Diterpenoid Alkaloids from *Delphinium yunnanense*

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Three new diterpenoid alkaloids, along with eleven known alkaloids, were isolated from the whole herbs of *Delphinium yunnanense*. The new alkaloids include a rearranged-type C_{19}-diterpenoid alkaloid, named yunnanenseine A (**1**), and two hetisine-type C_{20}-diterpenoid alkaloids, named yunnanenseine B and C (**2** and **3**, resp.). Their structures were elucidated by detailed NMR-spectroscopic studies.

Introduction. – The genus *Delphinium*, encompassing poisonous and medicinal plants, comprises *ca.* 350 species, half of them growing in China [1]. Diterpenoid alkaloids, a class of structurally complex compounds, are assumed to be the main bioactive components of the genus *Delphinium* [2–5]. They display a broad range of chemical and pharmacological properties, and also demonstrated an outstanding merit for chemotaxonomic considerations [2–5]. *Delphinium yunnanense* (FRANCH.) FRANCH. is endemic in the Yunnan Province of China. To the best of our knowledge, no previous study has been reported concerning the phytochemistry of this plant. As part of an ongoing research program to investigate the chemistry and biological activities of diterpenoid alkaloids from *Aconitum* and *Delphinium* plants, we have investigated whole plants of *D. yunnanense* and isolated a new rearranged-type C_{19}-diterpenoid alkaloid, yunnanenseine A (**1**), and two hetisine-type C_{20}-diterpenoid alkaloids, yunnanenseines B and C (**2** and **3**, resp.). The present investigation also led to the isolation of the following 15 known compounds: methyllycaconitine [6], 14-deacetylnudicauline [7], anhweidelphinine [8], postanisine F [9], delelatine [10], delbonine [11], delsemine A [12], delsemine B [12], 14-dehydrodecosine [13], deltaline [14], delcosine [15], blacknine [16], browniine [17], 14-dehydrobrowniine [12], and lycoctonine [12]. These known compounds were identified based on comparison of the respective spectroscopic data with those reported in the literature. Here, we report the isolation and structure elucidation of the three new compounds **1**–**3** (*Fig. 1*).

Results and Discussion. – Yunnanensine A (**1**) was obtained as an amorphous powder. Its molecular formula was determined as $C_{37}H_{48}N_2O_9$ based on its HR-ESI-MS, which showed a *quasi*-molecular-ion peak at *m/z* 665.3432 ($[M+H]^+$). The NMR spectra revealed the presence of an EtN group (δ(H) 0.90 (*t*, *J* = 7.2, 3 H); δ(C) 9.9 (*q*), 48.3 (*t*)), four MeO groups (δ(H) 3.27, 3.28, 3.39, 3.41 (4*s*, each 3 H); δ(C) 55.6 (*q*), 56.5 (*q*), 56.9 (*q*), 59.4 (*q*)), a ketone CO group (δ(C) 201.1 (*s*)), and a substituted

