Notes

C_{20}-Diterpenoid Alkaloids from *Delphinium trifoliolatum*

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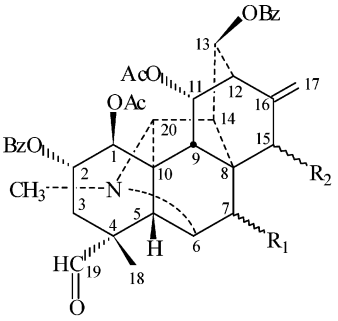
Three new C_{20}-diterpenoid alkaloids, trifoliolasines D−F (**1**−**3**), were isolated from the aerial parts of *Delphinium trifoliolatum*, and their structures were determined by the interpretation of spectroscopic data and by the single-crystal X-ray crystallographic analysis of **1**.

The roots of *Delphinium trifoliolatum* Finet et Gagnep (Ranunculaceae)[1] are used in Chinese traditional medicine for the treatment of rheumatism and neuralgia. In a continuation of our research on this plant, three new C_{20}-diterpenoid alkaloids, trifoliolasines D−F (**1**−**3**), were isolated. In this paper, we report the separation and structure elucidation of these new alkaloids and also propose the occurrence of a transannular effect in the vakognavine-type C_{20}-diterpenoid alkaloids.

Alkaloids **1**−**3** were assigned the molecular formulas of $C_{43}H_{45}NO_{13}$, $C_{39}H_{41}NO_{11}$, and $C_{39}H_{41}NO_{10}$, respectively, as calculated from their HRESIMS. Their NMR and mass spectra showed that they were vakognavine-type C_{20}-diterpenoid alkaloids.[2]



		R_1	R_2
1	trifoliolasine D	β-OAc	α-OAc
2	trifoliolasine E	β-OH	α-OH
3	trifoliolasine F	α-OH	H

Trifoliolasine D (**1**) was isolated as colorless sheet crystals (acetone−cyclohexane) with mp 238−240 °C. The HRESIMS at *m/z* 784.2962 corresponded to the protonated molecular ion $[M + H]^+$ ($C_{43}H_{46}NO_{13}$). The NMR spectra of **1** showed the presence of one NCH_3 group (δ_H 2.48, 3H, s; δ_C 33.5 q) and one $C-CH_3$ group (δ_H 1.03, 3H, s; δ_C 26.0 q), an aldehyde group (δ_H 9.05, 1H, brs; δ_C 186.4 d), an exocyclic double bond (δ_H 5.10, 5.26, each 1H, d, J = 2.0 Hz), four acetyl groups (δ_H 2.01, 2.10, 2.10, 2.13, each 3H, s; δ_C 169.5 s, 20.7 q; 169.7 s, 20.8 q; 170.6 s, 21.2 q; 170.6 s, 21.3 q), and two benzoyl groups (δ_H 7.10−7.76, 10H, m; δ_C see Table 2). The ^{13}C NMR signals of six oxygenated carbons at δ_C 69.7, 66.4, 65.0, 73.1, 72.6, and 65.9 could be assigned only at C-1, C-2, C-7, C-11, C-13, and C-15 as a result of HMQC data and HMBC correlations of H-1 (δ_H 5.86), H-2 (δ_H 5.80), H-7 (δ_H 5.28), H-11 (δ_H 5.45), H-13 (δ_H 5.35), and H-15 (δ_H 5.76) with their geminal ester carbonyl carbons OAc-1 (δ_C 169.7), OBz-2 (δ_C 165.6), OAc-7 (δ_C 169.5), OAc-11 (δ_C 170.6), OBz-13 (δ_C 164.5), and OAc-15 (δ_C 170.6) (Figure 1), respectively. In addition, all the ^1H and ^{13}C NMR signals for **1** could be assigned unambiguously (Tables 1 and 2) on the basis of 2D NMR (HMQC, $^1H-^1H$ COSY, HMBC) observations (Figure 1). However, no ester group could be assigned at C-3 due to the $^1H-^1H$ COSY interaction between H-1 (δ_H 5.86 d) and H-2 (δ_H 5.80 d), H-2 (δ_H 5.80 d), and H-3 (δ_H 1.72 dd, 2.05 d) (Figure 1). Similarly, a signal at δ_C 59.0 (d) was attributed to C-6 mainly on the basis of the correlations between H-6 (δ_H 3.15, d, J = 3.6 Hz) and C-5 (δ_C 56.4 d), C-8 (δ_C 51.7 s) in the HMBC spectrum of **1**. Owing to the presence of so many ester groups and their complex strereochemistry, X-ray diffraction analysis was needed to determine the structure and relative stereochemistry of trifoliolasine D as **1** (Figure 2).

