New C_{18}-Diterpenoid Alkaloids from *Delphinium anthriscifolium* var. *savatieri*

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Five new C_{18}-diterpenoid alkaloids, anthriscifolcines A (1), B (2), C (3), D (4), and E (5), together with a known C_{19}-diterpenoid alkaloid delcorine (6), were isolated from the whole herb of *Delphinium anthriscifolium* var. *savatieri*. The structures of these new alkaloids were established on the basis of spectral data (1D- and 2D-NMR, HR-ESI-MS).

Key words *Delphinium anthriscifolium* var. *savatieri*; Ranunuclaceae; C_{18}-diterpenoid alkaloid; anthriscifolcine

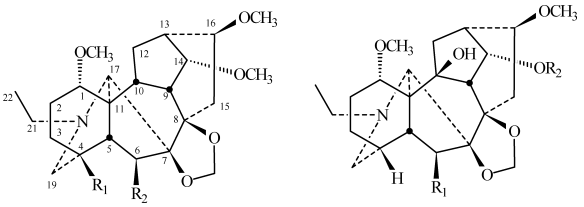
In the course of comparative research of new activities of alkaloids and evaluation of chemotaxonomy of the diterpenoid alkaloids from the *Aconitum* and *Delphinium* species,[1—3] we investigated the alkaloids of *D. anthriscifolium* var. *savatieri* (FRANCHET) MUNZ. The plant is endemic to China, especially the Sect. *Anthriscifolium* of Subgen. *Delphinium*,[4] of which no plants have been phytochemically reported yet, implying that the study is very important value for the chemotaxonomy of genera *Delphinium*. Our research of the whole herbs of *D. anthriscifolium* var. *calleryi* sevealed five new C_{18}-diterpenoid alkaloids, anthriscifolcines A, B, C, D, and E, together with a known C_{19}-diterpenoid alkaloid delcorine (**6**).[5] In this paper, we report the isolation and structural elucidation of these alkaloids.

Results and Discussion

Anthriscifolcine A (**1**) was isolated as an amorphous powder, mp 135—137 °C. Its molecular formula $C_{26}H_{39}NO_7$, was established based on HR-ESI-MS and ^{13}C-NMR. The IR (KBr) spectrum of **1** showed absorption bands at 1740 cm^{-1} ascribable to carbonyl groups. The NMR data showed the presence of an *N*-ethyl group (δ_H 1.03, 3H, t, J=7.2 Hz, 2.73, 2.78, 2H, m; δ_C 50.3 t, 13.8 q), three methoxyl groups (δ_H 3.27, 3.34, 3.45, each 3H, s; δ_C 55.7 q, 56.1 q, 57.7 q), an acetyl group (δ_H 2.04, 3H, s; δ_C 170.4 s, 21.6 q), and a methylenedioxyl group (δ_H 4.91, 2H, s; δ_C 93.5 t). The single non-oxygenated quaternary carbon signal (δ_C 49.9 s) suggested that compound **1** was a C_{18}-diterpenoid alkaloid from combined NMR data[6] and biogenesis. The three methoxyl groups were attributed to C-1, C-14, and C-16, respectively, based on the long-range correlations (1-OCH_3/C-1, 14-OCH_3/C-14, 16-OCH_3/C-16) in the HMBC spectrum (Fig. 1). The one-proton triplet signal at δ_H 3.66 (J=4.8 Hz) in the ^{1}H-NMR spectrum of (**1**) was assigned to H-14β based on the multiplicity and the coupling constant.[7] The only acetoxyl group was located at C-6 due to the HMBC correlation between 6-OAc (δ_C 170.4 s) and H-6 (δ_H 5.21 s), and its configuration was determined as β-orientation based on the multiplicity of H-6 (singlet) in the ^{1}H-NMR spectrum. Finally, the structure of anthriscifolcine A was established as **1** by careful analyses of the 1D-NMR and 2D-NMR (^{1}H–^{1}H COSY, HMQC and HMBC) spectra.

