Alkaloids from the Bulbs of *Lycoris aurea*

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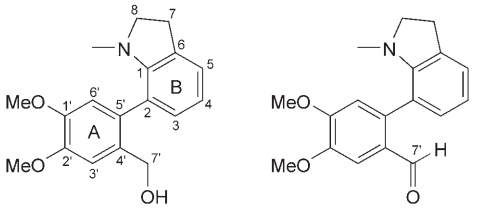
Two new and ten known alkaloids have been isolated from the bulbs of *Lycoris aurea* (Amaryllidaceae). The two new compounds, lycosinine A (= [2-(2,3-dihydro-1-methyl-1*H*-indol-7-yl)-4,5-dimethoxyphenyl]-methanol; **1**) and lycosinine B (= 2-(2,3-dihydro-1-methyl-1*H*-indol-7-yl)-4,5-dimethoxybenzaldehyde; **2**), were fully characterized by spectroscopic methods. In addition, a plausible biogenesis of homolycorine from **1** and **2** is proposed (*Scheme*).

1. Introduction. – Plants of the Amaryllidaceae are known to produce structurally unique alkaloids, covering a wide range of interesting physiological effects such as antitumor, antiviral, acetylcholinesterase-inhibitory, immunostimulatory, and antimalarial activities [1]. *Lycoris aurea* (Amaryllidaceae), a popular ornamental plant in China, is widely distributed in the tropics and warm-temperature regions.

In continuation of our search for new bioactive alkaloids, we investigated the chemical constituents of the bulbs of *L. aurea* (collected in Kunming, Yunnan province), since this plant has not been studied previously. Extensive column chromatography of the EtOH extract of *L. aurea* bulbs led to the isolation of two novel alkaloids, lycosinine A (**1**) and lycosinine B (**2**), together with ten known alkaloids: galanthamine [2][3], hippeastrine [3], haemanthidine [4], *N*-demethylgalanthamine [5], *O*-demethylgalanthamine [3], haemanthamine [6], homolycorine [4], *O*-demethyllycoramine [7], lycorine [8] and *O*-methyllycorenine [9]. The structures of these compounds were unambiguously established on the basis of spectroscopic methods. In addition, the biogenetic relationships of **1**, **2**, and homolycorine are discussed.



1 **2**

2. Results and Discussion. – Compound **1**, a colorless powder, had the molecular formula $C_{18}H_{21}NO_3$, as deduced by HR-ESI-MS (*m/z* 322.1420 ($[M+Na]^+$)) and

NMR. Its IR spectrum displayed absorptions at 3426*s* (br.), 1632, 1513, and 1465 cm^{-1}, associated to an oxygenated benzene ring. The ^{1}H-NMR spectrum of **1** (*Table*) revealed five aromatic H-atoms, two of which were *para*-oriented, appearing at δ(H) 6.99 (*s*, H−C(3′)) and 6.83 (*s*, H−C(6′)) on ring *A*[1]). The remaining three aromatic resonances appeared as an *AMX* system, typical of a 1,2,3-trisubstituented benzene ring (ring *B*) at δ(H) 7.14 (*d*, *J* = 7.2 Hz, 1 H), 6.93 (*d*, *J* = 7.2 Hz, 1 H), and 6.90 (*t*, *J* = 7.2 Hz, 1 H), as further confirmed by a ^{1}H,^{1}H-COSY spectrum.

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. At 500/125 MHz, resp, in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering. Assignements were confirmed by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments.

Position	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	–	151.0 (*s*)	–	151.8 (*s*)
2	–	126.1 (*s*)	–	121.2 (*s*)
3	6.93 (*d*, *J* = 7.2)	129.7 (*d*)	6.86 (*d*, *J* = 7.5)	130.7 (*d*)
4	6.90 (*t*, *J* = 7.2)	120.8 (*d*)	6.72 (*t*, *J* = 7.4)	118.3 (*d*)
5	7.14 (*d*, *J* = 7.2)	123.8 (*d*)	7.09 (*d*, *J* = 7.4)	124.3 (*d*)
6	–	132.1 (*s*)	–	131.6 (*s*)
7	3.09 (*dd*, *J* = 9.9, 5.5) 2.96–3.02 (*m*)	29.6 (*t*)	2.94–2.98 (*m*)	28.6 (*t*)
8	3.60 (*dd*, *J* = 14.3, 9.9) 2.92–2.99 (*m*)	56.6 (*t*)	3.32 (*dd*, *J* = 16.0, 8.3) 3.21 (*dd*, *J* = 17.1, 8.8)	57.0 (*t*)
1′	–	148.5 (*s*)	–	153.4 (*s*)
2′	–	148.6 (*s*)	–	148.7 (*s*)
3′	6.99 (*s*)	112.9 (*d*)	7.44 (*s*)	107.9 (*d*)
4′	–	132.0 (*s*)	–	139.8 (*s*)
5′	–	131.8 (*s*)	–	127.6 (*s*)
6′	6.83 (*s*)	112.7 (*d*)	6.83 (*s*)	113.0 (*d*)
7′	4.20 (*s*)	64.5 (*t*)	9.58 (s)	193.0 (*d*)
1′-MeO	3.93 (*s*)	56.9 (*q*)	3.94 (*s*)	56.3 (*q*)
2′-MeO	3.87 (*s*)	56.8 (*q*)	3.91 (*s*)	56.1 (*q*)
MeN	2.19 (*s*)	40.9 (*q*)	2.21 (*s*)	39.2 (*q*)

