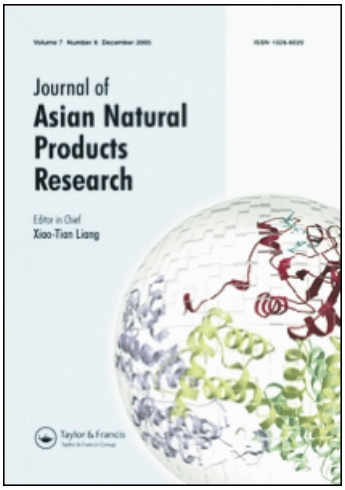

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C_{19}-Diterpenoid alkaloids from *Delphinium umbrosum*

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ORIGINAL ARTICLE

C_{19}-Diterpenoid alkaloids from *Delphinium umbrosum*

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Three new C_{19}-diterpenoid alkaloids, umbrosumines A–C (**1**–**3**), and 11 known compounds (**4**–**14**) were isolated from the roots of *Delphinium umbrosum*. Their structures were elucidated on the basis of the spectroscopic data interpretation.

Keywords: *Delphinium umbrosum*; C_{19}-diterpenoid alkaloids; umbrosumine A; umbrosumine B; umbrosumine C

1. Introduction

Diterpenoid alkaloids from the *Delphinium* species have spurred considerable interest of medicinal chemists due to their demonstrated pharmacological properties, such as analgesic, antiarrhythmic, anti-inflammatory, arrhythmogenic, curariform, hypotensive, neurotropic, psychotropic, and spasmolytic [1–5]. As part of our ongoing research program to comparatively study the diterpenoid alkaloids from the *Aconitum* and *Delphinium* species, we investigated the alkaloidal constituents of the roots of *Delphinium umbrosum* Var. *hispidum* W.T. Wang. The plant is endemic to the southwest Sichuan of China, and its roots are used for the treatment of arthritic pain in folk medicine [6]. To the best of our knowledge, no phytochemical information is currently available on this plant. The present study resulted in the isolation of 3 new C_{19}-diterpenoid alkaloids, umbrosumines A–C (**1**–**3**), and 11 known alkaloids (**4**–**14**) (see structures in Figure 1).

2. Results and discussion

The acid aqueous extracts of air-dried roots of *D. umbrosum* were basified with 10% NH_4OH and extracted with ethyl acetate. Repetitive column chromatography of the subsequent extracts yielded 3 new C_{19}-diterpenoid alkaloids (**1**–**3**) and 11 known compounds (**4**–**14**). Based on the comparisons of the respective spectroscopic data of each compound with those reported in the literature, the known compounds were identified as delsemine B (**4**) [7], delsemine A (**5**) [7], delavaine A (**6**) [8], delavaine B (**7**) [8], giraldine G (**8**) [9], ajacine (**9**) [10], methyliycaconitine (**10**) [7], lycoctonine (**11**) [7], 14-acetyldecosine (**12**) [11], delcosine (**13**) [10], and delectinine (**14**) [12].

Umbrosumine B (**1**) was obtained as an amorphous powder. Its positive-ion HR-ESI-MS showed a quasi-molecular ion peak at *m/z* 728.3778 $[M+H]^+$, corresponding to the molecular formula $C_{38}H_{53}N_3O_{11}$. Its NMR spectra showed the presence of an

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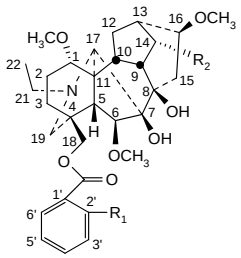
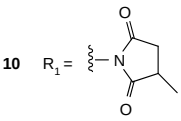
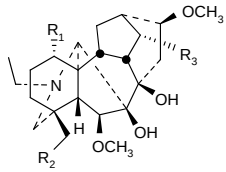


