Three New C_{19}-Diterpenoid Alkaloids from *Delphinium giraldii*

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Further investigation of the roots of *Delphinium giraldii* DIELS led to the isolation of three new C_{19}-diterpenoid alkaloids, giraldines G (1), H (2), and I (3). The structures of 1—3 were established based on spectroscopic evidence.

Key words *Delphinium giraldii*; Ranunculacene; C_{19}-diterpenoid alkaloid; giraldine G; giraldine H; giraldine I

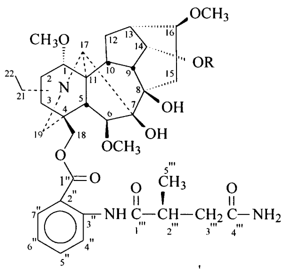
The isolation and structure elucidation of six new C_{19}-diterpenoid alkaloids as well as three known C_{19}-diterpenoid alkaloids from the roots of *Delphinium giraldii* (Ranunculaceae) have been reported in our previous papers.[1,2)] Our continuing investigations of the alkaloids of this plant resulted in the isolation of three new C_{19}-diterpenoid alkaloids, giraldines G (**1**), H (**2**), and I (**3**). This paper describes the isolation and structure determination of these new alkaloids.

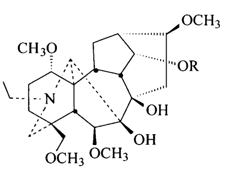
Results and Discussion

Giraldine G (**1**), $C_{40}H_{57}N_3O_{11}$ (HR-EI-MS), exhibited characteristic NMR features of a lycoctonine-type C_{19}-diterpenoid alkaloid,[3,4)] bearing an *N*-ethyl (δ_H 1.05, 3H, t, J=7.2 Hz; δ_C 50.9 t, 14.0 q), three methoxyl (δ_H 3.25, 3.28, 3.36, each 3H, s; δ_C 55.7 q, 55.9 q, 58.0 q), a substituted anthranoyl (δ_H 11.16, 1H, s, $\underline{N}H$, 7.07—8.71, 4H, m, 5.36, 5.80, each 1H, s, $\underline{N}H_2$; 1.35, 3H, d, J=7.0 Hz; δ_C see Table 1), and an isobutyryl (δ_H 1.16, 6H, d, J=7.0 Hz; δ_C see Table 1) groups. It's NMR spectrum indicated the presence of a C-18 ester moiety as delsemine A (**4**),[5)] and a C-14 ester residue as occidentalidine (**5**).[6)] The ^{13}C-NMR spectrum compares well with those of **4**[5)] (Table 1) except for the replacement of $C_{(14)}$-OMe in **4** with an isobutyryl group. These results led to the assignment of the structure of giraldine G as **1**. In addition, the stereochemistry of the methylsuccinimide moiety in methyllycaconitine has been assigned to be "*S*" by Blagbrough and his coworkers.[7)] Therefore the stereochemistry of C-2‴ in **1** could be deduced to be "*S*" based on comparisons of the ^{13}C-NMR data with those of **4**.

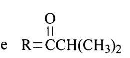
Giraldine H (**2**), $C_{41}H_{59}N_3O_{11}$ (HR-EI-MS), was also a lycoctonine-type C_{19}-diterpenoid alkaloid.[3,4)] The NMR spectra displayed signals at δ_H 1.06 (3H, t, J=7.2 Hz) and δ_C 14.0 q, 51.0 t, for an *N*-ethyl group, δ_H 3.25, 3.28, 3.36 (each 3H, s) and δ_C 55.8 q, 55.9 q, 58.1 q for three methoxyl groups, δ_H 11.16 (1H, s), 7.08—8.71 (1H, m), 5.34, 5.79 (each 1H, s), 1.35 (3H, d, J=7.0 Hz) and δ_C (see Table 1) for a substituted anthranoyl founctional group. Its ^{13}C-NMR spectrum also showed signals characteristic of the 2-methylbutyryl group (Table 1) which compare well with those of in jiufengdine (**6**)[8)] and glaucedine (**7**).[9)] Comparison of the ^{13}C-NMR data with those of **1** (Table 1) indicated that they differed only in the nature of the C-14 ester chain. This was also suggested by the difference of 14 mass units between the two compounds in their mass spectra. All available evidence strongly suggests the structure of giraldine H as depicted for **2**. Meanwhile, the stereochemistry of C-2′ in **2** could be deduced to be "*S*" based on comparisons of the ^{13}C-NMR data with those of glaucedine (**7**), in which the absolute configuration of C-2′ was firmly established by synthesis.[9)]

