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Isolation of N-Demethylgalanthamine from the Bulbs of *Crinum asiaticum* L. var. *japanicum* Baker (Amaryllidaceae)

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The bulbs of *Crinum asiaticum* L. var. *japonicum* Baker (Amaryllidaceae) were found to contain N-demethylgalanthamine (II) as well as lycorine (III) and crinamine (IV). This is the first time that II has been isolated directly, as an single base, from a member of the Amaryllidaceae.

The previous paper[2] reported the isolation of sanguinine (O-demethylgalanthamine) (I) from the bulbs of *Lycoris sanguinea* Maxim. var. *kiushiana* Makino (Amaryllidaceae).

This paper reports the isolation of N-demethylgalanthamine (II), as well as lycorine (III)[3] and crinamine (IV)[4,5] from the bulbs of *Crinum asiaticum* L. var. *japonicum* Baker (Japanese name, Hamaomoto).[4,6] Tanaka[4] isolated III and IV from the bulbs of this plant, and Kutani and Matsumoto[6] obtained III from the seeds. Narcissamine (V) isolated[7,8] from the bulbs of *Narcissus pseudonarcissus* L. was reported[7] to be II. However, later Laiho and Fales[9] found that the base (V) is a quasi-racemate of (−)-II and (+)-N-demethyldihydrogalanthamine ((+)-N-demethyllycoramine) (VI). Therefore, this is the first time that II has been isolated directly, as a single base, from a member of the Amaryllidaceae.

Bulbs were collected in Koochi Prefecture and crude basic materials were extracted from fresh bulbs by the method of Wildman and Bailey.[10] Crystals of lycorine (III) were formed on treatment of the crude extract obtained at pH 8 with chloroform. The base (III) was identified by elemental analysis, its mixed melting points, and comparison of its infrared (IR) spectrum with that of an authentic sample. The chloroform solution was then concentrated and the residue was triturated with acetone to give crinamine (IV).[4,5] The base (IV), mp 192—193°, was identified by elemental analysis and its nuclear magnetic resonance (NMR) spectrum (see Experimental). The acetone solution from which IV had been separated gave crude nonphenolic bases, which were submitted to preparative thin-layer chromatography (TLC) on silica gel to afford II, mp 152.5—153°, $[\alpha]_D^{22}$ −74.0°. Its melting point and optical rotation were identical with those of N-demethylgalanthamine (II)[9] isolated from narcissamine (V) by TLC. Furthermore, the base (II) was identified by elemental analysis and spectral

1) Location: *a) 1-78, Sho-machi, Tokushima, 770, Japan; b) Ikawadani, Tarumi-ku, Kobe, 673, Japan; c) 4-16, Edagawa, Nishinomiya, Hyogo, 663, Japan.*
2) S. Kobayashi, S. Takeda, H. Ishikawa, H. Matsumoto, M. Kihara, T. Shingu, A. Numata, and S. Uyeo, *Chem. Pharm. Bull.* (Tokyo), **24**, 1537 (1976).
3) See ref. 3 of our previous paper.
4) K. Tanaka, *Yakugaku Zasshi*, **56**, 652 (1937).
5) H.M. Fales and W.C. Wildman, *J. Am. Chem. Soc.*, **82**, 197 (1960).
6) N. Kutani and Y. Matsumoto, *Yakugaku Zasshi*, **64**, 239 (1944).
7) H.G. Boit and H. Ehmke, *Chem. Ber.*, **89**, 163 (1956); H.M. Fales, L.D. Giuffrida, and W.C. Wildman, *J. Am. Chem. Soc.*, **78**, 4145 (1956).
8) H.G. Boit and H. Ehmke, *Chem. Ber.*, **90**, 57 (1957); H.G. Boit, W. Döpke, and A. Beitner, *ibid.*, **90**, 2197 (1957).
9) S.M. Laiho and H.M. Fales, *J. Am. Chem. Soc.*, **86**, 4434 (1964).

