New C_{19}-Diterpenoid Alkaloids from *Delphinium trifoliolatum*

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Three new norditerpenoid alkaloids, trifoliolasines A (1), B (3), and C (5), were isolated from the whole plant of *Delphinium trifoliolatum* FINET *et* GAGNEP, and their structures were established based on the spectral data.

Key words *Delphinium trifoliolatum*; C_{19}-diterpenoid alkaloid; trifoliolasine A; trifoliolasine B; trifoliolasine C

The plant *Delphinium trifoliolatum* FINET *et* GAGNEP (Ranunculaceae) grows widely in southeastern Sichuan province and in western Hubei province in China at an elevation of 1600 m.[1)] To our knowledge, no phytochemical investigation of this plant has previously been undertaken. In the course of our comparative studies of diterpenoid alkaloids from *Aconitum* and *Delphinium* species, three new C_{19}-diterpenoid alkaloids, trifoliolasine A (**1**), trifoliolasine B (**3**), and trifoliolasine C (**5**), were isolated from the whole plants of *Delphinium trifoliolatum*. This paper describes the isolation and structural elucidation of these new alkaloids.

Results and Discussion

Trifoliolasine A (**1**) was isolated as a colorless granule crystal with mp 125—127 °C. The HR-ESI-MS showed $[M+H]^+$ at *m/z* 643.3595, corresponding to the pseudo molecular formula $C_{35}H_{51}N_2O_9$ $[M+H]^+$, which requires *m/z* 643.3594. The NMR spectra of trifoliolasine A (**1**) gave distinctive signals at δ_H 1.08 (3H, t, J=7.2 Hz), δ_C 14.0 q, and δ_C 50.9 t, for the *N*-ethyl group, δ_H 3.26, 3.30 and 3.39 (s, each 3H), δ_C 55.7 q, 57.9 q and 58.5 q for three methoxyl groups, δ_H 5.75 (2H, br s) and 6.68—7.83 (4H, m), δ_C see Table 1 for an anthranoyl group, as well as δ_H 1.18 (6H, d, J=7.2 Hz), δ_C see Table 1 for an isobutyryl group. The ^{13}C signals of seven oxygenated carbons at δ_C 68.4 t, 75.4 d, 77.3 s, 82.2 d, 83.9 d, 88.3 s, and 90.6 d suggested that **1** had two hydroxyl groups in addition to three methoxyl groups and two ester groups. Inspection of the NMR data (^1H, ^{13}C, DEPT, HMQC, and HMBC) indicated a lycoctonine-type C_{19}-diterpenoid alkaloid.[2,3)] Comparison of the ^{13}C-NMR data of **1** with those of jiufengdine (**2**)[4)] indicated that the only difference was that **1** had an isobutyryl group at C-14 instead of the 2-methylbutyryl group in **2** (see Table 1). This result was also suggested by the difference of 14 mass units between the two compounds in their MS spectra. The existence of a C-14 isobutyryl ester was confirmed by the long-range correlations between the proton signal of H-14 and the ester carbonyl carbon signal at δ 177.2 (C-1″) in the HMBC spectrum. These observations led to the assignment of the structure of trifoliolasine A as **1**.

