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A New Amaryllidaceae Alkaloid from the Bulbs of *Lycoris radiata*

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Lycoris radiata (red spider lily) is a bulbous perennial plant in Amaryllidaceae widely distributed in Korea, Japan and China. It is commonly used as an ornamental flower or sometimes as an antidote in Traditional Chinese Medicine (TCM).[1] The species is also known as a botanical source of galantamine, a clinically used medicine for the treatment of Alzheimer's diseases and various other memory impairments.[2] Actually, the bulbs of the species contains a lot of isoquinoline-based amine components, so called Amaryllidaceae alkaloids, which have been reported to possess a variety of pharmacological activities such as anti-cancer,[3] anti-viral,[4] anti-acetylcholinesterase[5] and anti-inflammatory[6] activities. Amaryllidaceae alkaloids are produced almost by plants of galanthus genus[7] in Amaryllidaceae and are classified with three distinct scaffolds, lycorine (**3**-**9**, **14**), haemanthamine (**1**-**2**, **10**, **15**-**18**) and galanthamine (**11**-**13**) series, which are classified depending on the pattern of oxidative phenolic coupling. For the purpose of searching for bioactive alkaloids from natural resources, extensive phytochemical investigation of the bulbs extract of *L. radiata* had undertaken and finally resulted in the isolation of a new amaryllidaceae alkaloid (**1**), together with seventeen related alkaloids (**2**-**18**). The chemical structures of isolated **1**-**18** (Fig. 1) were established by spectroscopic analyses as 6-hydroxytazettine (**1**), tazettine (**2**), lycorine (**3**), 2-*O*-acetyllycorine (**4**), homolycorine (**5**), 9-*O*-demethylhomolycorine (**6**), hippeastrine (**7**), *O*-methyllycorenine (**8**), 2-α-hydroxy-6-*O*-methyloduline (**9**), haemanthidine (**10**), galantamine (**11**), dihydrogalantamine (**12**), lycoramine N-oxide (**13**), lycosinineB (**14**), ismine (**15**), trisphaeridine (**16**), 3-epimacronine (**17**), and 6-*O*-methylpretazettine (**18**),[8-18] respectively. In the present paper, we describe briefly the isolation and identification of a new Amaryllidaceae alkaloid (**1**), as well as the inhibitory activities of isolated alkaloids (**1**-**18**)

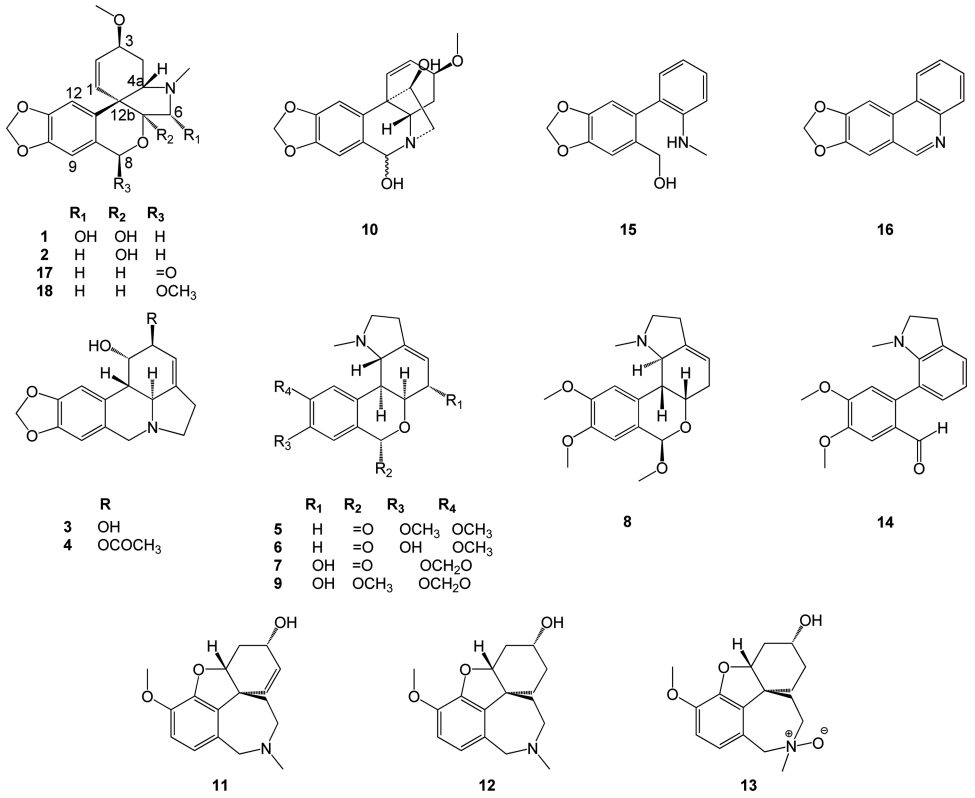

Figure 1. Structures of isolated compounds **1**-**18**.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **1** and **2**[a]