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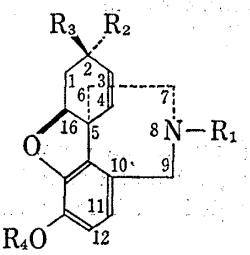
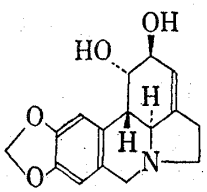
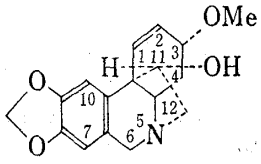
data: the IR spectrum showed absorptions for a hydroxyl group at 3600—3200 cm^{-1} and for an amino group at 3320 cm^{-1}. In the NMR spectrum of II, the signals at 3.36 (2H), 3.96 (2H), 5.40 (1H), 5.88 (1H), and 6.19 τ (3H) were assigned as shown in Table I, and were identical with those of the corresponding protons of galanthamine (VII), but different from those of the corresponding protons of epigalanthamine (VIII).

To confirm this assignment, the base (II) was methylated with formalin and sodium borohydride. Direct comparison showed that the methylated product was identical with an authentic sample of VII.





III



IV

I : R_1=Me, R_2=R_4=H, R_3=OH
II : R_1=R_2=H, R_3=OH, R_4=Me
VI: antipode of R_1=R_2=H, R_3=OH, R_4=Me (no double bond at C_3)
VII: R_1=R_4=Me, R_2=H, R_3=OH
VIII: R_1=R_4=Me, R_2=OH, R_3=H

Chart 1

TABLE I. Chemical Shifts of Galanthamine-type Alkaloids (in $CDCl_3$) (τ)

Compd.	C-11-H and C-12-H	C-3-H and C-4-H	C-16-H	C-2-H	C-9 H_2	OCH_3
N-Demethylgalanthamine(II)	3.36(s)	3.96(s)	5.40(m)	5.88(m)	6.04(m)	6.19(s)
Galanthamine(VII)	3.38(s)	3.98(s)	5.41(m)	5.88(m)	5.94(d), 6.36(d), J=14 Hz	6.19(s)
Epigalanthamine(VIII)	3.38(d), 3.46(d), J=8 Hz	3.79(d), 4.03(d), J=10 Hz	5.28—5.50(m)		5.93(d), 6.40(d), J=15 Hz	6.18(s)
Sanguinine(I)	3.45(s)	3.97(s)	5.45(m)	5.90(m)	5.95(d), 6.34(d), J=14 Hz	—

Experimental

All melting points are given as uncorrected values. The spectrophotometers used were a Hitachi, EPI-G2 model for IR spectra, a Yanagimoto, OR-50 model for optical rotations, and a JEOL, JNM-PS-100 or a Hitachi, R-22 model for NMR spectra using tetramethylsilane as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The signals were assigned by study of nuclear magnetic double resonance.

Isolation of Alkaloids——Following the method of Wildman and Bailey,[10] fresh bulbs (5.5 kg) of *C. asiaticum* L. var. *japonicum* BAKER were ground in 99% EtOH in a mixer. The insoluble material was extracted three times with 8.8 liters of 99% EtOH. The ethanolic extract was evaporated to approximately 1 liter *in vacuo*, made acidic (pH 4) with tartaric acid, and washed with ether until the ether layer was colorless, to remove neutral and acidic materials. The aqueous acidic solution was made basic (pH 8) with conc. NH_4OH and extracted five times with 400 ml-portions of $CHCl_3$. The extract was evaporated *in vacuo* to give crude alkaloids (4.876 g). The crude alkaloids gave 673 mg of $CHCl_3$-insoluble crystals [mp 228—232° (decomp.)] and $CHCl_3$-soluble material (4.2 g) when mixed with $CHCl_3$ (100 ml).

10) W.C. Wildman and D.T. Bailey, *J. Org. Chem.*, 33, 3749 (1968).

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Lycorine (III)——A sample (306 mg) of the crystals was recrystallized from EtOH to give 161 mg of III as white prisms, mp 256—258.5° (decomp.), undepressed on admixture with an authentic sample of III. The IR spectrum of III was also identical with that of the authentic sample. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 950 (OCH_2O). *Anal.* Calcd. for $C_{16}H_{17}O_4N$: C, 66.88; H, 5.96; N, 4.88. Found: C, 67.02; H, 6.03; N, 4.67.