Giraldine I (**3**) was obtained as an amorphous powder. Its molecular formula $C_{22}H_{35}NO_3$ was inferred from its HR-ESI-MS and 2D-NMR. The NMR spectral data showed the presence of an *N*-ethyl [δ_H 1.03 (3H, t, J=7.2 Hz); δ_C 13.6 q, 49.4 t], a methoxyl (δ_H 3.27, s; δ_C 56.3 q), and a tertiary methyl [δ_H 0.75 (3H, s); δ_C 26.2 q] group. Along with the above-mentioned signals, the ^{13}C-NMR spectrum displayed three oxygenated carbon signals (δ_C 74.7 d, 75.5 s, 86.5 d), suggesting the presence of two hydroxyl groups in addition to a methoxyl group. The spectral characteristics of **3** are indicative of an aconitine-type C_{19}-diterpenoid alkaloid.[3,4)] A triplet signal at δ_H 4.00 (J=4.8 Hz) was attributed to H-14β,[3)] implying the presence of a hydroxyl group at the C-14 position. The remaining hydroxyl group could be located at C-8 due to the ^{1}H–^{13}C long-range correlations (HMBC) between C-8 (δ_C 75.5 s) and H-14 (δ_H 4.00), H-17 (δ_H 3.42), and H-6 (δ_H 1.40, 1.86). Similarly, the methoxyl group was assigned to C-1 mainly based on the presence of correlations



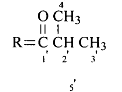


5 occidentalidine



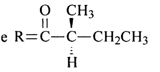
$R=\overset{O}{\overset{\|}{C}}CH(CH_3)_2$

1 giraldine **G**



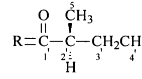
$R=\overset{O}{\overset{\|}{C}}\overset{\overset{4'}{CH_3}}{\overset{|}{CH}}CH_3$

7 glaucedine



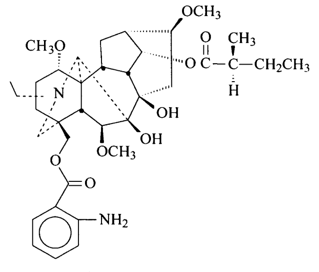
$R=\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\underset{H}{C}}-CH_2CH_3$

2 giraldine **H**

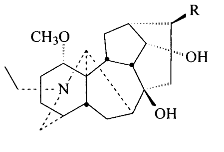


$R=\overset{O}{\overset{\|}{C}}-\overset{\overset{5'}{CH_3}}{\underset{H}{C}}-CH_2CH_3$

4 delsemine **A** $R=CH_3$



6 jiufengdine.



3 giraldine **I** R=H

8 genicunine **A** R=OH

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Table 1. ^{13}C-NMR Data of Compounds **1**—**7**

