Benzylphenethylamine Alkaloids from the Bulbs and Flowers of *Lycoris radiata*

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Abstract: **Objective** To study the benzylphenethylamine alkaloids from the bulbs and flowers of *Lycoris radiata*. **Methods** Alkaloids were isolated by various column chromatographic methods and their structures were identified by spectral data. **Results** Fifteen known benzylphenethylamine alkaloids were isolated and identified as lycoramine (**1**), *O*-demethyllycoramine (**2**), *N*-demethyllycoramine (**3**), galanthamine (**4**), lycorine (**5**), caranine (**6**), ungminorine (**7**), narciclasine (**8**), 5-hydroxy-10-*O*-demethyl-homolycorine (**9**), hippeastrine (**10**), ungerine (**11**), hippeastrine *N*-oxide (**12**), *O*-demethylhaemanthamine (**13**), haemanthidine (**14**), and 8-demethoxyhostasine (**15**). **Conclusion** Compound **15** is first isolated from the plants in Amaryllidaceae, compounds **3**, **6**, **9**, and **11** are first reported from the plants in *Lycoris* Herb., and compounds **2**, **7**, and **14** are isolated from *L. radiata* for the first time. The ^{13}C-NMR data of compouds **3**, **7**, and **12** are first reported in the present study. Furthermore, the galasine-type alkaloid is isolated from the plants of *Lycoris* Herb. for the first time.

Key words: Amaryllidaceae; benzylphenethylamine alkaloids; bulbs and flowers; 8-demethoxyhostasine; *Lycoris radiate*

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Introduction

Lycoris Herb. (Amaryllidaceae) is a genus endemic to East Asia, and plants in the genus are with medicinal values and ornamental values (Zhao *et al*, 2008). The plants of this genus are rich in benzylphenethylamine alkaloids, which have cytotoxic (Evidente and Kornienko, 2009), antimalarial (Sener, Orhan, and Satayavivad, 2003), antiviral (Szlávik *et al*, 2004), anticholinesterase (López *et al*, 2002), and other activities. *Lycoris radiate* (L' Her.) Herb., a traditional Chinese medicine, is used to treat and cure sore throat, bromatoxism, boils, rheumatoid arthritis, snake bites, and other diseases (Editorial Committee of the Flora of China of Chinese Academy of Science, 1985). In previous researches, some benzylphenethylamine alkaloids, such as lycorine, lycorenine, and galanthamine, had been isolated from *L. radiata* (Geng, Lv, and Zhang, 2008; Wang *et al*, 2009). In the present study, fifteen known benzylphenethylamine alkaloids were isolated and identified from the bulbs and flowers of *L. radiata*.

Materials and methods

General

Analytical TLC: pre-coated silica-gel-F_{254} plates (Qingdao Meigao Chemical Co.); Spots were detected under UV light (254 and 365 nm), and by spraying with Dragendorff's reagent or 5% H_2SO_4 in EtOH. Column chromatography (CC): silica gel G (80－100, 200－300, and 300－400 mesh; Qingdao Meigao Chemical Co.), C_{18} silica gel (40－75 μm, Fuji Silysia Chemical Ltd.), MCI gel (70－150 μm, Mitsubishi Chemical Corporation), D-101 resin (Qingdao Marine Chemical Ltd.), and Sephadex LH-20 gel (GE Healthcare Bio-Xciences AB). NMR spectra: Bruker AM—400, DRX—500,

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and AV—600 Spectrometers. MS: VG Auto Spec—3000 Magnetic-sector Instrument and API Qstar Pulsar Instrument.

Plant material

The bulbs and flowers of *Lycoris radiata* (L' Her.) Herb. were collected from Liping County, Guizhou Province of China, in September 2009. The plant material was identified by Dr. HU Guang-wan at the Kunming Institute of Botany, the Chinese Academy of Sciences (CAS). A voucher specimen (No. DS09130) was deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, CAS, China.

Extraction and isolation

The air-dried and powdered bulbs and flowers of *L. radiata* (4.5 kg) were exhaustively extracted with MeOH. The solvent was evaporated under reduced pressure to give a residue (120 g), which was suspended in water and extracted with petroleum ether. The pH value of water phase was adjusted to about 2 with 2 mol/L HCl, and extracted with EtOAc. The pH value of aqueous phase was adjusted to about 10 with 5% NaOH, and extracted with $CHCl_3$. Then the pH value of water part was adjusted to about 7 with 2 mol/L HCl.

