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THREE NEW LYCOCTONINE-TYPE C_{19}-DITERPENOID ALKALOIDS FROM *DELPHINIUM GIRALDII*

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Abstract - From the roots of *Delphinium giraldii*, three new lycoctonine-type C_{19}-diterpenoid alkaloids, giraldine D (**1**), giraldine E (**2**) and giraldine F (**3**), have been isolated. The structures of **1**~**3** were established by spectroscopic evidence including 2D NMR spectroscopy.

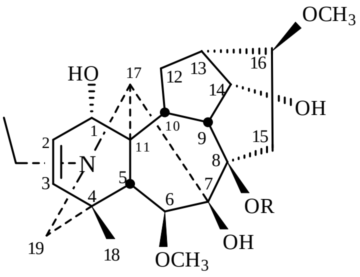
INTRODUCTION

The plant *Delphinium giraldii* Diels (Ranunculaceae) is a species endemic to Taibai mountain, Shanxi province in China.[1] In the previous paper,[2] we have isolated three new C_{19}-diterpenoid alkaloids, giraldines A, B and C, together with three known C_{19}-diterpenoid alkaloids, dihydrogadesine, tatsiensine and siwanine A. Continuing investigations on the roots of *Delphinium giraldii* have now led to the isolation of another three new lycoctonine-type C_{19}-diterpenoid alkaloids giraldines D (**1**), E (**2**), and F (**3**). This paper deals with the separation and structural elucidation of these new alkaloids (**1**~**3**).

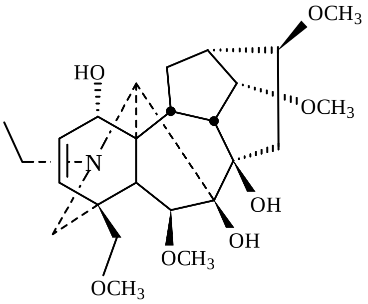
RESULTS AND DISCUSSION

The new bases, giraldines D (**1**), E (**2**) and F (**3**), were obtained as amorphous powder substances. Their molecular formulae, $C_{24}H_{37}NO_6$, $C_{25}H_{39}NO_7$ and $C_{23}H_{33}NO_6$, respectively, were inferred from their HRMS and 2D-NMR spectra. NMR and MS spectra showed that they were the lycoctonine-type C_{19}-diterpenoid alkaloids.[3]

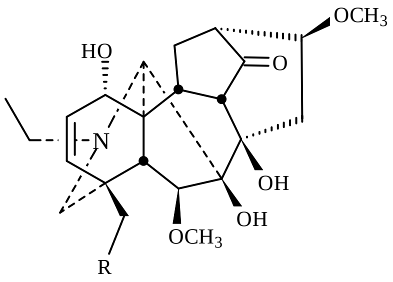
The NMR spectra of giraldine D (**1**) exhibited the signals at δ_H 1.06 (3H, t, J=7.2 Hz), δ_C 50.2 t, 13.7 q for an *N*-ethyl group; δ_H 3.39, 3.41, 3.48 (each 3H, s), δ_C 56.3 q, 57.5 q 59.4 q for three aliphatic methoxyl groups; δ_H 5.66 (1H, d, J=9.2 Hz), 5.77 (1H, dd, J=9.2, 4.8 Hz) and δ_C 130.4 d and 137.1 d for a disubstituted double bond, δ_H 1.07 (s, 3H) and δ_C 24.0 q for a tertiary-CH_3. Its IR and ^{13}C NMR spectra also showed distinctive signals at 3425 and 3429 cm^{-1}, δ_C 70.8 d and 74.8 d for two secondary hydroxyl groups, and δ_C 90.6 s for a tertiary hydroryl group. Comparison of the MS and NMR spectra of **1** with giraldine A (**4**)[2] showed that it had an additional methoxyl group instead of a hydroxyl group in **4**. The ^{13}C NMR spectra of **1** and **4** are very similar except for C-5, C-7, C-8, C-9, C-13 and C-15 (Table 1), indicating that the additional methoxyl group was located on C-8.[4] This assignment was further confirmed by the multibond correlations between 8-OCH_3 (δ_H 3.41 s) and C-8 (δ_C 81.1 s) (Table 1) in the HMBC (Figure 1) of **1**. Finally, the structure of giraldine D was established as **1** by careful analyses of the ^{1}H ^{13}(C) NMR and 2D-NMR (^{1}H-^{1}H COSY, HMQC, and HMBC) spectra.



