Diterpenoid Alkaloids from *Delphinium tatsienense*

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Three new C_{20}-diterpenoid alkaloids, along with twenty-two known alkaloids, were isolated from the whole herbs of *Delphinium tatsienense*. The new alkaloids include a vakognavine-type C_{20}-diterpenoid alkaloid, designated as tatsienenseine A (**1**), and two hetisine-type C_{20}-diterpenoid alkaloids, designated as tatsienenseines B (**2**) and C (**3**). Their structures were elucidated by IR, HR-ESI-MS, 1D- and 2D-NMR analyses.

Introduction. – Diterpenoid alkaloids are believed to be the major bioactive components of the genus *Delphinium* [1–4], a large genus within the Ranunculaceae family. As a class of structurally complex compounds, they possess a broad range of chemical and pharmacological properties, and also demonstrated a characteristic merit for chemotaxonomic considerations [1–4]. *Delphinium tatsienense* FRANCH grows mainly in southwest Sichuan and northwest Yunnan of China [5]. Previous investigations on the phytochemistry of this plant by the *Pelletier*'s research group resulted in the isolation of more than ten diterpenoid alkaloids [6–10]. Our present study on the whole plants of *Delphinium tatsienense* led to the identification of three new diterpenoid alkaloids, designated as tatsienenseines A–C (**1**–**3**), besides twenty-two known diterpenoid alkaloids, *i.e.*, majusine C [11], delbonine [12], 6-acetyldepheline [13][14], deltatsine [7], browniine [15], 14-acetyldelcosine [15], 14-acetylbrowniine [15], postanisine F [16], ajacine [15], delcosine [17], leroyine 14-*O*-acetate [18], 7-acetylbarbaline [19], acetyldelgrandine [20], deacetylambiguine [6], ajadelphine [21], 14-deacetylnudicauline [22], delsoline [23], barbaline [19], delsemine A [23], delsemine B [23], tatsinine [23], and delgrandine [20]. These known compounds were identified by comparing their spectroscopic data with those reported in the literature. Herein, we described the separation and structural elucidation of the three new alkaloids **1**–**3**.

Results and Discussion. – Tatsienenseine A (**1**; *Fig. 1*) was obtained as an amorphous powder. Its molecular formula was determined as $C_{43}H_{45}NO_{13}$ based on its HR-ESI-MS (*m*/*z* 784.2961 ($[M+H]^+$) and NMR spectra. Its NMR spectra (*Tables 1* and *2*) afforded evidence of a MeN group (δ(H) 2.43 (*s*); δ(C) 33.3 (*q*)), a tertiary Me group (δ(H) 1.02 (*s*); δ(C) 26.2 (*q*)), an exocyclic C=C bond (δ(H) 5.16 and 5.26 (2*s*, each 1 H); δ(C) 141.7 (*s*) and 115.3 (*t*)), four Ac groups (δ (H) 2.00, 2.09, 2.09, and 2.12 (4*s*, each 3 H); δ(C) see *Table 1*), two Bz groups (δ(H) 7.10–7.76 (*m*,

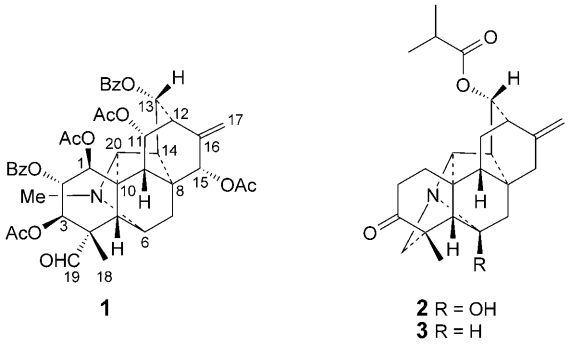

Fig. 1. *Compounds* **1**–**3** *isolated from* Delphinium tatsienense

10 H) (*Table 2*); δ(C) see *Table 1*), and a CH=O group (δ(H) 9.23 (br. *s*); δ(C) 196.0 (*d*)). Its ^{13}C-NMR spectrum, along with the HMQC and DEPT data, displayed four resonances of nonoxygenated quaternary C-atoms at δ(C) 141.7, 56.1, 51.8, and 43.2. These characteristic data, in combination with the biogenetic consideration, suggested that compound **1** should be a vakognavine-type C_{20}-diterpenoid alkaloid [1]. The NMR spectra of **1** are very similar to those of the known vakognavine-type C_{20}-diterpenoid alkaloid majusimine A [11], except for the absence of AcO–C(7) in compound **1**. The difference of 58 mass units in their molecular masses also supports the above-mentioned difference. Furthermore, the remaining four AcO groups at C(1), C(3),

Table 1. *^{13}C-NMR Data* (100 MHz, $CDCl_3$) *of Compounds* **1**–**3**. δ in ppm.

