HETEROCYCLES, Vol. 60, No. 3, 2003, pp. 631 - 636
Received, 14th November, 2002, Accepted, 8th January, 2003, Published online, 31st January, 2003

NEW C_{19}-DITERPENOID ALKALOIDS FROM *ACONITUM LILJESTRANDII*

Guang-Bo Xie, **Qiao-Hong Chen**, **Dong-Lin Chen**, **Xi-Xian Jian**, and **Feng-Peng Wang***

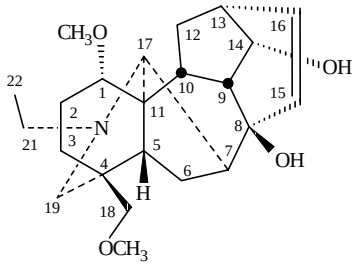
Department of Chemistry of Medicinal Natural Products, West China College of Pharmacy, Sichuan University, No. 17, Duan 3, Renmin Nan Road, Chengdu 610041, P. R. China (e-mail: wfp@wcums.edu.cn)

Abstract - Two new C_{19}-diterpenoid alkaloids, liljestrandinine (**1**) and *N*-deethyltalatisamine (**2**) were isolated from the roots of *Aconitum liljestrandii* along with five known C_{19}-diterpenoid alkaloids: chasmaconitine (**4**), forestine (**5**), pengshenine B (**6**), cammaconine (**7**), and 6-epi-foresticine (**8**). Structures were established by spectroscopic method including 2D NMR.

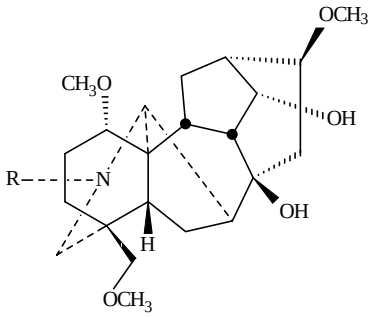
In the course of our comparative studies on diterpenoid alkaloids from *Aconitum* and *Delphinium* species,[1~5] we investigated the alkaloids of *Aconitum liljestrandii* (Ranunculaceae).[6] The plant is endemic to the China and its roots are used for the teatment of reuthmatic pain in folk medicine. Apart from a short report on the isolation of ten known compounds (indaconitine, yunaconitine, chasmanine, 14-debenzoylfranchetine, pseudaconine, chasmanine, ludaconitine, 8-deacetylyunaconitine, talatisamine, and genicunine A) from this plant species,[7] no further phytochemistry has been reported. In this Note we report the isolation and structure determination of two new C_{19}-diterpenoid alkaloids, liljestrandinine (**1**) and *N*-deethyltalatisamine (**2**) along with another five known alkaloids, chasmaconitine (**4**), forestine (**5**), pengshenine B (**6**), cammaconine (**7**), and 6-epiforesticine (**8**).

Liljestrandine (**1**), $C_{23}H_{35}NO_4$ [M^+] ion at *m/z* 389.2547 in HREIMS, exhibited characteristic NMR spectral feature of an aconitine-type C_{19}-diterpenoid alkaloid[8] bearing an *N*-ethyl group (δ_H 1.02, 3H, t, *J*=7.6 Hz; δ_C 13.4 q, 49.4 t), two methoxyl groups (δ_H 3.24, 3.30, each s; δ_C 56.2 s, 59.4 s), a disubstituted double bond (δ_H 5.63, 1H, dd, *J*=9.6; 1.6 Hz; δ_H 5.89 q, 1H, dd, *J*=9.2, 6.8 Hz; δ_C 131.7 d, 129.8 d). The MS displayed a base peak ion peak at *m/z* 358 (M^+-OCH_3) suggesting the presence of an 1α-OCH_3 group.[9] The remained methoxyl group could be located at C-18 due to the multibond correlation between