Anthriscifolcine B (**2**) was a white amorphous powder, mp 75—77 °C. The HR-ESI-MS of **2** exhibited a protonated molecular ion peak at *m/z* 436.2686 (Calcd 436.2694) corresponding to a molecular formula of $C_{24}H_{38}NO_6$ (42 mass units lower than that of **1**), suggesting that **2** is a hydrolytic derivative of **1**, the ^{13}C-NMR spectra of **2** were similar to those of **1** except for the lack of an acetyl group. Meanwhile, the signal of H-6 in **1** was shifted upfield from δ_H 5.21 to δ_H 4.25 in **2** indicting that 6-OAc was substituted for 6-OH. Finally, the structure of **2** was confirmed by NaOH–CH_3OH treatment of **1** to give the same alkamine with **2** and by full analysis of its NMR data (Table 1). Thus the structure of anthriscifolcine B was deduced as **2**.

Anthriscifolcine C (**3**) was isolated as needle crystals, mp 222—224 °C. The HR-ESI-MS showed $[M+H]^+$ at *m/z* 480.2590 corresponding to the pseudo molecular formula $C_{25}H_{38}NO_8$ $[M+H]^+$, which requires *m/z* 480.2592. The NMR spectra of anthriscifolcine C (**3**) gave distinctive signals at δ_H 1.07 (3H, t, J=7.2 Hz), δ_C 13.9 q, and δ_C 50.6 t, for the *N*-ethyl group, δ_H 3.26 and 3.35 (each 3H, s,), δ_C 55.7 q and 56.3 q for two methoxyl groups, δ_H 2.10 (3H, s), δ_C 21.7 q and 170.6 s for an acetyl group, and δ_H 4.98 and 5.01 (2H, s), δ_C 94.2 t for a methylenedioxy group. The ^{13}C signals of eight oxygenated carbons at δ_C 72.8 d, 77.2 d, 80.1 s, 81.1 d, 81.2 d, 83.1 s, 93.0 s, and 94.2 t suggested that **3** had two hydroxyl groups in addition to two methoxyl groups,



1 R_1 = H R_2 = OAc
2 R_1 = H R_2 = OH
6 R_1 = CH_2OCH_3 R_2 = OH

3 R_1 = OAc R_2 = H
4 R_1 = OAc R_2 = CH_3
5 R_1 = OH R_2 = CH_3

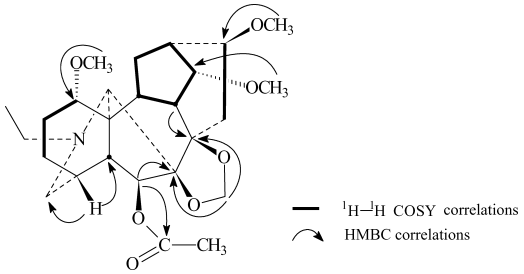


Fig. 1. Key ^{1}H–^{1}H COSY Correlations and Selected HMBC Correlations of Anthriscifolcine A (**1**)

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Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds (**1**, **2**) (^{1}H: 400 MHz, ^{13}C: 100 MHz; $CDCl_3$)

