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ALKALOID *N*-OXIDES FROM *LYCORIS SANGUINEA*

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Key Word Index—*Lycoris sanguinea*; Amaryllidaceae; bulbs; alkaloids; *N*-oxides.

Abstract—Three new alkaloids, galanthamine *N*-oxide, sanguinine *N*-oxide and lycoramine *N*-oxide were isolated from *Lycoris sanguinea* var. Kiushiana Makino.

INTRODUCTION

Over 100 alkaloids have been isolated from members of the Amaryllidaceae [1, 2]. Recently, Riguera and co-workers reported the first examples of naturally occurring *N*-oxides from this family [3]. Previously, we reported [4] the isolation of galanthamine (**1**) and sanguinine (**2**) from an ethanol extract of the bulbs of *Lycoris sanguinea* var. Kiushiana Makino. We now report the isolation of three new alkaloids, galanthamine *N*-oxide (**3**), sanguinine *N*-oxide (**4**) and lycoramine *N*-oxide (**5**) together with known alkaloids, galanthamine (**1**), sanguinine (**2**) and lycoramine (**6**) by further studies on this plant.

RESULTS AND DISCUSSION

The base (**3**), $C_{17}H_{21}NO_4$, $[\alpha]_D$ −122.9° (MeOH), was isolated as an amorphous powder. Its IR spectrum showed absorptions for a hydroxy group at 3300 cm^{-1} (br) and for a double bond at 1630 cm^{-1}. The ^1H NMR spectrum (Table 1) showed signals characteristic for a 1,2,3,4-tetrasubstituted aromatic ring and an olefinic group in addition to a deshielded *N*-methyl group and an aromatic methoxy group. The presence of an AB-type doublet (δ4.66 and 5.32) was assignable to a methylene group such as $C_6H_2CH_2N$(Me). The mass spectrum with its characteristic fragment pattern was similar to that of galanthamine (**1**) [4], except for the $[M]^+$ peak. From these and the following findings, the new base was concluded to be galanthamine *N*-oxide (**3**): (i) the molecular formula $C_{17}H_{21}NO_4$ for **3** with one more oxygen than in **1**, (ii) the fact that signals for protons (H-7, H-9 and NMe) attached to N-bearing carbon atoms in **3** were deshielded by *ca* 0.4–0.8 ppm in comparison with those in **1** [4] and (iii) in the ^{13}C NMR spectrum (Table 2) signals for the carbons (C-7, C-9 and NMe) attached to the N atom in **3** are shifted by *ca* 11–15 ppm downfield from the corresponding signals in **1**. This assignment was confirmed by conversion of **1** to its *N*-oxide (**3**); oxidation of the base **1** in chloroform with *m*-chloroperbenzoic acid (MCPBA) gave **3** as an amorphous powder, $C_{17}H_{21}NO_4$, $[\alpha]_D$ −115.2° (MeOH). The stereochemistry of the N atom in **3** was deduced from an NOE experiment. Observation of NOE between H-6α and the *N*-methyl proton in **3** showed an α configuration for the *N*-methyl group.

The base (**4**), mp 191–195°, $[\alpha]_D$ −106.1° (MeOH), gave a blue-violet colour with ferric chloride reagent. The IR spectrum showed absorptions for hydroxy groups at 3470 and 3160 cm^{-1} and for a double bond at 1630 cm^{-1}. The mass spectrum indicated the molecular formula $C_{16}H_{19}NO_4$ and its fragmentation pattern was similar to that of sanguinine (**2**) [4], except for the $[M]^+$ peak. The ^1H NMR spectrum (Table 1) was also similar to that of **2**. However, the signals of H-7, H-9 and NMe in the base **4** were deshielded in comparison with those in **2**. Furthermore, in the ^{13}C NMR spectrum (Table 2) of **4**, the signals for the carbons (C-7, C-9 and NMe) attached to the N atom are shifted by *ca* 10–15 ppm downfield

Table 1. ^1H NMR spectral data of bases **3–5** [200 MHz, pyridine-d_5–CD_3OD (3:1), −20°]