The $CHCl_3$ fraction (Fr. A, 15 g) was subjected to MCI gel CC, eluted with gradient MeOH-H_2O. The 80% MeOH fraction (Fr. A1) was recrystallized from MeOH to gain compound **5** (1.5 g). The 90% MeOH part (Fr. A2, 12 g) was chromatographed on RP-C_{18} CC to yield three fractions, Fr. A2a (5% MeOH, 50 mg), Fr. A2b (20% MeOH, 600 mg), and Fr. A2c (40% MeOH, 1.7 g). Fr. A2a was refined by preparative TLC ($CHCl_3$:MeOH = 2:1) to yield compounds **2** (8.0 mg) and **6** (2.8 mg). Fr. A2b was subjected to recrystallization (MeOH), Sephadex LH-20 (MeOH) and preparative TLC ($CHCl_3$:MeOH = 2:1) to yield compounds **7** (2.0 mg), **11** (2.1 mg), **13** (22.0 mg), and **14** (470 mg). Fr. A2c was separated by a Sephadex LH-20 column (MeOH), then by silica gel CC to obtain compounds **1** (70.5 mg), **3** (13.1 mg), **4** (285.0 mg), **9** (2.5 mg), and **10** (45.0 mg).

The H_2O fraction (Fr. B) was chromatographed on a D-101 resin column, eluted with H_2O and 95% EtOH. The EtOH eluent was collected and concentrated to yeild Fr. B′ (10 g). Fr. B′ was separated by silica gel CC to yield two parts, Fr. B′1 (2 g, $CHCl_3$:MeOH = 5:1) and Fr. B′2 (6 g, $CHCl_3$:MeOH = 1:2). Fr. B′1 was subjected to MCI gel CC and preparative TLC to obtain compounds **12** (5.1 mg) and **15** (11.0 mg). Fr. B′2 was chromatographed by MCI gel CC, silica gel CC, and recrystallization (MeOH) to give compound **8** (500 mg).

Results and discussion

Fifteen known benzylphenethylamine alkaloids were isolated and identified from the bulbs and flowers of *L. radiata*. These compounds belonged to six structure types, galanthamine- (**1**—**4**), lycorine- (**5**—**7**), narciclasine- (**8**), lycorenine- (**9**—**12**), crinine- (**13** and **14**), and galasine-type (**15**). The structures were in Fig. 1.

Compound **1**: white amorphous powder, was identified as lycoramine by comparison of the spectral data with the literature (Li *et al*, 1987).

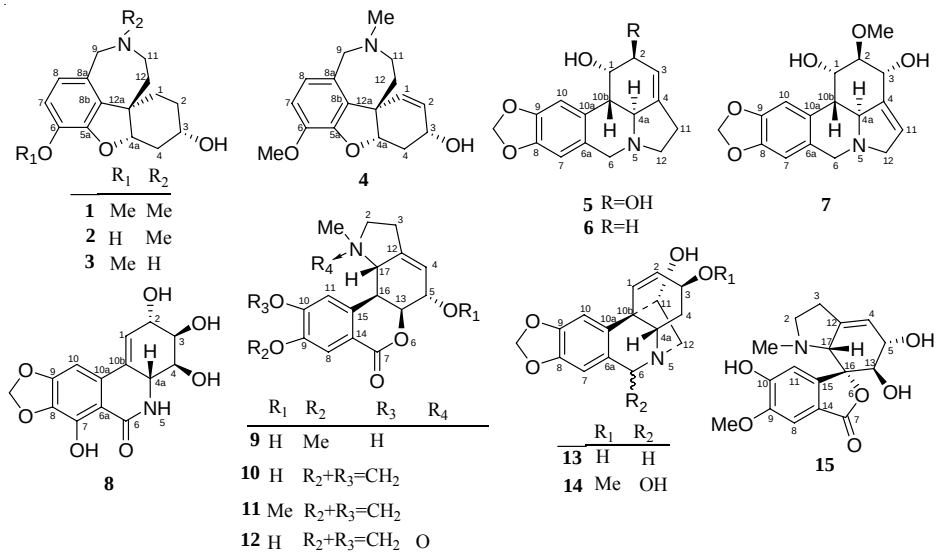

Fig. 1 Structures of benzylphenethylamine alkaloids from the bulbs and flowers of *L. radiata*

Compound **2**: white amorphous powder, was characterized as *O*-demethyllycoramine by comparison of the ^{1}H-NMR data with the literature (Kihara *et al*, 1991), and it was isolated from *L*. *radiata* for the first time.

Compound **3**: white amorphous powder. ^{13}C-NMR (100 MHz, CD_3OD) δ: 146.6 (C-6), 146.2 (C-5a), 135.1 (C-8b), 124.0 (C-8a), 118.1 (C-8), 111.8 (C-7), 89.4 (C-4a), 64.6 (C-3), 58.1 (C-9), 55.9 (*O*-Me), 51.9 (C-11), 46.3 (C-12a), 31.4 (C-4), 29.7 (C-1), 27.3 (C-2), 23.9 (C-12). It was defined as *N*-demethyllycoramine by comparison of the ^{1}H-NMR data with the literature (Kihara *et al*, 1987), and the ^{13}C-NMR data of this compound was first reported in the present study. Besides, compound **3** was first isolated from the plants in genus *Lycoris* Herb.

