Two New C_{19}-Diterpenoid Alkaloids from *Aconitum hemsleyanium* var. *circinacum*

Feng GAO, Dong-Lin CHEN, 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. Received July 4, 2005; accepted September 20, 2005

Two new C_{19}-diterpenoid alkaloids, circinadines A (1) and B (2), were isolated from the roots of *Aconitum hemsleyanium* var. *circinacum*. Their structures were elucidated by chemical evidence and spectral analyses, including ESI-MS, HR-EI-MS, 1D- and 2D-NMR.

Key words Ranunculaceae; *Aconitum hemsleyanium* var. *circinacum*; C_{19}-diterpenoid alkaloid; circinadine A; circinadine B

The plant *Aconitum hemsleyanium* var. *circinacum* (Ranunculaceae) is a species endemic to the Emei Mountains of Sichuan province in China and has been used as a falk remedy for the treatment of arthritic pain.[1)] The plant also plays an important role in the chemotaxonomy of *Aconitum* L., which could been learned from the deep phytochemistry study on it. In previous papers, seven C_{19}-diterpenoid alkaloids yunaconitine,[2)] hemsleyadine,[3)] hemsleyasine,[4)] hemsleyanisine, isohemsleyanisine,[5)] and hemsleyanines A, B[6)] have been reported. Continuing investigations seeking new bioactive compounds on the roots of *Aconitum hemsleyanium* var. *circinacum* have now led to the isolation of two other new aconitine-type C_{19}-diterpenoid alkaloids circinadines A (**1**) and B (**2**). This paper deals with the separation and structural elucidation of these new alkaloids (**1**, **2**).

Results and Discussion

Circinadine A (**1**) was isolated as an amorphous powder, mp 102—103 °C. It showed a positive reaction with Dragendorff's reagent. Its molecular formula, $C_{32}H_{45}NO_9$, was established based on HR-ESI-MS and ^{13}C-NMR. ^{1}H- and ^{13}C-NMR spectra of **1** showed one *N*-ethyl group (δ_H 1.10, 3H, t, J=7.2 Hz; δ_H 2.46, 2.54, each 1H, m; δ_C: 49.2 t, 13.5 q), three methoxyl groups (δ_H 3.28, 3.32, 3.36, each 3H, s; δ_C 56.2 q, 58.2 q, 59.4 q), and an *p*-methoxyl benzoyl ester group (δ_H 6.91, 7.95, each 2H, AA′BB′ system, J=8.4 Hz; 3.84, 3H, s; δ_C: see Table 1). The ^{1}H doublet (J=4.8 Hz) at δ_H 5.12 in the ^{1}H-NMR spectrum of **1** was assigned to H-14β

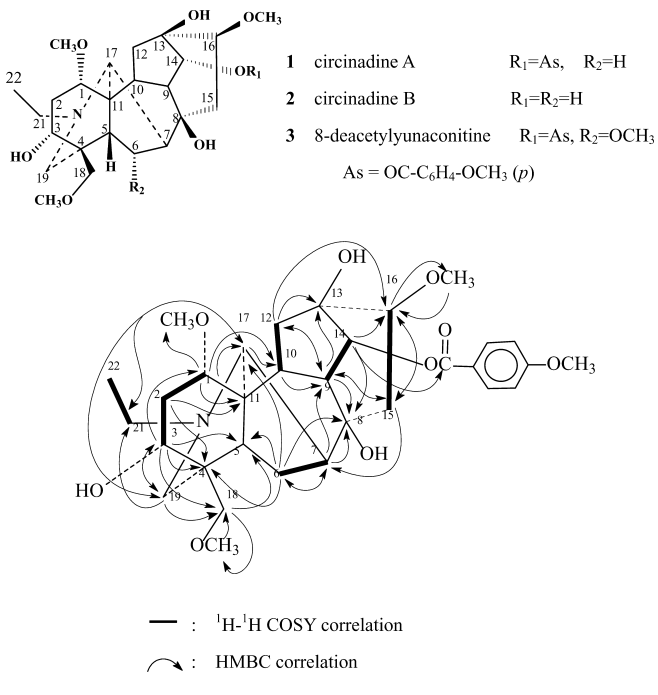

Fig. 1. Key ^{1}H–^{1}H COSY and HMBC Correlations of Circinadine A (**1**)