H	3	4	5
1α	2.73 *d* (16)	2.70 *d* (15)	2.57 *d* (16)
1β	2.12 *d*-like (16)	2.16 *d*-like (15)	2.09 *m*
2	4.46 *br s*	4.46 *t*-like (5)	*
3	6.28 *dd* (10, 4)	6.22 *dd* (10, 5)	*
4	6.43 *d* (10)	6.39 *d* (10)	*
6α	2.24 *t* (14)	2.23 *t* (16)	*
6β	1.90 *d* (14)	1.90 *d* (16)	1.73 *m*
7α	3.96 *d* (14)	3.89 *d* (14)	3.86 *d* (16)
7β	4.35 *t* (14)	4.26 *t* (14)	4.30 (16)
9α	4.66 *d* (14)	4.59 *d* (14)	4.57 *d* (14)
9β	5.32 *d* (14)	5.21 *d* (14)	5.07 *d* (14)
11	6.71 *d* (8)	6.78 *d* (8)	6.83 *s*
12	6.82 *d* (8)	6.95 *d* (8)	6.83 *s*
17	4.69 *br s*	4.67 *br s*	4.45 *br s*
NMe	3.20 *s*	3.16 *s*	3.12 *s*
OMe	3.71 *s*	—	3.80 *s*

*Obscured.

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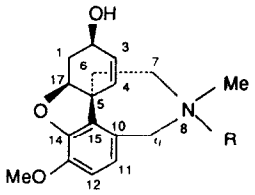
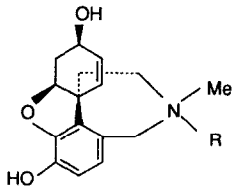
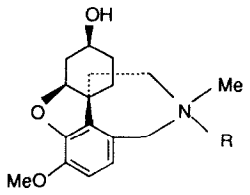


R
1 free base
3 O⁻



R
2 free base
4 O⁻



R
5 O⁻
6 free base

Table 2. ^{13}C NMR spectral data of bases **3–5** (50.10 MHz, −20°)

C	3*	4*	5†
1	31.6 *t*	31.2 *t*	32.6 *t*
2	62.2 *d*	62.2 *d*	64.2 *d*
3	127.0 *d*	127.0 *d*	27.6 *t*
4	130.3 *d*	129.9 *d*	32.7 *t*
5	†	‡	45.6 *s*
6	35.5 *t*	35.1 *t*	23.9 *t*
7	70.3 *t*	70.1 *t*	70.2 *t*
9	75.1 *t*	76.0 *t*	74.9 *t*
10	120.7 *s*	119.4 *s*	120.1 *s*
11	124.7 *d*	124.6 *d*	‡
12	114.1 *d*	117.3 *d*	112.0 *d*
13	148.4 *s*	147.4 *s*	147.8 *s*
14	147.6 *s*	144.6 *s*	146.3 *s*
15	133.8 *s*	133.5 *s*	135.3 *s*
17	89.0 *d*	88.7 *d*	89.8 *d*
NMe	53.6 *q*	53.3 *q*	52.3 *q*
OMe	56.8 *q*	—	56.0 *q*

*In methanol-d_4.
†In pyridine-d_5–methanol-d_4 (3:1).
‡Obscured.

from the corresponding signals in **2**. From these findings, the new base was assigned to be sanguinine *N*-oxide (**4**). This assignment was confirmed by oxidation of **2** with MCPBA to the *N*-oxide (**4**). The oxidation product, mp 192–195°, $C_{16}H_{19}NO_4$, $[\alpha]_D$ −133.6° (MeOH) was found to be identical with the natural amine (**4**) by direct comparison. The α-configuration of the *N*-methyl group in **4** was concluded as follows: methylation of **4** with diazomethane gave a methylated product, $[\alpha]_D$ −100.6° (MeOH), which was identical with the natural galanthamine *N*-oxide (**3**).