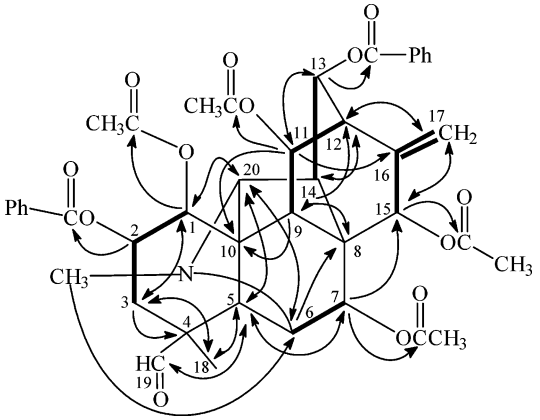


Figure 1. (—) $^1H-^1H$ COSY correlations (*W*-type coupling: H-7/H-9, H-12/H-14) and (↷) selected HMBC correlations of **1** (H→C) ($CDCl_3$).

The HRESIMS of **2** exhibited a protonated molecular ion peak at *m/z* 700.2755 (calcd 700.2757) corresponding to a molecular formula of $C_{39}H_{41}NO_{11}$ (84 mass units lower than

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Table 1. ^{1}H NMR Data of Trifoliolasines D−F (**1**−**3**) [400 MHz for ^{1}H, δ_H mult (J = Hz)]

	1	**2**	**3**
position	$CDCl_3$	$CDCl_3$+CD_3OD	$CDCl_3$
1	5.86 d (4.0)	5.93 d (3.2)	5.91 d (3.6)
2	5.80 d (3.2)	5.76 m	5.78 dd (6.4, 3.6)
3	1.72 dd (15.6, 3.6) (β)	1.81 dd (16.0, 4.0) (β)	1.71 dd (16.0, 3.6) (β)
	2.05 t (15.6) (α)	2.01 s (α)	2.01 m (α)
5	2.23 s	2.54 s	1.94 s
6	3.15 d (3.6)	3.18 d (4.0)	3.27 br.s
7	5.28 d (3.6)	4.37 d (4.0)	3.76 d (1.5)
9	2.90 dd (9.6, 2.4)	2.97 dd (9.2, 2.0)	2.41 dd (9.6, 2.0)
11	5.45 d (9.6)	5.35 dd (10.0, 2.4)	5.49 d (9.6)
12	2.69 d (2.0)	2.67 d (2.4)	2.56 d (2.4)
13	5.35 dt (10.0, 2.0)	5.37 d (9.6)	5.32 dt (10.0, 2.0)
14	3.25 dd (10.0, 2.0)	3.26 dt (10.0, 2.0)	3.32 dd (9.6, 2.0)
15	5.76 d (2.0)	4.58 t (2.0)	2.83 dt (18.0, 1.0) (α)
			2.18 m (β)
17	5.10 d (2.0), 5.26 d (2.0)	5.26 d (2.4), 5.41 d (1.2)	4.89 br.s, 5.04 br s
18	1.03 s	1.07 s	1.06 s
19	9.05 br s	8.93 br s[a]	8.87 s
20	3.93 s	3.90 s	3.92 s
N-CH_3	2.48 s	2.47 s	2.61 s
OAc	2.10 s (1)	2.02 s (OAc-1)	2.05 s (OAc-1)
	2.01 s (7)	2.11 s (OAc-11)	1.99 s (OAc-11)
	2.13 s (11)		
	2.10 s (15)		
$OCOC_6H_5$-2			
2′, 6′	7.76 dd (8.0, 1.2)	7.75 dd (8.4, 1.2)	7.79 d (8.4)
3′, 5′	7.10 t (8.0)	7.06 t (7.6)	7.06 t (8.0)
4′	7.30 m	7.30 m	7.26 m
$OCOC_6H_5$-13			
2′, 6′	7.55, dd (8.0, 1.2)	7.53 m	7.55 d (8.4)
3′, 5′	7.29 t (8.0)	7.33 m	7.28 t (8.0)
4′	7.47 m	7.51 m	7.46 m

[a] DMSO-d_6.

that of **1**), suggesting that **2** is a partial hydrolytic derivative of **1**. In addition, the ^{1}H NMR spectral data of **2** showed a close resemblance to those of **1** (Table 1) except for H-7 and H-15 ($\Delta\delta > 1$), indicating the substitution of the acetyl groups at C-7 and C-15 in **2** by hydroxyl groups. Finally, the structure of **2** was confirmed by NaOH treatment of both **1** and **2** to give the same alkamine and by full analysis of its 1D (Tables 1 and 2) and 2D NMR data (Figure S1, Supporting Information).