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1**, **2**, and **3**[9)] (^{1}H: 400 MHz, ^{13}C: 100 MHz; $CDCl_3$)

No.	**1**		**2**	**3**
	δ_H Mult (J=Hz)	δ_C	δ_C	δ_C
1	3.26 m	82.8 d	83.2 d	83.2
2	2.24 m (α)	34.4 t	34.1 t	33.7
	2.32 m (β)			
3	3.76 dd (12.0, 5.6)	71.7 d	72.1 d	71.9
4	—	43.2 s	43.3 s	43.3
5	2.08 m	43.8 d	43.9 d	47.7
6	1.54 m (α)	22.5 t	24.8 t	82.3
	1.86 m (β)			
7	1.96 m (hidden)	46.4 d	45.3 d	48.9
8	—	73.6 s	72.8 s	73.8
9	2.43 m	46.8 d	48.8 d	53.4
10	2.10 m	42.2 d	39.0 d	36.3
11	—	48.4 s	48.2 s	50.3
12	2.02 m (β)	35.7 t	35.1 t	36.0
	2.46 m (α)			
13	—	76.4 s	76.6 s	76.0
14	5.12 d (4.8)	80.2 d	79.6 d	79.9
15	2.35 m (α)	41.2 t	42.0 t	42.1
	2.42 m (β)			
16	3.28 m	83.5 d	84.8 d	83.2
17	3.12 br s	62.1 d	62.6 d	61.9
18	3.02 (ABq, 11.2)	77.0 t	77.3 t	77.3
	3.18 (ABq, 11.2)			
19	1.54 m (hidden)	46.5 t	46.5 t	48.9
	1.82 m (hidden)			
21	2.46 m	49.2 t	49.4 t	47.8
	2.54 m			
22	1.10 t (7.2)	13.5 q	13.6 q	13.5
1-OCH_3	3.28 s	56.2 q	56.3 q	56.1
16-OCH_3	3.36 s	58.2 q	57.7 q	57.5
6-OCH_3	—	—	—	58.3
18-OCH_3	3.32 s	59.4 q	59.5 q	59.1
ArCO	—	166.8 s	—	166.5
1′	—	122.5 s	—	122.4
2′, 6′	7.95 d (12.0)	131.7 d	—	131.8
3′, 5′	6.91 d (12.0)	113.7 d	—	113.8
4′	—	162.4 d	—	163.6
4′-OMe	3.84 s	55.3 q	—	55.4

* To whom correspondence should be addressed. e-mail: wfp@wcums.edu.cn

based on the multiplicity and the coupling constant, resulting in location of the ester group to C-14.[7] Three methoxyl groups could be located at C-1, C-16, and C-18 due to the ^{1}H–^{13}C long-range correlation (HMBC) between 1-OCH_3 (δ_H 3.28) and C-1 (δ_C 82.8), 16-OCH_3 (δ_H 3.36) and C-16 (δ_C 83.5), 18-OCH_3 (δ_H 3.32) and C-18 (δ_C 77.0) in the HMBC of **1**.