No.	**1**		**2**
	δ_C	δ_H mult. (J=Hz)	δ_C
1	82.4 d	3.01 t (9.6)	83.0 d
2	26.4 t	2.08 m	26.4 t
3	29.2 t	1.74 m	29.2 t
		1.36 m	
4	38.4 d	2.31 t (5.6)	37.9 d
5	50.2 d	1.46 s	51.0 d
6	81.1 d	5.21 s	81.5 t
7	92.0 s	—	92.9 d
8	83.5 s	—	84.5 s
9	48.0 d	2.10 m	47.6 d
10	39.7 d	3.47 m	40.2 d
11	49.9 s	—	50.2 s
12	28.3 t	1.77 m	28.3 t
		2.58 m	
13	34.2 d	2.11 m	34.7 d
14	83.3 d	3.66 t (4.8)	83.3 d
15	33.9 t	1.80 m (hidden)	33.5 t
		2.44 m	
16	81.7 d	3.20 m	81.9 d
17	64.4 d	3.12 s	64.4 d
19	50.5 t	2.70 m	50.8 t
		2.78 m	
21	50.3 t	2.73 m	50.8 t
		2.75 m	
22	13.8 q	1.03 t (7.2)	13.5 q
1-OCH_3	55.8 q	3.27 s	55.7 q
16-OCH_3	56.2 q	3.34 s	56.1 q
14-OCH_3	57.7 q	3.45 s	57.7 q
$\underline{CO}CH_3$	170.4 s	—	
$CO\underline{CH_3}$	21.6 q	2.04 s	
OCH_2O	93.5 t	4.91 s	92.9 t

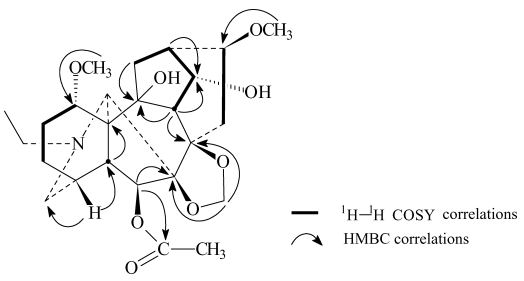

Fig. 2. Key ^{1}H–^{1}H COSY Correlations and Selected HMBC Correlations of Anthriscifolcine C (**3**)

an acetoxyl group, and a methylenedioxy group. Inspection of the single non-oxygenated quaternary carbon signal (δ_C 54.7 s) suggested that the compound **3** was a C_{18}-diterpenoid alkaloid.[6] The lowed-field 14-H signal at δ_H 4.64 (dd, J=10.8, 4.8 Hz, and t, J=4.8 Hz, in D_2O). indicated the location of hydroxyl groups in C-14 and C-10,[7] which was confirmed by the key corrections in HMBC (Fig. 2). The two methoxyl groups could be located at C-1, C-16 due to the ^{1}H–^{13}C long-range correlations 1-OCH_3 (δ_H 3.26 s)/C-1 (δ_C 77.2 d) and 16-OCH_3 (δ_H 3.35 s)/C-16 (δ_C 81.2 d) in the HMBC spectrum, and the acetoxyl group may be placed at C-6 based on the correlation of H-6 (δ_H 5.33 s)/$\underline{CO}CH_3$ (δ_C 170.6 s). It is worthy to note that the hydroxyl group at C-10 made the chemical shift of C-1 shift upfield *ca.* 5—6 ppm because of the γ-gauch effect. Thus, the structure of anthriscifolcine C was determined as compound **3**. The full assignment of anthriscifolcine C (**3**) was based on the 1D- and 2D-NMR spectral data (Table 2).

Anthriscifolcine D (**4**) was obtained as a white amorphous powder, mp 185—187 °C. The pseudo molecular formula $C_{26}H_{40}NO_8$ was inferred from its HR-ESI-MS and ^{13}C-NMR data. The ^{13}C-NMR data were much closer to those of anthriscifolcine C (**3**) except for an additional methoxyl group. The additional methoxyl group in compound **4** was attributed to C-14 to substitute the original hydroxyl group in **3**, which could be demonstrated by one-proton triplet (J=4.4 Hz) signal at δ_H 4.10 and the long-rang correlation between H-14 and the methoxyl carbon signal at δ_C 57.6 q in the HMBC spectrum (Fig. 3). In addition, this result was also confirmed by the additional 14 mass units in the molecular weight of compound **4** than in compound **3**. These observations led to the assignment of the structure of anthriscifolcine D as **4**.