The MS and ^{1}H NMR spectra of **3** as compared with **2** indicted the presence of the same ester groups, with **3** differing from **2** by the absence of one hydroxyl group. The HMBC spectrum of **3** showed key correlations for H-1/ OO*C*CH_3 (δ 169.6), H-2/OO*C*C_6H_5 (δ_C 165.8), H-11/OO*C*-CH_3 (δ_C 170.9), and H-13/OO*C*C_6H_5 (δ_C 164.5) (Figure S2, Supporting Information), leading to the location of the ester groups at C-1, C-2, C-11, and C-13, respectively, along with the assignment of the hydroxyl group at C-7. In addition, the close resemblance of the ^{13}C NMR spectra of **3** and delgrandine[3] (Table 2) was observed except for certain carbon signals, such as C-1, C-3, C-4, C-5, and C-18, mainly involving ring A. The stereochemistry of the ester and hydroxyl groups in **3** was established by observing the NOESY correlations for H-1/ H-20, H-2/ H-5β, H-7/H-5β or H-9, H-11/H-1α, and H-13/H-17 (Figure S2, Supporting Information), which indicated that H-2, H-7, and H-11 are β-oriented, while H-1 and H-13 possess the same α-orientation. The structure of trifoliolasine F, therefore, was assigned as **3**.

The ^{1}H NMR spectra of **3** obtained in $CDCl_3$ and pyridine-d_6 displayed some obvious solvent effects, as observed in the differences of the chemical shifts for H-1, H-2, H-6, H-7, H-9, H-11, H-12, H-13, H-15, H-19, and N-CH_3 (Tables 1 and S1, Supporting Information). Interestingly, no signals were observed for the C-19-aldehyde in the ^{1}H and ^{13}C NMR spectra (Tables S1, S2, Supporting Information) of **1** and **2** in $CDCl_3$ or $CDCl_3$−CD_3OD, but were observed in DMSO-d_6 or pyridine-d_5, probably due to the transannular effect between this aldehyde and the lone pair of the nitrogen atom. This has been demonstrated for the hetidine-type alkaloids, e.g., miyaconitine[4] and episcopalidine,[2,5−7] as well as the vakognavine-type alkaloids, e.g., vakognavine.[8] This led to the conclusion that the measured δ values of the aldehyde groups of these alkaloids under incomplete alkaline conditions, in the presence of slight amounts of HCl in $CDCl_3$[9] or DMSO-d_6, are an average value between the aldehyde (A) and the *N,O*-acetal (B) as depicted in canonical forms in Scheme 1. This interpretation is supported by the fact that in the ^{1}H and ^{13}C NMR spectra of **3** the δ values of the C-19-aldehyde obtained in pyridine-d_5 are larger than those in $CDCl_3$ (Tables 1 and S2, Supporting Information), as well as because its chemical shift occurs at δ_H 8.36 ($CDCl_3$) and is shifted downfield by 0.51 ppm after complete alkalization with anhydrous Na_2CO_3. As shown in Table S3 (Supporting Information), there are apparent differences of the δ values of the C-19-aldehyde group reported in the literature[3,8,10,11] because of this transannular effect, indicating that the true δ values might fall in the range 9.2−9.8 ppm for H-19 and 193−200 ppm for C-19. In 1970, Ichinohe et al.[4] first reported the presence of the transannular effect in miyaconitine based on the IR spectrum. This effect was confirmed latter by our group in the IR and ^{13}C NMR spectra of episcopalidine and its analogues.[5,6] Pelletier et al.[8] have also earlier described this phenomenon in vakognavine. Thus, it is concluded that there is an obvious transannular effect among the vakognavine- and 6-keto-containing hetidine-type C_{20}-diterpenoid alkaloids.

Table 2. ^{13}C NMR Data of Trifoliolasines D−F (**1**−**3**) (100 MHz)