A double doublet (J=12.0, 5.6 Hz) signal at δ_H 3.76 was attributed to H-3 due to the presence of multi-bond ^{1}H–^{13}C correlation between H-3 and C-1, C-2, C-4, C-18 in the heteronuclear multiple bond connectivity (HMBC) spectrum of **1**. Meanwhile, the stereochemistry of H-3 in **1** was deduced as the β-orientation, as in many cases, *e.g.*: hemsleyatine (δ_H 3.70, dd, J=10.0, 4.8 Hz),[8] based on the coupling constants (δ_H 3.76, dd, J=12.0, 5.6 Hz) in the ^{1}H-NMR spectra. Two remaining hydroxyl groups were assigned to C-8 and C-13 based on the correlations between the C-8 (δ_C 73.6) and H-6 (δ_H 1.54, 1.86), H-7 (δ_H 1.96), H-9 (δ_H 2.43), as well as C-13 and H-14 (δ_H 5.12, d, J=4.8 Hz), H-16 (δ_H 3.28), in the HMBC of **1**. Comparison of the NMR spectra of **1** with those of 8-deacetylyunaconitine (**3**)[9] showed that the latter had an additional methoxyl group. The ^{13}C-NMR spectra of **1** and **3** were very similar except for C-5, C-6, C-7, C-9, C-10, and C-11 (Table 1), indicating that the two compounds are different only at the substitution of C-6. Structure of circinadine A, therefore, was established as **1**. All the ^{1}H- and ^{13}C-NMR spectra data obtained for circinadine A (Table 1) supported structure **1**. Circinadine A (**1**) is the first natural aconitine-type C_{19}-diterpenoid alkaloid possessing the hydroxyl group at C-3 but without the oxygenous substitution at C-6.

Circinadine B (**2**) was obtained as an amorphous powder, mp 92—93 °C. It also showed a positive reaction with the Dragendorff's reagent. The protonated molecular formula $C_{24}H_{39}NO_7$ was inferred from HR-ESI-MS and 2D-NMR data. The NMR spectra of **2** exhibited an *N*-ethyl group (δ_H 1.09, 3H,t, J=7.2 Hz; δ_C 13.6 q, 49.4 t) and three methoxyl groups (δ_H 3.25, 3.32, 3.41, each 3H, s; δ_C 56.3 q, 57.7 q, 59.5 q), one-proton double signal (J=4.8 Hz) at δ_H 4.20 was attributed to be H-14β implying the appearance of the hydroxyl group at C-14.[7] Its ^{13}C-NMR spectrum is very similar to those of circinadine A (**1**) (Table 1). As expected, there are only minor difference mainly restricted to the vicinity of the C-14 function. Coupling compared with the co-TLC (silica gel GF_{254}, $CHCl_3$–CH_3OH=98 : 2, cyclohexane–acetone=3 : 2) and NMR spectrum of circinadine B with those of the hydrolytic product of circinadine A (**1**), thus, the structure of circinadine B was established as **2**.

Experimental

Genernal Experimental Procedures Melting points were performed on a thermal values analytical microscope and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectrum was recorded on a Nicolet FI-IR 200SXY 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. ESI-MS and HR-EI-MS were measured by a VG Auto spec 3000 or Finnigan MAT 90 instrument. Silica gel GF_{254} and H (Qindao Sea Chemical Factory, China) were used for TLC, and column chromatography, respectively. 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, China) was used for the extraction of total alkaloids.

Plant Material The *Aconitum hemsleyanium* var. *circinacum* W. T. Wang was collected in the Emei Mountains of 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 the method reported in the literature,[10] powdered roots (4.0 kg) of *Aconitum hemsleyanium* var. *circinacum* were percolated with 0.05 mol/l 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 a yellowish amorphous powder substance. The crude alkaloids (38.2 g) were chromatographed over silica gel (200 g) column eluting with $CHCl_3$–MeOH (200 : 1→7 : 1) gradient system to give hemsleyadine (**3**)[3,6] (2.6 g), fractions A (3.2 g), B (10.8 g), C (9.6 g), and D (6.2 g). Fraction B (10.8 g) was chromatographed on a silica gel (100 g) column eluting with $CHCl_3$–CH_3OH (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). Fraction B-2 was separated on a silica gel H (20 g) column eluting with ether–acetone (3 : 1) to give four subfractions, B-2-1 (120 mg), B-2-2 (650 mg), B-2-3 (400 mg) and B-2-4 (700 mg). Further silica gel (5.0 g) chromatography of fraction B-2-1 eluting with cyclohexane–acetone (3 : 1) produced circinadine A (74 mg). Fraction B-4 was chromatographed over a silica gel (50 g) column with petroleum ether–acetone (2 : 1) to give three fractions B-4-1 (1.2 g), B-4-2 (1.6 g), and B-4-3 (800 mg). Column chromatography (silica gel: 30 g) of fraction B-4-2 with cyclohexane–acetone–diethylamine (80 : 20 : 1) as eluent gave fractions B-4-2-1 (76 mg), B-4-2-2 (180 mg), B-4-2-3 (560 mg), and B-4-2-4 (320 mg). In addition, fraction B-4-2-3 was chromatographed on a silica gel (10 g) column (petroleum ether–acetone–diethylamine, 80 : 20 : 1) to provide circinadine B (12 mg).