Trifoliolasine B (**3**) was obtained as an amorphous powder, mp 103—105 °C. The pseudo molecular formula $C_{36}H_{51}N_3O_{10}$ was inferred from its HR-ESI-MS and ^{13}C-NMR. The $^1H(^{13}C)$-NMR spectra of trifoliolasine B (**3**) exhibited characteristic NMR features of a lycoctonine-type norditerpenoid alkaloid[2,3)] bearing an *N*-ethyl (δ_H 1.08, 3H, t, J=7.2 Hz, δ_C 14.1 q, 51.0 t), three methoxyl (δ_H 3.26, 3.37, 3.39, each 3H, s; δ_C 55.9 q, 56.4 q, 58.3 q) and a substituted anthranoyl (δ_H 11.08, 1H, s, NH; 7.11—8.68, 4H, m; 5.57, 6.09, each 1H, s, NH_2, 1.26, 3H, d; δ_C see Table 2). The ^1H triplet (J=4.8 Hz) signal at δ_H 4.00 was assigned to H-14β, suggesting the presence of an OH-14α.[2,3)] The ^{13}C-NMR spectra of **3** and delsemine B (**4**)[5)] are very close except that the chemical shift of C-14 in the ^{13}C-NMR spectra of **3** (Table 2) was shifted upfield from 83.9 to 75.2. Further comparison of the NMR and MS data of **3** with those of **4** showed that **3** differed from **4** by the presence of a hydroxyl group in place of a methoxyl group at C-14. The structure of **3**, thus, was determined to be trifoliolasine B. In addition, the stereochemistry of the methylsuccinimide moiety in methyllycaconitine has been assigned as "*S*" by Blagbrough and his co-workers.[6)] Therefore, the stereochemistry of C-3‴ in **3** could be deduced as "*S*" based on comparison of the ^{13}C-NMR data with delsemine B (**4**).

The pseudo molecular formula of trifoliolasine C (**5**) ($C_{40}H_{57}N_3O_{11}$) was determined by HR-ESI-MS. The NMR data strongly suggested a lycoctonine-type C_{19}-diterpenoid alkaloid for trifoliolasine C (**5**).[2,3)] An *N*-ethyl group, three methoxyl groups, a substituted anthranoyl group and an isobutyryl group were present in its $^1H(^{13}C)$-NMR Spectra (see Experimental and Table 2). The ^{13}C-NMR spectrum of **5** and trifoliolasine B (**3**) are very similar except that **5** had an additional isobutyryl group. This extra isobutyryl functional group could be assigned to C-14 due to the triplet (J=4.8 Hz) signal at δ_H 4.75 (H-14β) in the ^1H-NMR spectrum of **5**.[2,3)] The above findings led us to determine the structure of trifo-

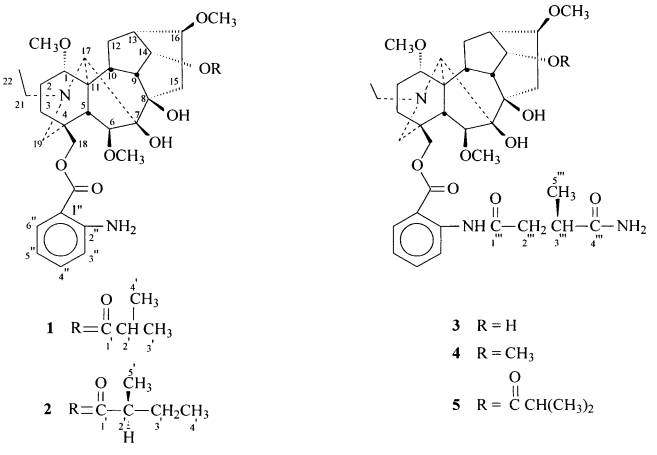

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Table 1. NMR Data of Trifoliolasine A (**1**) and Jiufengdine (**2**)