	R_1	R_2
1	$NHCOCH_2CH(CH_3)CONH_2$ (5″; 1″ 2″ 3″ 4″)	OAc
2	$NHCOCH(CH_3)CH_2CONH_2$ (5″; 1″ 2″ 3″ 4″)	OAc
3	$NHCOCH_2CH(CH_3)COOH$ (5″; 1″ 2″ 3″ 4″)	OAc
4	$NHCOCH_2CH(CH_3)CONH_2$	OCH_3
5	$NHCOCH(CH_3)CH_2CONH_2$	OCH_3
6	$NHCOCH(CH_3)CH_2COOH$	OCH_3
7	$NHCOCH_2CH(CH_3)COOH$	OCH_3
8	$NHCOCH(CH_3)CH_2CONH_2$	$OCOCH(CH_3)_2$
9	$NHCOCH_3$	OCH_3
10	R_1 = 	OCH_3



	R_1	R_2	R_3
11	OCH_3	OH	OCH3
12	OH	OCH_3	OAc
13	OH	OCH_3	OH
14	OCH_3	OCH_3	OH

Figure 1. Chemical structures of compounds **1**–**14**.

N-ethyl group [δ_H 1.07 (3H, t, $J = 7.2$ Hz); δ_C 14.0 q, 51.0 t], three methoxyl groups [δ_H 3.26, 3.33, 3.37 (each 3H, s); δ_C 55.8 q, 56.3 q, 58.1 q], an acetyl group (δ_H 2.06, 3H, s; δ_C 171.9 s, 21.5 q), and a substituted anthranoyl group [(δ_H 11.0, 1H, s, *NH*; 7.14–8.67, 4H, m, Ar-H; 5.35, 5.91, each 1 H, br s, NH_2; 1.26, 3H, d, $J = 6.8$ Hz, $CHCH_3$); δ_C, see Table 1]. Its ^{13}C NMR spectrum displayed seven oxygenated carbon signals at δ_C 69.7, 75.9, 77.4, 82.3, 83.7, 88.3, and 90.7, suggesting that **1** possesses two hydroxyl groups in addition to three methoxyl groups and two ester groups. All of the available evidence indicated that **1** is a lycoctonine-type C_{19}-diterpenoid alkaloid [13]. The three methoxyl groups were readily assigned to C-1, C-6, and C-16, respectively, according to the related HMBC correlations shown in Figure 2. A triplet signal at δ_H 4.75 ($J = 4.4$ Hz) was attributed to H-14β [14], implying the presence of an ester substituent at the C-14 position. Comparison of the NMR data of **1** (Table 1) with those of delsemine B [7] revealed the existence of similar structures between them. The only difference between them was that the methoxyl group at C-14 in delsemine B was replaced by an acetoxyl group in **1**. The existence of the acetoxyl group at C-14 in **1** was confirmed by the observation of the HMBC cross-signal between H-14β (δ_H 4.75) and the carbonyl carbon of the acetyl ester (δ_C 171.9) (Figure 2). The planar structure of **1** was assigned as shown in Figure 1, which was corroborated by the HMBC correlations (Figure 2). The configuration of **1** was deducted based on the related NOESY correlations. The methoxyl group at C-1 was placed in α-orientation based on the NOESY correlation between H-1β and H-10β. Similarly, the NOESY correlations between H-6 and H-19α and H-16 and H-12α suggested β-orientation of the methoxyl groups at C-6 and C-16; while the NOESY correlation between H-14 and H-10β suggested α-orientation of the acetyl ester group at C-14. In addition, the configuration of C-5″ in **1** could be deduced as '*S*' based on the comparison of the ^{13}C NMR spectral data with those of delsemine B [8,9].

Umbrosumines B (**2**) and C (**3**) were obtained as amorphous powders. The molecular formulas of **2** ($C_{38}H_{53}N_3O_{11}$) and **3** ($C_{38}H_{52}N_2O_{12}$) were determined by their HR-ESI-MS and ^{13}C NMR spectra.

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1**–**3**.