1 R=CH_3
4 R=H



2



3 R=H
5 R=OCH_3

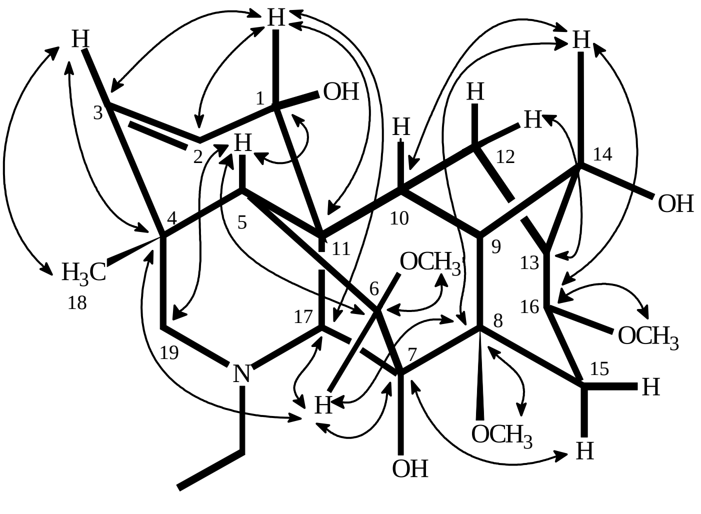


Figure 1 The key HMBC correlations of **1**

The NMR spectra of giraldine E (**2**) exhibited an *N*-ethyl group [δ_H 1.06 (3H, t, *J*=7.2 Hz), δ_C 50.2 t, 13.5 q], four methoxyl groups (δ_H 3.36, 3.36, 3.38, 3.42, each 3H, s; δ_C 56.2 q, 57.2 q, 57.6 q, 59.1 q) and a disubstituted double bond [δ_H 5.82 (1H, dd, *J*=9.6, 4.4 Hz); 5.86 (1H, d, *J*=9.6 Hz); δ_C 131.6 d, 134.2 d]. Its IR spectra (3427 cm^{-1}) and ^{13}C NMR spectra (δ_C 71.0 d, 78.5 s, 86.7 s) also showed the presence of a secondary hydroxyl group and two tertiary hydroxyl groups. The HMBC spectrum (Figure 2) of **2**

