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; Ranunulaceae; C_{18}-diterpenoid alkaloid; anthriscifoline

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 revealed five new C_{18}-diterpenoid alkaloids, anthriscifolines A, B, C, D, and E, together with a known C_{19}-diterpenoid alkaloid delcorine (6). 5,6 In this paper, we report the isolation and structural elucidation of these alkaloids.

Results and Discussion

Anthriscifoline A (1) was isolated as an amorphous powder, mp 135−137°C. Its molecular formula C_{37}H_{54}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 (\(\delta_{\text{H}}\) 1.03, 3H, t, J=7.2 Hz, 2.73, 2.78, 2H, m; \(\delta_{\text{C}}\) 50.3 t, 13.8 q), three methoxyl groups (\(\delta_{\text{H}}\) 3.27, 3.34, 3.45, each 3H, s; \(\delta_{\text{C}}\) 55.7 q, 56.1 q, 57.7 q), and a methylenedioxy group (\(\delta_{\text{H}}\) 4.91, 2H, s; \(\delta_{\text{C}}\) 93.5 t). The single non-oxygenated quaternary carbon signal (\(\delta_{\text{C}}\) 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 \(\delta_{\text{H}}\) 3.66 (J=4.8 Hz) in the ^{1}H-NMR spectrum of 1 was assigned to H-14\(\beta\) 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 (\(\delta_{\text{C}}\) 170.4 s) and H-6 (\(\delta_{\text{H}}\) 5.21 s), and its configuration was determined as \(\beta\)-orientation based on the multiplicity of H-6 (singlet) in the ^{1}H-NMR spectrum. Finally, the structure of anthriscifoline A was established as 1 by careful analyses of the 1D-NMR and 2D-NMR (\(^{1}H\)−^{1}H COSY, HMHQ and HMBC) spectra.

Anthriscifoline 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_{32}H_{50}NO_{4} (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 \(\delta_{\text{H}}\) 5.21 to \(\delta_{\text{H}}\) 4.25 in 2 indicating that 6-OAc was substituted for 6-OH. Finally, the structure of 2 was confirmed by NaOH−CH_{3}OH treatment of 1 to give the same alkamid with 2 and by full analysis of its NMR data (Table 1). Thus the structure of anthriscifoline B was deduced as 2.

Anthriscifoline 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_{32}H_{50}NO_{4} [M+H]^+, which requires \(m/z\) 480.2592. The NMR spectra of anthriscifoline C (3) gave distinctive signals at \(\delta_{\text{H}}\) 1.07 (3H, t, J=7.2 Hz), \(\delta_{\text{C}}\) 13.9 q, and \(\delta_{\text{C}}\) 50.6 t, for the N-ethyl group, \(\delta_{\text{H}}\) 3.26 and 3.35 (each 3H, s), \(\delta_{\text{C}}\) 55.7 q and 56.3 q for two methoxyl groups, \(\delta_{\text{H}}\) 2.10 (3H, s), \(\delta_{\text{C}}\) 21.7 q and 170.6 s for an acetyl group, and \(\delta_{\text{H}}\) 4.98 and 5.01 (2H, s), \(\delta_{\text{C}}\) 94.2 t for a methylenedioxy group. The ^{13}C signals of eight oxygenated carbons at \(\delta_{\text{C}}\) 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,

![Fig. 1. Key ^{1}H−^{1}H COSY Correlations and Selected HMBC Correlations of Anthriscifoline A (1)](image)

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an acetoxy 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. The low-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, which was confirmed by the key corrections in HMBC (Fig. 2). The two methoxy groups could be located at C-1, C-16 due to the δ_H 4.10 and the long-rang correlation between H-14 and the methoxy 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 anthriscifoline D as 4.

Anthriscifoline 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 and 13C-NMR data. The 13C-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 anthriscifoline D (4) with 5% NaOH–CH_3OH gave the hydrolytic derivative, which had the same TLC (S1: CHCl_3–CH_2OH (95:5); S2: cyclohexane–acetone (2:1)) behavior as the compound 5, implying that they were the same alkaline. Therefore the structure of anthriscifoline 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 1H-NMR spectra. Because of the rigid skeleton of these alkaloids, the dihedral angle between 5–6-H and 6α-H approaches to 90°, which led to the multiplicity of H-6 being singlet. While the multiplicity of 6β-H should be doublet, and the coupling constant of H-6 was ca. J = 6—7 Hz, like most of aconitine-type alkaloids. 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 delatamine, deltamine, and dictyocarpine.

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, and also similar to those of Subgen. Delphinastrum due to the nortriterpenoid alkaloids having the 7,8-methylenedioxy groups. 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 polarimeter. IR spectra were obtained on a Nicolet FT-IR 200SXY spectrophotometer. 1H- and 13C-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.
Plant Material  *D. anthriscifolium* var. *savatieri* (FRANCHET) MUNZ was collected in August 2004 in Pengzhou city of Sichuan Province, China, and authenticated by Wen-Jiang 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 alloyed with 28% aqueous NH₄OH (1:2) 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 (60 mg), fraction C-4 (180 mg), which was recrystallized with acetone to afford compound 2 (330 mg) and fraction A-2, which 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).