The third new *N*-oxide (**5**), $C_{17}H_{23}NO_4$, $[\alpha]_D$ −91.8° (MeOH), was isolated as an amorphous material. Its structure was assigned as lycormaine *N*-oxide (**5**) in the same way as **3** and **4** on the basis of the IR, mass, ^{1}H and ^{13}C NMR spectral data (see Tables 1 and 2). This assignment was confirmed by conversion of lycoramine (**6**) with MCPBA to its *N*-oxide (**5**), which was identical with the natural *N*-oxide (5) by comparison of their spectral data. However, the stereochemistry of the quaternary N atom in **5** could not be deduced by NOE.

Galanthamine (**1**), sanguinine (**2**) and lycoramine (**6**) were isolated and their structures confirmed by direct comparisons with authentic samples [4, 5, 6]. It is interesting to note that the *N*-oxides **3–5** were isolated together with the corresponding free bases **1**, **2** and **6**.

EXPERIMENTAL

Mps: uncorr. MS were determined at 70 eV. ^{1}H NMR were recorded at 200 MHz, ^{13}C NMR at 50 or 100 MHz. Prep. TLC was carried out on silica gel 60 (PF_{254}, Merck) and aluminium oxide 60 (PF_{254}, Merck) with solvent systems as follows: a, $CHCl_3$–MeOH (8:1); b, $CHCl_3$–MeOH–H_2O (70:15:2); c, $CHCl_3$–MeOH–H_2O (12:7:12); d, $CHCl_3$–EtOAc–MeOH (21:9:2); e, $CHCl_3$–EtOAc–MeOH (7:3:1); f, C_6H_6–EtOAc (1:1).

Plant material. Lycoris sanguinea Maxim. was collected at our Faculty Herbary (Faculty of Pharmaceutical Sciences, The University of Tokushima) in May 1980. A voucher specimen is available at the Herbary in Kokufu-cho, Tokushima 770, Japan.

Extraction and isolation. Following the method of ref. [7], fr. bulbs (7 kg) were ground in 99% EtOH in a mixer. The insol. material was extracted ×3 with 24 l 99% EtOH. The EtOH ext was evapd to *ca* 1.6 l *in vacuo*, acidified (pH 4) with tartaric acid and washed with Et_2O. The acidic aq. soln was made basic (pH 10) with conc NH_4OH and extracted ×3 with 800 ml $CHCl_3$. The extract was evapd *in vacuo* to give crude alkaloids (5.5 g).

Crude alkaloids (3.885 g) were subjected to prep. TLC (silica gel–solvent a) to give 6 frs: A, R_f 0–0.03; B, R_f 0.03–0.09; C, R_f 0.09–0.18; D, R_f 0.18–0.30; E, R_f 0.30–0.39 and F, R_f 0.39–0.54. Fr. A was subjected to prep. TLC (alumina–solvent b) to give fr. A–1 (R_f 0.06–0.25). This fr. was purified by prep. TLC (silica gel–solvent c) to give sanguinine *N*-oxide (**4**) as a powder (41 mg, R_f 0.04–0.25). Fr. B was subjected to prep. TLC (silica gel–solvent d) to afford fr. B–1 (R_f 0.18–0.29), which was purified by prep. TLC (silica gel–solvent b) to give lycoramine *N*-oxide (**5**) as an amorphous powder (7 mg, R_f 0.35–0.47). On prep. TLC (alumina–solvent d), fr. C gave crude material (R_f 0.15–0.25), which was purified by prep. TLC (silica gel–solvent b) to afford galanthamine *N*-oxide (**3**) as an amorphous powder (53 mg, R_f 0.35–0.47). Fr. D was purified by prep. TLC (alumina–solvent e) to give sanguinine (**2**) as a powder (159 mg, R_f 0.25–0.56). On prep. TLC (alumina–$CHCl_3$), fr. E afforded lycoramine (**6**) as crystals (73 mg, R_f 0.82–0.88). Fr. F was subjected to prep. TLC (alumina–solvent f) to afford galanthamine (**1**) as needles (136 mg, R_f 0.29–0.59).