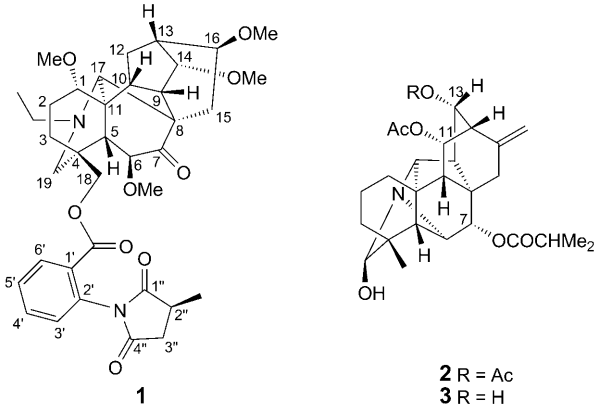

Fig. 1. *The structures of compounds* **1**–**3**

anthranoyl group ((δ(H) 7.26–8.20 (*m*, 4 arom. H)); δ(C), see *Table 1*). In addition, five additional O-bearing C-atoms (δ(C) 70.7 (*t*), 79.4 (*d*), 83.1 (*d*), 83.4 (*d*), 84.0 (*d*)) and three additional quaternary C-atoms ((δ(C) 39.0 (*s*), 51.2 (*s*), 58.9 (*s*)) were indicated by the ^{13}C-NMR data. All of the above-mentioned evidences, in conjunction with biogenetic considerations, suggested that **1** is a C_{19}-diterpenoid alkaloid [4]. The upfield chemical shift ((δ(C) 9.9 (*q*)) of C(22) of compound **1**, as well as the presence of the ketone CO group, indicated that compound **1** is a C(7)-oxo rearranged-type C_{19}-diterpenoid alkaloid [4]. Anhydrolycaconitine is a known rearranged-type C_{19}-diterpenoid alkaloid, whose structure was established by X-ray crystallographic analysis [18]. The NMR data of **1** (*Tables 1* and *2*) were very similar to those of anhydrolycaconitine except for the presence of a MeCH moiety instead of a CH_2 moiety in the substituted anthranoyl moiety in anhydrolycaconitine. It was thus concluded that the succinimide moiety in anhydrolycaconitine was replaced by a methylsuccinimide moiety in **1**. Correlations between MeO–C(1) and C(1), MeO–C(6) and C(6), MeO–C(14) and C(14), MeO–C(16) and C(16), and CH_2(18) with the benzoate ester CO group (164.0, *s*) in the HMBC spectrum (*Fig. 2*) confirmed the assignment of the four MeO groups and the substituted anthranoyl unit. In the NOESY spectrum, correlations between H_β–C(1) and H_β–C(10), and H_β–C(14) and H_β–C(10) indicated the β-orientation of H–C(1) and H–C(14). Similarly, correlations between H_α–C(6), and CH_2(19), H–C(16), and H_α–C(12) suggested the α-orientation of H–C(6) and H–C(16). The configuration of C(2″) could be deduced as (*S*) based on comparison of the ^{13}C-NMR data of methylsuccinimide moiety of **1** with those of methyllycaconitine [19]. Thus, the structure of yunnanensine A (**1**) was determined as (1α,6β,14α,16β)-20-ethyl-1,6,14,16-tetramethoxy-7-oxo-8,17-cyclo-7,17-secoaconitan-18-yl 2-(2β-2-methylsuccinimide)benzoate.

Yunnanensine B (**2**) was isolated as amorphous powder, and the molecular formula was determined to be $C_{28}H_{37}NO_7$ according to the HR-ESI-MS. Compound **2** exhibited characteristic NMR features of a C_{20}-diterpenoid alkaloid [20][21], which bears a tertiary Me group (δ(H) 1.02 (*s*, 3 H); δ(C) 22.5 (*q*)), an exocyclic C=C bond (δ(H)

Table 1. *^{13}C-NMR Data of Compounds* **1**–**3**. Recorded at 100 MHz in $CDCl_3$; δ in ppm.