	1	**2**	**3**		**1**	**2**	**3**
C(1)	69.8 (*d*)	54.1 (*t*)	55.7 (*t*)	*Ac*O–C(1)	169.8 (*s*), 21.6 (*q*)		
C(2)	66.5 (*d*)	51.9 (*t*)	51.4 (*t*)	*Ac*O–C(3)	169.6 (*s*), 21.3 (*q*)		
C(3)	65.1 (*d*)	212.6 (*s*)	209.0 (*s*)	*Ac*O–C(11)	170.7 (*s*), 20.9 (*q*)		
C(4)	51.8 (*s*)	42.3 (*s*)	42.2 (*s*)	*Ac*O–C(15)	170.7 (*s*), 20.9 (*q*)		
C(5)	56.6 (*d*)	54.1 (*d*)	50.2 (*d*)	*PhC*OO–C(2):			
C(6)	58.7 (*d*)	98.0 (*s*)	58.2 (*d*)	C=O	164.5 (*s*)		
C(7)	29.5 (*d*)	33.8 (*t*)	25.4 (*t*)	C(1′)	129.0 (*d*)		
C(8)	43.8 (*s*)	45.4 (*s*)	41.1 (*s*)	C(2′,6′)	129.4 (*d*)		
C(9)	49.1 (*d*)	47.9 (*d*)	48.9 (*d*)	C(3′,5′)	128.3 (*d*)		
C(10)	56.1 (*s*)	56.3 (*s*)	47.9 (*s*)	C(4′)	133.3 (*d*)		
C(11)	73.2 (*d*)	38.8 (*t*)	47.7 (*t*)	*PhC*OO–C(13):			
C(12)	44.9 (*d*)	40.8 (*d*)	38.7 (*d*)	C=O	165.7 (*s*)		
C(13)	72.7 (*d*)	72.1 (*d*)	73.5 (*d*)	C(1′)	129.0 (*d*)		
C(14)	39.0 (*d*)	40.9 (*d*)	43.2 (*d*)	C(2′,6′)	129.3 (*d*)		
C(15)	66.0 (*d*)	29.9 (*t*)	35.3 (*t*)	C(3′,5′)	128.2 (*d*)		
C(16)	141.7 (*s*)	145.6 (*s*)	146.4 (*s*)	C(4′)	133.3 (*d*)		
C(17)	115.3 (*t*)	108.4 (*t*)	107.1 (*t*)	^{i}*PrC*OO–C(13):			
C(18)	26.2 (*q*)	30.1 (*q*)	29.7 (*q*)	C=O		176.7 (*s*)	176.2 (*s*)
C(19)	196.0 (*d*)	62.4 (*t*)	63.0 (*t*)	CH		33.8 (*d*)	34.0 (*d*)
C(20)	63.5 (*d*)	66.0 (*d*)	69.0 (*d*)	Me		18.8 (*q*)	19.0 (*q*)
MeN	33.3 (*q*)			Me		19.0 (*q*)	19.0 (*q*)