	1	**2**	**3**
position	$CDCl_3$	$CDCl_3$+CD_3OD	$CDCl_3$
1	69.7 d	69.5 d	70.2 d
2	66.4 d	66.3 d	66.8 d
3	29.6 t	29.9 t	29.9 t
4	43.4 s	42.1 s	43.6 s
5	56.4 d	54.8 d	57.2 d
6	59.0 d	61.5 d	62.5 d
7	65.0 d	61.8 d	73.0 d
8	51.7 s	54.9 s	49.8 s
9	49.0 d	46.5 d	52.7 d
10	56.0 s	54.0 s	55.3 s
11	73.1 d	73.2 d	74.8 d
12	44.9 d	44.8 d	45.9 d
13	72.6 d	73.2 d	73.8 d
14	39.1 d	38.2 d	39.3 d
15	65.9 d	64.1 d	29.3 t
16	141.7 s	146.3 s	141.8 s
17	115.3 t	112.4 t	111.1 t
18	26.0 q	23.4 q	25.7 q
19	186.4[a]		184.9 d
20	63.7 d	63.6 d	64.2 d
N-CH_3	33.5 q	31.6 q	34.8 q
OAc	169.7 s (O_2CCH_3-1)	169.7 s (O_2CCH_3-1)	169.6 s (O_2CCH_3-1)
	20.8 q (O_2CCH_3-1)	20.2 q (O_2CCH_3-1)	21.4 q (O_2CCH_3-1)
	169.5 s (O_2CCH_3−7)	170.5 s (O_2CCH_3-11)	170.9 s (O_2CCH_3-11)
	20.7 q (O_2CCH_3-7)	19.9 q (O_2CCH_3-11)	21.2 q (O_2CCH_3-11)
	170.6 s (O_2CCH_3-11)		
	21.3 q (O_2CCH_3-11)		
	170.6 s (O_2CCH_3-15)		
	21.2 q (O_2CCH_3-15)		
$OCOC_6H_5$-2	165.6 s	165.4 s	165.8 s
1′	128.2 s	128.2 s	129.3 s
2′, 6′	129.1 d	128.5 d	129.3 d
3′, 5′	128.1 d	127.4 d	128.1 d
4′	133.2 d	132.3 d	132.9 d
$OCOC_6H_5$-13	164.5 s	164.1 s	164.5 s
1′	128.9 s	128.2 s	129.1 s
2′, 6′	129.3 d	128.6 d	129.1 d
3′, 5′	128.2 d	127.5 d	128.2 d
4′	132.9 d	132.5 d	133.0 d

[a] C_5D_5N.

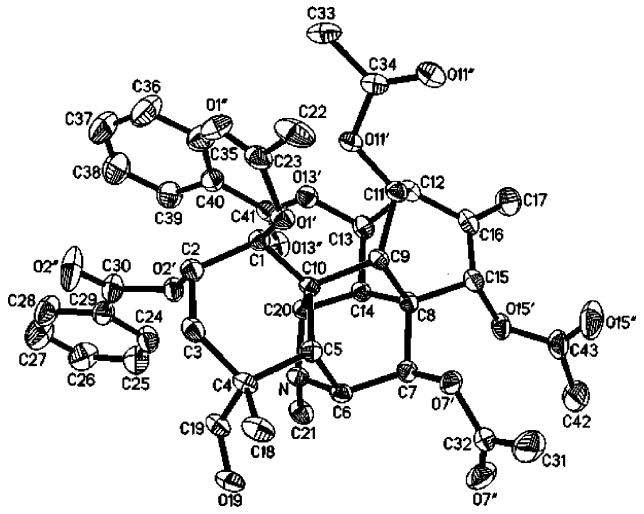
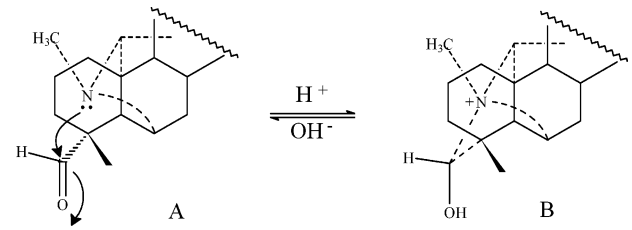


Figure 2. ORTEP drawing of trifoliolasine D (**1**).

Scheme 1



Experimental Section

General Experimental Procedures. Melting points were determined on a Thermal instrument 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. ^1H and ^{13}C NMR spectra were taken on a Varian Unity INOVA 400/45 NMR spectrometer, in $CDCl_3$, C_5D_5N, DMSO-d_6, or $CDCl_3$−CD_3OD, with TMS as the internal standard. FABMS and HRESIMS were recorded on a VG Auto Spec 3000 or Finnigan-MAT 90 instrument. Silica gel H (Qingdao Sea Chemical Factory, Qingdao, People's Republic of China) was used for column and radial chromatography. Spots on TLC (silica gel G) were detected with modified Dragendorff's reagent. A polyvinyl sulfonic ion-exchange resin (H-form, cross linking 1 × 1, Chemical Factory of Nankai University, Tianjin, People's Republic of China) was used in the extraction of the crude alkaloids.

Plant Material. The plant *Delphinium trifoliolatum* was collected in Chongqing City, Nanchuan County, People's Republic of 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 (No. 01-7-2) has been deposited.

