Amaryllidaceae Alkaloids from *Lycoris radiata*

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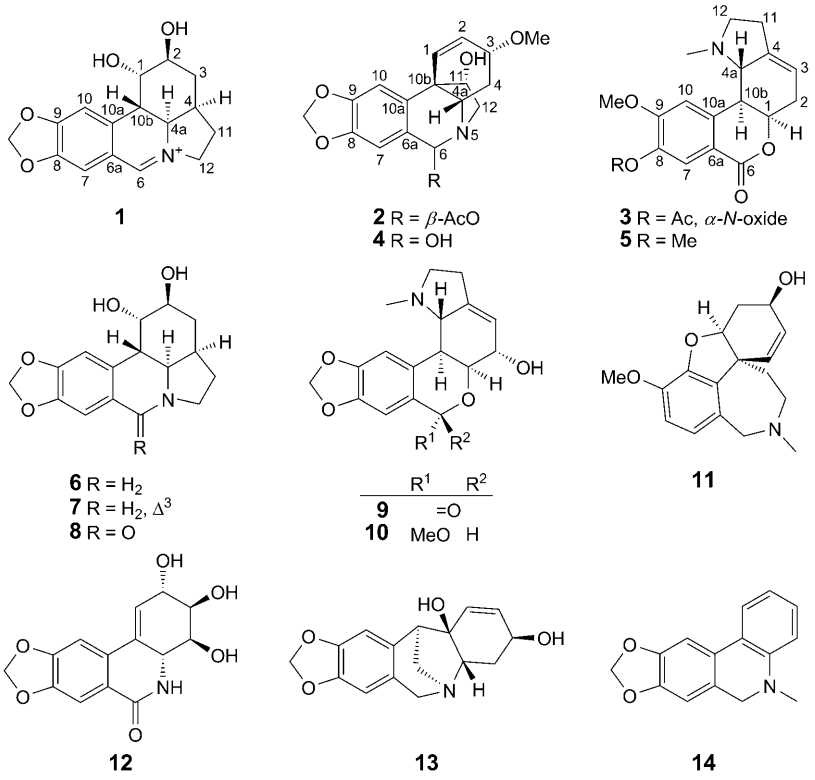
A phytochemical investigation on bulbs of *Lycoris radiata* resulted in the isolation of three new Amaryllidaceae alkaloids, named 5,6-dehydrodihydrolycorine (**1**), 6β-acetoxycrinamine (**2**), and (+)-8-*O*-acetylhomolycorine α-*N*-oxide (**3**), together with eleven known alkaloids, **4**–**14**. The structures of the new alkaloids were established by means of spectroscopic methods, and the known compounds were identified by comparison of their data with those in the literature. Compound **2** showed cytotoxicity against HL-60, A-549, and MCF-7 cells, with IC_{50} values of 8.1, 24.3, and 15.0 μM, respectively.

Introduction. – Amaryllidaceae alkaloids have for a long time attracted great interest of synthetic organic chemists because of their several biological activities and their potential diversity in pharmacology [1–9]. So far, more than 100 alkaloids have been isolated from Amaryllidaceae plants [10], which exhibited diverse bioacitivities, such as antiviral, insect antifeedant, antineoplastic, and acetylcholinesterase inhibitory activities [10–14]. As part of our search for novel and bioactive compounds, we isolated three new Amaryllidaceae alkaloids, 5,6-dehydrodihydrolycorine (**1**), 6β-acetoxycrinamine (**2**), and (+)-8-*O*-acetylhomolycorine α-*N*-oxide (**3**), together with eleven known analogs from bulbs of *Lycoris radiata*, a Chinese folk medicine famous for the treatment of poliomyelitis [15]. The new structures were elucidated by means of spectroscopic methods, and the known compounds were identified as 6-hydroxycrinamine (**4**) [16], homolycorine (**5**) [17], dihydrolycorine (**6**) [18], lycorine (**7**) [18], 7-oxodihydrolycorine (**8**) [19], (+)-hippeastrine (**9**) [20], 2α-hydroxy-6-*O*-methoxyloduline (**10**) [21], galanthamine (**11**) [22], 7-deoxynarciclasine (**12**) [23], pancratinine C (**13**) [24], and 5,6-dihydrobicolorine (**14**) [25]. In addition, compounds **1**–**3** were evaluated for their cytotoxic activities against five human cancer cell lines.