Anthriscifolcine E (**5**) was a white amorphous powder, mp 150—152 °C. Its molecular formula $C_{24}H_{37}NO_7$ was derived from HR-ESI-MS (m/z 452.2644 $[M+H]^+$) and ^{13}C-NMR data. The ^{13}C-NMR data of **5** were very similar to those of **4** except for lacking a signal for acetyl group. Besides, the proton signal δ_H 5.27 in compound **4** was shifted upfield to δ_H 4.28 in compound **5** suggesting that 6-OAc in **5** was substituted by a hydroxyl group, which was confirmed by the difference of 42 mass units between those two compounds. Treatment of anthriscifolcine D (**4**) with 5% NaOH–CH_3OH gave the hydrolytic derivative, which had the same TLC (S1: $CHCl_3$–CH_3OH (95 : 5); S2: cyclohexane–acetone (2 : 1)) behavior as the compound **5**, implying that they were the same alkamine. Therefore the structure of anthriscifolcine F was determined as **5**.

The stereochemistry of C-6 and C-16 in **1**—**5** was determined by NMR data. The configurations of 6-OAc or 6-OH were all deduced to be β-orientation based on the multiplicity of H-6 in the ^{1}H-NMR spectra. Because of the rigid skeleton of these alkaloids, the dihedral angle between 5β-H and 6α-H approaches to 90°, which led to the multiplicity of H-6 being singlet. While the multiplicity 6β-H should be doublet, and the coupling constant of H-6 was *ca.* J=6—7 Hz, like most of acontine-type alkaloids.[8] The 16-OCH_3 was determined to be β-orientation in **1**—**5** due to the δ values (81—83 ppm) of C-16 as compared with known compounds such as deltaline,[9] deltamine,[9] and dictyocarpine.[9]

Finally, from a chemotaxonomy view, *D. anthriscifolium* var. *savatieri* (Franchet) Munz is close to the Subgen. *Lycoctonum* (DC.) Peterm, which contains almost all of the C_{18}-diterpenoid alkaloids,[3] and also similar to those of Subgen. *Delphinastrum* due to the norditerpenoid alkaloids having the 7,8-methylenedioxy groups.[3] This is useful for the chemotaxonomy of *Delphinium* species.

Experimental

General Experimental Procedures Melting points were assessed by a thermal values analysis with microscope and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 341 polarimenter. IR spectra were obtained on a Nicolet FT-IR 200SXY spectrophotomer. ^{1}H- and ^{13}C-NMR were measured in $CDCl_3$, with TMS as internal standard, on a Varian Unity INOVA 400/54 NMR spectrometer. MS spectra were measured on Finnigan LCQ and Micromass Auto Ultima-Tof spectrometer. Silica gel H (Qindao sea Chemical Factory, People's Republic of China) was used for TLC, and column chromatography, respectively.

Table 2. ^{1}H- and ^{13}C-NMR Data of Compounds (**3**, **4**, **5**) (^{1}H: 400 MHz, ^{13}C: 100 MHz; $CDCl_3$)