	1		**2**		**3**	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	83.7 d	2.96 hidden	83.7 d	3.06 hidden	83.7 d	3.03 t ($J = 5.2$)
2	26.0 t	2.21 m	26.0 t	2.11 m	26.0 t	2.16 m
3	32.1 t	1.78 m	32.1 t	1.80 m, 1.89 m	32.1 t	1.83 m
4	37.6 s	–	37.6 s	–	37.5 s	–
5	50.1 d	1.75 s	50.1 d	1.79 s	50.1 d	1.82 s
6	90.7 d	3.90 s	90.7 d	3.91 s	90.7 d	3.92 s
7	88.3 s	–	88.3 s	–	88.3 s	–
8	77.4 s	–	77.5 s	–	77.5 s	–
9	42.5 d	3.23 hidden	42.5 d	3.25 hidden	42.5 d	3.16 dd ($J = 6.4, 4.8$)
10	45.7 d	2.08 hidden	45.7 d	2.04 hidden	45.7 d	2.11 hidden
11	49.0 s	–	49.0 s	–	49.0 s	–
12	28.1 t	2.42 m, 2.53 m	28.1 t	2.56 m, 2.63 m	28.7 t	2.45 m
13	38.2 d	2.57 m	38.1 d	2.51 m	38.1 d	2.50 m
14	75.9 d	4.75 dd ($J = 4.4$)	75.9 d	4.76 dd ($J = 4.8$)	75.9 d	4.76 dd ($J = 4.8$)
15	33.7 t	1.54 hidden	33.7 t	1.60 hidden	33.7 t	1.58 dd ($J = 16.0, 8.4$)
		1.75 hidden	33.7 t	1.86 hidden	33.7 t	1.92 dd ($J = 16.0, 8.4$)
16	82.3 d	3.24 hidden	82.2 d	3.28 hidden	82.3 d	3.29 hidden
17	64.4 d	2.94 s	64.4 d	2.96 s	64.4 d	2.96 s
18	69.7 t	4.13, 4.20	69.6 t	4.15, 4.21	69.7 t	4.14, 4.23
		ABq (11.2)		ABq (11.2)		ABq (11.2)
19	52.3 t	2.66 m, 2.81 m	52.2 t	2.50 m, 2.79 m	52.2 t	2.51 m, 2.75 m
21	51.0 t	2.68 hidden	51.0 t	2.85 m, 3.08 m	51.0 t	2.78 m
22	14.0 q	1.07 t ($J = 7.2$)	14.0 q	1.07 t ($J = 7.2$)	14.0 q	1.07 t ($J = 7.2$)
CH_3O-1	55.8 q	3.26 s	55.7 q	3.26 s	55.8 q	3.27 s
CH_3O-6	58.1 q	3.37 s	58.0 q	3.37 s	58.1 q	3.37 s
CH_3O-16	56.3 q	3.33 s	56.2 q	3.33 s	56.2 q	3.33 s
AcO-14	171.9 s		171.9 s		171.9 s	
	21.5 q	2.06 s	21.5 q	2.06 s	21.5 q	2.06 s
18-COO	167.9 s	–	167.9 s	–	168.0 s	–
1′	114.7 s	–	114.8 s	–	114.9 s	–

2′	141.5 s	–	141.7 s	–	141.7 s	–
3′	120.7 d	7.96 d ($J = 8.0$)	120.7 d	7.97 d ($J = 8.4$)	120.8 d	7.96 d ($J = 8.0$)
4′	134.9 d	7.56 t ($J = 8.0$)	134.9 d	7.57 t ($J = 8.4$)	134.9 d	7.56 t ($J = 8.0$)
5′	122.8 d	7.14 t ($J = 8.0$)	122.7 d	7.14 t ($J = 8.4$)	122.7 d	7.13 t ($J = 8.0$)
6′	130.3 d	8.67 d ($J = 8.0$)	130.3 d	8.70 d ($J = 8.4$)	130.3 d	8.70 d ($J = 8.0$)
1″	174.5 s	–	174.6 s	–	174.8 s	–
2″	42.0 t	2.62 hidden 2.99 hidden	39.4 d	3.29 hidden	39.0 d	3.38 hidden
3″	36.3 d	3.03 m	39.2 t	3.23 hidden	38.0 t	3.27 hidden
4″	177.8 s	–	173.4 s		174.6 s	
5″	17.7 q	1.26 d ($J = 6.8$)	18.2 q	1.35 d ($J = 7.2$)	17.9 q	1.34 d ($J = 7.2$)
N*H*CO	–	11.0 s	–	11.2 s	–	–
NH_2	–	5.39 br s 5.91 br s	–	5.45 br s 5.88 br s	–	– –

Note: Recorded in $CDCl_3$ at 400 MHz for ^1H, 100 MHz for ^{13}C, δ in ppm, J in Hz.

