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TWO NORDITERPENOID ALKALOIDS FROM *DELPHINIUM SIWANENSE*

SUOMING ZHANG, GUILING ZHAO* and GUOQIANG LIN†

State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, People's Republic of China; *Department of Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China

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Key Word Index—*Delphinium siwanense*; Ranunculaceae; norditerpenoid alkaloid; siwanine E and F.

Abstract—The aerial parts of *Delphinium siwanense* var. *leptogen* yielded two new norditerpenoid alkaloids siwanine E and siwanine F. Their structures were elucidated as 2,3-dehydro-16-acetylelasine and 2,3-dehydrodictyocarpine, respectively, on the basis of spectral methods including 1D and 2D NMR experiments. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

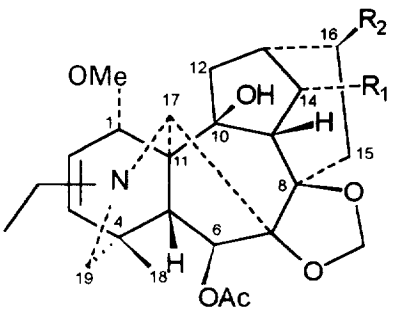
Aconitum and *Delphinium* species have been used in medicine for many centuries. *Aconitum* preparations have been used as cardiotonics, febrifuges, sedatives, and anodynes. *Delphinium* extracts have been employed in analgesic balms and also as sedatives, emetics, and antihelminthics [1, 2]. S. Zhang and Q. Ou (unpublished work) have isolated the new norditerpenoid alkaloids siwanine B–D from the aerial parts of *Delphinium siwanense* var. *leptogen* (H.-M.) W. T. Wang. Further investigation on the alkaloids of this plant resulted in the isolation of two additional new norditerpenoid alkaloids, siwanine E (**1**) and siwanine F (**2**). In this paper, we report on the isolation and structural elucidation of **1** and **2**.

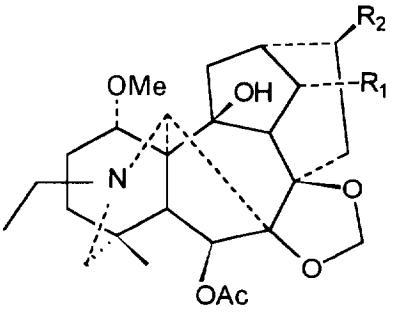
RESULTS AND DISCUSSION

The crude extracts of the air-dried and powdered aerial parts of *D. siwanense* var. *leptogen* were subjected to column chromatography on Al_2O_3 (neutral, 200–300 mesh) to afford eight fractions I–VIII. Fraction VII was further separated to yield siwanine E (**1**) as a resin. Fraction IV was further purified to give siwanine F (**2**) as an amorphous powder.

The FAB-mass spectrum of **1** contained the molecular ion peak at *m/z* (rel. int.) 534 (55). The ^{13}C NMR spectrum and DEPT of **1** indicated the presence of 28 carbon atoms, corresponding to seven quaternary, six methyl, five methylene, and ten methine carbon atoms in the molecule. An IR absorption band at 3529 cm^{-1} showed the presence of hydroxyl group in **1**. The ^1H and ^{13}C NMR spectra (Table 1) of **1** showed the presence of a tertiary methyl group (δ_H 0.99 *s*; δ_C 23.1 *q*), two methoxyl groups (δ_H 3.35 *s*, 3.38 *s*; δ_C 55.9 *q*, 57.5 *q*), two acetoxyl groups (δ_H 2.02 *s*, 2.04 *s*; δ_C 21.2 *q*, 169.5 *s*, 21.6 *q*, 170.5 *s*), a *N*-ethyl group (δ_H 1.07 *t*, J = 7.2 Hz; δ_C 12.8 *q*), a methylenedioxy group (δ_H 4.89 *s*, 4.95 *s*; δ_C 94.2 *t*), and an unsubstituted olefin (δ_H 5.68 *d*, 5.99 *dd*; δ_C 137.8 *d*, 125.2 *d*). These spectral data suggested that the molecular formula of **1** was $C_{28}H_{39}NO_9$, referring to the chemical formula of $C_{19}H_{19}(OCH_3)_2(OH)(OCOCH_3)_2(N\text{-}CH_2CH_3)(OCH_2O)$, which agreed well with those of other related norditerpenoid alkaloids identified in the other species [3–5]. The long-range correlations of two ester carbonyl carbons (δ_C 169.5 *s*, 170.5 *s*) with H-6α (δ 5.49, *br s*) and H-16α (δ_H 4.70 *dt*, J = 4.5, 9.0 Hz), respectively, were observed in an HMBC experiment, indicating that two acetoxyl groups are located at C-6 and C-16. The NMR spectrum of **1** was similar to those of 16-acetylelasine [5], except that the two methylene groups in 16-acetylelasine (**3**) were replaced by an unsubstituted olefin in **1**. The one-proton signal at δ_H 5.49 (*s*) was assigned to the C-6 methine because of its three-bond connectivities with C-11 and C-4 observed in the HMBC spectrum. As no coupling was observed between H-6 and H-5 in the ^1H NMR spectrum, the presence of an β-oriented acetoxyl group at the C-6 position was established, because the torsional angle of H-5 and H-6α in a norditerpenoid alkaloid is about 92 [3]. Also in the HMBC spectrum of **1**, both the methoxyl proton signals gave connectivities with carbon signals at δ_C 75.6 (*d*, C-1) and