No.	**1**	**2**	**4**	**5**	**6**	**7**
1	83.7 d	83.8 d	83.9 d	84.2	83.9 d	84.3
2	25.9 t	26.0 t	26.1 t	26.2	26.0 t	26.2
3	32.1 t	32.2 t	32.2 t	32.4	32.2 t	32.4
4	37.5 s	37.7 s	37.6 s	38.0	37.7 s	37.1
5	50.0 d	50.2 d	50.5 d[a]/43.3[b]	49.5[a]/43.0[b]	51.5 d	51.1[a]/43.2[b]
6	90.6 d	90.8 d	91.0 d	90.4	90.7 d	90.5
7	88.4 s	88.4 s	88.6 s	88.3	88.4 s	88.4
8	77.3 s	77.3 s	77.5 s	77.4	77.0 s	77.4
9	42.8 d	43.1 d	43.3[a]/50.5 d[b]	4.30[a]/49.5[b]	43.1 d	43.2[a]/51.1[b]
10	45.8 d	45.7 d	46.1 d[a]/38.2 d[b]	45.6	45.7 d	45.7[a]/38.1[b]
11	48.9 s	49.0 s	49.1 s	48.8	49.0s	49.6
12	28.0 t	28.2 t	28.7 t	28.1	28.2 t	28.3
13	37.7 d	37.6 d	38.2 d[a]/46.1 d[b]	37.7	37.6 d	38.1[a]/45.7[b]
14	75.5 d	75.4 d	83.9 d	75.6	75.3 d	75.6
15	33.6 t	33.7 t	33.7 t	33.7	33.7 t	33.8
16	82.1 d	82.2 d	82.6 d	82.2	82.2 d	82.3
17	64.3 d	64.4 d	64.5 d	64.7	64.4 d	64.8
18	69.6 t	69.7 t	69.8 t	78.4	68.5 t	78.1
19	52.2 t	52.3 t	52.4 t	52.6	52.4 t	52.8
21	50.9 t	51.0 t	50.9 t	51.1	51.0 t	48.9
22	14.0 q	14.0 q	14.0 q	14.1	14.0 q	14.2
1-OCH_3	55.9 q	55.9 q	55.7 q	55.7	55.7 q	55.8
6-OCH_3	58.0 q	58.1 q	57.8 q	57.2	58.0 q	57.4
14-OCH_3	—	—	58.1 q	—	—	—
16-OCH_3	55.7 q	55.8 q	56.3 q	55.8	55.8 q	55.8
18-OCH_3	—	—	—	58.9	—	59.0
1′	177.3 s	176.9 s	—	177.2	176.8 s	176.9
2′	34.1 d	41.2 d	—	34.2	41.2 d	41.3
3′	18.7 q	26.2 t	—	18.8	26.2 t	26.2
4′	18.8 q	11.4 q	—	18.8	11.5 q	11.6
5′	—	16.1 q	—	—	16.1 q	16.2
1″	167.9 s	167.9 s	168.1 s	—	167.7 s	—
2″	114.8 s	114.9 s	114.7 s	—	110.3 s	—
3″	141.7 s	141.7 s	141.9 s	—	150.7 s	—
4″	120.5 d	120.7 d	120.7 d	—	116.7 d	—
5″	134.8 d	134.9 d	134.9 d	—	134.3 d	—
6″	122.6 d	122.7 d	122.5 d	—	116.3 d	—
7″	130.2 d	130.3 d	130.3 d	—	130.6 d	—
1‴	174.6 s	174.6 s	174.1 s	—	—	—
2‴	39.3 d	39.4 d	39.3 d	—	—	—
3‴	39.0 t	39.2 t	39.0 t	—	—	—
4‴	173.3 s	173.3 s	172.4 s	—	—	—
5‴	18.1 q	18.2 q	18.0 q	—	—	—

a) Revised data, *b*) Original data. ^{13}C chemical shift assignments for C-5, C-9, C-10, and C-13 of compounds **4**, **5**, and **7** were revised based on the comparison with those of jiufengdine (**6**),[8] in which the ^{13}C-NMR signals were assigned unambiguously base on 2D-NMR spectra.

between 1-OCH_3 (δ_H 3.27) and C-1 (δ_C 86.5) in the HMBC of **3**. A tertiary methyl could be located on C-4 due to the observation of long-range ^1H–^{13}C correlations between H_3-18 (δ_H 0.75) with C-4 (δ_C 34.6 s) in the HMBC of **3**. In addition, the NMR spectra of **3** lacked a hydroxyl group at C-16 when compared with genicunine A (**8**).[10] ^{13}C-NMR spectra of the two alkaloids are very close, especially in rings A and B, except for C-8, C-12, C-13, C-15, and C-16. Finally, comparisons of ^{13}C-NMR (Table 2) and MS data between **3** and **8**,[10] especially in its 2D-NMR, led to determine the structure of **3**.

Experimental

General Experimental Procedures Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectra were obtained on a Nicolet FT-IR 200 SXV spectrophotometer. ^{1}H- 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. EI-MS and HR-EI-MS were measured from a VG Auto Spec 3000 or Finnegan MAT 90 instrument. Silica gel GH_{254} and H (Qindao Sea Chemical Factory, China) were used for TLC, and Chromatotron and column chromatography, respectively. Spots on TLC 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, Chemical Factory of Nankai University, China) was used for the extraction of total alkaloids.