Crinamine (IV)——The $CHCl_3$-soluble material was triturated with acetone to give IV (1.28 g) as colorless needles mp 192—193° (from acetone). $[\alpha]_D^{22}$ +148.8° (c=0.813, $CHCl_3$) (lit.[11] $[\alpha]_D^{28}$ +156.6° (c=1.65, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400—3200 (OH), 930 (OCH_2O). NMR ($CDCl_3$) τ: 3.21 (1H, s, C-10-H), 3.53 (1H, s, C-7-H), 3.76 (2H, s, C-1-H and C-2-H), 4.12 (2H, s, OCH_2O), 5.76 and 6.32 (each 1H, d, J=17 Hz, AB type of C-6 H_2), 5.92—6.10 (2H, m, C-3-H and C-11-H), 6.62 (3H, s, OCH_3), 6.70—6.93 (3H, m, C-4a-H and C-12 H_2), 7.84—8.06 (2H, m, C-4 H_2), 7.62 (1H, s, OH). *Anal.* Calcd. for $C_{17}H_{19}O_4N$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.60; H, 6.40; N, 4.61.

N-Demethylgalanthamine (II)——The mother liquid (the acetone solution) from which IV had been separated was concentrated and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was treated with 5% NaOH to give nonphenolic bases (2.01 g), which were submitted to preparative TLC using SiO_2-[$CHCl_3$–MeOH (5: 1)]. Elution of material of *Rf* 0.53—0.63 with MeOH–acetone–$CHCl_3$ (1: 1: 1) gave an additional 646 mg of IV (total 1.926 g, 0.035% from fresh bulbs). Elution of material of *Rf* 0.34—0.50 gave 204 mg of II as white needles, mp 152.5—153°, after recrystallization from acetone (lit.[9] mp 156—158°). $[\alpha]_D^{22}$ −74.0° (c=0.277, $CHCl_3$) [lit.[9] $[\alpha]_D^{23}$ −62° (c=0.28, $CHCl_3$)], $[\alpha]_D^{22}$ −90.7° (c=0.717, EtOH). NMR ($CDCl_3$) τ: 6.49—6.97 (2H, m, C-7 H_2), 7.17—7.43 (1H, br d, C-1-H), 7.73—8.45 (5H, m, OH, NH, C-1-H, and C-6 H_2); (pyridine-d_5) τ: 3.36 [2H, overlapping s(J=8.5 Hz), C-11-H and C-12-H], 3.88 (center) (2H, m, C-3-H and C-4-H), 5.46 (1H, diffuse t, C-16-H), 5.67 (center) (1H, m, C-2-H), 5.90 and 6.00 (each 1H, d, J=15.5 Hz, AB type of C-9 H_2), 6.36 (3H, s, OCH_3), 6.76 (center) (2H, m, C-7 H_2), 7.32 [1H, br d, J=15.5 Hz, C-1-H (equatorial)], 7.98 [1H, d of q, J=3.2, 5.2, and 15.5 Hz, C-1-H(axial)], 8.31 (center) (2H, m, C-6 H_2). *Anal.* Calcd. for $C_{16}H_{19}O_3N$: C, 70.31; H, 7.01; N, 5.13. Found: C, 70.00; H, 7.00; N, 4.85.

Conversion of II to Galanthamine (VII)——To a mixture of H_3BO_3 (12 mg), MeOH (2 ml), formalin (0.12 ml), and II (11 mg) $NaBH_4$ (37 mg) was added in small portions with stirring at room temperature for 30 min. Then AcOH (0.12 ml) and H_2O (10 ml) were added and the mixture was concentrated under reduced pressure. The residue was made basic (pH 10) with conc. NH_4OH and extracted with $CHCl_3$. The extract gave 6.5 mg of VII as white prisms, mp 125.5—126.5° (from acetone–ether). $[\alpha]_D^{22}$ −107.2° (c=0.681, EtOH). *Anal.* Calcd. for $C_{17}H_{21}O_3N$: C, 71.05; H, 7.37; N, 4.87. Found: C, 70.91; H, 7.47; N, 4.64. This was shown to be identical with an authentic sample of VII by the mixed melting point test and comparison of IR spectra.

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11) L.H. Mason, E.R. Pushett, and W.C. Wildman, *J. Am. Chem. Soc.*, 77, 1253 (1955).

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