† Author to whom correspondence should be addressed.





1 R_1 = OMe, R_2 = OAc

2 R_1 = OH, R_2 = OMe

3 R_1 = OMe, R_2 = OAc

4 R_1 = OH, R_2 = OMe

δ_C 81.3 (*d*, C-14), respectively, and the olefinic proton (H-3) signal correlated with the carbon signal at δ_C 75.6 (*d*) attributable to C-1. The coupling of Me-18 with the carbon signal at δ_C 138.8 (*d*, C-3), indicated that the two methoxyl groups were located at C-1 and C-14, while the double bond was located between C-2 and C-3. In the ^{1}H NMR spectrum of **1**, the triplet at δ_H 4.21 (*t*, J = 4.8 Hz) attached to C-14 revealed that C-9 and C-13 were not oxygenated. This finding, together with the signals for C-10, C-11, C-12 and C-9 appearing at δ_C 83.5 (*s*), 56.3 (*s*), 34.3 (*t*) and 49.9 (*d*) in the ^{13}C NMR spectrum, respectively, suggested that a tertiary hydroxyl group was located at C-10. In the ^{1}H NMR spectrum of **1**, two oxygenated quaternary carbon signals at δ_C 90.9 (*s*) and 82.2 (*s*) attributable to C-7 and C-8, respectively, indicated that the methylenedioxy group was located at C-7 and C-8. Accordingly, the structure of siwanine E was assigned as 2,3-dehydro-16-acetylelasine (**1**) [5].

The molecular formula of **2** was deduced as $C_{26}H_{37}NO_8$ from its FAB-mass spectrum. ^{1}H NMR, ^{13}C NMR and DEPT data which were similar to those

Table 1. ^{13}C and ^{1}H NMR chemical shift assignments and HMBC correlations of siwanine E (**1**) ($CDCl_3$, 400 MHz)

C	δ_C	H (HMQC)	δ_H	Mult. J (Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C long range correlation
1	75.6 *d*	H-1	3.85	*d*, 4.0	H-2	H-3, 1-OC$\underline{H}_3$
2	125.2 *d*	H-2	5.99	*dd*, 4.0, 10.0	H-1, H-3	H-1
3	138.8 *d*	H-3	5.68	*d*, 10.0	H-2	H-18, H-1
4	34.5 *s*	—	—			H-6α, H-2, H-3
5	50.5 *d*	H-5	1.90	*s*		H-18, H-6, H-19
6	78.8 *d*	H-6	5.49	*br s*		
7	91.0 *s*	—	—			H-6, OCH_2O
8	81.5 *s*	—	—			H-6, OCH_2O
9	49.9 *d*	H-9	3.45	*d* 4.8	H-14	H-12, H-13, H-15
10	83.5 *s*	—	—			H-1, H-12, H-13
11	56.3 *s*	—	—			H-6α, H-1, H-2, H-17
12	34.3 *t*	H-12	1.90, 2.65	*m*, *m*	H-13	
13	38.9 *d*	H-13	2.55	*m*	H-12, H-14	H-12, H-15
14	81.3 *d*	H-14	4.21	*t*, 4.8	H-13, H-9	14-OC$\underline{H}_3$, H-12, H-16
15	37.9 *t*	H-15	1.95, 2.95	*m*, *m*	H-16	
16	73.9 *d*	H-16	4.70	*dt*, 4.5, 9.0	H-15, H-13	H-12, H-14, H-15
17	60.9 *d*	H-17	3.20	*s*		H-1
18	23.1 *q*	H-18	0.99	*s*		H-3
19	57.5 *t*	H-19	2.47, 2.65	*m*, *m*		H-18
N-$\underline{C}H_2$	48.8 *t*	N-$\underline{C}H_2$	2.55	*m*		
$\underline{C}H_3$	12.8 *q*	$\underline{C}H_3$	1.07	*t*, 7.2		
6-$\underline{C}O$	169.5 *s*	—	—			H-6α, 6-OCOCH$_3$
$\underline{C}H_3$	21.6 *q*	COC$\underline{H}_3$	2.07	*s*		
16-$\underline{C}O$	170.5 *s*	—	—			H-16α, 16-OCOCH$_3$
$\underline{C}H_3$	21.2 *q*	COC$\underline{H}_3$	2.02	*s*		
$O\underline{C}H_2O$	93.9 *t*	$OC\underline{H}_2O$	4.89, 4.95	*s*, *s*		
1-O$\underline{C}H_3$	56.0 *q*	1-OC$\underline{H}_3$	3.30	*s*		
14-O$\underline{C}H_3$	57.8 *q*	14-OC$\underline{H}_3$	3.42	*s*		