No.	**1**			**2**
	δ_H (J=Hz)	δ_C	HMBC (H→C)	δ_C
1	3.01 dd (7.2, 5.2)	83.9 d	C-2, C-10, C-11, C-17, 1-OCH_3	83.9
2	2.08 m (α)	26.0 t	C-1, C-4, C-11	26.0
	2.16 m (β)		C-1, C-3, C-4	
3	1.59 dd (14.4, 6.0) (α)	32.1 t	C-2, C-4, C-19	32.2
	1.74 m (β)		C-1, C-2, C-4, C-18	
4	—	37.5 s	—	37.7
5	1.81 s	50.0 d	C-1, C-4, C-10, C-11, C-17, C-19	50.1
6	3.91 s	90.6 d	C-4, C-7, C-8, C-11, 6-OCH_3	90.7
7	—	88.3 s	—	88.4
8	—	77.3 s	—	77.0
9	3.17 dd (10.0, 7.2)	42.9 d	C-8, C-10, C-12, C-13, C-14	43.1
10	2.04 m	45.6 d	C-8, C-9, C-11	45.7
11	—	48.9 s	—	49.0
12	1.89 m (β)	28.1 t	C-10, C-11, C-13, C-16	28.2
	2.46 m (α)		C-10, C-11, C-13, C-14, C-16	
13	2.42 m	37.7 d	C-9, C-10, C-14, C-16	37.6
14	4.80 t (4.8)	75.4 d	C-8, C-9, C-13, C-16, C-1′	75.3
15	1.56 dd (13.6, 5.2) (β)	33.7 t	C-7, C-8, C-16	33.7
	2.64 dd (13.6, 9.2) (α)		C-7, C-8, C-9, C-13, C-16	
16	3.26 (hidden)	82.2 d	C-12, C-13, C-14, C-15, 16-OCH_3	82.2
17	2.96 d (2.4)	64.4 d	C-6, C-8, C-10, C-11, C-19	64.4
18	4.10 d (11.6)	68.4 t	C-3, C-4, C-19, CO	68.5
	4.15 d (11.6)		C-3, C-4, C-19, CO	
19	2.43 ABq (10.4, hidden)	52.3 t	C-4, C-17	52.4
	2.73 ABq (10.4)		C-3, C-4, C-17	
21	2.82 m	50.9 t	C-22	51.0
	2.93 m		C-22	
22	1.08 t (7.2)	14.0 q	C-21	14.0
1-OCH_3	3.26 s	55.7 q	C-1	55.7
6-OCH_3	3.39 s	57.9 q	C-6	58.0
16-OCH_3	3.30 s	55.8 q	C-16	55.8
1′	—	177.2 s	—	176.8
2′	2.57 m	34.1 d	C-1′, C-3′	41.2
3′	1.18 d (7.2)	18.7 q	C-1′, C-2′, C-3′, C-5′	26.2
4′	1.18 d (7.2)	18.8 q	C-1′, C-2′, C-4′, C-5′	
CO	—	167.7 s	—	167.7
1″	—	110.2 s	—	110.3
2″	—	150.7 s	—	150.7
3″	6.67 dd (8.0, 1.2)	116.7 d	C-1″, C-2″, C-4″, C-5″	116.7
4″	6.67 ddd (8.0, 7.2, 1.2)	134.3 d	C-2″, C-6″	134.3
5″	7.29 ddd (8.0, 7.6, 1.6)	116.3 d	C-1″, C-3″, C-4″, C-6″	116.3
6″	7.80 dd (7.8, 1.2)	130.6 d	CO, C-2″, C-4″, C-5″	130.6
2″-NH_2	5.75 br s		C-1″, C-3″	
8-OH	3.81 s		C-8, C-15	

$CDCl_3$, ^{1}H: 400 MHz; ^{13}C: 100 MHz.

liolasine C as (**5**).

Experimental

General Experimental Procedures Melting points were determined by thermal values analysis with a microscope (uncorrected). 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 INOVA 400/45 NMR spectrometer, in $CDCl_3$ with TMS as the internal standard. FAB-MS and HR-ESI-MS were recorded on a VG Auto Spec 3000 or Finnegan MAT 90 instrument. Silica gel GH_{254} and H (Qindao Sea Chemical Factory, China) were used for TLC, Chromatotron and CC, respectively. Spots on TLC were detected with modified Dragendorff's reagent. A polyvinyl sulfonic ion exchange resin (H-form, cross linking 1×1, Chemical Factory of Nankai University, China) was used in the extraction of crude alkaloids.

Plant Material The plant *Delphinium trifoliolatum* was collected in Nanchuan county Chongqing City, China, in July 2001. The plant was identified by Professor W. T. Wang of the Beijing Institute of Botany, Chinese Academy of Sciences, where a voucher specimen has been deposited.