Plant Material The *Delphinium giraldii* was collected on Taibai Mountain, 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 According to method reported in the literature,[11] 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 percolate, followed by repeated washing on a suction filter with deionized H_2O. The air-dried resin was then alkalized with 10% aqueous NH_4OH (total amount 5.6 l) and extracted sequentially in a specially designed extractor[11] with ether (9 l), chloroform (400 ml), and 95% ethanol (2 l) under reflux until no alkaloid could be detected with Dragendorff's reagent to give the crude alkaloids I (ether extract: 36 g), II ($CHCl_3$ extract: 3.5 g) and 95% ethanol extract, respectively. The 95% ethanol extract was dissolved in 15% aqueous HCl solution and filtered. Then the filtrate was basified to pH 10 with concentrated NH_4OH and extracted with chloroform to produce crude alkaloid III (8.5 g).

Table 2. NMR Data of Compounds **3** and **8**

No.	**3**		**8**
	δ_H (J=Hz)	δ_C	δ_C
1	3.08 dd (10.4, 6.4)	86.5 d	86.3
2	1.81 m (α) 2.22 m (β)	26.2 t	26.2
3	1.28 m 1.63 m	37.9 t	37.5
4	—	34.6 s	34.2
5	1.42 d (6.8)	51.3 d	50.6
6	1.40 dd (14.4, 6.8) 1.86 dd (14.4, 7.6)	25.1 t	24.9
7	2.08 m	46.8 d	45.6
8	—	75.5 s	73.5
9	2.14 m	46.4 d	46.3
10	1.60 m	45.7 d	46.0
11	—	49.4 s	48.7
12	2.08 m (α) 2.14 t (5.2) (β)	29.8 t	27.8
13	2.04 m	35.2 d	45.9
14	4.00 t (4.8)	74.7 d	75.5
15	1.57 m	26.2 t	42.2
16	1.32 m 2.12 d (5.6)	22.6 t	72.3
17	3.42 s	62.8 d	62.5
18	0.75 s	26.2 q	26.0
19	2.07 hidden 2.45 d (11.2)	56.9 t	56.7
21	2.48 m	49.4 t	49.3
22	1.03 t (7.2)	13.6 q	13.5
1-OCH_3	3.27 s	56.3 q	56.2

The crude alkaloid I (23 g) was chromatographed on a 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 (33 mg), A-2 (550 mg), and A-3 (640 mg). Fraction A-3 was chromatographed on a silica gel H column eluting with petroleum ether–acetone–diethylamine (90 : 15 : 1) to furnish fractions A-3-1 (234 mg), A-3-2 (122 mg), A-3-3 (31 mg), and A-3-4 (23 mg). Further column chromatography of fraction A-3-2 eluting with chloroform–methanol–concentrated ammonia (300 : 2 : 3) followed by HPLC purification (RP-C18, 10 μm, 1.0×20 cm; mobile phase: CH_3OH–H_2O, 75 : 25; Waters 2410 refraction detector) provided giraldines G (**1**) (8 mg) and H (**2**) (7 mg). In addition, crude alkaloids II, III and part F of crude alkaloid I were combined and subjected to MPLC ($CHCl_3$–CH_3OH, 100 : 5—1 : 1) followed by column chromatography eluting with chloroform–acetone–diethylamine (80 : 20 : 1) to produce three fractions, A′ (1.2 g), B′ (500 mg), and C′ (120 mg). Part A′ was separated on a silica gel H column eluting with petroleum ether–acetone–diethylamine (50 : 50 : 1) to yield two fractions, A′-1 (240 mg) and A′-2 (700 mg). Fraction A′-1 was chromatographed on a Chromatotron (cyclohexane–ethyl acetate–acetone, 4 : 1 : 1) and a silica gel H column (cyclohexane–acetone–diethylamine, 60 : 40 : 1) to afford giraldine I (**3**) (26 mg).

