Two New Amaryllidaceae Alkaloids from the Bulbs of *Lycoris radiata*

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Two new Amaryllidaceae alkaloids, named lycoranines A (1) and B (2), were isolated from the bulbs of *Lycoris radiata*. Their structures were elucidated on the basis of extensive spectroscopic analysis. Compound 2 was a new-type alkaloid, which provided a new insight into the biosynthesis of alkaloids in Amaryllidaceae plants.

Key words *Lycoris radiata*; Amaryllidaceae; alkaloid; biosynthetic pathway; lycoranine A; lycoranine B

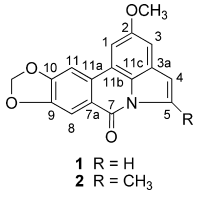
Amaryllidaceae alkaloids are typically found in some species of Amaryllidaceae, which is one of the most widely applied medicinal plant families. To date, nearly 500 alkaloids have been isolated from some Amaryllidaceae plants. These alkaloids exhibit acetylcholinesterase-inhibitory, immunostimulatory, antitumor, antiviral, and antimalarial activities.[1,2] The bulbs of *Lycoris radiata* (L'HER.) HERB. (Amaryllidaceae) have long been used as folk medicines to treat laryngeal trouble, furuncle, carbuncle, and suppurative wounds. Previous phytochemical studies of this plant have led to the isolation of >20 Amaryllidaceae alkaloids.[3] In our search for bioactive constituents from the bulbs of *L. radiata*, we herein describe the isolation and structural elucidation of two new Amaryllidaceae alkaloids, lycoranines A (**1**) and B (**2**) (Fig. 1).

Results and Discussion

The total alkaloids extracted from the bulbs of *L. radiata* were sequentially subjected to column chromatography on silica gel and Sephadex LH-20 to afford compounds **1** and **2**.

Compound **1** was obtained as yellow needles. The molecular formula of **1** was determined as $C_{17}H_{11}NO_4$ on the basis of a quasi-molecular ion at *m*/*z* 316.0582 $[M+Na]^+$ (Calcd 316.0586) in its HR-ESI-MS spectrum. The UV spectrum showed maxima absorptions at 253, 303, and 371 nm. The IR spectrum of **1** revealed the presence of carbonyl (1675 cm^{-1}), methylenedioxy (1368, 940 cm^{-1}), and aromatic ring (1622, 1489 cm^{-1}). The ^{1}H-NMR and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra indicated the presence of two sets of aromatic protons coupled in AX and AB system [δ 8.01 (d, J=3.6 Hz), 6.82 (d, J=3.6 Hz), 7.49 (d, J=1.9 Hz), and 7.30 (d, J=1.9 Hz)], respectively, as well as two aromatic singlets at δ 7.98 (1H) and 7.59 (1H). The ^{1}H-NMR spectrum also showed signals for a methoxyl [δ 3.96 (3H, s)] and a methylenedioxy [δ 6.17 (2H, s)] group. The ^{13}C-NMR and DEPT spectra displayed 17 signals, including a methoxyl [δ 56.4 (q)] and a methylenedioxy [δ 102.3 (t)] groups, which suggested that **1** possessed a skeleton with 15 C-atoms. With the aid of 1D and 2D NMR experiments, all the ^{1}H- and ^{13}C-NMR signals of **1** were assigned as shown in Table 1. Comparison of the NMR data of **1** with those of the known compound hippadine[4,5] indicated that their NMR signals were very similar, except for a methoxyl group [δ_C 56.4 (q); δ_H 3.96 (3H, s)] instead of an aromatic proton [δ 7.44 (1H, t, J=7.6 Hz)] in **1**. Thus **1** was proposed a methoxyl-substituted derivative of hippadine. The substituted location could be deduced by an HMBC experiment. Hence in the HMBC spectrum, correlation between methoxyl protons (δ 3.96) and C-2 (δ 157.6) was observed (Fig. 2), suggesting that the methoxyl group was located at C-2. The ROESY correlations between methoxyl protons (δ 3.96) and H-1 [δ 7.49 (d, J=1.9 Hz)] and H-3 [δ 7.30 (d, J=1.9 Hz)] (Fig. 2) further confirmed the above result. On the basis of these results, the structure of **1** was unambiguously identified as 2-methoxy-7*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-7-one, and named as lycoranine A.