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

	1	**2**	**3**
H–C(1) or CH_2(1)	5.85 (*d*, $J = 4.0$)	2.33, 2.39 (overlapped)	2.32, 2.68 (overlapped)
H–C(2) or CH_2(2)	5.80 (*t*, $J = 4.0$)	2.44–2.48 (*m*)	2.14, 2.38 (overlapped)
H–C(3)	5.27 (*d*, $J = 4.0$)	–	–
H–C(5)	2.21 (*s*)	2.41 (*s*)	2.46 (*s*)
H–C(6)	3.10 (*d*, $J = 4.0$)	–	2.02 (br. *s*)
CH_2(7)	1.71 (*dd*, $J = 16.0, 4.0$), 2.15 (overlapped)	2.30 (overlapped)	1.92 (*dt*, $J = 10.8, 2.0$)
H–C(9)	2.88 (*d*, $J = 10.0$)	2.23–2.28 (*m*)	2.42–2.46 (*m*)
H–C(11) or CH_2(11)	5.44 (br. *d*, $J = 9.6$)	2.08–2.12, 2.20–2.25 (2*m*)	2.20–2.25, 2.64–2.68 (2*m*)
H–C(12)	2.69 (*d*, $J = 3.0$)	2.13–2.18 (*m*)	2.25–2.30 (*m*)
H–C(13)	5.34 (*dt*, $J = 9.6, 3.0$)	4.81 (*t*, $J = 13.2$)	4.97 (*dt*, $J = 9.6, 2.0$)
H–C(14)	3.23 (*dd*, $J = 10.0, 3.0$)	2.19–2.23 (*m*)	2.08–2.13 (*m*)
H–C(15) or CH_2(15)	5.78 (*s*)	1.23, 1.96 (*AB*, $J = 13.2$)	2.40–2.44, 2.24–2.28 (2*m*)
CH_2(17)	5.16, 5.26 (2*s*)	4.64, 4.79 (2*s*)	4.70, 4.87 (2 br. *s*)
Me(18)	1.02 (*s*)	1.38 (*s*)	1.49 (*s*)
H–C(19) or CH_2(19)	9.23 (*s*)	2.09, 3.11 (*AB*, $J = 12.0$)	1.73, 2.60 (*AB*, $J = 12.4$)
H–C(20)	3.91 (*s*)	2.20 (*s*)	2.12 (*s*)
MeN	2.43 (*s*)		
AcO–C(1)	2.00 (*s*)		
AcO–C(3)	2.12 (*s*)		
AcO–C(11)	2.09 (*s*)		
AcO–C(15)	2.09 (*s*)		
PhCOO–C(2):			
H–C(2′,6′)	7.76 (*d*, $J = 7.6$)		
H–C(3′,5′)	7.10 (*t*, $J = 7.6$)		
H–C(4′)	7.35 (*t*, $J = 7.6$)		
PhCOO–C(13):			
H–C(2′,6′)	7.53 (*d*, $J = 7.2$)		
H–C(3′,5′)	7.29 (*t*, $J = 7.2$)		
H–C(4′)	7.46 (*t*, $J = 7.2$)		
^{i}PrCOO–C(13):			
CH		2.30–2.35 (*m*)	2.30–2.35 (*m*)
Me		1.08 (*d*, $J = 7.2$)	1.21 (*d*, $J = 7.2$)
Me		1.10 (*d*, $J = 7.2$)	1.22 (*d*, $J = 7.2$)

C(11), and C(15) in compound **1** were apparent from the HMBCs H–C(1)/*Ac*O–C(1) (δ(C) 169.8), H–C(3)/*Ac*O–C(3) (δ(C) 169.6), H–C(11)/*Ac*O–C(11) (δ(C) 170.7), and H–C(15)/*Ac*O–C(15) (δ(C) 170.7) (*Fig. 2*). Two Bz groups at C(2) and C(13) could be supported by the HMBCs H–C(2)/*Bz*O–C(2) (δ(C) 164.5) and H–C(13)/*Bz*O–C(13) (δ(C) 165.7) (*Fig. 2*). In addition, the relative configurations of these ester groups were evident from the NOESY correlations summarized in *Fig. 3*. The NOEs H_α–C(1)/H–C(20) and H_α–C(3)/H_α–C(1) indicated the α-orientation of H–C(1) and H–C(3); similarly, the NOEs H_β–C(2)/H_β–C(5), H_α–C(1)/AcO–C(11), H_β–C(13)/H–C(17), H_β–C(13)/AcO–C(15), and H_β–C(15)/H_β–C(9), suggested the β-orienta-

tion of H–C(2), H–C(11), H–C(13), and H–C(15). Therefore, the structure of tatsienenseine A (**1**) was assigned as (1β,2α,3β,11α,13α,15α)-1,2,3,11,13,15-hexahydroxy-21-methyl-19,21-secohetisan-19-al 1,3,11,15-tetraacetate 2,13-dibenzoate.