Results and Discussions. – Compound **1** was isolated as a yellow powder ($[\alpha]_D^{22} = +399.7$ ($c = 0.1$, MeOH)). The HR-ESI-MS (positive-ion mode) displayed a molecular ion at m/z 288.1228 (M^+), corresponding to the molecular formula $C_{16}H_{18}NO_4$. The UV absorption bands at 373, 308, 253, and 211 nm suggested an extended chromophore, and a O–CH_2–O-substituted benzene ring [26], whereas the IR absorption bands at 3405, 3358, 1646, 1605, and 922 cm^{-1} indicated two OH groups and a phenyl function. The ^{1}H-NMR spectrum (*Table*) displayed two *singlet*s for two *para*-positioned aromatic H-atoms (δ(H) 7.30, 7.16), signal of a OCH_2O group (δ(H) 6.18), and a downfield *singlet* corresponding to the H-atom of an iminium salt (δ(H) 8.86) [27]. The ^{13}C-NMR spectrum (*Table*) displayed 16 C-atom resonances ascribable to four CH_2 and eight CH



groups (including three sp^3-C-atoms bearing a heteroatom), and four sp^2 quaternary C-atoms. The above data suggested that compound **1** was an amaryllidaceae alkaloid similar to dihydrolycorine (**6**) [18], except for an imine moiety located between N(5) and C(6) (δ(C) 163.0) in **1**, as supported by HMBCs of δ(H) 8.86 (*s*, H–C(6)) with δ(C) 61.5 (*d*, C(4a)), 139.3 (*s*, C(6a)), 122.3 (*s*, C(10a)), and 57.5 (*t*, C(12)) (*Fig.*). The relative configuration of **1** was elucidated by a ROESY experiment. For biogenetic

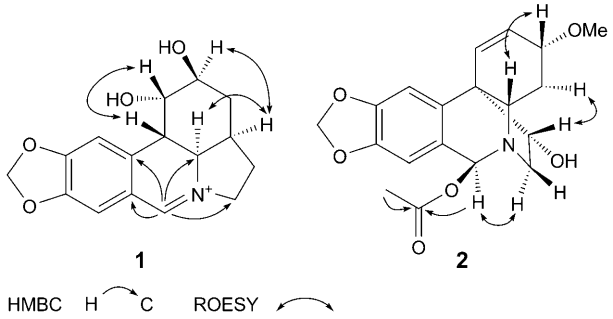


Figure. *Key HMBC and ROESY Correlations of* **1** *and* **2**

reasons, H–C(10b) is considered to be β-oriented, and H–C(4a) α-oriented [18]. Subsequently, the ROESY correlation H–C(10b)/H–C(1) suggested the α-orientation for the OH group at C(1), and the ROESY correlations of H–C(4a)/H–C(4) and H–C(4)/H–C(2) indicated also α-orientation for both H–C(4) and H–C(2) (*Fig.*). Finally, detailed analysis of 2D-NMR data established the structure of **1** as 5,6-dehydrodihydrolycorine.

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 100 MHz, resp.) *of* **1**–**3** (δ in ppm, J in Hz)