	1	**2**	**3**
C(1)	83.4 (*d*)	35.4 (*t*)	35.5 (*t*)
C(2)	26.1 (*t*)	33.9 (*t*)	33.8 (*t*)
C(3)	29.6 (*t*)	36.9 (*t*)	36.9 (*t*)
C(4)	39.0 (*s*)	42.2 (*s*)	42.2 (*s*)
C(5)	42.5 (*d*)	61.2 (*d*)	60.8 (*d*)
C(6)	84.0 (*d*)	60.5 (*d*)	59.8 (*d*)
C(7)	201.1 (*s*)	69.8 (*d*)	69.5 (*d*)
C(8)	51.2 (*s*)	44.7 (*s*)	44.6 (*s*)
C(9)	46.6 (*d*)	49.6 (*d*)	50.0 (*d*)
C(10)	49.2 (*d*)	50.3 (*s*)	50.3 (*s*)
C(11)	58.9 (*s*)	75.6 (*d*)	75.2 (*d*)
C(12)	20.3 (*t*)	44.6 (*d*)	48.2 (*d*)
C(13)	39.8 (*d*)	73.4 (*d*)	74.1 (*d*)
C(14)	79.4 (*d*)	53.0 (*d*)	55.0 (*d*)
C(15)	31.6 (*t*)	29.9 (*t*)	31.9 (*t*)
C(16)	83.1 (*d*)	142.6 (*s*)	142.0 (*s*)
C(17)	66.1 (*d*)	110.4 (*t*)	109.4 (*t*)
C(18)	70.7 (*t*)	22.5 (*q*)	22.7 (*q*)
C(19)	55.5 (*t*)	91.1 (*d*)	91.5 (*d*)
C(20)		64.9 (*d*)	64.9 (*d*)
C(21)	48.3 (*t*)		
C(22)	9.9 (*q*)		
*Me*O–C(1)	55.6 (*q*)		
*Me*O–C(6)	59.4 (*q*)		
*Me*O–C(14)	56.9 (*q*)		
*Me*O–C(16)	56.5 (*q*)		
Anthranoyloxy			
C=O	164.0 (*s*)		
C(1′)	126.7 (*s*)		
C(2′)	132.9 (*s*)		
C(3′)	129.2 (*d*)		
C(4′)	133.3 (*d*)		
C(5′)	129.7 (*d*)		
C(6′)	131.2 (*d*)		
C(1″)	179.8 (*s*)		
C(2″)	35.0 (*d*)		
C(3″)	37.1 (*t*)		
C(4″)	175.8 (*s*)		
C(5″)	16.6 (*q*)		
Isobutyryloxy–C(7)		175.6 (*s*)	175.9 (*s*)
		34.4 (*d*)	34.3 (*d*)
		18.6 (*q*)	18.6 (*q*)
		18.9 (*q*)	19.3 (*q*)
*Ac*O–C(11)		169.7 (*s*)	169.9 (*s*)
		21.3 (*q*)	21.4 (*q*)
*Ac*O–C(13)		170.4 (*s*)	
		21.3 (*q*)	

Table 2. *^{1}H-NMR Data of Compounds* **1**–**3**. Recorded at 400 MHz in $CDCl_3$; δ in ppm, J in Hz.

Position	**1**	**2**	**3**
1	3.50–3.55 (*m*)	1.65–1.69 (*m*), 1.77–1.82 (*m*)	
2	2.18–2.22 (*m*), 2.32–2.35 (*m*)	2.14–2.19 (*m*), 2.30–2.34 (*m*)	
3	1.40–1.45 (*m*), 1.76–1.80 (*m*)	1.55–1.60 (*m*), 2.02–2.05 (*m*)	
5	3.42 (*s*)	1.56 (*s*)	1.56 (*s*)
6	3.81 (*s*)	3.65 (br. *s*)	3.64 (br. *s*)
7	–	5.18 (br. *s*)	5.22 (br. *s*)
9	2.09 (overlapped)	2.34 (overlapped)	
10	2.19–2.23 (*m*)		2.00–2.03 (*m*)
11	–	5.12 (*d*, *J* = 9.2)	5.12 (*d*, *J* = 8.8)
12	2.40–2.44 (*m*), 2.50–2.55 (*m*)	2.60 (*d*, *J* = 2.4)	
13	2.55–2.60 (*m*)	5.05 (br. *d*, *J* = 9.6)	4.30 (br. *d*, *J* = 8.8)
14	4.75 (*t*, *J* = 4.4)	2.19 (br. *d*, *J* = 9.6)	
15	1.50–1.54 (*m*), 2.26–2.30 (*m*)	1.58, 2.83 (*AB*, *J* = 15.2)	
16	3.55–3.60 (*m*)	–	–
17	3.49 (*s*)	4.80 (*s*), 4.98 (*s*)	4.75 (*s*), 4.94 (*s*)
18	3.90, 4.27 (*AB*, *J* = 11.6)	1.02 (*s*)	1.02 (*s*)
20		3.61 (*s*)	3.52 (*s*)
21	2.56–2.60 (*m*)		
22	0.90 (*t*, *J* = 7.2)		
MeO–C(1)	3.28 (*s*)		
MeO–C(6)	3.41 (*s*)		
MeO–C(14)	3.27 (*s*)		
MeO–C(16)	3.39 (*s*)		
2″	3.03–3.08 (*m*)		
3″	2.45–2.50 (*m*), 3.14–3.18 (*m*)		
Isobutyryloxy–C(7)		2.45–2.50 (*m*)	
		1.16 (*d*, *J* = 5.2)	1.16 (*d*, *J* = 7.2)
		1.18 (*d*, *J* = 5.2)	1.19 (*d*, *J* = 7.2)
AcO–C(11)		2.03 (*s*)	2.05 (*s*)
AcO–C(13)		2.04 (*s*)	