Compound **2** was also isolated as yellow needles. The HR-ESI-MS of **2** exhibited a quasi-molecular ion $[M+H]^+$ at *m*/*z* 308.0922, consistent with the molecular formula $C_{18}H_{13}NO_4$ (Calcd 308.0923; $C_{18}H_{14}NO_4^+$), suggesting that **2** has a CH_2 mass unit more than **1**. Detailed examination of 1D and 2D NMR spectra of **2** and comparison with those of **1** revealed their considerable structural similarity. The main difference was that **2** was substituted by a methyl group instead of a hydrogen atom at the C-5 position. The HMBC correlations between methyl protons (δ 2.87) and C-5 (δ 140.1) and C-4 (δ 109.1), as well as the ROESY correlations between methyl protons and H-4 (δ 6.47) and between H-4 (δ 6.47) and H-3 (δ 7.17), further suggested that the methyl group was attached to the C-5 position. Assignment of the ^{1}H- and ^{13}C-NMR data (Table 1) of **2** was completed with the aid of ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY data. Consequently, the structure of **2** was characterized as 2-methoxy-5-methyl-7*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-7-one, and named as lycoranine B.



1 R = H
2 R = CH_3

Fig. 1. Chemical Structures of Compounds **1** and **2**

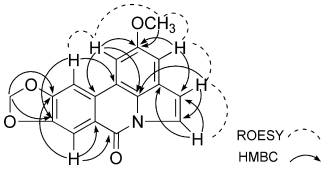


Fig. 2. Key HMBC and ROESY Correlations of **1**

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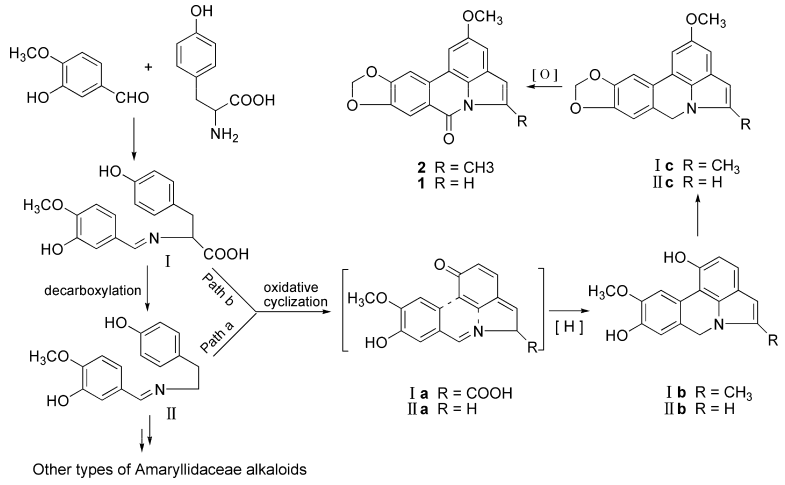

Chart 1. Proposed Biosynthetic Pathways for **1** and **2**

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and **2** ($CDCl_3$, *J* in Hz)

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
1	7.49 (1H, d, 1.9)	106.0	7.38 (1H, d, 1.9)	103.9
2	—	157.6	—	157.4
3	7.30 (1H, d, 1.9)	107.4	7.17 (1H, d, 1.9)	106.1
3a	—	128.9	—	128.2
4	6.82 (1H, d, 3.6)	110.7	6.47 (1H, s)	109.1
5	8.01 (1H, d, 3.6)	124.1	—	140.1
7	—	157.9	—	159.6
7a	—	122.9	—	123.5
8	7.98 (1H, s)	108.2	7.96 (1H, s)	108.0
9	—	148.7	—	148.5
10	—	152.5	—	152.4
11	7.59 (1H, s)	101.8	7.56 (1H, s)	101.5
11a	—	131.3	—	131.3
11b	—	116.9	—	116.5
11c	—	126.3	—	126.9
OCH_3	3.96 (3H, s)	56.4	3.95 (3H, s)	56.3
OCH_2O	6.17 (2H, s)	102.3	6.16 (2Hß, s)	102.2
5-CH_3	—	—	2.87 (3H, s)	15.9

Compound **2** was a new-type alkaloid found in nature. The structural features of **2** provide new insight into the biosynthesis of alkaloids in Amaryllidaceae plants. Previously, the biosynthetic pathways of Amaryllidaceae alkaloids were clearly proposed all derived from the aldimine (II) or its derivatives, which are produced in plants from aromatic aldehydes and tyrosine with a decarboxylation procedure,[2] such as **1** could be biosynthesized through 'Path a' (Chart 1). However, the occurrence of **2** might reveal that oxidative cyclization through the intermediate (I) without decarboxylation procedure (Path b) may be another biogenetic pathway for the alkaloids occurring in Amaryllidaceae plants.