Position	**1**[a])		**2**[b])		**3**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	4.48 (br. *s*)	67.3 (*d*)	6.38 (*d*, *J* = 10.1)	126.3 (*d*)	4.95 (*d*, *J* = 2.0)	78.0 (*d*)
2	3.83 (*ddd*, *J* = 8.5, 4.0, 2.6)	69.1 (*d*)	6.41 (*dd*, *J* = 10.1, 1.2)	132.7 (*d*)	2.52 (*d*, *J* = 16.8), 2.88–2.91 (*m*)	31.5 (*t*)
3	1.91 (*dd*, *J* = 11.6, 4.0), 2.14 (*dd*, *J* = 11.6, 2.6)	28.4 (*t*)	3.87–3.89 (*m*)	72.4 (*d*)	5.79 (br. *s*)	125.9 (*d*)
4	2.85–2.88 (*m*)	35.1 (*d*)	1.99 (*dd*, *J* = 13.3, 5.1), 2.11 (*dd*, *J* = 13.3, 4.5)	27.6 (*t*)		141.4 (*s*)
4a	4.26–4.28 (*m*)	61.5 (*d*)	3.63 (*dd*, *J* = 13.3, 4.5)	58.2 (*d*)	4.14 (*d*, *J* = 10.0)	79.0 (*d*)
6	8.86 (*s*)	163.0 (*d*)	6.13 (*s*)	87.0 (*d*)		166.6 (*s*)
6a		139.3 (*s*)		125.0 (*s*)		118.1 (*s*)
7	7.30 (*s*)	112.6 (*d*)	6.65 (*s*)	108.8 (*d*)	7.15 (*s*)	121.3 (*d*)
8		149.0 (*s*)		146.4 (*s*)		143.3 (*s*)
9		157.1 (*s*)		148.1 (*s*)		157.0 (*s*)
10	7.16 (*s*)	107.1 (*d*)	6.83 (*s*)	103.0 (*d*)	7.52 (*s*)	114.1 (*d*)
10a		122.3 (*s*)		137.1 (*s*)		135.4 (*s*)
10b	3.09 (br. *s*)	41.8 (*d*)		49.9 (*s*)	3.62 (*dd*, *J* = 10.0, 2.0)	38.1 (*d*)
11	2.20–2.22 (*m*), 2.28–2.31 (*m*)	33.5 (*t*)	3.93 (*dd*, *J* = 6.7, 3.0)	78.2 (*d*)	2.69–2.72 (*m*, 2 H)	26.4 (*t*)
12	4.08 (*dt*, *J* = 10.0, 4.4), 4.23 (*t*, *J* = 10.0)	57.5 (*t*)	3.27 (*dd*, *J* = 13.5, 3.0), 3.44 (*dd*, *J* = 13.5, 6.7)	58.7 (*t*)	3.51 (*t*, *J* = 9.3), 3.70 (*t*, *J* = 9.3)	70.8 (*t*)
OCH_2O	6.18 (br. *s*)	104.7 (*t*)	5.94 (br. *s*)	101.2 (*t*)		
MeN					2.97 (*s*)	56.0 (*q*)
MeO			3.44 (*s*)	56.6 (*q*)	3.98 (*s*)	57.2 (*q*)
MeCO_2			2.12 (*s*)	21.5 (*q*)	2.28 (*s*)	20.3 (*q*)
Me*C*O_2				170.4 (*s*)		170.2 (*s*)

[a]) Recorded in CD_3OD. [b]) Recorded in $CDCl_3$.

Compound **2** was isolated as a colorless oil. The positive-ion-mode HR-ESI-MS displayed an $[M+H]^+$ peak at m/z 360.1447, corresponding to the molecular formula $C_{19}H_{21}NO_6$. The IR absorption bands at 3386 and 1711 cm^{-1} are ascribable to a OH group and an ester CO group, respectively. In the ^{1}H-NMR spectrum, two *singlet*s at δ(H) 6.65 (*s*) and 6.83 (*s*) were assigned to two *para*-positioned aromatic H-atoms

H–C(7) and H–C(10), respectively. A broad *singlet* at δ(H) 5.94 (br. *s*, 2 H) was ascribed to the OCH_2O H-atoms. Two signals at δ(H) 6.38 (*d*, $J = 10.1$) and 6.41 (*dd*, $J = 10.1, 1.2$) were assigned to the olefinic H-atoms H–C(1) and H–C(2), respectively. Two *singlet*s at δ(H) 3.44 (*s*) and 2.12 (*s*) were ascribed to H-atoms of a MeO group and a Me group, respectively. The ^{13}C-NMR spectrum exhibited 19 C-atom resonances which revealed the existence of a phenyl (δ(C) 103.0, 108.8, 125.0, 137.1, 146.4, 148.1), an Ac (δ(C) 21.5, 170.4), a MeO (δ(C) 56.6), and a OCH_2O group (δ(C) 101.2), two O-bearing CH groups (δ(C) 72.4, 78.2), an sp^3 quaternary C-atom (δ(C) 49.9), and a downfield sp^3-CH group (δ(C) 87.0) bearing two heteroatoms. The above data resembled those of 6-hydroxycrinamine (**4**) [16] except for an additional AcO group (δ(C) 21.5 (*q*), 170.4 (*s*); δ(H) 2.12 (*s*, 3 H)) in **2**. The key HMBC of δ(H) 6.13 (*s*, H–C(6)) with δ(C) 170.4 (*s*, Me*C*O_2) suggested that the AcO group was at C(6) (*Fig.*). The ROESY correlations H–C(4a)/H–C(3) and H–C(11)/H–C(4α) suggested both H–C(3) and H–C(11) to be β-oriented, and the ROESY correlation of H–C(6)/H–C(12α) indicated α-orientation for H–C(6) (*Fig.*). Detailed analysis of 2D-NMR data established compound **2** to be 6β-acetoxycrinamine.

