

New C₁₉-Diterpenoid Alkaloids from the Roots of *Delphinium Giraldii*[†]

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Three new C₁₉-diterpenoid alkaloids, giraldines A (1), B (3) and C (4), were isolated from the roots of *Delphinium giraldii* Diels, together with three known C₁₉-diterpenoid alkaloids, dihydrogadesine (5), tatsiensine (6) and siwanine A (7), as well as their structures were elucidated by chemical evidence and spectral analyses, including IR, MS, 1D NMR and 2D NMR.

Keywords *Delphinium giraldii*, Ranunculaceae, C₁₉-diterpenoid alkaloid, giraldine A, giraldine B, giraldine C

Introduction

In the course of our studies on the alkaloids of *Aconitum* and *Delphinium*,^{1,4} we investigated the alkaloids of *Delphinium giraldii* (Ranunculaceae),⁵ a species endemic to Taibai mountain, Shaanxi province in China. No phytochemistry on the plant has been reported so far. The total alkaloids (36 g) were obtained by using an ion-exchange resin method⁶ from the dry powdered roots (12.5 kg) and subjected to silica gel column chromatography to give three new C₁₉-diterpenoid alkaloids, giraldines A (1), B (3) and C (4), in addition to the known C₁₉-diterpenoid alkaloids, dihydrogadesine (5), tatsiensine (6) and siwanine A (7). We described here the structure elucidation of these new alkaloids by applying chemical methods and spectral analyses, including 2D NMR (¹H-¹H COSY, HMQC, HMBC).

Results and discussion

Giraldine A (1) was obtained as white amorphous powder (CHCl₃-MeOH), m. p. 79—80 °C, [α]_D²⁰ + 75.3 (c 0.55, CHCl₃). The formula C₂₃H₃₅NO₆ was confirmed by its HRFABMS (*m/z* 422.2534 [M⁺ + 1]) and 2D NMR data. The IR and ¹H (¹³C) NMR spectra of 1 showed the presence of two methine protons and carbons bearing the hydroxyl groups [3472, 3430 cm⁻¹; δ _H 3.74

(d, *J* = 4.8 Hz), 4.12 (t, *J* = 4.4 Hz); δ _C 71.1, 75.5], two quaternary carbons bearing the hydroxyl groups [3472, 3430 cm⁻¹; δ _C 78.1 (s), 86.7 (s)], one *N*-ethyl group [δ _H 1.06 (t, *J* = 7.2 Hz, 3H), 2.75—2.85 (m), 3.02 (s, each 1H); δ _C 13.6 (q), 50.1 (t)], two methoxyl groups [δ _H 3.37, 3.38 (s, each 3H); δ _C 56.2 (q), 57.9 (q)], a *C*-methyl group [δ _H 1.12 (s), 3H; δ _C 24.4 (q), 18-CH₃] and a disubstituted double bond [1639 cm⁻¹; δ _H 5.66 (d, *J* = 9.6 Hz), 5.82 (dd, *J* = 9.6, 4.8 Hz), δ _C 137.0 (d), 130.5 (d)]. Its NMR and MS spectra showed that it was the lycoctonine-type C₁₉-diterpenoid alkaloid.^{7,8} The HMBC spectra of 1 showed the multiple bond correlations among the H-2 (δ 5.82) and C-1, C-4, C-11; the H-3 (δ 5.66) and C-4, C-5, C-18, C-19, indicated the presence of double bond $\Delta^{2(3)}$ with the OH-1. The one signal at δ 4.12 (t, *J* = 4.4 Hz, 1H, 14 β -H) in the ¹H NMR spectrum of 1 showed the presence of 14-hydroxyl group.⁷ Two methoxyl groups in 1 should be located at C-6 and C-16 respectively⁷ by showing the oxygenated methine signals at δ _C 91.2 d, 82.0 d in the ¹³C NMR of 1, and the multiple bond correlations between the OCH₃-6 (δ 3.38) and C-6, the OCH₃-16 (δ 3.37) and C-16 (Table 1) in the HMBC of 1.