Table 2. ^{13}C and ^{1}H NMR chemical shift assignments and HMBC correlations of siwanine F (**2**) ($CDCl_3$, 400 MHz)

C	δ_C	H (HMQC)	δ_H	Mult. *J* (Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C long range correlation
1	76.0 *d*	H-1	3.86	*d*, 4.0	H-2	H-3, 1-OC<u>H</u>$_3$
2	125.1 *d*	H-2	6.00	*dd*, 4.0, 10.0	H-1, H-3	H-1
3	136.7 *d*	H-3	5.70	*d*, 10.0	H-2	H-18, H-1
4	34.5 *s*	—	—			H-6α, H-3, H-2
5	52.2 *d*	H-5	1.84	*s*		H-18, H-6, H-19
6	78.7 *d*	H-6	5.50	*br s*		
7	91.4 *s*	—	—			H-6, OC<u>H</u>$_2$O
8	81.5 *s*	—	—			H-6, OC<u>H</u>$_2$O
9	49.7 *d*	H-9	3.32	*m*	H-14	H-12, H-13, H-15
10	83.9 *s*	—	—			H-1, H-13, H-12
11	55.8 *s*	—	—			H-6α, H-1, H-2, H-12, H-17
12	36.9 *t*	H-12	1.86, 2.66	*m*, *m*	H-10, H-13	
13	38.9 *d*	H-13	2.70	*m*	H-12, H-14	H-12, H-15
14	73.0 *d*	H-14	4.67	*t*, 4.8	H-13, H-9	14-OC<u>H</u>$_3$, H-12, H-16
15	35.4 *t*	H-15	1.90, 2.70	*m*, *m*	H-16, H-15	
16	80.6 *d*	H-16	3.35	*m*	H-15	H-12, H-14, H-15
17	61.2 *d*	H-17	3.21	*s*		H-1
18	23.1 *q*	H-18	0.99	*s*		H-3
19	57.6 *t*	H-19	2.50, 2.66	*m*, *m*		H-18
N-C<u>H</u>$_2$	48.6 *t*	*N*-C<u>H</u>$_2$	2.54	*m*		
C<u>H</u>$_3$		C<u>H</u>$_3$	1.10	*t*, 7.2		
6-C<u>O</u>	169.9 *s*	—	—			H-6α, COCH$_3$
C<u>H</u>$_3$	21.6 *q*	COC<u>H</u>$_3$	2.10	*s*		
OC<u>H</u>$_2$O	94.1 *t*	OC<u>H</u>$_2$O	4.92, 5.01	*s*, *s*		
1-OC<u>H</u>$_3$	55.8 *q*	1-OC<u>H</u>$_3$	3.36	*s*		
16-OC<u>H</u>$_3$	56.1 *q*	16-OC<u>H</u>$_3$	3.38	*s*		