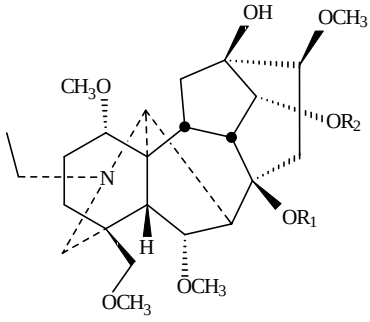
OCH_3 (δ_H 3.30) and C-18 (δ_C 79.6 t) (HMBC). On the other hand, a triplet signal at δ_H 4.05 (J=4.4 Hz) was attributed to H-14β,[8] implying the presence of a hydroxyl group at C-14 position. The disubstituted double bond was assigned at C-15 and C-16 ($\triangle^{15,16}$) mainly based on the presence of correlations between C-9 (δ_C 46.3 d), C-13 (δ_C 38.9 d) and H-15 (δ_H 5.63), as well as C-8 (δ_C 74.3 s), C-13 (δ_C 38.9 d) and H-16 (δ_H 5.89) in HMBC. Finally, unambiguous assignments of the ^1H and ^{13}C chemical shifts for liljestrandine (**1**) (Table 1) were accomplished using the 2D NMR techniques (^1H-^1H COSY, HMQC, HMBC). All available evidence suggested the structure of liljestrandine as depicted in **1**.



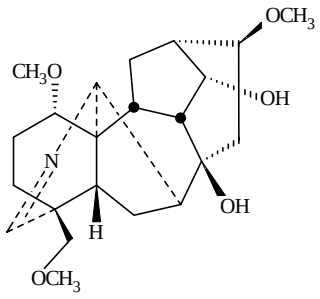
1 liljestrandinine



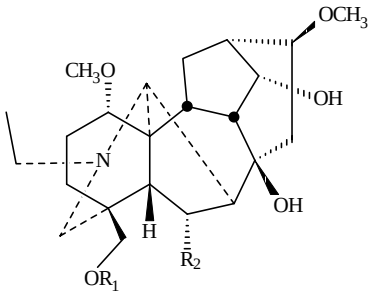
2 *N*-deethyltalatisamine R=H
3 talatisamine R=Et



4 chasmaconitine R_1=Ac R_2=B
5 forestine R_1=H
R_2=CO-C_6H_4-OCH_3 (*p*)



6 pengshenine B



7 cammaconine R_1=R_2=H
8 6-epiforesticine R_1=CH_3 R_2=OH

The molecular formula of *N*-deethyltalatisamine (**2)** ($C_{22}H_{35}NO_5$) was determined by HREIMS (M^+*m/z* 393.2492). The NMR spectral data strongly suggested an aconitine-type structure for **2**.[8] Three methoxyl groups were present in structure of **2**. In its ^1H NMR spectrum, a triplet (J=5.2 Hz) at δ_H 4.16 could be assigned to H-14β,[8] suggesting the presence of a 14-hydroxyl group. The MS exhibited the base peak at *m/z* 362 (M^+-OCH_3) indicating the presence of a 1α-OCH_3 group.[9] Other two methoxyl groups (δ_H 3.34 s, δ_C 56.3 q; δ_H 3.29 s, δ_C 59.5 q, HMQC) could be located at C-16 ad C-18, respectively, on the basis of the presence of the correlations between 16-OCH_3 (δ_H 3.34) and C-16 (δ_C 81.9 d), 18-OCH_3 (δ_H 3.29) and C-18 (δ_C 78.9 t) in HMBC spectrum. In the NMR spectra of **2**, no *N*-ethyl group was showed, and comparisons of the ^{13}C NMR (Table 2) and MS data between **2** and talatisamine (**3**),[10] especially in its 2D NMR (Table 2), led to the structural determination of *N*-deethyltalatisamine (**2**).

During the course of isolation, five known alkaloids chasmaconitine (**4**),[11] forestine (**5**),[12] pengshenine B (**6**),[13] cammaconine (**7**),[14] and 6-epiforesticine (**8**),[12, 15] were obtained and their structures were established by comparison of the NMR data with reported values and co-TLC behavior with an authentic samples.