Giraldine G (**1**): White amorphous powder, mp 108—110 °C; $[\alpha]_D^{20}$ +35.4° (c=0.42, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3438, 3365, 1723, 1682, 1605, 1587, 1295, 1257; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.05 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{CH_3}$), 1.16 (6H, d, J=7.0 Hz, $COCH(\underline{CH_3})_2$), 1.35 (3H, d, J=7.0 Hz, $CH\underline{CH_3}$), 3.25, 3.28, 3.36 (each 3H, s, 3×OCH_3), 4.75 (1H, t, J=4.8 Hz, H-14β), 5.36, 5.80 (each 1H, br s, $\underline{N}H_2$), 7.07—8.71 (4H, m, Ar-H), 11.16 (1H, s, $\underline{N}HCO$); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: see Table 1; EI-MS m/z (%): 755 (M^+, 3), 724 (M-31, 43), 611 (30), 492 (33), 214 (24), 188 (73), 126 (24), 110 (67), 70 (66); HR-EI-MS m/z: 755.4012, Calcd for $C_{40}H_{57}N_3O_{11}$, 755.3993.

Giraldine H (**2**): White amorphous powder, mp 122—124 °C; $[\alpha]_D^{20}$ +34.6° (c=0.35, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3465, 3427, 1721, 1685, 1628, 1606, 1588, 1526, 1297, 1256; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.89 (3H, t, J=7.2 Hz, $COCH(CH_3)CH_2\underline{CH_3}$), 1.06 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{CH_3}$), 1.14 (3H, d, J=6.8 Hz, $COCH(\underline{CH_3})CH_2CH_3$), 1.35 (3H, d, J=7.0 Hz, $CH\underline{CH_3}$), 3.25, 3.28, 3.36 (each 3H, s, 3×OCH_3), 4.78 (1H, t, J=4.6 Hz, H-14β), 5.34, 5.79 (each 1H, br s, $\underline{N}H_2$), 7.08—8.71 (4H, m, Ar-H), 11.16 (1H, s, $\underline{N}HCO$); ^{13}C-NMR (50 MHz, $CDCl_3$): see Table 1; EI-MS m/z (%): 769 (M^+, 1), 738 (M-31, 1), 625 (100), 577 (5), 522 (6), 506 (27), 476 (13). 188 (28), 137 (17), 120 (41); HR-EI-MS m/z: 769.4132, Calcd for $C_{41}H_{59}N_3O_{11}$, 769.4149.

Giraldine F (**3**): White amorphous powder, mp 78—80 °C; $[\alpha]_D^{20}$ −15.6° (c=1.0, $CHCl_3$). IR_{max}^{KBr} cm^{-1}: 3466, 2924, 2875, 2815; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 2; FAB-MS m/z (%): 362 (M^++1, 100), 344 (3), 58 (9); HR-ESI-MS m/z: 362.2696 (M^++H), Calcd for $C_{22}H_{36}NO_3$ (M^++H), 362.2695.

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References

1) Zhou X. L., Chen Q. H., Chen D. L., Wang F. P., *Chin. J. Chem.*, **21**, 871—874 (2003).
2) Zhou X. L., Chen Q. H., Wang F. P., *Heterocycles*, **63**, 123—128 (2004).
3) Pelletier S. W., Mody N. V., Joshi B. S., Schramm L. C., "Alkaloids: Chemical and Perspectives," Vol. 2, ed. by Pelletier S. W., Wiley, New York, 1984, pp. 206—264.
4) Pelletier S. W., Joshi B. S., "Alkaloids: Chemical and Perspectives," Vol. 7, ed. by Pelletier S. W., Wiley, New York, 1991, pp. 297—564.
5) Pelletier S. W., Harraz F. M., Badawi M. M., *Heterocycles*, **24**, 1853—1865 (1986).
6) Kulanthaivel P., Pelletier S. W., Olsen J. D., *Heterocycles*, **27**, 339—342 (1988).
7) Coates P. A., Blagbrough I. S., Hardick D. J., Rowan M. G., Wonnacott S., Potter B. V. L., *Tetrahedron Lett.*, **35**, 8701—8704 (1994).
8) Shen X. L., Zhou X. L., Chen Q. H., Chen D. L., Wang F. P., *Chem. Pharm. Bull.*, **50**, 1265—1267 (2002).
9) Pelletier S. W., Dailey O. D., Jr., Mody N. V., *J. Org. Chem.*, **46**, 3284—3293 (1981).
10) Wang F. P., Li Z. B., Wang J. Z., *Acta Chim. Sin.*, **58**, 576—579 (2000).
11) Fang Q. C., Hou Z. M., *Acta Pharm. Sinica*, **13**, 577—588 (1966).