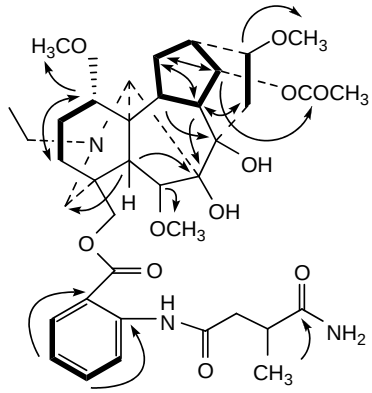


Figure 2. Key ^{1}H–^{1}H COSY (▬) and HMBC (↷) correlations of **1**.

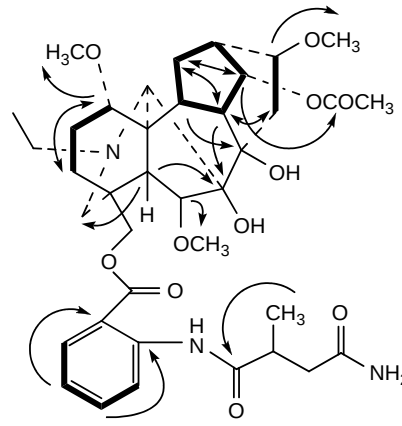


Figure 3. Key ^{1}H–^{1}H COSY (▬) and HMBC (↷) correlations of **2**.

They exhibited characteristic NMR features of the lycoctonine-type C_{19}-diterpenoid alkaloids by bearing an *N*-ethyl group [δ_H 1.07 (3H, t, $J = 7.2$ Hz); δ_C 14.0 q, 51.0 t], three methoxyl groups [δ_H 3.26, 3.33, 3.37 (each 3H, s) and δ_C 55.7 q, 56.2 q, 58.0 q for **2**; δ_H 3.27, 3.33, 3.37 (each 3H, s) and δ_C 55.8 q, 56.2 q, 58.1 q for **3**], an acetyl ester group (δ_H 2.06, 3H, s; δ_C 171.9 s, 21.5 q), and a substituted anthranoyl group (for NMR spectral data, see Table 1) in each structure [13]. In addition, the presence of two hydroxyl groups attached to quaternary carbons was also evident from the ^{13}C NMR spectral data [δ_C 88.3 s, 77.5 s] and IR absorbance (3452 cm^{-1} for **2**; 3453 cm^{-1} for **3**). In their ^{1}H NMR spectra, each triplet at δ_H 4.76 ($J = 4.8$ Hz) could be assigned to H-14β [14], suggesting the presence of an ester group at each C-14 of **2** and **3**. The assignments of the acetyl ester group at C-14 were evidenced by the observation of a long-range coupling signal between H-14β and the acetyl carbonyl carbon in the HMBC spectra of both compounds. Comparison of the NMR spectral data of **2** and **3** (Table 1) with those of **1** revealed that they share the same basic skeleton. The differences between the three sets of spectra were the substituted anthranoyl groups that were located at C-18. Finally, comparisons of the NMR spectral data of **2** (Table 1) with those of delsemine A [7], as well as **3** with those of delavaine A [8], led to the structural determination of the respective substituted anthranoyl group indicated for umbrosumine B (**2**) and umbrosumine C (**3**). The structures of **2** and **3** were confirmed by their HMBC and ^{1}H–^{1}H COSY correlations (Figures 3 and 4).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were obtained on a Nicolet FT-IR 200 SXV spectrophotometer. ^{1}H and ^{13}C NMR spectra were taken on a Varian Unity

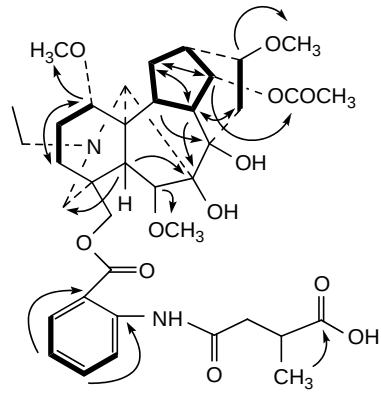


Figure 4. Key ^{1}H–^{1}H COSY (▬) and HMBC (↷) correlations of **3**.