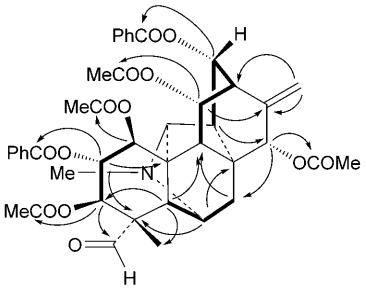

Fig. 2. *Key $^1H,^1H$–COSY* (—) *and HMBC* (H → C) *features of* **1**

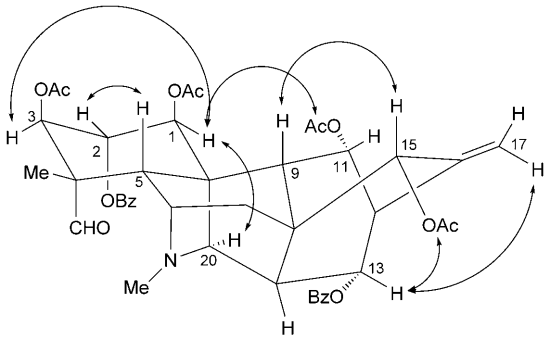

Fig. 3. *Key NOESY* (H ↔ H) *correlations of* **1**

Tatsienenseine B (**2**; *Fig. 1*) was isolated as an amorphous powder, and its molecular formula was determined as $C_{24}H_{31}NO_4$ according to the HR-ESI-MS and NMR. Compound **2** exhibited characteristic NMR features (*Tables 1* and *2*) of a C_{20}-diterpenoid alkaloid [1] bearing a tertiary Me group (δ(H) 1.38 (*s*); δ(C) 30.1 (*q*)), an exocyclic C=C bond (δ(H) 4.64 and 4.79 (2*s*, each 1 H); δ(C) 108.4 (*t*) and 145.6 (*s*)), an isobutyryl group (δ(H) 1.08 and 1.10 (*d*, J = 7.2 Hz, each 3 H); δ(C) see *Table 1*), and a ketone carbonyl group (δ(C) 212.6 (*s*)). The resonances of four nonoxygenated quaternary C-atoms in the ^{13}C NMR spectrum (δ(C) 42.3, 45.4, 56.3, and 145.6 (4*s*)) are indicative of the 'finger-print' characteristics of a hetisine-type C_{20}-diterpenoid alkaloid [1]. The existence of an oxygenated quaternary C-atom at δ(C) 98.0 strongly suggested that compound **2** possesses an OH group at C(6) in addition to the above-mentioned ester. This assignment was supported by the HMBCs C(6)/H–C(7) and C(6)/H–C(5) (*Fig. 4*). The isobutyryloxy group could be positioned at C(13) due to the HMBC H–C(13)/Me_2CHCOO (δ(C) 176.7). The ketone carbonyl was located at C(3) based on the HMBCs H–C(1)/C(3), H–C(5)/C(3), Me(18)/C(3), and CH_2(19)/C(3). In the NOESY plot, the correlations H–C(15)/H–C(13) suggested the β-orientation of

H–C(13). Accordingly, the structure of compound **2** was elucidated as (6β,13α)-6-hydroxy-3-oxohetisan-13-yl 2-methylpropanoate, named tatsienenseine B.

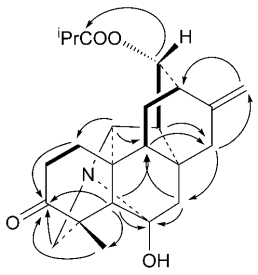

Fig. 4. *Key* $^1H,^1H$*–COSY* (—) *and HMBC* (H → C) *features of* **2**

Tatsienenseine C (**3**; *Fig. 1*) gave a molecular formula $C_{24}H_{31}NO_3$, as determined by HR-ESI-MS (*m*/*z* 382.2378 ([M + H]$^+$)) and supported by NMR data. It showed similar NMR spectroscopic patterns to those of **2** (*Tables 1* and *2*), except for H–C(6) and C(6), indicating that both compounds are of the same type of C_{20}-diterpenoid alkaloids and that the only difference between these two compounds is the absence of an OH group at C(6) in compound **3**. This hypothesis was further evidenced by the disappearance of OH absorptions in the IR spectrum of **3** and the difference of 16 mass units in **3** found by mass spectroscopy. The proposed structure of compound **3** was also supported by 2D-NMR experiments, and the unambiguous assignments of the ^{1}H- and ^{13}C-NMR data of **3** were completed by 2D-NMR techniques (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY). Thus, the structure of tatsienenseine C (**3**) was elucidated as (13α)-3-oxohetisan-13-yl 2-methylpropanoate.

It is uncommon for the hetisine-type diterpenoid alkaloids to possess a ketone C=O group at the C(3) position. Following majusidine B [10], tatsienenseines B and C represent the second and third examples of this type of alkaloids.