In the NOE spectrum of the acetate 2 of 1 irradiation of H-1 (δ 5.08), OAc-1 (δ 2.08) and CH₃-22 (δ 1.10) led to the enhancement of the signals of H-12 β [δ (m, 2.08—2.17)], H-12 α [δ (m, 1.50—1.58)] and OAc-1 (negative NOE), respectively, indicating that the OH-1 was α -oriented. In addition, irradiation of H-14 β (δ 4.75) and H-17 (δ 2.85) resulted in an enhancement of H-12 β and H-15 α (δ 1.62), respectively (Fig. 1). The structure of giraldine A was determined as 1. Assignment of ¹H NMR and ¹³C NMR data of 1 and 2 (Table 1) were made by their ¹H-¹H COSY, HMQC, HMBC and NOE spectra. It is worthy to note that H-6 showed a singlet due

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to dihedral angles of 90° between H-5 and H-6 and that a "W"-type coupling between H-5 (d , $J = 1.6$ Hz) and H-17 (d , $J = 2.0$ Hz) was observed in the ^1H NMR of **1**. There is a lot of difference by comparison of the ^{13}C NMR data of **1** with that of **2**, such as C-1, C-2, C-3, C-11, C-17, and C-10, C-13, C-16, possibly attributing the conformational change of the ring A from the boat form in **1** to the chair form in **2**, as well as the shift effects of substitution group.⁹

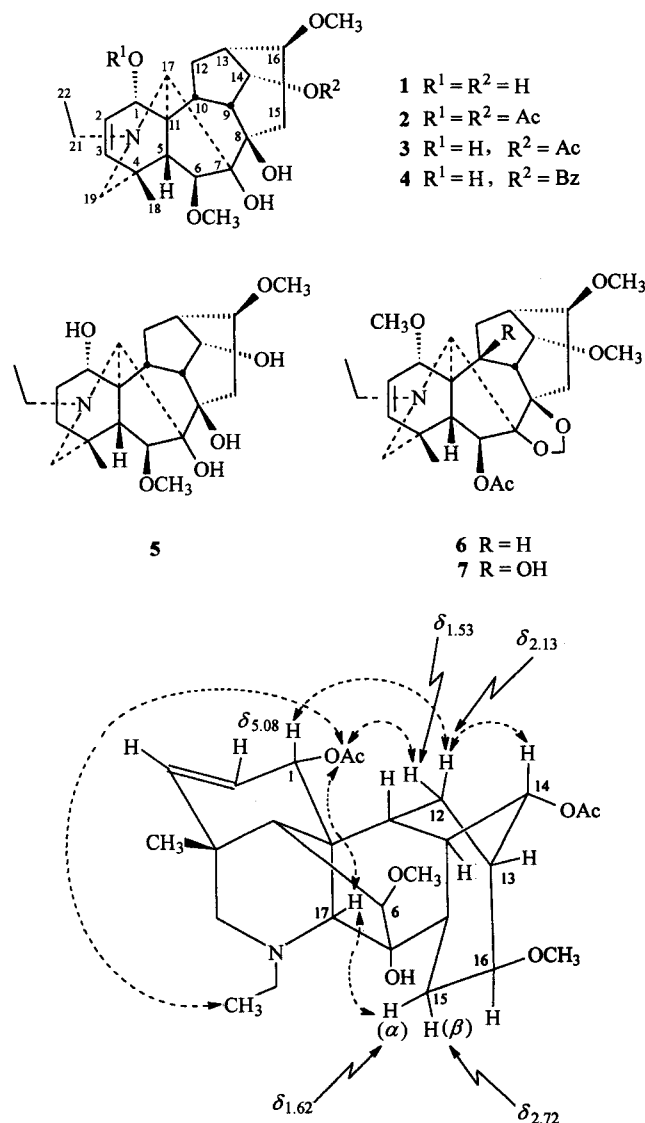


Fig. 1 NOE correlations of **2**.