The ^{1}H-NMR spectrum of **1** displayed signals at δ(H) 3.93 (*s*, 3 H), 3.87 (*s*, 3 H), and 2.19 (*s*, 3 H), characteristic of two MeO and one MeN group, respectively. Further, a CH_2OH group was identified from the signals at δ(H) 4.20 (*s*, 2 H) and δ(C) 64.5 (*t*) in the ^{1}H- and ^{13}C-NMR spectra, respectively. From the ^{1}H,^{1}H-COSY spectrum of **1**, a CH_2CH_2 fragment was evident, which was attached to ring *B* at C(6) (δ(C) 132.1 (*s*)) because of HMBC correlations of both CH_2(7) and CH_2(8) with C(6), between H−C(4) and C(6), and between H−C(5) and C(7) (*Figure*).

The N-atom of the MeN group was connected to both C(1) at δ(C) 151.0 (*s*) and C(8), at 56.6 (*t*), which was supported by HMBC correlations between C(1), C(8), and MeN. Ring *A* was joined to ring *B* through C(5′) at δ(C) 131.8 (*s*) and C(2) at 126.1 (*s*), in accord with HMBC correlations between H−C(3) and C(5′), and between H−C(6′) and C(2). The CH_2OH group was attached to ring *A* at C(4′) (δ(C) 132.0 (*s*)), as confirmed by HMBC correlations between CH_2(7′) and C(3′), C(4′), and C(5′), respectively. The two MeO groups were placed in 1′- and 2′-position of ring *A*, as

[1]) Arbitrary atom numbering. For systematic names, see *Exper. Part*.

deduced from HMBC correlations between 1-MeO and C(1′), and between 2′-MeO and C(2′). From these data, the structure of lycosinine A (**1**) was determined as [2-(2,3-dihydro-1-methyl-1*H*-indol-7-yl)-4,5-dimethoxyphenyl]methanol.

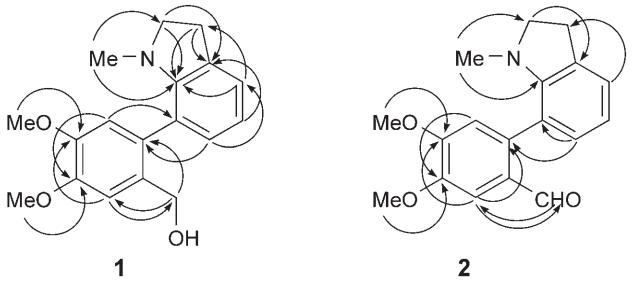
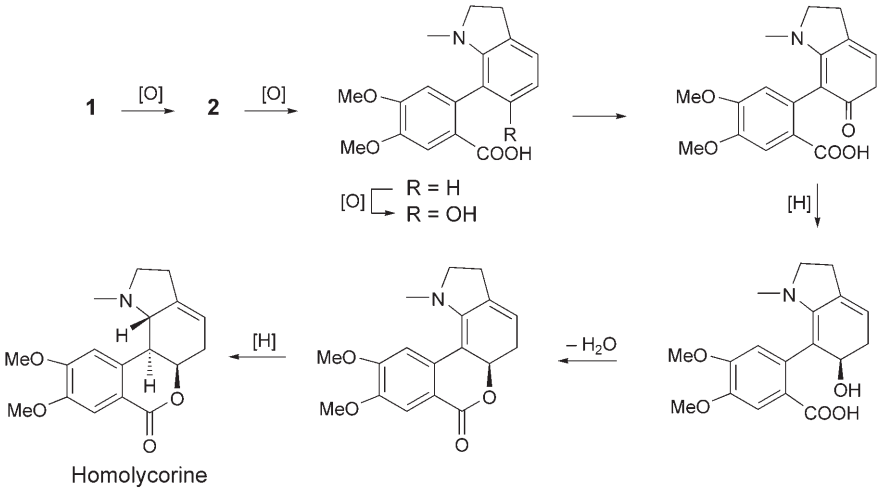


Fig. 1. *Key HMBC correlations for compounds* **1** *and* **2**

Compound **2** was isolated as a colorless powder. It had the molecular formula $C_{18}H_{19}NO_3$ according to HR-ESI-MS (*m/z* 298.1438 ($[M+H]^+$)) and NMR (*Table*). Its IR spectrum displayed strong absorptions at 1677, 1596, 1510, and 1446 cm^{-1}, indicating a C=O group and a benzene ring. The spectroscopic data of **1** and **2** were similar, but **2** was lacking the CH_2OH group, which was replaced with a CHO function (δ(C) 193.0 (*d*); δ(H) 9.58 (*s*, 1 H)). HMBC Correlations (*Figure*) further confirmed that lycosinine B (**2**) was an oxidized congener of lycosinine A, and corresponds to 2-(2,3-dihydro-1-methyl-1*H*-indol-7-yl)-4,5-dimethoxybenzaldehyde.

