12.0)		
21	48.7 t	2.16 m	49.2 t	49.1 t
		3.37 m		
22	13.2 q	0.92 t (7.2)	13.5 q	13.5 q
1-OCH_3	56.0 q	3.12 s	56.6 q	56.4 q
16-OCH_3	57.8 q	3.20 s	58.4 q	57.7 q
18-OCH_3	59.1 q	3.17 s	59.6 q	59.4 q
O=C	166.5 s	—	167.2 s	—
1'	122.3 s	—	121.7 s	—
2', 6'	131.3 d	78.0 AA'BB' (8.4)	131.8 d	—
3', 5'	113.2 d	6.74 AA'BB' (8.4)	115.9 d	—
4'	162.9 s	—	160.9 s	—
4'-OCH_3	54.9 q	3.68 s	—	—

Hemsleyadine (3). White amorphous powder, mp. 106~107℃; $[\alpha]_D^{20}$ +56.4° (c1.0, $CHCl_3$); ^{1}H- and

^{13}C-NMR see Table 1. *Crystal structure for* **3**: a colorless monoclinic crystal from hexane-acetone was mounted on the a P_4 four circle diffractometer and exposed to graphite-monochromated MoKα irradiation. The unit cell parameters are a=14.580 (4) Å, b=14.129 (3) Å, c=15.246 (3) Å, β=103.33 (2) deg in space group PZ_1/m, of the 8481 measured, 7861 were independently observed at the level of $F_0>4\sigma$ (F_0). The structure was solved by the directed method using the program SHELXTL and the atomic parameters were refined by the full-matrix least squares on F^2 method. The final R index [$I>2\sigma$ (I)] was R1=0.0451, WR2=0.0797.

Hemsleyanine A (6). White amorphous powder, mp 126.5-127℃; $[\alpha]_D^{20}$ +23.6° (c 1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3447, 2925, 1667; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.06 (3H, t, *J*=7.2 Hz, *N*-CH_2CH_3); 3.27, 3.31, 3.34 (each 3H, s, 3×OCH_3); 5.19 (1H, d, *J*=4.8 Hz, H-14β), 6.80, 7.84 (each 2H, AA'BB' system, *J*=8.4 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1; ESI-MS: *m/z* (%): 574 (M^+, 100), 542 (28), 496 (12); HR-EI-MS *m/z*: 573.2906 [M^+], calcd for $C_{31}H_{43}NO_9$, 573.2938.

Hemsleyanine B (7). White amorphous powder, mp 92-93℃; $[\alpha]_D^{20}$ -35.2° (c 1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3422, 2924; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.98 (3H, t, *J*=7.2 Hz, *N*-CH_2CH_3); 3.18, 3.27, 3.35 (each 3H, s, 3×OCH_3); 4.05 (1H, d, *J*=4.8 Hz, H-14β), ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1; ESI-MS: *m/z* (%): 454 (M^+, 100), 422 (M^+-OCH_3-H, 5); HR-EI-MS *m/z* 453.2707 [M^+] calcd for $C_{24}H_{39}NO_7$, 453.2726.

ACKNOWLEDGMENTS

This work was supported by the Doctoral Foundation of the Ministery of Education, P. R. China (2002-2004).

REFERENRES

1. S. Y. Chen, *Acta Chim. Sinica*, 1980, **37**, 142.
2. Q. Y. Xu and F. P. Wang, *Chin. Chem. Lett*., 1996, **7**, 723.
3. Q. Y. Xu and F. P. Wang, *Chin. Chem. Lett*., 1997, **8**, 137.
4. Q. Y. Xu and F. P. Wang, *Heterocycles*, 1996, **43**, 1243.
5. S. W. Pelletier, N. V. Mody and A. P, Venkov, *Tetrahedron Lett*, 1978, 5045.
6. Q. P. Jiang and W. L. Sung, *Heterocycles*, 1984, **22**, 2429.
7. Q. C. Fan and Z. M. Hu, *Acta Pharm. Sinica*, 1966, **13**, 577.