Circinadine A (**1**): White amorphous powder, mp 102—103 °C, $[\alpha]_D^{20}$ −69.2° (c=1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3448, 1668, 1299, 1262, 1199, 1054; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; ESI-MS: m/z (%): 588 $[M+H]^+$ (100), 556 (12); HR-ESI-MS m/z: 588.4216 $[M+H]^+$, Calcd for $C_{32}H_{45}NO_9$, 588.4229.

Circinadine B (**2**): White amorphous powder, mp 92—93 °C, $[\alpha]_D^{20}$ −32.3° (c=1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3446, 2919; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.09 (3H, t, J=7.2 Hz; N-CH_2-$\underline{CH_3}$), 3.25, 3.32, 3.41 (each 3H, s, 3×OCH_3); 4.20 (1H, d, J=4.8 Hz, H-14β); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; ESI-MS: m/z (%): 454 $[M+H]^+$ (100), 422 (5); HR-ESI-MS m/z: 454.3526 $[M+H]^+$, Calcd for $C_{24}H_{39}NO_7$, 454.3556.

Hydrolysis of **1**: To a solution of 5% methanolic sodium hydroxide (1 ml) 10 mg of circinadine A (**1**) was added. The solution was allowed to stand at room temperature overnight. Removal of the solvent under reduced pressure provided a residue, to which 10 ml of water was added and extracted with $CHCl_3$ (10 ml×3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give an amorphous powder showing a single spot on tlc.

Acknowledgment This work was supported by the Doctoral Foundation of the Ministry of Education, P. R. China (2002—2005).

References

1) Institute of Botany, Chinese Academy of Science & Institute of Materia Medica, Chinese Academy of Medical Science, "Flora Reipublicae Popularis Sinicae," Vol. 27, Science Press, Beijing, 1979, pp. 235—236.
2) Chen S. Y., *Acta Chimica Sinica*, **37**, 15—19 (1977).
3) Xu Q. Y., Chen D. L., Wang F. P., *Chin. Chem. Lett.*, **7**, 723—724 (1996).
4) Xu Q. Y., Wang F. P., Che C. T., *Chin. Chem. Lett.*, **8**, 137—140 (1997).
5) Xu Q. Y., Li Z. B., Wang F. P., Che C. T., *Heterocycles.*, **43**, 1243—1250 (1996).
6) Gao F., Wang F. P., *Heterocycles.*, **65**, 365—370 (2005).
7) Pelletier S. W., Mody N. V., Joshi B. S., Schramm L. C., "Alkaloids: Chemical and Perspectives," Vol. 2, ed. by Pelletier S. W., Wiley, New York, 1984, pp. 206—264.
8) Zhou X. L., Chen Q. H., Chen D. L., Wang F. P., *Chem. Pharm. Bull.*, **51**, 592—594 (2003).
9) Chen S. Y., Liu Y. Q., *Acta Botanica Yunn*, **6**, 338—340 (1984).
10) Fang Q. C., Hou Z. M., *Acta Pharm. Sinica*, **13**, 577—588 (1966).