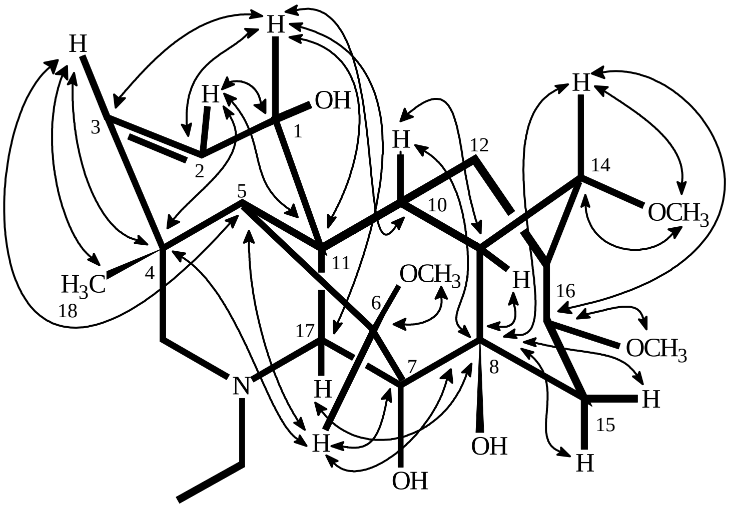


Figure 2 The key HMBC correlations of **2**

showed the multiple bond correlations among the H-2 (δ_H 5.82) and C-1 (δ_C 71.0), C-4 (δ_C 39.9 s), C-11 (δ_C 49.1 s); as well as the H-3 (δ_H 5.86) and C-1 (δc 71.0), C-4 (δc 39.9 s), C-5 (δc 48.8 d), C-18 (δc 75.8 t), supporting the presence of $\Delta^{2(3)}$ double bond with 1-OH. Two tertiary hydroxyl groups were assigned to C-7, C-8 based on the presence of correlations between the C-8 (δ_C 78.5 s) and H-6 (δ_H 3.93), H-9 (δ_H 2.98), H-10 (δ_H 2.00 m), H-15 (δ_H 1.74, 2.64), H-17 (2.84) as well as the C-7 (86.7 s) and H-6 (δ_H 3.93), H-15 (δ_H 1.74, 2.64) in the HMBC of **2**. One signal at δ 3.64 (1H, t, *J*=4.6) in the ^{1}H NMR spectrum of **2** showed the presence of 14α–OCH$_3$.[3] The other three methoxyl groups in **2** could be located at C-6, C-16 and C-18, respectively by showing two oxygenated methane signals at δ_C 91.1 d, 82.9 d and one oxygenated methylene at δ_C 75.8 t in the ^{13}C NMR of **2**,[3] and the multiple bond correlations between the 6-OCH$_3$ (δ_H 3.38) and C-6 (δ_C 91.1), the 16-OCH$_3$ (δ_H 3.36) and C-16 (δ_C 82.9), the 18-OCH$_3$ (δ_H 3.36) and C-18 (δ_C 75.8) in the HMBC of **2**. The structure of giraldine E, thus, was determined as **2**. Assignments based on ^{1}H and ^{13}C NMR data of **2** (Tables 1) were carried out by their ^{1}H-^{1}H COSY, HMQC, and HMBC spectra.

The NMR spectra of giraldine F (**3**) displayed signals at δ_H 1.09 (3H, t, *J*=7.2 Hz), δ_C 50.3 t, 13.6 q for an N-ethyl, δ_H 3.35, 3.37 (each 3H, s), δ_C 56.0 q, 57.9 q for two methoxyl groups, δ_H 5.84 (1H, dd, *J*=9.2, 4.8 Hz), 5.69 (1H, d, *J*=9.2 Hz), δ_C 130.5 d, 137.1 d for an disubstituted double bond, and δ_H 1.13 (3H, s), δ_C 24.4 q for a tertiary methyl group. Its IR and ^{13}C NMR spectra showed distinctive signals at 3462 and 3427 cm^{-1}, δ_C 70.8 d, 83.0 s, 86.3 s for one secondary hydroxyl group and two tertiary hydroxyl groups. Comparison with takaonine (**5**)[4] showed that **3** had an additional tertiary methyl group but lacked an oxygenated methylene group and a methoxyl group. In addition, the ^{13}C NMR spectra data of both alkaloids are very similar except for C-3, C-4, C-5, C-18 and C-19, indicating that **3** had a tertiary methyl

Table 1 NMR data of Compounds (**1, 2, 3, 4,** and **5**) (400 MHz for ^{1}H, 100 MHz for ^{13}C, CDCl$_3$)