INOVA 400/45 NMR spectrometer in $CDCl_3$ with TMS as the internal standard. The ESI-MS and HR-ESI-MS were recorded on a VG Auto Spec 3000 or a Finnigan-MAT 90 instrument. Silica gel H (Qingdao Marine Chemical Factory, Qingdao, China) was used for column chromatography. Zones on thin layer chromatography (TLC) (silica gel G) were detected with modified Dragendorff's reagent.

3.2 Plant material

D. umbrosum was collected from Yuexi County, Sichuan Province, China, in September 2007. The plant was identified by Prof. Q.E. Yang at the Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (200709-1) has been deposited.

3.3 Extraction and isolation

Air-dried and powdered roots of *D. umbrosum* (450 g) were percolated with diluted HCl (0.1 M, 6 liter). The subsequent percolate was basified with 10% NH_4OH aqueous solution to pH 10 and then extracted with ethyl acetate (6 liter × 3). Removal of the solvent under reduced pressure afforded the total crude alkaloids (3.0 g) as a yellowish amorphous powder, which was chromatographed over a silica gel column, eluted with cyclohexane–acetone (9:1 → 1:2) gradient system, to give fractions A (200 mg), B (566 mg), C (533 mg), D (268 mg), E (280 mg), F (720 mg), and G (254 mg). Fraction B (566 mg) was further chromatographed on a silica gel column employing cyclohexane–acetone (6:1 → 3:1) as eluent to afford ajacine (**9**, 26 mg) and methyliycaconitine (**10**, 18 mg). Column chromatography of fraction C over silica gel eluted with cyclohexane–acetone (6:1 → 2:1) provided 14-acetyldecosine (**12**, 130 mg) and lycoctonine (**11**, 200 mg). Fraction D was purified over a silica gel H column, eluted with cyclohexane–acetone (5:1 → 1:1), to yield delectinine (**14**, 9.1 mg), delcosine (**13**, 20 mg), and giraldine G (**8**, 11.5 mg). Fraction E was subjected to column chromatography over silica gel, employing cyclohexane–acetone (5:1 → 1:1) as eluent, to yield umbrosumine A (**1**, 10 mg). Fraction F was separated on a silica gel H column, eluted with cyclohexane–acetone (5:1 → 1:1), to yield delsemine A (**5**, 26 mg), delsemine B (**4**, 13 mg), and umbrosumine B (**2**, 15 mg). Separation of fraction G on a silica gel H column, employing cyclohexane–acetone (4:1 → 1:1) for elution, yielded delavaine A (**6**, 14 mg), delavaine B (**7**, 14 mg), and umbrosumine C (**3**, 8.5 mg).

3.3.1 *Umbrosumine A (**1**)*

White amorphous powder; $[\alpha]_D^{20}$ +21.7 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max} 3543, 3320, 2937, 1680, 1588, 1526, 1253 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 1; ESI-MS *m/z*: 728 $[M+H]^+$; HR-ESI-MS *m/z*: 728.3778 $[M+H]^+$ (calcd for $C_{38}H_{54}N_3O_{11}$, 728.3758).

3.3.2 *Umbrosumine B (**2**)*

White amorphous powder; $[\alpha]_D^{20}$ +48.6 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max} 3452, 3320, 2934, 1680, 1588, 1528, 1255 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 1; ESI-MS *m/z*: 728 $[M+H]^+$; HR-ESI-MS *m/z*: 728.3779 $[M+H]^+$ (calcd for $C_{38}H_{54}N_3O_{11}$, 728.3758).

3.3.3 *Umbrosumine C (**3**)*

White amorphous powder; $[\alpha]_D^{20}$ +30.6 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max} 3453, 3319, 2935, 1679, 1588, 1527, 1254 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 1; ESI-MS *m/z*: 729 $[M+H]^+$; HR-ESI-MS *m/z*: 729.3581 $[M+H]^+$ (calcd for $C_{38}H_{53}N_2O_{12}$, 729.3599).

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