Compound **3** was obtained as a colorless oil. The IR absorption bands at 1703 and 1657 cm^{-1} indicated the existence of CO moieties, while the UV absorption bands at 265 and 212 nm suggested a conjugated moiety. The HR-ESI-MS (positive-ion mode) displayed an $[M + H]^+$ peak at *m*/*z* 360.1457, 16 mass units higher than that of (+)-8-*O*-acetylhomolycorine [27]. Compound **3** was readily identified as (+)-8-*O*-acetylhomolycorine *N*-oxide from the ^{1}H- and ^{13}C-NMR data (*Table*), in particular with the characteristic downfield signals of the C-atom resonances for C(4a) (δ(C) 79.0), C(12) (δ(C) 70.8), and MeN (δ(C) 56.0) with respect to those of (+)-8-*O*-acetylhomolycorine [27]. The ROESY correlation MeN/H–C(4a) suggested α-orientation of the *N*-oxide [28]. Hence, compound **3** was established as (+)-8-*O*-acetylhomolycorine α-*N*-oxide.

Compounds **1**–**3** were evaluated for their cytotoxicity against five human cancer cell lines. Compound **2** showed cytotoxicity against HL-60, A-549, and MCF-7 cells, with IC_{50} values of 8.1, 24.3, and 15.0 μM, respectively, while the positive control cisplatin gave IC_{50} values of 2.4, 17.6, and 18.7 μM. Compounds **1** and **3** were inactive (IC_{50} values > 40 μM).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China), *RP-18* gel (20–45 μm; *Fuji Silysia Chemical Ltd.*, Japan), and *Sephadex LH-20* (*Pharmacia Fine Chemical Co., Ltd.*, Sweden). TLC: *GF 254* plates (*Qingdao Haiyang Chemical Co., Ltd.*, P. R. China). Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrophotometer. IR Spectra: *Tenor 27* spectrophotometer with KBr pellets. 1D- and 2D-NMR spectra: a *Bruker DRX-500* and an *AM-400* spectrometers with TMS as internal standard, δ in ppm and *J* in Hz. HR-ESI-MS: *API-Qstar-Pulsar-1* spectrometer.

Plant Material. Bulbs of *L. radiata* were bought from Juhuacun Chinese Traditional Medicine Market, Kunming, Yunnan Province, P. R. China, and identified by *X.-D. L.* A voucher specimen has been deposited with the Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air-dried and powdered sample (15 kg) was powdered and extacted with MeOH at r.t. (3×24 h) to give a crude extract (950 g). The extract was partitioned between AcOEt and 0.5% HCl soln. The acidic H_2O-soluble material was adjusted to pH 9–10 with 10% aq. NH_3 soln. and then extracted with AcOEt to give an alkaloidal extract (68 g). The alkaloidal extract was subjected