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Experimental Part

General. TLC: silica gel plates; detection by spraying with *Dragendorff*'s reagent. Column chromatography (CC): silica gel (300–400 mesh, 10–40 μm; *Qingdao 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 400/100 or 200/50 MHz, resp.; δ in ppm with $SiMe_4$ as the internal standard; *J* in Hz. ESI-MS: *Finnigan-LCQ* spectrometer; in *m*/*z* (rel.%). HR-ESI-MS: *VG-Auto-Spec-3000* spectrometer.

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

Extraction and Isolation. Air-dried and powdered whole herbs (3.0 kg) were percolated with 0.1M HCl (40 l). The acid aq. soln. was basified with 10% aq. NH_4OH soln. to pH 9 and then extracted with AcOEt (3 × 20 l). Evaporation of the solvent afforded the total crude alkaloids (12.1 g) as a yellowish amorphous powder, which was subjected to CC (silica gel, cyclohexane/acetone 8:1 → 1:2): *Frs. A* (94 mg), *B* (399 mg), *C* (2.3 g), *D* (2.2 g), *E* (2.1 g), *F* (1.3 g), and *G* (2.8 g). *Fr. B* was further subjected

to CC (silica gel, cyclohexane/acetone 8:1 → 4:1): *majusine C* (68 mg), *delbonine* (29 mg), and *6-acetyldelpheline* (94 mg). CC of *Fr. C* (silica gel, cyclohexane/acetone 7:1 → 2:1) provided *deltatsine* (300 mg), *browniine* (50 mg), *14-acetyldelcosine* (10 mg), *14-acetylbrowniine* (9 mg), *tatsienenseine A* (**1**; 33 mg), and *postanisine F* (68 mg). *Fr. D* was subjected CC (silica gel, cyclohexane/acetone 5:1 → 1:2): *ajacine* (45 mg), *delcosine* (10 mg), *leroyine 14*-O-*acetate* (10 mg), *7-acetylbarbaline* (10 mg), *acetyldelgrandine* (10 mg), and *deacetylambiguine* (10 mg). Separation of *Fr. E* by CC (silica gel *H*, $CHCl_3$/MeOH 80:1 → 20:1) yielded *ajadelphine* (16 mg) and *14-deacetylnudicauline* (18 mg). Separation of *Fr. F* by CC (silica gel, $CHCl_3$/MeOH 80:1 → 10:1) yielded *delsoline* (28 mg), *barbaline* (7.9 mg), *tatsienenseine B* (**2**; 16 mg), and *tatsienenseine C* (**3**; 9 mg). Further purification of *Fr. G* by CC (silica gel, $CHCl_3$/MeOH 80:1 → 10:1) gave *delsemine A* (16 mg), *delsemine A* (12 mg), *tatsinine* (14 mg), and *delgrandine* (20 mg).

Tatsienenseine A (=*(1β,2α,3β,11α,13α,15α)-1,2,3,11,13,15-Hexahydroxy-21-methyl-19,21-secohetisan-19-al 1,3,11,15-Tetraacetate 2,13-Dibenzoate*; **1**): White amorphous powders. $[\alpha]_D^{20} = +27.1$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3736, 2932, 1748, 1541, 1232. NMR: *Tables 1* and *2*. ESI-MS: 784 ($[M+H]^+$). HR-ESI-MS: 784.2961 ($[M+H]^+$, $C_{43}H_{46}NO_{13}^+$; calc. 784.2969).

Tatsienenseine B (=*(6β,13α)-6-Hydroxy-3-oxohetisan-13-yl 2-Methylpropanoate*; **2**): White amorphous powder. $[\alpha]_D^{20} = +21.9$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3726, 2932, 1746, 1236. NMR: *Tables 1* and *2*. ESI-MS: 398 ($[M+H]^+$). HR-ESI-MS: 398.2326 ($[M+H]^+$, $C_{24}H_{32}NO_4^+$; calc. 398.2331).

Tatsienenseine C (=*(13α)-3-Oxohetisan-13-yl 2-Methylpropanoate*; **3**): White amorphous powders. $[\alpha]_D^{20} = +9.9$ ($c = 1.0$, $CHCl_3$). IR (KBr): 2939, 1748, 1233. NMR: *Tables 1* and *2*. ESI-MS: 382 ($[M+H]^+$). HR-ESI-MS: 382.2378 ($[M+H]^+$, $C_{24}H_{32}NO_3^+$; calc. 382.2382).

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