Extraction and Isolation Dried whole plants of *Delphinium trifoliolatum* Finet *et* Gagnep (5.0 kg) were milled and percolated with 0.05 mol/l HCl (75 l). Wet resin (dry weight 1.2 kg) was added to the percolates, followed by washing repeatedly on a suction filter with deionized water. The air-dried resin was well mixed with 10% aqueous NH_4OH (4 l) and extracted in a specially designed extractor[7] with Et_2O (7000 ml) and $CHCl_3$ (4000 ml) under reflux until no alkaloid could be detected, to furnish crude alkaloids I (23 g) and II (15 g). Further extraction of the resin with 95% EtOH (2000 ml) provided a brownish residue which was dissolved in 5% HCl and filtered. The filtrate was alkalized to pH 11 with concentrated NH_4OH and extracted with chloroform. Evaporation of the organic solvents gave crude alkaloid III (2.6 g).

Crude alkaloids I, II and III were combined and subjected to column chromatography eluting with increasing polarity of the $CHCl_3$–MeOH mixtures to afford nine parts (A—I). Part A was chromatographed over silica gel H (100 mg) eluting with petroleum ether–acetone (4 : 1) to provide fractions A-1 (474 mg), A-2 (473 mg), A-3 (392 mg) and A-4 (365 mg). Fraction A-1 was further separated repeatedly on a Chromatotron eluting with petroleum ether–ethyl acetate–acetone (8 : 1 : 1) and cyclohexane–ethyl acetate–acetone

Table 2. The ^{13}C-NMR Data of Compounds **3**—**5**

No.	**3**	**4**[a)]	**5**	No.	**3**	**4**[a)]	**5**
1	84.7 d	83.9	84.1 d	1-OCH_3	55.9 q	55.7	55.7 q
2	25.2 t	26.1	25.9 t	6-OCH_3	58.3 q	57.8	58.1 q
3	32.1 t	32.2	32.1 t	14-OCH_3	—	58.1	—
4	37.8 s	37.6	37.5 s	16-OCH_3	56.4 q	56.3	55.9 q
5	45.0 d	43.3	42.9 d	1′	—	—	177.3 s
6	90.4 d	91.0	90.3 d	2′	—	—	34.1 d
7	89.1 s	88.6	88.3 s	3′	—	—	18.8 q
8	76.2 s	77.5	77.3 s	4′	—	—	18.9 q
9	50.4 d	50.5	50.5 d	CO	167.9 s	168.1	167.8 s
10	36.3 d	38.2	37.7 d	1″	114.7 s	114.7	114.8 s
11	48.3 s	49.1	48.9 s	2″	141.4 s	141.9	141.6 s
12	27.4 t	28.7	28.2 t	3″	120.5 d	120.7	120.3 d
13	45.9 d	46.1	45.6 d	4″	134.8 d	134.9	134.5 d
14	75.2 d	83.9	75.5 d	5″	122.7 d	122.5	122.5 d
15	33.0 t	33.7	33.7 t	6″	130.3 d	130.3	130.8 d
16	81.6 d	82.6	82.1 d	1‴	170.5 s	170.0	170.5 s
17	64.9 d	64.5	64.7 d	2‴	41.8 t	41.4	41.8 t
18	69.7 t	69.8	69.5 t	3‴	39.1 d	39.0	39.3 d
19	52.3 t	52.4	52.2 t	4‴	177.6 s	177.8	177.3 s
21	51.0 t	50.9	51.1 t	5‴	17.6 q	17.9	18.2 q
22	14.1 q	14.0	14.0 q				

a) $CDCl_3$.

(5 : 1 : 1), successively, to give trifoliolasine A (**1**, 26 mg). In addition, chromatography of part B with petroleum ether–acetone–diethylamine (100 : 20 : 1—90 : 30 : 1) as an eluent yielded fractions B-1 (590 mg), B-2 (800 mg), B-3 (550 mg), B-4 (782 mg) and B-5 (240 mg). Fraction B-2 was further chromatographed on silica gel H eluting with petroleum ether–acetone–diethylamine (100 : 20 : 1) and recrystallized from petroleum ether–acetone to provide trifoliolasine A (**1**, 600 mg). Chromatography of fraction B-5 with petroleum ether–acetone–diethylamine (60 : 30 : 1) as an eluent furnished trifoliolasine B (**3**, 20 mg). Column chromatography of fraction B-3 eluting with petroleum ether–acetone–diethylamine (90 : 30 : 1), followed by purification over semipreparative HPLC (RP-C18, 10 μm, 1.0×20 cm, motive phase: MeOH–H_2O 75 : 25; Water's 2410 refraction Detector) produced trifoliolasine C (**5**, 6 mg).