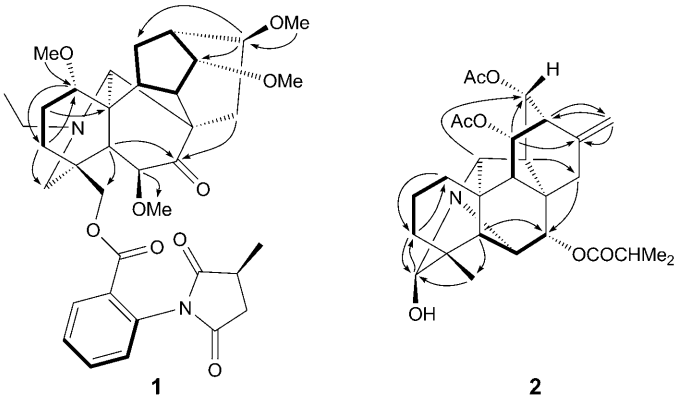

Fig. 2. *Key ^{1}H,^{1}H-COSY* (—) *and HMBC* (H → C) *correlations of compounds* **1** *and* **2**

4.80, 4.98 (2 br. *s*, each 1 H)), two AcO groups (δ(H) 2.03, 2.04 (2*s*, each 3 H); δ(C), see *Table 1*), and an isobutyryl group (δ(H) 1.16, 1.18 (2*d*, J = 5.2, each 3 H); δ(C), see *Table 1*). The δ values (42.2 (*s*); 44.7 (*s*); 50.3 (*s*); 142.6 (*s*)) of four non-O-bearing, quaternary C-atoms in the ^{13}C-NMR spectrum are indicative of the 'finger-print' characteristics for a hetisine-type C_{20}-diterpenoid alkaloid [2]. The location of two AcO groups and of an isobutyryl group could be assigned at C(11), C(13), and C(7), respectively, due to the correlations H–C(11)/AcO–C(11) (δ(C) 169.7), H–C(13)/AcO–C(13) (δ(C) 170.4), and H–C(7)/OC(O)CHMe$_2$–C(7) (δ(C) 175.6), respectively (*Fig. 2*). The presence of a *doublet* at δ(C) 91.1, but absence of a *triplet*, usually observed at δ(C) 65 – 70, characteristic for CH_2(19), strongly suggested that compound **2** contains a OH group at C(19), in addition to the above-mentioned three ester groups. This assignment was confirmed by the related correlations in the HMBC spectrum (*Fig. 2*). The relative configurations of these ester groups and OH group were evident from the NOESY correlations depicted in *Fig. 3*. In the NOESY experiment, correlations between H_β–C(7) and H_β–C(5), H–C(1) and AcO_α–C(11), and H_β–C(13) and H–C(17) indicated the β-orientations of H–C(7), H–C(11), and H–C(13). Similarly, correlation between H_α–C(19) and H_α–C(20) suggested the α-orientation of H–C(19). Therefore, the structure of yunnanensine B (**2**) was established as (7α,11α,13α,19β)-11,13-bis(acetyloxy)-19-hydroxy-7-(2-methylpropanoyloxy)hetisane.