of **1**. The spectral data also showed that **2** possessed the same olefin and methylenedioxyl group as **1**. The IR spectrum of siwanine F displayed the absorption bands of hydroxyl (3530 cm^{-1}) and an ester carbonyl (1740 cm^{-1}) group. The ^{1}H and ^{13}C NMR spectrum (Table 2) of siwanine F indicated the presence of the following functional groups: two methoxyl (δ_H 3.36 *s*, 3.38 *s*; δ_C 55.8 *q*, 56.1 *q*), a tertiary methyl (δ_H 0.99 *s*; δ_C 23.1 *q*), an acetoxyl (δ_H 2.10 *s*; δ_C 21.6, 169.9), and a methylenedioxy (δ_H 4.92 *s*, 5.01 *s*; δ_C 94.1 *t*). These spectral data suggested that the chemical formula of siwanine F was $C_{19}H_{19}(OCH_3)_2(OH)_2(OCOCH_3)(N\text{-}CH_2CH_3)(OCH_2O)$, co-relating to those of norditerpenoid alkaloids. The NMR spectra of siwanine F gave similar signals to those reported for dictyocarpine (**4**) [4], except for the presence of an unsubstituted olefin (δ_H 6.00, *dd*, J = 10.0, 4.0 Hz; δ_H 5.70, *d*, J = 10.0 Hz); (δ_C 125.1 *d*, 138.8 *d*). Long-range couplings between C-1 and H-3, as well as between C-3 and H-18 were observed in the HMBC experiment, thus the location of the double-bond between C-2 and C-3 was established. The two methoxyl groups were assigned at C-1 and C-16 respectively, on the basis of the three-bond connectivities of the 1-OCH_3 protons with C-1 (δ_C 76.0 *d*) and the 16-OCH_3 protons with C-16 (δ_C 80.6 *d*) in the HMBC experiment. In lycoctine-type alkaloids, a methylenedioxy group is usually found at the C-7 and C-8 positions [4]. In the HMBC spectrum, the three-bond long range correlations of H-6 (δ_H 5.50, *br s*) with C-4 and C-11 were observed, and indicated that the acetoxyl group at C-6 was β-oriented. The signal at δ_H 4.67 (*t*, J = 5.0 Hz) was assigned to the C-14 methine on the basis of the ^{1}H-^{1}HCOSY, HMQC and HMBC spectra. These data suggested that a hydroxyl group was located at C-14, and that C-9 and C-13 positions do not have an oxygen function. Thus the remaining hydroxyl group had to be located at C-10 [6], and the structure of siwanine F was determined as 2,3-dehydrodictyocarpine (**2**).

EXPERIMENTAL

General. Mp: uncorr.; OR: $CHCl_3$; IR: KBr; FAB-MS: VG ZAB-HS mass spectrometer; ^{1}H, ^{13}C and 2D NMR: Bruker AM-400 spectrometer, $CDCl_3$, with TMS as int. standard. All solvents used were analytical grade. Al_2O_3 (neutral, 200–300 mesh) was used for column chromatography.

Plant material. The aerial parts of *D. siwanense* var. *leptogen* was collected from Yu-zhong County, Gansu Province, People's Republic of China, in August 1992. It was identified by Dr Ji Ma, Department of Biology, Lanzhou University, People's Republic of China. A voucher specimen is deposited in the herbarium of the Department of Biology, Lanzhou University.

Extraction and isolation. The aerial parts of the

plant (2 kg) were percolated with 95% EtOH at room temp. The filtrates were combined and concd under red. pres. (cyclone evaporator) to a dark green syrup. This was then partitioned between $CHCl_3$ (500 ml) and aq. HCl (2%, 500 ml). The $CHCl_3$ phase was further extracted with aq. HCl (3 × 300 ml) until the last extracts showed only negative reaction when tested with Dragendorff's reagent. The acidic soln. was adjusted with NH_4OH to pH 11 and extracted thoroughly with $CHCl_3$ to give the alkaloids (2 g). The crude alkaloid mixt. was chromatographed over Al_2O_3 (neutral) and eluted with petrol–Et_2O–EtOAc to afford eight frs I–VIII. Fr. IV was further sepd by CC on Al_2O_3 (petrol–Et_2O–Me_2CO 7:3:0.5) to afford **2** (30 mg). Fr. VII was subjected to repeated chromatography on Al_2O_3 (neutral) to yield **1** (20 mg).

Siwanine E (**1**). $[\alpha]_D$: +20.8° ($CHCl_3$, *c* 0.7). FAB-MS *m/z* (rel. int.): 534 $[M+1]^+$ (55), 532 (40), 518 (10), 504 (12), 490 (100), 474 (20), 111 (45) and 77 (50); IR ν_{max}^{KBr} cm^{-1}: 1740, 1742 (OAc), 1452, 1364, 1245, and 1082; ^1H and ^{13}C NMR ($CDCl_3$): Table 1.

Siwanine F (**2**). $[\alpha]_D$: +34.1° ($CHCl_3$, *c* 1.5). mp: 208–210°. FAB-MS *m/z* (rel. int.): 492 $[M+1]^+$ (30), 490 (15), 476 (15), 464 (5), 448 (80), 434 (20), 111 (30) and 77 (80); IR ν_{max}^{KBr} cm^{-1}: 3559, 3400 (OH), 1740 (OAc), 1450, 1368, 1245 and 1085 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): Table 2.

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