Galanthamine N-oxide (**3**). Amorphous powder, $[\alpha]_D^{26}$ −122.9° (MeOH; *c* 0.38). IR ν_{max}^{KBr} cm^{-1}: 3300 (br, OH), 1630 (C=C), 1520, 1440, 1280, 1080, 1020. HRMS: $[M]^+$ (found: 303.1479; $C_{17}H_{21}NO_4$ requires 303.1470). EIMS *m/z* (rel. int.): 303 $[M]^+$ (25), 287 (81), 286 (100), 272 (18), 244 (22), 230 (49), 226 (12), 216 (35), 174 (34). ^{1}H and ^{13}C NMR: see Tables 1 and 2.

Galanthamine (**1**). Needles (from Me_2CO), mp 120–125° (ref. [4]; mp 123–127°). $[\alpha]_D^{23}$ −109.2° (EtOH; *c* 0.85) (ref. [4]: $[\alpha]_D^{27}$

$-118°$ (EtOH; *c* 0.24). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1620 (C=C), 1595, 1515, 1420, 1280, 1040. EIMS *m/z* (rel. int.): 287 $[M]^+$ (91), 286 (100), 272 (4), 244 (28), 230 (17), 226 (10), 216 (35), 174 (34). ^{13}C NMR: see ref. [8]. ^1H NMR: see ref. [4]. This sample was identical with authentic **1** [4] by direct comparison.

Synthesis of **3** *from* **1**. A soln of MCPBA (12.2 mg) in $CHCl_3$ (1 ml) was added to a soln of **1** (14.8 mg) in $CHCl_3$ (0.5 ml) and stirred at 0° for 1.5 hr. The mixt. was subjected to CC [alumina, $CHCl_3$–MeOH (3:1)] to give a crude product (16.6 mg). This was purified by prep. TLC (silica gel–solvent b) to give **3** as an amorphous powder (14.7 mg), $[\alpha]_D^{26}$ $-115.2°$ (MeOH; *c* 0.42). HRMS: $[M]^+$ (found: 303.1470; $C_{17}H_{21}NO_4$ requires 303.1470). This compound was identical with natural **3** in all respects (IR, ^1H and ^{13}C NMR).

Sanguinine N-oxide (**4**). Prisms (from MeOH), mp 191–195°, $[\alpha]_D^{25}$ $-106.1°$ (MeOH; *c* 0.25). IR ν_{max}^{KBr} cm^{-1}: 3470 (br, OH), 3160 (OH), 1630 (C=C), 1595, 1505, 1465, 1315, 1170, 1075. HRMS: $[M]^+$ (found: 289.1321; $C_{16}H_{19}NO_4$ requires 289.1314). EIMS *m/z* (rel. int.): 289 $[M]^+$ (15), 273 (100), 272 (89), 258 (7), 256 (20), 230 (15), 216 (33), 212 (24), 202 (37), 198 (17), 160 (56). ^1H and ^{13}C NMR: see Tables 1 and 2. (Found: C, 63.0; H, 7.2; N, 4.3. $C_{16}H_{19}NO_4 \cdot 4/5H_2O$ requires: C, 63.3; H, 6.9; N, 4.6%).

Sanguinine (**2**). Prisms (from MeOH), mp 209–212° (dec.) (ref. [4]; mp 210.5–213° (dec.)). $[\alpha]_D^{23}$ $-144.7°$ (EtOH; *c* 0.5) (ref. [4]; $[\alpha]_D^{27}$ $-133°$ (EtOH; *c* 0.23)). HRMS: $[M]^+$ (found: 273.1349; $C_{16}H_{19}NO_3$ requires 273.1365). EIMS *m/z* (rel. int.): 273 $[M]^+$ (80), 272 (66), 256 (19), 230 (16), 216 (21), 212 (25), 202 (40), 160 (70). ^{13}C NMR (100 MHz, CD_3OD): δ31.5 (*t*, C-1), 61.7 (*d*, C-2), 128.3 (*d*, C-3), 128.7 (*d*, C-4), 35.6 (*t*, C-6), 55.3 (*t*, C-7), 61.6 (*t*, C-9), 127.9 (*s*, C-10), 123.2 (*d*, C-11), 116.8 (*d*, C-12), 146.6 (*s*, C-13), 142.3 (*s*, C-14), 134.3 (*s*, C-15), 88.9 (*d*, C-17), 43.2 (*q*, NMe). ^1H NMR: see ref. [4]. This sample was identical with authentic **2** by direct comparison.