No.	**3**		**4**		**5**
	δ_C	δ_H mult. (J=Hz)	δ_C	δ_H mult. (J=Hz)	δ_C
1	77.2 d	3.62 t (8.0)	77.1 d	3.50 t (8.8)	77.0 d
2	25.8 t	2.06 m (α)	26.1 t	2.06 m (α)	26.0 t
		2.12 m (β)		2.12 m (β)	
3	29.6 t	1.35 m (α)	28.3 t	1.37 m (α)	28.9 t
		1.78 m (β)		1.80 m (β)	
4	33.6 d	2.10 m	33.5 d	2.09 m	34.3 d
5	44.8 d	1.83 m	44.7 d	1.84 m	45.5 d
6	81.1 d	5.33 s	81.5 d	5.27 s	82.0 d
7	93.0 s	—	91.3 s	—	92.2 s
8	80.1 s	—	81.6 s	—	82.3 s
9	52.1 d	3.32 m	50.1 d	3.29 m	50.5 d
10	83.1 s	—	83.6 s	—	83.0 s
11	54.7 s	—	55.1 s	—	55.3 s
12	36.9 t	2.55 m	34.9 t	2.48 m	34.4 t
13	37.5 d	1.72 m	36.0 d	2.51 m	37.4 d
14	72.8 d	4.64 dd (10.0, 4.8)	81.5 d	4.10 t (4.4)	81.5 d
15	37.5 t	1.83 m (α)	39.5 t	1.85 m (α)	38.7 t
		2.64 m (β)		2.69 m (β)	
16	81.2 d	3.46 d (8.4)	81.5 d	3.18 d (8.0)	81.6 d
17	65.0 d	3.30 br s	63.9 d	3.05 br s	63.9 d
19	50.6 t	2.80 m	50.2 t	2.75 m	50.5 t
21	50.6 t	2.90 m	50.2 t	2.85 m	50.7 t
22	13.9 q	1.07 t (7.2)	13.4 q	1.04 t (7.2)	13.4 q
1-OCH_3	55.7 q	3.26 s	55.4 q	3.25 s	55.6 q
14-OCH_3	—	—	57.6 q	3.43 s	57.7 q
16-OCH_3	56.3 q	3.35 s	56.3 q	3.31 s	56.1 q
OCH_2O	94.2 t	4.99 s, 5.01 s	93.9 t	4.92, 4.94 s	93.2 t
OAc	170.6 s	—	170.2 s	—	
	21.7 q	2.10 s	21.6 q	2.06 s	

Plant Material *D. anthriscifolium* var. *savatieri* (Franchet) Munz was collected in August 2004 in Pengzhou city of Sichuan Province, China, and authenticated by Wen-Jin Zhang of Pengzhou County Centre of Disease Prevention and Control. The voucher specimen (No. 20030418-1) has been deposited at West China College of Pharmacy, Sichuan University.

Extraction and Isolation The powder (4.0 kg) of *D. anthriscifolium* var. *calleryi* was percolated with 0.1 mol/l HCl (40 l). The filtrate was then alkalized with 28% aqueous NH_4OH (1.2 l) to pH >9 and extracted with ethyl acetate (each 20 l) for three times, and evaporated to give the total crude alkaloids (17.0 g). The crude alkaloids (17 g) were chromatographed over silica gel column eluting with chloroform–methanol (100 : 1→95 : 5) gradient system to give fractions A (7.2 g), B (1.5 g), C (2.9 g), and D (2.7 g). Fraction A (7.2 g) was chromatographed on a silica gel column eluting with cyclohexane–acetone (5 : 1) to give compound **1** (40 mg), compound **4** (330 mg) and fraction A-2, which was chromatographed on a silica gel column eluting with cyclohexane–acetone (10 : 1) to afford compound **2** (18 mg) and **6** (9 mg). Fraction C (2.9 g) was chromatographed on a silica gel column eluting with cyclohexane–acetone (4 : 1) to give compound **5** (60 mg), fraction C-4 (180 mg), which was recrystallized with acetone to give compound **3** (16 mg).

Anthriscifolcine A (**1**): White amorphous powder, mp 135—137 °C; $[\alpha]_D^{20}$ −12.2° (c=0.5, $CHCl_3$); IR (KBr) cm^{-1}: 1740, 962; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1; HR-ESI-MS m/z: 478.2811 $[M+H]^+$, Calcd for $C_{26}H_{40}NO_7$ $[M+H]^+$ 478.2799.

Hydrolysis of Anthriscifolcine A (**1**): Anthriscifolcine A (**1**) (10 mg) was dissolved in 5 ml of 5% NaOH–CH_3OH and stirred at 50 °C for 30 min, then extracted with $CHCl_3$ to yield corresponding hydrolytic derivative anthriscifolcine B (**2**) (7 mg).