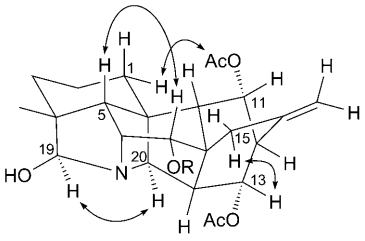

Fig. 3. *Key NOESY* (↔) *correlations of compound* **2**

HR-ESI-MS (positive-ion mode) of yunnanensine C (**3**) exhibited a *pseudo*-molecular-ion peak at *m*/*z* 458.2539 ([M + H]$^+$), corresponding to the molecular formula $C_{26}H_{35}NO_6$. This compound also exhibited characteristic NMR features of a hetisine-subtype C_{20}-diterpenoid alkaloid [2], *i.e*, signals for a tertiary Me group (δ(H) 1.04 (*s*, 3 H); δ(C) 22.7 (*q*)), an exocyclic C=C bond (δ(H) 4.75, 4.94 (2 br. *s*, each 1 H)), an AcO group (δ(H) 2.05 (*s*, 3 H); δ(C), see *Table 1*), and an isobutyryl group (δ(H 1.16, 1.19 (*d*, J = 7.2, each 3 H); δ(C), see *Table 1*). The presence of two O-bearing CH groups (δ(C) 74.1 (*d*); δ(H) 4.30 (br. *d*, J = 8.8, 1 H), and δ(C) 91.5 (*d*); δ(H) 4.71 (*s*, 1 H)) implied that compound **3** possesses two OH groups in addition to the above-mentioned two ester groups. Compound **3** exhibited nearly identical ^{1}H- and ^{13}C-NMR resonances to those of **2**. Differences between the two sets of spectra were consisted in the absence of an AcO group in **3**, thus accounting for the 42 mass units lower [M + H]$^+$ in **3**, as found by mass spectrometry. The signal of H–C(13) was shifted upfield from δ(H) 5.05 in **2** to δ(H) 4.30 in **3**, suggesting that the AcO group at C(13) in **2** is replaced by a OH group in **3**. The structure of yunnanensine C (**3**) was thus deduced as (7α,11α,13α,19β)-11-acetyloxy-13,19-dihydroxy-7-(2-methylpropanoyloxy)hetisane.

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Experimental Part

General. TLC: silica-gel plates; detection by spraying with *Dragendorff*'s reagent. Column chromatography (CC): silica gel (SiO_2; 300–400 mesh, 10–40 μm; *Qindao Marine Chemical, Inc.*, Qingdao, P. R. China). Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet FT-IR 200S* spectrometer. 1D- and 2D-NMR Spectra: *Varian Unity-INOVA-400/54* spectrometers: at 400 and 100, or 200 and 50 MHz, resp.; δ in ppm with TMS as the internal standard. ESI-MS: *Finnigan LCQ*; in *m/z* (rel. %). HR-ESI-MS: *VG Auto Spec 3000* spectrometer.

Plant Material. The whole herbs of *Delphinium yunnanense* FRANCH. were collected in YueQi County, Sichuan Province, P. R. China, in September 2007. The plant was authenticated by Prof. *Q. E. Yang* of the Beijing Institute of Botany of Chinese Academy of Sciences, where a voucher specimen (No. 200709-1) has been deposited.