	1		**2**		**3**		**4**	**5**
No.	δ_{H} (J=Hz)	δc	δ_{H} (J=Hz)	δc	δ_{H} (J=Hz)	δc	δc	δc
1	3.70 d (4.8)	70.8 d	37.5 d (4.4)	71.0 d	3.87 d (4.8)	70.8 d	71.1 d	70.8
2	5.77 dd (4.8, 9.2)	130.4 d	5. 82 dd (4.4, 9.6)	131.6 d	5.84 dd (4.8, 9.2)	130.5 d	130.5 d	131.3
3	5.66 d (9.2)	137.1 d	5.86 d (9.6)	134.2 d	5.69 (9.2)	137.1 d	137.0 d	134.5
4	—	34.7 s	—	39.9 s	—	34.8 s	34.8 s	40.0
5	3.49 s	51.6 d	2.06 d (1.6)	48.8 d	3.08 d (2.0)	53.2 d	53.8 d	49.0
6	3.69 s	91.6 d	3.93 s	91.1 d	3.93 s	91.2 d	91.2 d	90.4
7	—	90.6 s	—	86.7 s	—	86.3 s	86.7 s	86.2
8	—	81.1 s	—	78.5 s	—	83.0 s	78.1 s	83.0
9	3.31 m	40.3 d	2.98 m	43.5 d	2.20 m	53.9 d	45.4 d	53.2
10	2.28 m	40.0 d	2.00 m	44.7 d	2.23 m	41.7 d	39.7 d	41.6
11	—	48.4 s	—	49.1 s	—	49.8 s	48.9 s	49.6
12	1.92 m (α) 2.08 m (β)	26.3 t	1.92 m (α) 2.20 dd (4.0, 13.2) (β)	28.6 t	2.50 m	25.8 t	27.6 t	25.8
13	2.04 m	46.0 d	2.40 m	38.7 d	2.44 m	47.1 d	44.5 d	47.1
14	4.00 t (4.8)	74.8 d	4.80 t (4.8)	84.4 d	—	215.4 s	75.5 d	215.3
15	1.83 m (β) 2.67 dd (18.8, 7.6) (α)	31.1 t	1.74 dd (8.8, 14.8) (β) 2.64 dd (8.8, 14.8) (α)	33.6 t	1.35 m (β) 2.72 dd (15.6, 8.0) (α)	35.0 t	34.4 t	35.1
16	3.40 m	82.2 d	3.30 t (8.8)	82.9 d	3.86 m	86.7 d	82.0 d	86.7
17	2.86 s	65.2 d	2.84 d (2.8)	65.0 d	3.25 s	65.3 d	65.0 d	65.7
18	1.07 s	24.0 q	3.17 ABq (6.8) 3.47 ABq (6.8)	75.8 t	1.13 s	24.4 q	24.4 q	75.5
19	2.43 ABq (11.6) 2.47 ABq (11.6)	56.2 t	2.33 ABq (11.2) 2.41 ABq (11.2)	51.8 t	2.40 m 2.52 m	56.0 t	56.0 t	51.6
21	2.88 m 3.00 m	50.2 t	2.80 m 3.02 m	50.2 t	3.02 m 2.88 m	50.3 t	50.1 t	50.5
22	1.06 t (7.2)	13.7 q	1.06 t (7.2)	13.5 q	1.09 t (7.2)	13.6 q	13.6 q	13.8
6-OCH$_3$	3.48 s	59.4 q	3.38 s	57.2 q	3.37 s	57.9 q	57.9 q	57.3
8-OCH$_3$	3.41 s	57.5 q	—	—	—	—	—	—
14-OCH$_3$	—	—	3.42 s	57.6 q	—	—	—	—
16-OCH$_3$	3.39 s	56.3 q	3.36 s	56.2 q	3.35 s	56.0 q	56.2 q	56.1
18-OCH$_3$	—	—	3.36 s	59.1 q	—	—	—	59.3

group at C-4 instead of -CH_2OCH_3 group in **5**. This assignment was supported by the observation of an HMBC cross signal between H_3-18 (δ_H 1.13) and the C-4 (δ_C 34.8 s). Finally, unambiguous assignments of the ^1H and ^{13}C chemical shifts for giraldii F (**3**) (Table 1) were accomplished by the 2D NMR techniques (^1H-^1H COSY, HMQC, HMBC). All available evidence indicated the structure of giraldine F to be as depicted in **3**.

EXPERIMENTAL

General Experimental Procedure. Melting points were determined with a Thermal values analysis with microscope (uncorrected). Optical rotation was taken on a Perkin-Elmer 341 polarimeter. IR spectrum was recorded on a Nicolet FT-IR 200 SXV spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Varian Unity INOVA 400/45 NMR spectrometer, in $CDCl_3$ with TMS as the internal standard. EIMS and HREIMS were obtained from a VG Auto Spec 3000 or Finnegan MAT 90 instrument. Silica gel G_{254} and H (Qindao Sea Chemical Factory, China) were used for TLC, Chromatotron and CC, respectively. Spots on TLC (silica gel GF_{254}) were detected under UV light (245 nm) and 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 total alkaloids.