Experimental

Experimental General Experimental Procedures All melting points were recorded on a XT-4 micro melting point apparatus without correction. NMR spectra were measured on a Bruker AV-400 spectrometer (^{1}H-NMR, 400 MHz; ^{13}C-NMR, 100 MHz). Chemical shift values (δ) were recorded in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. COSY, HSQC, HMBC, and ROESY were performed using standard Bruker pulse programs. IR spectra were determined on a Jasco FT/IR-480 plus infrared spectrometer. UV spectra were obtained on a Jasco V-550 UV/VIS spectrophotometer. HR-ESI-MS data were detected on an Agilent 6210 ESI/TOF mass spectrometer. ESI-MS spectra were recorded on a Finnigan LCQ Advantage Max ion trap mass spectrometer. Column chromatographies (CC) were carried out using silica gel (200—400 mesh, Qingdao Marine Chemical Factory, China) and Sephadex LH-20 (Pharmacia Biotec AB, Sweden).

Plant Material The bulbs of *L. radiata* were collected in October 2006 in Lanxi City, Zhejiang Province of China, and identified by Prof. Min-jian Qin, Department of Pharmacognosy, China Pharmaceutical University, Nanjing, P. R. China. A voucher specimen (No. 2006101201) was deposited in the herbarium of China Pharmaceutical University.

Extraction and Isolation The air-dried and finely powdered bulbs of *L. radiata* (15 kg) were exhaustively extracted at room temperature (25±5°) with 95% EtOH (60 l×4). The solution was evaporated under reduced pressure to afford a brownish residue, which was dissolved in water and basified to pH 9—10 with 10% aqueous ammonia then extracted with chloroform. The chloroform solution was further extracted with 5% hydrochloric acid. The acidic solution was basified to pH 8 with Na_2CO_3 and extracted with EtOAc, which was concentrated to yield total alkaloids (60 g). The alkaloid fraction (58 g) was subjected to CC over silica gel (800 g), eluting with $CHCl_3$–MeOH (100 : 0—100 : 100) to give 10 fractions (A—J). Fraction B (1.11 g) was further separated by CC over silica gel using *n*-hexane–EtOAc (8 : 2) as eluent to afford four subfractions (B1—B4). Fractions B2 and B3 (110 and 80 mg, respectively) were further purified using Sephadex LH-20 column eluting with $CHCl_3$–MeOH (1 : 1) to give compounds **1** (4 mg) and **2** (3 mg), respectively.

2-Methoxy-7*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-7-one (Lycoranine A, **1**): Yellow needles (MeOH); mp 230—232 °C; UV λ_{max} ($CHCl_3$) nm (log ε): 253 (4.67), 303 (4.46), 371 (4.15); IR (KBr), cm^{-1}: 2924, 1675, 1622, 1489, 1368, 1306, 1131, 1026, 940; HR-ESI-MS *m/z*: 316.0582 $[M+Na]^+$ (Calcd for $C_{17}H_{11}NO_4Na^+$, 316.0586). ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

2-Methoxy-5-methyl-7*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-7-one (Lycoranine B, **2**): Yellow needles (MeOH); mp 220—222 °C; UV λ_{max} ($CHCl_3$) nm (log ε): 245 (4.65), 307 (4.36); IR (KBr), cm^{-1}: 2922, 1672, 1632, 1482, 1362, 1299, 1105, 1040, 946; HR-ESI-MS *m/z*: 308.0922 $[M+H]^+$ (Calcd for $C_{18}H_{14}NO_4^+$, 308.0923). ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

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References

1) Jin Z., *Nat. Prod. Rep.*, **20**, 606—614 (2003).
2) Jin Z., *Nat. Prod. Rep.*, **24**, 886—905 (2007).
3) Kihara M., Konishi K., Xu L., Kobayashi S., *Chem. Pharm. Bull.*, **39**, 1849—1853 (1991).
4) Ghosal S., Rao P. H., Jaiswal D. K., Kumar Y., Frahm A. W., *Phytochemistry*, **20**, 2003—2007 (1981).
5) Torres J. C., Pinto A. C., Garden S. J., *Tetrahedron*, **60**, 9889—9900 (2004).