Giraldine B (**3**) was obtained in an amorphous powder (CHCl_3 -MeOH), m.p. 76 – 78°C , $[\alpha]_{\text{D}}^{20} + 37.5$ (c 0.45 , CHCl_3). Its molecular formula $\text{C}_{25}\text{H}_{37}\text{NO}_7$ was derived from HREIMS (m/z 463.2554 [M^+]) and 2D NMR analyses. The NMR spectral data of **3** revealed signals at δ_{H} 1.06 (t, $J = 7.2$ Hz, 3H), 2.80 – 2.89 , 2.95 – 3.05 (m, each 1H), δ_{C} 13.5 (q), 50.1 (t) for an *N*-ethyl group; δ_{H} 3.35 , 3.37 (s, each 3H), δ_{C} 56.2 (q), 57.9 (q) for two methoxyl groups; δ_{H} 2.07 (s, 3H), δ_{C} 21.4 (q), 171.4 (s) for the acetyl group.

Its IR and ^{13}C NMR spectra [3462 cm^{-1} ; δ_{C} 86.5 (s), 78.4 (s), 71.0 (d)] showed the presence of two tertiary hydroxyl groups and a secondary hydroxyl group. Comparison of the NMR spectra of **3** with those of **1** showed that **3** had an additional acetyl group. The signal at δ 4.80 (t, $J = 5.2$ Hz, 1H) in the ^1H NMR spectrum of **3** indicated that the presence of an acetyl group at C-14.⁷ Finally, the structure of giraldine B was established as **3** by careful analyses of the ^1H NMR, ^{13}C NMR and 2D NMR (^1H - ^1H COSY, HMQC and HMBC) spectra of **3**.

Giraldine C (**4**), m.p. 58 – 59°C , $[\alpha]_{\text{D}}^{20} + 36.5$ (c 0.40 , CHCl_3), a white amorphous powder (CHCl_3 -MeOH), had a molecular formula $\text{C}_{30}\text{H}_{39}\text{NO}_7$ by HREIMS (m/z 525.2721 [M^+]). Its NMR spectra showed the presence of an *N*-ethyl group [δ_{H} 1.08 (t, $J = 7.2$ Hz, 3H); δ_{C} 50.1 (t), 13.6 (q)], two methoxyl groups [δ_{H} 3.36 , 3.38 (s, each 3H); δ_{C} 56.1 (q), 57.9 (q)], and a benzoyl group [δ_{H} 7.44 – 8.15 (m, 5H); δ_{C} : see *Experimental* section]. Comparison of the ^1H NMR spectrum of **4** with those of **3** indicated that the presence of the benzoyl group instead of an acetyl group at C-14 in **4**. Thus, the structure of giraldine C was established as **4**.

In addition, the identification (MS, ^1H NMR and ^{13}C NMR) of the known alkaloids, dihydrogadesine (**5**),¹⁰ tatsiensine (**6**)¹¹ and siwanine A (**7**),¹² were carried out. The assignment of the ^1H NMR and ^{13}C NMR data (Table 1) of **5** with no ^{13}C NMR and partial ^1H NMR data in Ref. 10, were made by its 2D NMR spectra (^1H - ^1H COSY, HMQC, HMBC).

To the best of our knowledge, there are only ten double bond $\Delta^{2(3)}$ -containing lycoctonine-type C_{19} -diterpenoid alkaloids, *e.g.*, tatsiensine (**6**),¹¹ deacetyltatsiensine,¹¹ siwanines A-D,¹² takaonine,¹³ ibukinamine,¹⁴ 2, 3-dehydrodelcosine¹⁵ and caeruleine,¹⁶ our research resulted in enhancing additionally number of this group alkaloids.