Extraction and Isolation. Air-dried and powdered whole plants (3.0 kg) of *Delphinium yunnanense* FRANCH. were percolated with 0.1M HCl (30 l). The aq. acidic soln. was basified with 10% aq. $NH_3 \cdot H_2O$ to pH 9–10 and then extracted with AcOEt (3 × 15 l). Removal of the solvent under reduced pressure afforded a total of 8.0 g of crude alkaloids as a yellowish amorphous powder, which was chromatographed over a SiO_2 column, eluting with a cyclohexane/acetone (9:1 → 1:2) gradient system, to give *Frs. A–E*. *Fr. B* (2.08 g) was further subjected to CC (SiO_2; cyclohexane/acetone 8:1 → 2:1) to yield *yunnanenseine A* (**1**; 23 mg), *yunnanenseine B* (**2**; 18 mg), methyllycaconitine (20 mg), 14-deacetylnudicauline (76 mg), anhweidelphinine (23 mg), postanisine F (38 mg), delelatine (26 mg), and delbonine (31 mg). Further purification of *Fr. C* (2.20 g) by CC (silica gel; cyclohexane/acetone 7:1 → 1:1) provided *yunnanenseine C* (**3**, 5 mg), 14-dehydrodecosine (13 mg), deltanine (18 mg), delcosine (21 mg), blacknine (23 mg), browniine (6 mg), and 14-dehydrobrowniine (4 mg). *Fr. D* was subjected to CC (SiO_2; cyclohexane/acetone 5:1 → 1:2) to yield delsemine A (24 mg), delsemine B (7 mg), and lycoctonine (40 mg).

Yunnanenseine A (*=(1α,6β,14α,16β)-20-Ethyl-1,6,14,16-tetramethoxy-7-oxo-8,17-cyclo-7,17-seco-aconitan-18-yl 2-(2β-2-Methylsuccinimide)benzoate=[(1*R,*5*S,*8*S,*10*R,*11*S,*12*R,*13*R,*14*S,*17*S,*18*R*)-3-Ethyl-8,12,14,17-tetramethoxy-16-oxo-3-azahexacyclo[7.6.3.1^{10,13}.0^{1,11}.0^{2,9}.0^{5,18}]nonadec-5-yl]methyl 2-[(3*S*)-3-Methyl-2,5-dioxopyrrolidin-1-yl]benzoate*; **1**). White amorphous powder. $[\alpha]_D^{20} = +27.1$ ($c = 1.0$, $CHCl_3$). IR (KBr): 2935, 1683, 1588, 1522, 1258. NMR: see *Tables 1* and *2*. ESI-MS: 665 ($[M+H]^+$). HR-ESI-MS: 665.3432 ($[M+H]^+$, $C_{45}H_{48}NO_{15}^+$; calc. 665.3438).

Yunnanenseine B (*=(7α,11α,13α,19β)-11,13-Bis(acetyloxy)-19-hydroxy-7-(2-methylpropanoyloxy)hetisane=(7α,11α,13*S*,19*R*)-11,13-Bis(acetyloxy)-19-hydroxyhetisan-7-yl 2-Methylpropanoate*; **2**). White amorphous powder. $[\alpha]_D^{20} = -24.7$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3748, 3556, 2936, 1701, 1152. NMR: see *Tables 1* and *2*. ESI-MS: 499 ($[M+H]^+$). HR-ESI-MS: 499.2565 ($[M+H]^+$, $C_{45}H_{48}NO_{15}^+$; calc. 499.2570).

Yunnanenseine C (*=(7α,11α,13α,19β)-11-(Acetyloxy)-13,19-dihydroxy-7-(2-methylpropanoyloxy)hetisane=(7α,11α,13*S*,19*R*)-11-(Acetyloxy)-13,19-dihydroxyhetisan-7-yl 2-Methylpropanoate*; **3**). White amorphous powder. $[\alpha]_D^{20} = -15.7$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3747, 3553, 2935, 1700, 1150. NMR: see *Tables 1* and *2*. ESI-MS: 500 ($[M+H]^+$). HR-ESI-MS: 457.2539 ($[M+H]^+$, $C_{45}H_{48}NO_{15}^+$; calc. 457.2543).

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