Table 1 ^{1}H- and ^{13}C-NMR Data of compound (**1**)

	δ(C)(DEPT)	δ(H)	^{1}H-^{1}H COSY	HMBC(H→C)
CH(1)	85.7(CH)	3.09 (dd, *J*=10.8, 6.8)	CH_2(2)	C(10), C(11), C(17), C(1')
CH_2(2)	22.6(CH_2)	1.97 (m) (α)	CH(1), CH(2β), CH_2(3)	C(4)
		2.24 (m) (β)	CH(1), CH(2α), CH_2(3)	
CH_2(3)	32.6(CH_2)	1.43 (td, *J*=13.2, 2.8) (β)	CH_2(2), CH(3α)	C(19)
		1.76 (m) (α)	CH_2(2), CH(3β)	C(4), C(5)
C(4)	38.5(C)			
CH(5)	45.6(CH)	1.64 (d, *J*=7.2)	CH(6), CH(7)	C(4), C(7), C(10), C(11), C(17), C(18), C(19)
CH_2(6)	23.4(CH_2)	1.54 (dd, *J*=14.8, 8.0) (α)	CH(6β), CH(7)	C(4), C(5), C(7), C(8), C(11)
		1.92 (m) (β)	CH(5), CH(6α)	C(4), C(7), C(8), C(17)
CH(7)	42.3(CH)	2.09 (d, *J*=8.0)	CH(6α)	C(8), C(9), C(11), C(15), C(17)
C(8)	74.3(C)			
CH(9)	46.3(CH)	2.27 (m)	CH(10), CH(14)	C(8), C(10), C(12), C(13), C(15)
CH(10)	45.8(CH)	1.92 (m)	CH(9), CH(12α)	C(5), C(9), C(11), C(17)
C(11)	48.2(C)			
CH_2(12)	33.0(CH_2)	1.86 (m) (α)	CH(12β), CH(13)	C(10), C(16)
		2.39 (m) (β)	CH(12α)	C(9), C(10), C(14)
CH(13)	38.9(CH)	2.41(m)	CH(12α)	C(15), C(16)
CH(14)	74.5(CH)	4.05 (t, *J*=4.4)	CH(9), CH(13)	C(8), C(16)
CH(15)	131.7(CH)	5.63 (dd, *J*=9.6, 1.6)	CH(16)	C(9), C(13)
CH(16)	129.8(CH)	5.89 (dd, *J*=9.2, 6.8)	CH(15), CH(13)	C(8), C(13)
CH(17)	63.2(CH)	2.94 (s)		C(6), C(8), C(10), C(11), C(19), C(21)
CH_2(18)	79.6(CH_2)	3.00 (ABq, *J*=8.0)	CH(18)(3.13)	C(3), C(4), C(5), C(19), C(18')
		3.13 (ABq, *J*=8.0)	CH(18)(3.00)	C(3), C(4), C(5), C(19), C(18')
CH_2(19)	53.1(CH_2)	1.98 (hidden)	CH(19)(2.51)	C(18), C(21)
		2.51 (hidden)	CH(19)(1.98)	C(17)
CH_2(21)	49.4(CH_2)	2.41 (m)	CH_3(22)	C(17), C(19), C(22)
CH_3(22)	13.4(CH_3)	1.02 (t, *J*=7.6)	CH_2(21)	C(21)
OCH_3(1')	56.2(CH_3)	3.24 (s)		C(1)
OCH_3(18')	59.4(CH_3)	3.30 (s)		C(18)

EXPERIMENTAL

General Experimental Procedure. Melting points were uncorrected. IR spectrum was recorded on a

Nicolet FT-IR 200SXV spectrophotometer. Optical rotation was taken on a Perkin-Elmer 341 polarimeter.

Table 2 ^{1}H- and ^{13}C-NMR Data of compounds (**2**) and (**3**)