Experimental

General procedures

Melting point was performed on a Thermal values analysis with microscope and is uncorrected. Optical rotation was measured by a Perkin-Elmer 341 polarimeter, CHCl_3 , 1 cm cell. IR spectrum was recorded on a Nicolet FT-IR 200 SXV spectrophotometer with KBr pellets. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Unity INOVA 400/45 or Bruker AC-E 200 NMR spectrometer, TMS as internal standard. EIMS and HREIMS were taken on a VG Auto Spec 3000 or Finnegan MAT 90 instrument. Silica gel GF₂₅₄ and H (Qindao Sea Chemical Factory, China) were used for TLC, Chromatotron and CC, respectively. Spots on chromatograms were detected under UV light (254 nm) and with modified Dragendorff's reagent. A polyvinyl sulfonic ion exchange resin (H-form, cross linking 1×1 , Nankai University Chemical Factory, China) was used in the extraction of total alkaloids.

Table 1 NMR data of giraldine A (1), 2, giraldines B (3), C (4), and 5 (400 MHz for ^1H , 100 MHz for ^{13}C ; CDCl_3)

No.	1		2		3		4 ^a	5
	δ_{H} (J, Hz)	δ_{C}	δ_{H} (J, Hz)	δ_{C}	δ_{H} (J, Hz)	δ_{C}	δ_{C}	δ_{C}
1	3.74 d (4.8)	71.1 d	5.08 d (2.8)	75.3 d	3.75 d (4.8)	71.0 d	71.1 d	72.7 d
2	5.82 dd (9.6, 4.8)	130.5 d	5.70 dd (9.6, 2.8)	124.6 d	5.82 dd (9.6, 4.8)	130.6 d	130.7 d	29.2 t
3	5.66 d (9.6)	137.0 d	5.71 d (9.6)	138.8 d	5.66 d (9.6)	136.9 d	137.0 d	32.1 t
4	—	34.8 s	—	34.6 s	—	34.7 s	34.8 s	33.0 s
5	1.72 d (1.6)	53.8 d	1.81 d (1.6)	53.8 d	1.70 d (1.6)	53.6 d	53.8 d	50.7 d
6	3.92 s	91.2 d	3.90 s	90.6 d	3.81 s	91.3 d	91.4 d	90.9 d
7	—	86.7 s	—	87.1 s	—	86.5 s	86.8 s	87.8 s
8	—	78.1 s	—	78.0 s	—	78.4 s	78.5 s	77.9 s
9	2.90—3.02 m	45.4 d	1.88—1.96 m	44.8 d	2.00—2.08 m	44.2 d	43.2 d	45.2 d
10	2.30—2.40 m	39.7 d	2.32—2.43 m	38.6 d	2.37—2.47 m	38.5 d	38.4 d	39.2 d
11	—	48.9 s	—	47.9 s	—	49.2 s	49.4 s	49.0 s
12 α	1.96—2.05 m	27.6 t	1.50—1.58 m	27.8 t	1.95—2.05 m	28.0 t	27.9 r	29.1 t
12 β	2.00—2.10 m		2.08—2.17 m		2.19—2.28 m			
13	1.90—2.03 m	44.5 d	3.22—3.33 m	42.5 d	3.10—3.22 m	42.6 d	43.2 d	44.0 d
14	4.12 t (4.4)	75.5 d	4.75 t (4.8)	75.8 d	4.80 t (5.2)	76.3 d	76.4 d	75.6 d
15 α	1.62 dd (12.0, 6.4)	34.4 t	1.58—1.68 m	33.6 t	1.55—1.66 m	33.9 t	34.3 t	34.3 t
15 β	2.74—2.84 m		2.67—2.77 m		2.66—2.76 m			
16	3.30—3.42 m	82.0 d	3.20 t (8.0)	83.2 d	3.28—3.38 m	82.6 d	82.5 d	81.9 d
17	2.85 d (2.0)	65.0 d	2.85 d (2.8)	62.5 d	2.79 d (2.0)	64.7 d	64.8 d	65.6 d
18	1.12 s	24.4 q	1.08 s	23.9 q	1.11 s	24.4 q	24.4 q	27.6 q
19	2.40 ABq (12.0)	56.0 t	2.36 ABq (hidden)	56.1 t	2.40 ABq (hidden)	56.0 t	56.0 t	60.7 t
	2.44 ABq (12.0)		2.58 ABq (11.6)		2.46 ABq (hidden)			
21	2.75—2.85 m 3.02 s	50.1 t	2.61—2.72 m 3.05—3.15 m	49.5 t	2.80—2.89 m 2.95—3.05 m	50.1 t	50.1 t	50.1 t
22	1.06 t (7.2)	13.6 q	1.10 t (7.2)	13.9 q	1.06 t (7.2)	13.5 q	13.6 q	13.6 q
6'	3.38 s	57.9 q	3.38 s	57.9 q	3.37 s	57.9 q	57.9 q	57.9 q
16'	3.37 s	56.2 q	3.34 s	56.9 q	3.35 s	56.2 q	56.1 q	56.2 q
			2.08 s	170.6 s	2.07 s	171.4 s		
			(OAc-1)	21.6 q	(OAc-14)	21.4 q		
			2.07 s	171.3 s	3.89 s	—		
			(OAc-14)	21.3 q	(OH-1)			