Trifoliolasine A (**1**): Colorless granule crystals, mp 125—127 °C; $[\alpha]_D^{20}$ +44.2° (c=0.53, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3457, 3371, 2966, 2930, 2873, 2820, 1723, 1711, 1690, 1619, 1590, 1563, 1487, 1482, 1384, 1319, 1295, 1243, 1193, 1161, 1088, 1029, 983, 953, 860, 751, 702, 669, 637, 529; ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1; FAB-MS m/z (%): 643 $[M+1]^+$, (100); HR-ESI-MS $[M+H]^+$ m/z: 643.3595, calcd for $C_{35}H_{51}N_2O_9$ $[M+H]^+$, 643.3594.

Trifoliolasine B (**3**): White amorphous powder, mp 103—105 °C; $[\alpha]_D^{20}$ +36.6° (c=0.48, $CHCl_3$). cm^{-1}: 3437, 2928, 2857, 1682, 1605, 1587, 1525, 1448, 1386, 1294, 1252, 1190, 1162, 1088, 982, 952, 757, 701, 667, 602, 521; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.08 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{CH_3}$), 1.26 (3H, d, J=7.0 Hz, $CH\underline{CH_3}$), 3.26, 3.37, 3.39 (each 3H, s, 3×OCH_3), 4.00 (1H, t, J=4.8 Hz, H-14β), 5.57, 6.09 (each 1H, br s, NH_2), 7.13 (1H, dd, J=8.0, 8.0, 1.2 Hz, H-5″), 7.56 (1H, ddd, J=7.6, 7.6, 1.2 Hz, H-4″), 7.97 (1H, dd, J=8.0, 1.6 Hz, H-3″), 8.67 (1H, dd, J=7.6, 1.2 Hz, H-6″), 11.08 (1H, s, $\underline{NH}$CO); ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 2; FAB-MS m/z (%): 686 $[M+1]^+$ (100), 557 (7), 452 (8), 391 (17), 227 (17), 149 (7), 114 (5). 74 (66); HR-ESI-MS $[M+1]^+$ m/z: 686.3654, calcd for $C_{36}H_{52}N_3O_{10}$ $[M+1]^+$, 686.3652.

Trifoliolasine C (**5**): White amorphous powder, mp 117—118 °C; $[\alpha]_D^{20}$ +24.0° (c=0.3, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3465, 2964, 2931, 1722, 1685, 1606, 1589, 1527, 1448, 1384, 1296, 1259, 1193, 1162, 1143, 1089, 956, 802, 759, 702, 671; ^{1}H-NMR (200 MHz, $CDCl_3$). δ: 1.10 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{CH_3}$), 1.16 (6H, d, J=7.0 Hz, $CH(\underline{CH_3})_2$), 1.25 (3H, d, J=6.4 Hz, $CH\underline{CH_3}$), 3.25, 3.28, 3.36 (each 3H, s, 3×OCH_3), 4.75 (1H, t, J=4.8 Hz, H-14β), 5.35, 5.93 (each 1H, br s, NH_2), 7.08 (1H, t, J=7.8 Hz, H-5″), 7.55 (1H, t, J=7.8 Hz, H-4″), 7.95 (1H, d, J=8.0 Hz, H-3″), 8.67 (1H, d, J=8.0 Hz, H-6″), 11.0 (1H, s, $\underline{NH}$CO); ^{13}C-NMR (50 MHz, $CDCl_3$): see Table 2; FAB-MS m/z (%): 756 $[M+1]^+$ (100), 522 (85), 265 (18), 114 (7); HR-ESI-MS m/z: 756.4085 $[M+1]^+$, calcd for $C_{40}H_{58}N_3O_{11}$ $[M+1]^+$, 756.4071.

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