	2				**3**
	δ(C)(DETP)	δ(H)	^{1}H-^{1}H COSY	HMBC(H→C)	δ(C)
CH(1)	83.9(CH)	3.25 (m)	CH_2(2)	C(3), C(17), C(1')	86.1
CH_2(2)	24.2(CH_2)	1.88 (m) (α)	CH(1)		25.7
		1.94 (m) (β)	CH(1), CH(3β)	C(1), C(3), C(11)	
CH_2(3)	30.4(CH_2)	1.56 (m) (β)	CH(2β), CH(3α)	C(2)	32.6
		1. 67 (m) (α)	CH(3β)	C(1), C(2), C(18)	
C(4)	38.2(C)				38.6
CH(5)	43.6(CH)	1.78 (m)	CH(6)	C(3)	37.7
CH_2(6)	24.8(CH_2)	1.76 (m) (α)	CH(6β), CH(7)	C(4), C(5), C(7), C(17)	24.8
		2.00 (t, *J*=7.6) (β)	CH(6α), CH(5)	C(4), C(7), C(8), C(17)	
CH(7)	52.1(CH)	2.09 (m)	CH(6β)	C(5), C(8), C(9), C(11), C(15), C(17)	45.7
C(8)	73.2(C)				72.7
CH(9)	46.3(CH)	2.23 (t, *J*=5.2)	CH(10), CH(14)	C(8), C(10), C(12), C(13), C(14)	46.9
CH(10)	45.5(CH)	1.72 (m)	CH(9)	C(1), C(8), C(9), C(12), C(17)	45.7
C(11)	48.7(C)				48.6
CH_2(12)	27.9(CH_2)	1.61 (m) (α)	CH(10), CH(12β)	C(10), C(13), C(14), C(16)	28.6
		1.82 (m) (β)	CH(12α), CH(13)	C(13), C(16)	
CH(13)	38.5(CH)	2.34 (t, *J*=6.8)	CH(12α), CH(14), CH(16)	C(9), C(14), C(15), C(16)	45.7
CH(14)	75.4(CH)	4.16 (t, *J*=5.2)	CH(9), CH(13)	C(8), C(16)	75.7
CH_2(15)	39.2(CH_2)	2.06 (m) (α)	CH(15β), CH(16)	C(16)	39.2
		2.55 (dd, *J*=17.2, 8.4) (β)	CH(15α), CH(16)	C(7), C(8), C(9), C(16)	
CH(16)	81.9(CH)	3.39 (d, *J*=8.0)	CH_2(15), CH(13)	C(8), C(14), C(16')	82.2
CH(17)	57.4(CH)	3.24 (s)		C(5), C(6), C(8), C(10), C(19)	62.8
CH_2(18)	78.9(CH_2)	2.97 (ABq, *J*=8.8)	CH(18)(3.10)	C(3), C(4), C(19), C(18')	79.4
		3.10 (ABq, *J*=8.8)	CH(18)(2.97)	C(3), C(4), C(19),C(18')	
CH_2(19)	48.2(CH_2)	2.52 (ABq, *J*=13.6)	CH(19)(2.80)	C(3), C(4), C(17)	53.1
		2.80 (ABq, *J*=13.6)	CH(19)(2.52)	C(3), C(4), C(18)	
CH_2(21)					49.1
CH_3(22)					13.6
OCH_3(1')	55.8(CH_3)	3.27 (s)		C(1)	56.1
OCH_3(16')	56.3(CH_3)	3.34 (s)		C(16)	56.3
OCH_3(18')	59.5(CH_3)	3.29 (s)		C(18)	59.3

^{1}H- and ^{13}C-NMR spectra were measured on Bruker AC-E 200 and Varian Unity INOVA 400/45 spectrometers, in $CDCl_3$ with TMS as the internal standard. HREIMS was obtained from an

AutoSpec-3000 spectrometer. Silica gel GF_{254} and H (Qindao Sea Chemical Factory, China) were used for TLC, Chromatotron and CC, respectively. Spots on chromatograms were detected under UV light (254 nm) and with modified Dragendorff's reagent. A polyvinyl sulfonic ion exchange resin (H-form, cross linking 1×3, Nankai University Chemical Factory, China) was used in the extraction of total alkaloids.

Plant Material. The *Aconitum liljestrandii* was collected in the Yu Lin Gong mountain in Gan Zi area of Sichuan Province, China, in August, 2000. The voucher specimen was deposited at the herbarium of the West China College of Pharmacy, Sichuan University.