^a 4: 166.6 s (OCO), 129.8 s (C-1''), 129.8 d (C-2'', 6''), 128.3 d (C-3'', 5''), 132.6 d (C-4'').

Plant material

Plants were collected in Taibai mountain of Shaanxi province, China, and authenticated by Professor W. T. Wang, Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (No. 98091501) has been deposited.

Extraction and isolation

According to the reported method,⁸ 12.5 kg of dry powdered roots of *Delphinium giraldii* Diels were percolated with HCl (0.05 mol/L, 250 L). After ion-exchanging (resin: dry weight 1.8 kg) and basifying with NH_4OH (5.6 L), successive extractions with ether (9000 mL) and CHCl_3 (4000 mL) under reflux until no alkaloid by detec-

tion of Dragendorff's reagent gave the crude alkaloids I (36 g) and II (3.5 g), respectively. The crude alkaloid I (23 g) was chromatographed successively on silica gel column eluting with CHCl_3 -MeOH (99:1—3:1, $V:V$) according to combination of the similar fractions by TLC detection to give seven parts, A (4.77 g), B (1.28 g), C (3.31 g), D (3.09 g), E (3.10 g), F (1.21 g) and G (2.56 g). Part A was separated on a silica gel H column eluting with cyclohexane-ethyl acetate-acetone-diethylamine (100:8:4:1—50:10:10:1, $V:V:V:V$) to afford giraldine A (**1**) (500 mg) and other fractions A-1 (67 mg), A-2 (270 mg) and A-3 (230 mg). Further centrifugal thin layer chromatograph of fraction A-1 eluting with petroleum ether-ethyl acetate-acetone (40:20:1, $V:V:V$) produced giraldine C (**4**, 8 mg). Fraction A-2 was chromatographed over silica gel H column (petroleum ether-acetone-ammonia solution 100:1:1, $V:V:V$) to afford tatsiensine (**6**, 34 mg) and giraldine B (**3**) (51 mg). Fraction A-3 was chromatographed on a Chromatotron eluting with petroleum ether-ethyl acetate-acetone (15:5:1, $V:V:V:V$) to afford siwanine A (**7**) (37 mg). Part C was chromatographed over silica gel H column (cyclohexane-acetone 3:1—1:1, $V:V$) to afford dihydrogadesine (**5**) (30 mg).

Giraldine A (1) White amorphous powder, m.p. 79—80 °C, $[\alpha]_D^{20} + 75.3$ (c 0.55, CHCl_3); ^1H NMR and ^{13}C NMR data see Table 1; IR (KBr) ν : 3472, 3430, 2934, 2902, 2821, 1639, 1457, 1393, 1219, 1090, 1049, 999, 949, 736, 672, 611 cm^{-1} ; FABMS m/z (%): 422 ($\text{M}^+ + 1$, 100), 406 (14), 391 (65). HRFABMS calcd for $\text{C}_{23}\text{H}_{35}\text{NO}_6$ [$\text{M}^+ + 1$] 422.2542, found 422.2534.