**Anthriscifolice A (1):** White amorphous powder, mp 135 – 137°C; [α]₂⁰o −12.2° (c=0.5, CHCl₃); IR (KBr) cm⁻¹: 1740, 962; [H-NMR (400 MHz, CDCl₃)] and [¹³C-NMR (100 MHz, CDCl₃)]; see Table 1; HR-ESI-MS m/z: 478.2811 [M+H][⁺], Calcd for C₂₄H₃₈NO₆ [M+H][⁺] 478.2799.

Hydrolysis of Anthriscifolice A (1): Anthriscifolice A (1) (10 mg) was dissolved in 5 ml of 5% NaOH–CH₂OH and stirred at 50°C for 30 min, then treated with CHCl₃ to yield corresponding hydrolytic derivative anthriscifolice B (2) (7 mg).

**Anthriscifolice B (2):** White amorphous powder, mp 75 – 77°C; [α]₂⁰o −27.7° (c=0.5, CHCl₃); IR (KBr) cm⁻¹: 3408, 962; [H-NMR (400 MHz, CDCl₃)], δ₁ (3H, t, J=7.2 Hz, NCH₂CH₃); 2.38 (1H, d, J=5.6 Hz, H-4), 3.27, 3.35, 3.43 (each 3H, s, 3xOCH₃), 3.66 (1H, t, J=4.8 Hz, H-14), 4.25 (1H, s, H-6), 5.06, 5.12 (each 1H, s, OCH₃); [¹³C-NMR (100 MHz, CDCl₃)]; see Table 1; HR-ESI-MS m/z: 436.2686 [M+H][⁺], Calcd for C₁₉H₂₄NO₇ [M+H][⁺] 436.2694.

**Anthriscifolice C (3):** Colorless needle crystals, mp 222 – 224°C; [α]₂⁰o −11.4° (c=0.5, CHCl₃); IR (KBr) cm⁻¹: 3509, 1717, 960; [H-NMR (400 MHz, CDCl₃)] and [¹³C-NMR (100 MHz, CDCl₃)]; see Table 2; HR-ESI-MS m/z: [M+H][⁺] 480.2590, Calcd for C₂₅H₃₉NO₇ [M+H][⁺] 480.2592.

**Anthriscifolice D (4):** White amorphous powder, mp 185 – 187°C; [α]₂⁰o −41.3° (c=0.5, CHCl₃); IR (KBr) cm⁻¹: 3444, 1739, 943; [H-NMR (400 MHz, CDCl₃)] and [¹³C-NMR (100 MHz, CDCl₃)]; see Table 2; HR-ESI-MS m/z: 494.2744 [M+H][⁺], Calcd for C₂₅H₃₉NO₇ [M+H][⁺] 494.2748.

Hydrolysis of Anthriscifolice D (4): Anthriscifolice D (4) (10 mg) was dissolved in 5 ml of 5% NaOH–CH₂OH and stirred at 50°C for 30 min, then extracted with CHCl₃ to yield hydrolytic anthriscifolice E (5) (7 mg).

**Anthriscifolice E (5):** White amorphous powder, mp 150 – 152°C; [α]₂⁰o −34.5° (c=0.5, CHCl₃); IR (KBr) cm⁻¹: 3446, 950; [H-NMR (400 MHz, CDCl₃)], δ₁ (3H, t, J=7.2 Hz, NCH₂CH₃); 3.26, 3.35, 3.45 (each 3H, s, 3xOCH₃), 4.15 (1H, t, J=4.8 Hz, H-14), 4.28 (1H, s, H-6), 5.07, 5.13 (each 1H, s, OCH₃); [¹³C-NMR (100 MHz, CDCl₃)]; see Table 1; HR-ESI-MS m/z: 452.2644 [M+H][⁺], Calcd for C₂₅H₃₈NO₇ [M+H][⁺] 452.2643.

**Dulcoric (6):** White amorphous powder, mp 200 – 202°C; [α]₂⁰o −18.0° (c=0.5, CHCl₃); [H-NMR (400 MHz, CDCl₃)], δ₁ (3H, t, J=7.2 Hz, NCH₂CH₃); 3.26, 3.33, 3.34 (each 3H, s, 4xOCH₃), 3.68 (1H, t, J=4.8 Hz, H-14), 4.27 (1H, s, H-6), 5.05, 5.12 (each 1H, s, OCH₃); [¹³C-NMR (100 MHz, CDCl₃)], 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 (s, C-18), 53.7 (t, C-19), 50.7 (t, NCH₂CH₃), 14.0 (q, NCH₂CH₃), 55.5 (q, 1-OCH₃), 57.8 (q, 14-OCH₃), 56.3 (q, 16-OCH₃), 59.6 (q, 18-OCH₃), 92.9 (t, OCH₃); ESI-MS m/z: 480.6 [M+H][⁺] (100).

**References**