Anthriscifolcine B (**2**): White amorphous powder, mp 75—77 °C; $[\alpha]_D^{20}$ −27° (c=0.5, $CHCl_3$); IR (KBr) cm^{-1}: 3408, 962; ^{1}H-NMR (400 MHz, $CDCl_3$), δ_H 1.05 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{CH_3}$), 2.36 (1H, d, J=5.6 Hz, H-4), 3.27, 3.35, 3.43 (each 3H, s, 3×OCH_3), 3.66 (1H, t, J=4.8 Hz, H-14), 4.25 (1H, s, H-6), 5.06, 5.12 (each 1H, s, OCH_2O); ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1; HR-ESI-MS m/z: 436.2686 $[M+H]^+$, Calcd for $C_{24}H_{38}NO_6$ $[M+H]^+$ 436.2694.

Anthriscifolcine C (**3**): Colorless needle crystals, mp 222—224 °C; $[\alpha]_D^{20}$ −11.4° (c=0.5, $CHCl_3$); IR (KBr) cm^{-1}: 3509, 1717, 960; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 2; HR-ESI-MS m/z: $[M+H]^+$ 480.2590, Calcd for $C_{25}H_{38}NO_8$ $[M+H]^+$ 480.2592.

Anthriscifolcine D (**4**): White amorphous powder, mp 185—187 °C; $[\alpha]_D^{20}$ −41.3° (c=0.5, $CHCl_3$); IR (KBr) cm^{-1}: 3444, 1739, 943; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 2; HR-ESI-MS m/z: 494.2744 $[M+H]^+$, Calcd for $C_{26}H_{40}NO_8$ $[M+H]^+$ 494.2748.

Hydrolysis of Anthriscifolcine D (**4**): Anthriscifolcine D (**4**) (10 mg) was dissolved in 5 ml of 5% NaOH–CH_3OH and stirred at 50 °C for 30 min, then extracted with $CHCl_3$ to yield anthriscifolcine E (**5**) (7 mg).

Anthriscifolcine E (**5**): White amorphous powder, mp 150—152 °C; $[\alpha]_D^{20}$ −34.5° (c=0.5, $CHCl_3$); IR (KBr) cm^{-1}: 3446, 950; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 1.06 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{CH_3}$), 3.26, 3.35, 3.45 (each 3H, s, 3×OCH_3), 4.15 (1H, t, J=4.8 Hz, H-14), 4.28 (1H, s, H-6), 5.07, 5.13 (each 1H, s, OCH_2O); ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1; HR-ESI-MS m/z: 452.2644 $[M+H]^+$, Calcd for $C_{24}H_{38}NO_7$ $[M+H]^+$ 452.2643.

Delcorine (**6**): White amorphous powder, mp 200—202 °C; $[\alpha]_D^{20}$ −18.0° (c=0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 1.05 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{CH_3}$), 3.26, 3.33, 3.35, 3.43 (each 3H, s, 4×OCH_3), 3.68 (1H, t, J=4.8 Hz, H-14), 4.27 (1H, s, H-6), 5.05, 5.12 (each 1H, s, OCH_2O); ^{13}C-NMR (100 MHz, $CDCl_3$): 83.1 (d, C-1), 26.4 (t, C-2), 31.8 (t, C-3), 38.1 (s, C-4), 52.6 (d, C-5), 78.9 (d, C-6), 92.7 (s, C-7), 83.9 (s, C-8), 48.1 (d, C-9), 40.3 (d, C-10), 50.2 (s, C-11), 28.1 (t, C-12), 37.9 (d, C-13), 82.5 (d, C-14), 33.3 (t, C-15), 81.8 (d, C-16), 63.9 (d, C-17), 78.9 (t, C-18), 53.7 (t, C-19), 50.7 (t, $\underline{N}CH_2CH_3$), 14.0 (q, $\underline{N}CH_2\underline{CH_3}$), 55.5 (q, 1-$OCH_3$), 57.8 (q, 14-$OCH_3$), 56.3 (q, 16-$OCH_3$), 59.6 (q, 18-$OCH_3$), 92.9 (t, OCH_2O); ESI-MS m/z: 480.6 $[M+H]^+$ (100).

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