Extraction and Isolation. According to a literature method,[13] dried whole plants (5.0 kg) were milled and percolated with 0.05 mol/L HCl (75 L). Wet cation-exchange 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 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 alkaloid fractions I (23) 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 alkalinized to pH 11 with concentrated NH_4OH and extracted with chloroform. Evaporation of the organic solvents gave crude alkaloid fraction III (2.6 g).

Crude alkaloid fractions I, II, and III (40 g) were combined and subjected to column chromatography (silica gel H, 400 g) eluting with $CHCl_3$–MeOH (100:1 to 1:1) mixtures of increasing polarity to afford nine further fractions (A–I). Fraction A (2.9 g) was chromatographed over silica gel H (100 g) eluting with petroleum ether–acetone (4:1) to afford fractions A-1 (474 mg), A-2 (473 mg), A-3 (392 mg), and A-4 (365 mg). Fraction A-3 was further separated repeatedly on a Chromatotron (radial chromatography) eluting with cyclohexane–ethyl acetate–acetone (8:1:1) to provide A-3-1 (96 mg) and then by recrystallization with petroleum ether–acetone to give trifoliolasine D (**1**, 31 mg). In addition, chromatography (silica gel H, 200 g) of fraction I (4.0 g) eluted with $CHCl_3$–acetone (3:1 to 1:1) yielded fractions I-1 (150 mg) and I-2 (1.2 g). Fraction I-2 was recrystallized with $CHCl_3$ to provide trifoliolasine E (**2**, 300 mg). Column chromatography of fraction H (2.3 g) eluting with $CHCl_3$–acetone–diethylamine (80:20:1) gave fractions H-1 (330 mg) and H-2 (1.0 g). Fraction H-2 was separated by column chromatography (silica gel H, 100 g) with petroleum ether–acetone–NH_4OH (66:33:1) to produce trifoliolasine F (**3**, 100 mg).

Trifoliolasine D (1): sheet crystals; mp 238–240 °C; $[\alpha]_D^{20}$ −15.0° (*c* 0.40, $CHCl_3$); IR (KBr) ν_{max} 1741, 1728, 1601, 1450, 1275, 1239, 926, 866 cm^{-1}; FABMS *m/z* 784 $[M + H]^+$, 678; HRESIMS *m/z* 784.2962 $[M + H]^+$ (calcd for $C_{43}H_{45}NO_{13}$, 784.2969).

Single-Crystal X-ray Crystallography of 1. A colorless sheet crystal from acetone–cyclohexane was mounted on a P_4 four-circle diffractometer and exposed to graphite-monochromated Mo Kα irradiation. The unit cell parameters are a = 14.819(2) Å, b = 14.944(2) Å, c = 18.277(3) Å in space group $P2_12_12_1$ ($Z = 4$), $D_x = 1.286$ mg·cm^{-3}. The structure was solved by direct methods with the program SHELX 97[14] and refined by full-matrix least-squares on F^2. The final R indexes were $R_1 = 0.0357$ and $wR_2 = 0.0565$. CCDC 267464 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 IEZ, UK, fax: +44 1223 336033.

Trifoliolasine E (2): white amorphous powder; mp 226–227 °C; $[\alpha]_D^{20}$ −60.4° (*c* 0.45, $CHCl_3$); IR (KBr) ν_{max} 3545, 1738, 1719, 1601, 1584, 1449, 1421, 1275, 1239, 911, 888 cm^{-1}; FABMS *m/z* 700 $[M + H]^+$; HRESIMS *m/z* 700.2755 $[M + H]^+$ (calcd for $C_{39}H_{41}NO_{11}$, 700.2757).

Trifoliolasine F (3): white amorphous powder; mp 138–139 °C; $[\alpha]_D^{20}$ −32.6° (*c* 0.54, $CHCl_3$); IR (KBr) ν_{max} 3434, 3071, 1737, 1718, 1655, 1600, 1490, 1450, 1273, 1238, 910, 884 cm^{-1}; FABMS *m/z* 684 $[M + H]^+$; HRESIMS *m/z* 684.2785 $[M + H]^+$ (calcd for $C_{39}H_{41}NO_{10}$, 684.2808).

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Supporting Information Available: ^1H and ^{13}C NMR data (Tables S1 and S2) in diverse solvents for compounds **1**–**3**, Figures S1 and S2 showing COSY correlations of **2** and **3**, as well as Table S3 summarzing NMR data for diterpenoid alkaloids with a C-19 aldehyde group. These materials are available free of charge via the Internet at http://pubs.acs.org.

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