Position	**1** (δ_H)	**2** (δ_H)	**1** (δ_C)	**2** (δ_C)
1	5.64 (1H, d, J = 10.4 Hz)	5.64 (1H, d, J = 10.4 Hz)	127.6	128.7
2	6.00 (1H, d, J = 10.4 Hz)	6.16 (1H, d, J = 10.4 Hz)	129.5	130.6
3	4.06 (1H, m)	4.16 (1H, ddd, J = 10.4, 5.8, 1.8 Hz)	72.6	72.9
4	2.23 (1H, m)	2.24 (1H, dddd, J = 13.5, 5.8, 4.0, 1.1 Hz)	25.7	26.7
	1.68 (1H, m)	1.65 (1H, ddd, J = 13.5, 10.4, 2.3 Hz)		
4a	3.01 (1H, m)	2.88 (1H, m)	67.4	70.0
6	4.41 (1H, s)	3.32 (1H, d, J = 10.6 Hz)	93.0	65.6
		2.70 (1H, d, J = 10.6 Hz)		
6a			101.4	102.1
8α	5.03 (1H, J = 14.5 Hz)	4.98 (1H, d, J = 14.7 Hz)	62.1	62.1
8β	4.63 (1H, J = 14.5 Hz)	4.65 (1H, d, J = 14.7 Hz)		
8a			125.6	125.5
9	6.48 (1H, s)	6.52 (1H, s)	103.8	103.9
10			146.5	146.4
11			146.6	146.6
12	6.91 (1H, s)	6.88 (1H, s)	109.6	109.4
12a			127.2	128.0
12b			47.9	49.9
-OCH_2O-	5.92 (2H, d, J = 3.7 Hz)	5.92 (2H, s)	100.9	100.9
-OCH_3	3.45 (3H, s)	3.48 (3H, s)	56.1	56.1
-NCH_3	2.62 (3H, s)	2.42 (3H, s)	38.8	41.9

[a]Assignments are based on DEPT, HSQC, and HMBC experiments, and chemical shifts are given in ppm

on acetylcholinesterase, *in vitro* assay.

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was established as $C_{18}H_{21}NO_6$, *m/z* 347.1424 (calcd. 347.1369) by HREIMS. The ^{1}H-NMR data, particularly signals in lower field were quite similar with those of tazettine (**2**), which suggested that **1** is a kind of congener of **2** (Table 1). **2** has been isolated previously from other plants in Amaryllidaceae family, *i.e.*, *Narcissus tazetta* and *Pancratium maritimum*, and the chemical structure of **2** as well as the absolute configuration was established by X-ray diffraction analysis.[19]

The ^{1}H-NMR data of **1** implied the presence of a tetra-substituted benzene ring (δ_H 6.91, 6.48), a pair of olefin (δ_H 6.00, 5.64), a methylendioxy group (δ_H 5.92), and another methylene group (δ_H 5.03, 4.63). However, when the ^{1}H-NMR spectrum of **1** was compared with that of **2,** a pair of methylene signals of H-6 observed at δ_H 3.32 and 2.70 in ^{1}H-NMR of **2** was disappeared, instead, a singlet proton signal at δ_H 4.41 was observed in ^{1}H-NMR of **1**. These ^{1}H-NMR results suggested that one of methylene protons of **2** was replaced with hydroxyl group to yield **1**, and this assumption was supported by the mass differences (Δ 16) between **1** and **2**.

Thus, All proton and carbon signals of **1** were completely assigned by the aid of two-dimensional NMR experiments such as COSY, DEPT, HSQC, HMBC, and NOESY, which led to the conclusion that one of methylene protons at C-6 of **2** was replaced with hydroxyl group to become **1**, thus **1** is established as 6-hydroxytazettine. In HMBC experiment of **1**, the H-6 (δ_H 4.41) signal was observed to give correlations with C-12b, C-6a, and *N*-methyl carbon, respectively (Fig. 2). Supposing that the stereochemistry of C-4a, C-12b and C-6a of **2** were identical with those of **1**, the relative configuration of **1**, particularly the C-6 chirality was proposed by the NOESY experiment; the signal of H-6 was correlated with H-4a and H-8α, which strongly suggested a β-orientation of the hydroxyl group attached at C-6 (Fig. 2) and the *R* configuration of C-6. Thus, **1** was proposed as 6β-hydroxytazettine as illustrated in Figure 1 and Figure 2. The other purified components (**2**-**18**) were identified by direct comparison of their spectral data with those in the literatures.[8-18] All isolated Amaryllidaceae alkaloids (**1**-**18**) were evaluated for the inhibitory effect on acetylcholinesterse (EC 3.1.1.7. electric eel) using acetylcholine as substrate *in vitro*[20] and it was observed that galantamine (**11**) and ismine (**15**) exhibited a significant inhibitory effect on the acetylcholinesterase with IC_{50} values of 27.9 and 109.7 μM, when other isolated compounds showed poor inhibitions (IC_{50} > 300 μM) on acetylcholinesterse, *in vitro* assay.