Acetylation of 1 A solution of **1** (150 mg) was dissolved in 5 mL of pyridine, then treated with 2 mL of Ac_2O for 72 h at room temperature. Usual work-up of the reaction mixture gave an acetate **2** (88 mg). **2**: white amorphous powder, m.p. 178—180 °C, ^1H NMR and ^{13}C NMR data see Table 1; FABMS m/z (%): 506 ($\text{M}^+ + 1$, 100), 490 (54), 446 (30), 122 (10). HRFABMS calcd for $\text{C}_{27}\text{H}_{39}\text{NO}_8$ [$\text{M}^+ + 1$] 506.2753, found 506.2738, NOE see Fig. 1.

Giraldine B (3) White amorphous powder, m.p. 76—78 °C, $[\alpha]_D^{20} + 37.5$ (c 0.45, CHCl_3); ^1H NMR and ^{13}C NMR data see Table 1; IR (KBr) ν : 3462, 2928, 1736, 1457, 1382, 1248, 1091, 1055, 999, 951, 737, 664, 599 cm^{-1} ; EIMS m/z (%): 463 (M^+ , 6), 448 (100). HREIMS calcd for $\text{C}_{25}\text{H}_{37}\text{NO}_7$ 463.2570, found 463.2554.

Giraldine C (4) White amorphous powder, m.p. 58—59 °C, $[\alpha]_D^{20} + 36.5$ (c 0.40, CHCl_3); ^1H NMR (CDCl_3 , 200 MHz) δ : 1.08 (t, $J = 7.2$ Hz, 3H, $\text{N-CH}_2\text{CH}_3$), 1.12 (s, 3H, 18- CH_3), 3.36, 3.38 (s, each 3H, 2 \times OCH₃), 5.09 (t, $J = 4.6$ Hz, 1H, H-14 β), 5.70 (d, $J = 9.4$ Hz, 1H, H-3), 5.80 (dd, $J = 9.4$, 4.6 Hz, 1H, H-2), 7.44—8.15 (m, 5H, ArH); IR (KBr) ν : 3458, 3433, 2927, 1717, 1642, 1454, 1379, 1279, 1220, 1182, 1124, 1091, 1030, 950, 715 cm^{-1} ;

EIMS m/z (%): 525 (M^+ , 5), 510 (88), 494 (7), 105 (100), 77 (24), 58 (52). HREIMS calcd for $\text{C}_{30}\text{H}_{39}\text{NO}_7$ 525.2726, found 525.2721.

Dihydrogadesine (5) ^1H NMR (CDCl_3 , 400 MHz) δ : 3.58—3.72 (m, 1H, H-1), 1.83—1.97 (m, 1H, H-2 α), 2.00—2.10 (m, 1H, H-2 β), 1.52—1.64 (m, 1H, H-3), 1.54 (d, $J = 1.6$ Hz, H-5), 3.95 (s, 1H, H-6), 2.88—3.00 (m, 1H, H-9), 2.31—2.41 (m, 1H, H-10), 1.54—1.68 (m, 1H, H-12 α), 1.60—1.69 (m, 1H, H-12 β), 1.75—1.84 (m, 1H, H-13), 4.10 (t, $J = 4.8$ Hz, 1H, H-14 β), 1.65—1.75 (m, 1H, H-15 β), 2.70—2.80 (m, 1H, H-15 α), 3.30—3.42 (m, 1H, H-16), 2.86 (d, $J = 2.0$ Hz, H-17), 1.11 (s, 3H, H₃-18), 2.47, 2.51 (ABq, $J = 12.0$ Hz, each 1H, H₂-19), 2.76—2.87, 2.93—3.02 (m, each 1H, H₂-21), 1.10 (t, $J = 7.2$ Hz, H₃-22), 3.37 (s, 3H, H₃-6'), 3.36 (s, 3H, H₃-16').

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