to CC (SiO_2; $CHCl_3$/Me_2CO gradient 1:0, 15:1, 10:1, 5:1, 3:1, 1:1) to give six fractions *Frs. 1–6*. *Fr. 1* (3 g) was subjected to CC (SiO_2; petroleum ether (PE)/Me_2CO 15:1 → 10:1) to yield **5** (6 mg) and **14** (80 mg). *Fr. 2* (16 g) was subjected to CC (SiO_2; PE/Me_2CO 10:1 → 2:1) to yield **2** (12 mg), **9** (110 mg) and **11** (2 g). *Fr. 3* (7 g) was subjected to CC (SiO_2; PE/Me_2CO 5:1 → 1:1), then purified by CC (*RP-18* gel; MeOH/H_2O 6:4) to yield **4** (130 mg) and **10** (60 mg). *Fr. 4* (9 g) was subjected to CC (*RP-18* gel; MeOH/H_2O 5:5) to yield **7** (80 mg), **8** (12 mg), and a mixture (2 g). Compound **6** (1.7 g) precipitated from the mixture. *Fr. 5* (11 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 10:1 → 5:1) and further purified by CC (*RP-18* gel; MeOH/H_2O 4:6) to yield **3** (6 mg), **13** (20 mg), and a mixture (5.8 g). Compound **12** (3.2 g) precipitated from the mixture. *Fr. 6* (7 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 5:1) and further purified by CC (*Sephadex LH-20*; MeOH) to yield **1** (8 mg).

5,6-Dehydrodihydrolycorine (=*(1S,2S,3aR,12bS,12cR)-1,2,3,3a,4,5,12b,12c-Octahydro-1,2-dihydroxy[1,3]dioxolo[4,5-j]pyrrolo[3,2,1-de]phenanthridin-6-ium*; **1**). Yellow powder. $[\alpha]_D^{22} = +399.7$ ($c =$ 0.1, MeOH). UV (MeOH): 373 (3.95), 308 (3.80), 253 (4.32), 211 (4.73). IR (KBr): 3405, 3358, 1646, 1605, 1589, 1499, 1270, 1033, 922. ^{1}H- and ^{13}C-NMR: see *Table*. HR-ESI-MS (pos.): 288.1228 (M^+, $C_{16}H_{18}NO_4^+$; calc. 288.1235).

6β-Acetoxycrinamine (=*(3α,6β,11S,13β,19α)-11-Hydroxy-3-methoxy-1,2-didehydrocrinan-6-yl Acetate*; **2**). Colorless oil. $[\alpha]_D^{22} = +22.1$ ($c = 0.1$, $CHCl_3$). UV ($CHCl_3$): 291 (3.79), 240 (3.88). IR (KBr): 3386, 2899, 1711, 1484, 1248, 1060, 932, 868. ^{1}H- and ^{13}C-NMR: see *Table*. HR-ESI-MS (pos.): 360.1447 ($[M+H]^+$, $C_{19}H_{20}NO_6^+$; calc. 360.1447).

(+)-8-O-Acetylhomolycorine α-N-oxide (=*(1R,5aR,11bS,11cS)-1,2,3,5,5a,7,11b,11c-Octahydro-10-methoxy-1-methyl-1-oxido-7-oxoisochromeno[3,4-g]indol-9-yl Acetate*; **3**). Colorless oil. $[\alpha]_D^{22} = +113.5$ ($c = 0.1$, MeOH). UV (MeOH): 379 (2.67), 307 (3.51), 265 (3.80), 212 (4.61). IR (KBr): 2944, 1703, 1657, 1600, 1451, 1309, 1226, 1066, 909. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 360.1457 ($[M+H]^+$, $C_{19}H_{20}NO_6^+$; calc. 360.1447).

Cytotoxicity Assay. Five human cancer cell lines (*Sigma*, USA), *i.e.*, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer W480, and lung cancer A-549 cells, were used in the cytotoxic assay. Cells were cultured in *RPMI-1640* or in DMEM medium (*Dulbecco*'s Modified Eagle Medium; *Hyclone*, USA), supplemented with 10% fetal bovine serum (*Hyclone*, USA) in 5% CO_2 at 37°. The cytotoxicity assay was performed according to the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method in 96-well microplates [29]. Briefly, 100 µl of adherent cells were seeded into each well of 96-well cell-culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 µM in triplicates for 48 h, with cisplatin (*Sigma*, USA) as a pos. control. After compound treatment, cell viability was detected, and a cell growth curve was graphed. The IC_{50} values were calculated by *Reed* and *Muench*'s method [30].

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