Extraction and Isolation. The total alkaloids (A: 59.87 g; B: 7.71 g) were obtained from 8.15 kg of dried powdered roots of *Aconitum liljestrandii* according to the literature.[7] Part A was fractionated by a pH gradient method to four portions Ⅰ(pH 2, 20.75 g); Ⅱ(pH 7, 3.49 g); Ⅲ(pH 9, 10.71 g); Ⅳ(pH 10,680 g). Part I was chromatographed successively on silica gel H column eluting with petroleum ether-acetone (4.5:1) → cyclohexane-acetone (4:1 → 7:1) to give liljestrandinine (**1**) (27 mg), chasmaconitine (**4**) (16 mg), forestine (**5**) (50 mg), and pengshenine B (**6**) (60 mg). The part B was chromatographed on silica gel H column eluting with $CHCl_3$-MeOH (93:7)→petroleum ether-acetone (1.5:1→1:1) to give cammaconine (**7**) (26 mg), *N*-deethyltalatisamine (**2**) (44 mg), and 6-epi-foresticine (**8**) (22 mg).

Liljestrandinine (1). white amorphous powder, mp 54-56°C; $[\alpha]_D$ –10 ° (c 0.5, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3431, 2923, 1639, 1456, 1095; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): Table 1; EIMS *m/z* 9%): 389 (M^+, 15), 358 (M^+-OCH_3, 100); HREIMS *m/z*: 389.2547, calcd for $C_{23}H_{35}NO_4$, 389.2539.

***N*-Deethyltalatisamine (2).** white amorphous powder. mp 59-60°C; $[\alpha]_D$ +10 ° (c 0.5, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3428, 2931, 1638, 1458, 1091; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): see Table 2; EIMS *m/z* (%): 393 (M^+, 5), 362 (M^+-OCH_3, 100); HREIMS *m/z*: 393.2492, calcd for $C_{22}H_{35}NO_5$, 393.2515.

ACKNOWLEDGMENT

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

REFERENCES

1 F. P. Wang, Z. B. Li, X. P. Dai, and C. S. Peng., *Phytochemistry*, 1997, **45**, 4539.

2 F. P. Wang and Z. B, Li, *J. Nat. Prod*., 1998, **61**, 1555.

3 Z. B. Li and F. P. Wang, *J. Asian Nat. Prod. Res*., 1998, **1**, 87.

4 F. P. Wang, C. S. Peng, and K. B. Yu, *Tetrahedron*, 2000, **56**, 7443.

5 F. P. Wang, C. S. Peng, X. X. Jian, and D. L. Chen, *J. Asian Nat. Prod. Res*., 2001, **3**, 15.

6 Institute of Botany, Chinese Academy of Sciences & Institute of Meteria Medica, Chinese Academy of Medicinal Sciences, in 'The Flora Reipublicae Popularis Sinicae', Vol. 27, pp. 113, 303, Science Press, Beijing, 1979.

7 G. B. Xie and F. P. Wang, *Natural Product R&D*., 2002, **14**(1), 32.

8 S. W. Pelletier, N. V. Mody, B. S. Joshi, and L. C. Schramm, In 'Alkaloids: Chemical and Biological Perspectives', ed. by S. W. Pelletier, Vol. 2, p. 205, Wiley, New York, 1984.

9 M. S. Yunnusov, Y. V. Rashkes, V. A. Tel'nov, and S. Y. Yunnusov, *Khim. Priod. Soedin*., 1969, **5**, 515.

10 C. Konno, M. Shirasaka, and H. Hikino, *J. Nat. Prod*., 1982, **45**, 128.

11 J. R. Achmatowicz and L. Marion, *Can. J. Chem*., 1964, **42**, 154.

12 S. W. Pelletier, S. Y. Chen, B. S. Joshi, and H. K. Desai, *J. Nat. Prod*., 1984, **47**, 474.

13 C. S. Peng, F. P. Wang, and X. X. Jian, *Chin. Chem. Lett*., 2002, **13**, 233.

14 O. E. Edward, R. J. Kolt, and K. K. Purushothaman, *Can. J. Chem*., 1983, **61**, 1194.

15 F. P. Wang, Z. B. Li, J. J. Chen, and J. S. Yang, *Chin. Chem. Lett*., 2000, **11**, 1003.