Plant Material. The *Delphinium giraldii* was collected in Taibai mountain of Shanxi province, China, and authenticated by Professor W. T. Wang of the Beijing Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (No. 98091501) has been deposited.

Extraction and Isolation. Powdered roots (12.5 kg) of *Delphinium giraldii* Diels were percolated with 0.05 mol/L HCl (250 L). Wet resin (dry weight 1.8 kg) was added to the percolates followed by washing repeatedly on a suction filter with deionized H_2O. The air-dried resin was then mixed with 10% aqueous NH_4OH (total amount 5.6 L), and extracted sequentially in a specially designed extractor[6] with ether (9000 mL) and chloroform (400 mL) under reflux until no alkaloid could be detected with Dragendorff’s reagent to give the crude alkaloids **I** (36 g) and **II** (3.5 g), respectively.

The crude alkaloid **I** (23 g) was subsequently chromatographed successively on silica gel H column eluting with $CHCl_3$-MeOH (99:1~3:1) to afford six parts, A (4.77 g), B (1.28 g), C (3.3 g), D (1.45 g), E (3.3 g) and F (5.1 g). Part A was subjected to silica gel H column chromatography eluting with cyclohexane-ethyl acetate-acetone-diethylamine (100:8:4:1~50:10:10:1) to provide three fractions, A-1 (67 mg), A-2 (270 mg), and A-3 (230 mg). Fraction A-2 was separated on a silica gel H column eluted with petroleum ether-acetone-diethylamine (100:10:1) to give four subfractions, A-2-1 (68 mg), A-2-2 (188 mg), A-2-3 (151 mg) and A-2-4 (23 mg). Further centrifugal thin layer chromatography of fraction A-2-2 eluting with $CHCl_3$-MeOH (200:1) produced giraldine D (**1**) (18 mg). Fraction A-2-3 was chromatographed over a Chomatotron with petroleum ether-ether-acetone (4:4:1) as solvent system to provide giraldine F (**3**) (64 mg). Column chromatography of fraction A-2-4 with cyclohexane-acetone-diethylamine (80:20:1) as eluent gave giraldine E (**2**). In addition, Fraction A-3 was chromatographed on a silica gel H Column (petroleum ether-acetone-diethylame, 90:15:1) and a Chomatotron (petroleum ether-ethyl acetate-acetone, 15:5:1), successively to provide additional giraldine D (**1**) (14 mg).

Giraldine D (1). White amorphous powder, mp 159-160 °C; $[\alpha]_D^{20}$ +38.5° (c 0.30, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3449, 3425, 2927, 1460, 1379, 1260, 1092, 1017, 949, 804, 675, 607; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): Table 1; EIMS *m/z* (%): 435 (M, 6), 420 (M-15, 100), 404 (M-31, 19), 97 (21), 71 (29), 58 (73); HREIMS *m/z*: 435.2619, calcd for $C_{24}H_{37}NO_6$, 435.2620.

Giraldine E (2). white amorphous powder, mp 51-53 °C; $[\alpha]_D^{20}$ +16.0° (c 0.80, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3471, 2917, 2850, 1728, 1635, 1463, 1400, 1299, 1261, 1199, 1054, 1027, 1004, 948, 875, 802, 752, 675, 605, 541, 501, 484; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): Table 1; FABMS *m/z* (%): 466 (M^++H), 391 (11); HRFABMS *m/z*: 466.2809 (M^++H), calcd for $C_{25}H_{39}NO_7$ (M^++H), 466.2804.

Giraldine F (3). white amorphous powder, mp 55-57 °C; $[\alpha]_D^{20}$ +12.3° (c 0.70, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3451, 3427, 2929, 1748, 1659, 1459, 1379, 1213, 1090, 1051, 999, 948, 738, 678; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): Table 1; EIMS *m/z* (%): 419 (M^+, 7), 404 (M-15, 100), 388 (M-31, 18), 343 (12), 122 (12), 105 (16), 91 (25), 58 (71); HREIMS *m/z*: 419.2306, calcd for $C_{23}H_{33}NO_6$, 419.2307.

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