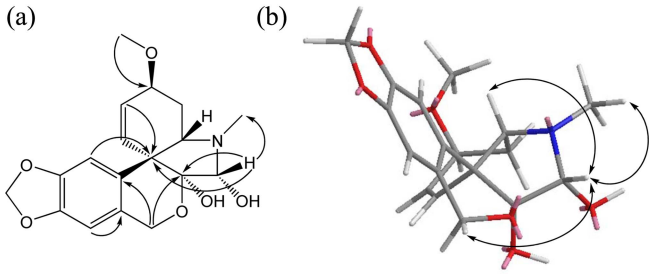

Figure 2. Key HMBC (H→C) (a) and NOESY (H↔H) (b) correlations of **1**.

Experimental Section

General Experimental Procedures. NMR spectra were obtained by a Brucker AM 300 and 500 spectrometers using TMS as an internal standard for ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, HMBC, and NOESY. Preparative-HPLC was performed on a Futecs P-4000 system with a Shim-pack prep-ODS(H) kit column (5 μm, 20 mm × 25 cm). Isolation and purification was also carried out using a medium-pressure liquid chromatographic (MPLC) system [BUCHI pump Module C-601, silica gel 60 (230-400 mesh, Merck), ODS (Cosmosil 140 C_{18})].

Plant Material. The bulbs of *L. radiata* were collected on November 2013 at the flower garden of Korea Research Institute of Chemical Technology (KRICT) and were authenticated by Dr. Young sup Kim. A voucher specimen (KR1315) was deposited at the herbarium of the KRICT.

Extraction and Isolation. The dried bulbs (6 Kg) of *L. radiata* were soaked twice in 90 L of methanol at room temperature for 7 days. The concentrated methanol extract (700 g) was suspended in 4 L of distilled water and adjusted to pH 12.0 with 1 N NaOH, and then extracted successively with equal volume of ethylacetate (EtOAc) and *n*-butanol (BuOH). The resultant EtOAc layer and BuOH layer was collected and extracted with equal volume 5% citric acid, respectively. The 5% citric acid layer was pooled up and neutralized with 1 N NaOH to pH 12.0 and then extracted with BuOH, which gave 39 g of the alkaloids rich fraction. The alkaloids rich fraction (39 g) was subjected to column chromatography on silica gel eluted with dichloromethane : MeOH (0.5 to 100% MeOH gradient) to yield four fractions (Fr. 1 - Fr. 4). Fr. 3 (6.2 g) was dissolved in methanol and allowed to crystallize at room temperature to give compound **3** (2.33 g). Fr. 1 (1.6 g) was subjected to column chromatography with NH_2 gel (Merck Lichroprep NH_2) eluted with a hexane : EtOAc (0.5 to 100% EtOAc gradient) to give seven fractions (Fr. 11 - Fr. 17). Fr. 12 afforded compound **18** (2.3 mg) by repeated preparative HPLC (50 to 100% MeOH). Fr. 13 was further purified through sephadex LH-20 chromatography and preparative HPLC (30 to 90% MeOH) to yield compounds **17** (4.5 mg), **1** (2.2 mg), **16** (3.4 mg). Fr. 14 was chromatographed on a sephadex LH-20 columns to give compounds **4** (5.1 mg) and **15** (7.5 mg). Fr. 2 (5.5 g) was further purified to column chromatography on silica gel eluted with dichloromethane : MeOH (1 to 100% MeOH gradient) to give four fractions (Fr. 21 - Fr. 24). Fr. 22 was purified further by preparative HPLC (50-90% MeOH) to yield compound **5** (38.7 mg) and **14** (3.1 mg). Compound **2** (27.4 mg) was crystallized from Fr. 24 in MeOH. Fraction 3 (6.17 g) was isolated to five fractions (Fr. 31 - Fr. 35). Fr. 33 was repeatedly chromatographed by preparative HPLC (30 - 90% MeOH) to afford compounds **8** (125.0 mg), **9** (5.0 mg), **11** (14.1 mg) and **7** (44.2 mg). Fr. 35 was subjected to column chromatography on silica gel eluted with dichloromethane : MeOH (1 to 20% MeOH gradient) to yield compound **6** (150.0 mg). Fr. 4 was subjected to column chromatography with silica gel eluted with dichloromethane : MeOH (2 to 50% MeOH gradient) to give five fractions (Fr. 41 - Fr. 45). Fr. 44 was isolated by preparative HPLC (10 to 80% MeOH) and finally purified through sephadex LH-20 to afford compounds **10** (34.2 mg), **12** (74.0 mg) and **13** (4.8 mg).

6-Hydroxytazettine (1): White amorphous powder; $[\alpha]_D^{20}$ +35.9 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} 240, 291 nm; ^{1}H NMR ($CDCl_3$, 500 MHz); ^{13}C NMR ($CDCl_3$, 125 MHz) (Table 1); HREIMS *m/z* 347.1424 (calcd. for $C_{18}H_{21}NO_6$, 347.1369).

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