Compound **4**: white amorphous powder, was determined as galanthamine, for its NMR data was in agreement with literatures (Li *et al*, 1987; Zetta, Gatti, and Fuganti, 1973).

Compound **5**: white crystals (MeOH), was identified as lycorine by contrasting the spectral data with the literature (Wang, 2006).

Compound **6**: white crystals (MeOH), was identified as caranine by comparison of the ^{1}H-NMR data with the literature (Lamoral-Theys *et al*, 2009), and it was isolated from the plants in genus *Lycoris* Herb. for the first time.

Compound **7**: white amorphous powder. ^{13}C-NMR (100 MHz, CD_3OD) δ: 150.1 (C-9), 148.1 (C-8), 141.1 (C-4), 125.6 (C-6a, 10a), 122.8 (C-11), 109.2 (C-7), 106.6 (C-10), 102.9 (OCH_2O), 81.1 (C-2), 72.5 (C-4a), 65.6 (C-3), 63.1 (C-12), 60.1 (C-1), 58.7 (*O*-Me), 53.8 (C-6), 38.5 (C-10b). Compound **7** was defined as ungminorine by comparison of the ^{1}H-NMR data with the literature (Ingkaninan *et al*, 2000). This compound was isolated from *L. radiata* for the first time, and its ^{13}C-NMR data was first reported in the present study.

Compound **8**: white amorphous powder, was identified as narciclasine by comparison of the spectral data with the literature (Evidente, 1991).

Compound **9**: white solid, was identified as 5-hydroxy-10-*O*-demethyl-homolycorine, for its NMR data was in accordance with the literature (Bastida *et al*, 1988). Furthermore, it was first isolated from the plants in genus *Lycoris* Herb.

Compound **10**: colorless crystals (MeOH), was identified as hippeastrine by comparison of the NMR data with the literature (Evidente *et al*, 2004).

Compound **11**: colorless solid, was determined as ungerine by comparison of the ^{1}H-NMR data with the literature (Yagudaev, Abduazimov, and Yunusov, 1970), and it was isolated from plants in the genus *Lycoris* Herb. for the first time.

Compound **12**: white amorphous powder. ^{13}C-NMR (100 MHz, CD_3OD) δ: 165.1 (C-7), 152.2 (C-10), 150.0 (C-9), 139.6 (C-12), 139.0 (C-15), 123.6 (C-4), 119.8 (C-14), 110.6 (C-8), 110.3 (C-11), 104.1 (OCH_2O), 83.2 (C-17), 78.3 (C-13), 70.8 (C-2), 67.0 (C-5), 56.3 (*N*-Me), 34.3 (C-16), 26.5 (C-3). It was defined as hippeastrine *N*-oxide by comparison of the ^{1}H-NMR data with the literature (Kihara *et al*, 1991), and this compound was the first report for its ^{13}C-NMR data.

Compound **13**: white solid, was identified as *O*-demethylhaemanthamine by comparison of the NMR data with literatures (Tato, Castedo, and Riguera, 1988; Wang, 2006).

Compound **14**: colorless needles (MeOH). According to the literature (Hohmann *et al*, 2002), it was identified as haemanthidine. And this alkaloid was isolated from *L*. *radiata* for the first time.

Compound **15**: white crystals (MeOH), was identified as 8-demethoxyhostasine by comparison of the spectral data with the literature (Wang *et al*, 2007). This compound was isolated from the plants in Amaryllidaceae for the first time, and the galasine-type alkaoid was first reported from plants in the genus *Lycoris* Herb.

Many researches had been done to study the bioactivities of these alkaloids from the plants in Amaryllidaceae. Lycorine-, narciclasine-, and crinine-type alkaloids had noteworthy cytotoxic activities (Evidente *et al*, 2009; Evidente and Kornienko, 2009), whereas galanthamine- (Jokhadze *et al*, 2007) and lycorenine-type (Evidente *et al*, 2009; Evidente and Kornienko, 2009) alkaloids only had weak activities. Galanthamine- (López *et al*, 2002), lycorine- (López *et al*, 2002), and galasine-type (Wang *et al*, 2007) alkaloids had remarkable anticholinesterase activity, but lycorenine- and crinine-type alkaloids were inactive (López *et al*, 2002). Lycorine- and crinine-type alkaloids had stronger antimalarial activities than

galanthamine-type alkaloids (Campbell *et al*, 2000; Sener, Orhan and Satayavivad, 2003; Toriizuka *et al*, 2008). Besides, lycorine- and crinine-type alkaloids had notable antiviral activities (Li *et al*, 2005; Szlávik *et al*, 2004). As stated previously, these benzylphenethylamine alkaloids have excellent bioactivities, and their structure-activity relationship should deserve our attention.

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