Based on the structures of compounds **1** and **2**, and considering the isolation of homolycorine from *L. aurea*, we propose that homolycorine is biosynthesized from **1** and **2**, as shown in the *Scheme*.

Scheme 1. *Proposed Biogenesis of Homolycorine from the New Constituents Lycosinin A* (**1**) *and Lycosinin B* (**2**)



Experimental Part

General. Petroleum ether (PE) for chromatography had a b.p. range of 60–90°. Column chromatography (CC) was performed on silica gel (100–200 mesh; *Qingdao Marine Chemical, Inc.*, China) and silica gel *H* (10–40 μm, *Qingdao*). Fractions were monitored by TLC, and spots were visualized by spraying with *Dragendorff* reagent. UV Spectra: *Shimadzu 210A* double-beam spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrophotometer, KBr discs; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* instruments; chemical shifts δ in ppm rel. to residual solvent signals, *J* in Hz. EI-MS and HR-ESI-MS: *VG AutoSpec-3000* and *Finnigan MAT-90* spectrometers, resp.; in *m/z* (rel. %).

Plant Material. Fresh bulbs of *L. aurea* were collected in Kunming, Yunnan Province, China, in March 2004, and were identified by Prof. *Xiao Chen*, Kunming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (KIB L00401) was deposited.

Extraction and Isolation. The Air-dried bulbs of *L. aurea* (4.8 kg) were extracted with 95% EtOH at r.t. for 5 × 24 h. The extract was concentrated in *vavuo*. The resulting residue was dissolved in H_2O, basified pH Ph 8–9 with 10% aq. NH_3 soln., and extracted with $CHCl_3$ (3×) and then $CHCl_3$/MeOH 3:2. The extracts were combined and evaporated. The crude residue was purified by CC (SiO_2 (300 g), 200–300 mesh; PE/AcOEt/Et_2NH 90:5:5 → 20:75:5): three fractions (Fr.). *Fr. 1* was subjected to CC (SiO_2; PE/$CHCl_3$/Et_2NH 50:45:5), which afforded galanthamine (560 mg) after recrystallization from $CHCl_3$/MeOH 4:1. Further CC (SiO_2; cyclohexane/acetone/Et_2NH 90:8:2) of *Fr. 1* afforded **1** (4 mg), **2** (3 mg), and hippeastrine (3 mg). *Fr. II* was purified by VLC (SiO_2; PE/acetone/Et_2NH 60:35:5), which afforded hippeastrine (120 mg) after crystallization from $CHCl_3$/MeOH 1:1. A second recrystallization afforded a mixture of haemanthidine (21 mg) and *N*-demethylgalanthamine (30 mg), which were further separated by CC (*RP-18*; MeOH/H_2O 7:3). *Fr. 3* was purified by CC (SiO_2; cyclohexane/i-PrOH/Et_2NH 80:15:5 → 30:65:5): *Fr. 3a–c*. *Fr. 3a* was subjected to CC (*RP-18*; MeOH/H_2O 7:3), and then recrystallized from MeOH to afford *O*-demethylgalanthamine (23 mg) and haemanthamine (15 mg). *Fr. 3b* was purified as *Fr. 3a* to afford homolycorine (498 mg) and *O*-demethyllycoramine (60 mg). *Fr. 3c* was purified by CC (SiO_2; PE/i-PrOH/Et_2NH 50:45:5) to afford lycorine (9 mg) and *O*-methyllycorenine (24 mg).

Lycosinine A (= *[2-(2,3-Dihydro-1-methyl-1*H*-indol-7-yl)-4,5-dimethoxyphenyl]methanol*; **1**). Yield: 4 mg. Colorless powder. UV ($CHCl_3$): 283 (3.28), 240 (3.57). IR (KBr): 3426, 2923, 2481, 1632, 1513, 1464, 1062, 805. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 299 (29, M^+), 238 (11), 137 (20), 58 (100), 57 (75). HR-ESI-MS: 322.1420 ($[M+Na]^+$, $C_{18}H_{21}NNaO_3^+$; calc. 322.1419).

Lycosinine B (= *2-(2,3-Dihydro-1-methyl-1*H*-indol-7-yl)-4,5-dimethoxybenzaldehyde*; **2**). Yield: 3 mg. Colorless powder. UV ($CHCl_3$): 312 (3.97), 280 (4.13), 242 (4.39). IR (KBr): 2923, 2850, 1677, 1596, 1510, 1446, 1349, 1282, 1260, 1139, 746. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 297 (29, M^+), 296 (54), 268 (21), 236 (13), 57 (100). HR-ESI-MS: 298.1438 ($[M+H]^+$, $C_{18}H_{20}NO_3^+$; calc. 298.1443).

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