Synthesis of compound **4** *from* **2**. A soln of MCPBA (12 mg) in $CHCl_3$ (2 ml) was added to a soln of **2** (16 mg) in $CHCl_3$ (1 ml) and stirred for 30 min at room temp. Work-up gave a crude product (33 mg). This was subjected to prep. TLC (silica gel–solvent c) to give a solid (11 mg). Recrystallization from MeOH gave **4** as cubes, mp 192–195°, $[\alpha]_D^{25}$ $-113.6°$ (MeOH; *c* 0.1). HRMS: $[M]^+$ (found: 289.1312; $C_{16}H_{19}NO_4$ requires 289.1312). This compound was identical with natural **4** in all respects (IR, ^1H and ^{13}C NMR).

Synthesis of compound **3** *from* **4**. A soln of **4** (5.4 mg) in MeOH (1 ml) was added to a satd soln (1 ml) of CH_2N_2 in Et_2O at 0°. The mixt. was stood at room temp. for 2 days and work-up gave an oil. This crude product was purified by prep. TLC (silica gel–solvent c) to give **3** as an amorphous powder (2.7 mg). ^1H NMR of this product was identical with that of natural **3**.

Lycoramine N-*oxide* (**5**). Amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1595, 1515, 1440, 1280, 1075. HRMS: $[M]^+$ (found: 305.1651; $C_{17}H_{23}NO_4$ requires 305.1626). EIMS *m/z* (rel. int.): 305 $[M]^+$ (9), 289 (61), 288 (100), 232 (40), 228 (50), 216 (10), 202 (18), 187 (23), 174 (14). ^1H and ^{13}C NMR: see Table 1 and 2.

Lycoramine (**6**). Plates (from Me_2CO), mp 115–116° (ref. [5]; mp 120–122°). $[\alpha]_D^{26}$ $-77.9°$ (MeOH; *c* 0.35) (ref. [6]; $[\alpha]_D^{22}$ $-96.0°$ (EtOH; *c* 0.71)). HRMS: $[M]^+$ (found: 289.1321; calc. for $C_{16}H_{19}NO_4$: 289.1314). ^{13}C NMR (50.10 MHz, $CDCl_3$): δ31.4 (*t*, C-1), 65.3 (*d*, C-2), 27.7 (*t*, C-3), 31.6 (*t*, C-4), 46.8 (*s*, C-5), 23.8 (*t*, C-6), 54.7 (*t*, C-7), 60.5 (*t*, C-9), 129.1 (*s*, C-10), 121.7 (*d*, C-11), 110.8 (*d*, C-12), 146.0 (*s*, C-13), 144.0 (*s*, C-14), 136.3 (*s*, C-15), 89.9 (*d*, C-17), 41.9 (*q*, NMe), 55.9 (*q*, OMe). EIMS: see ref. [6]. ^1H NMR: see refs [5, 6]. This compound was identical with authentic **6** [6] by direct comparison.

Synthesis of compound **5** *from* **6**. A soln of MCPBA (25.9 mg) in $CHCl_3$ (1 ml) was added to a soln of 6 (25 mg) in $CHCl_3$ (1 ml) and stirred at 0° for 1 hr. Work-up gave a crude product. This product was purified by prep. TLC (silica gel–solvent b) to give **5** as an amorphous powder (20.7 mg). $[\alpha]_D^{26}$ $-111.6°$ (MeOH; *c* 0.98). HRMS: $[M]^+$ (found: 305.1635; $C_{17}H_{23}NO_4$ requires 305.1626). This compound was identical with natural **5** in all respects (IR, ^1H and ^{13}C NMR).

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