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Isolation of Carinatine and Pretazettine from the Bulbs of *Zephyranthes carinate* Herb. (Amaryllidaceae)

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The bulbs of *Zephyranthes carinata* Herb. (Amaryllidaceae) were found to contain pretazettine (I) and a new phenolic base, carinatine (II), as well as lycorine (III), galanthine (IV), and haemanthamine (V). Carinatine (II), $C_{17}H_{21}NO_4$, was established to be O-demethylgalanthine.

Keywords——carinatine; pretazettine; lycorine; galanthine; haemanthamine; *Zephyranthes carinata* Herb.; Amaryllidaceae; alkaloid

Previously we reported[2] the isolation of pretazettine (I)[3] from the bulbs of *Lycoris radiata* Herb. (Amaryllidaceae).

This paper reports the isolation of I and a new phenolic base, named carinatine (II), as well as lycorine (III),[2] galanthine (IV),[4] and haemanthamine (V)[5] from the bulbs of *Zephyranthes carinata* Herb. (Japanese name, Safuranmodoki), and the structural assignment of II. Boit, *et al.*[6] isolated III, IV, V, and tazettine (VI)[2] from the bulbs of this plant.

Bulbs were collected in Tokushima Prefecture and crude basic materials were extracted from fresh bulbs by the method of Wildman and Bailey.[3] Lycorine (III), sparingly soluble in chloroform, was isolated by the difference of the solubility in the solvent from the chloroform solution of the materials, and identified by direct comparison of the base with an authentic sample of III. The chloroform solution was submitted to preparative thin-layer chromatography (TLC) using silica gel–chloroform–methanol–diethylamine to give three fractions, *Rf* 0.47—0.56, *Rf* 0.37—0.44, and *Rf* 0.19—0.30: the first fraction gave IV and V, the second fraction I, and the third fraction II.

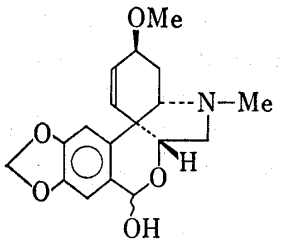
Galanthine (IV), mp 128—130.5°, and haemanthamine (V), mp 196.5—197°, were identified by elemental analyses and their spectral data.

Pretazettine (amorphous) (I) was characterized as its hydrochloride, mp 223—223.5° (dec.), and picrate, mp 204—205° (dec.), which were identical with those of authentic samples, respectively, by direct comparison.

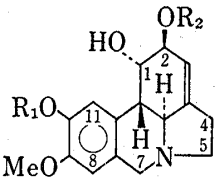
A new amorphous phenolic base, carinatine (II), has $[\alpha]_D^{27}$ −68.97° (c=0.696, chloroform), and was characterized as its picrate, mp 195—197° (dec.), $C_{17}H_{21}NO_4 \cdot C_6H_3N_3O_7$. This

1) Location: *a) 1-78, Sho-machi, Tokushima, 770, Japan; b) Ikawadani, Tarumi-ku, Kobe, 673, Japan; c) Yamashiro-cho, Tokushima, 770, 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) W.C. Wildman and D.T. Bailey, *J. Org. Chem.*, **33**, 3749 (1968).
4) *a)* N.F. Proskurnina and L. Ya. Areshkina, *J. Gen. Chem.* (USSR), **17**, 1216 (1947) [*C.A.*, **42**, 1595 (1948)]; *b)* H.M. Fales, L.D. Giuffrida, and W.C. Wildman, *J. Am. Chem. Soc.*, **78**, 4145 (1956); *c)* H.G. Boit and H. Ehmke, *Chem. Ber.*, **89**, 163 (1956).
5) *a)* H.G. Boit, *Chem. Ber.*, **87**, 1339 (1954); *b)* W.C. Wildman and C.J. Kaufman, *J. Am. Chem. Soc.*, **77**, 1248 (1955); *c)* F.L. Warren and W.G. Wright, *J. Chem. Soc.*, **1958**, 4696.
6) H.G. Boit, W. Döpke, and W. Stender, *Chem. Ber.*, **90**, 2203 (1957).

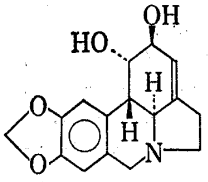
base (II) was suggested to be a lycorine type alkaloid from its mass spectrum [*m/e* 303 (M^+)], since the characteristic and diagnostic fragment ion peak[7] of lycorine type alkaloids appeared at *m/e* 229 (the base peak, M^+-74), 228 (the second largest peak, M^+-75), and 173.5 (the metastable peak, $m_c^*=173.07$ for 303→229) (see Fig. 2. and Chart 2). The base (II) gave a red-brown and a blue-violet color with ferric chloride and Gibbs[8] reagents, respectively. The infrared (IR) spectrum of the base showed absorptions for hydroxyl groups at 3540 cm^{-1} and for a double bond at 1620 cm^{-1}. The ultraviolet (UV) spectrum, having $\lambda_{max}^{ethanol}$ 206 (log ε, 4.47) and 283 nm (log ε, 3.71), and the optical rotatory dispersion (ORD) curve were similar to those of IV, respectively, as shown in Fig. 1. In the nuclear magnetic resonance (NMR) spectrum of the base (II), the assignment of two aromatic protons, C–11–H and C–8–H, and



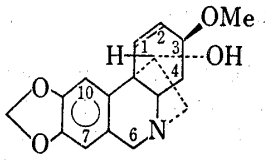
I



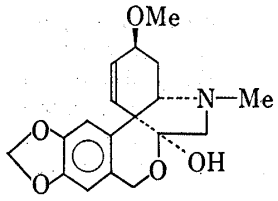
II : R_1=H, R_2=Me
IV : R_1=Me, R_2=Me
VIII : R_1=H, R_2=H



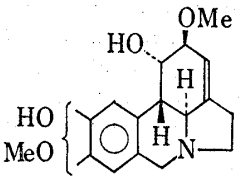
III



V



VI



VII

Chart 1

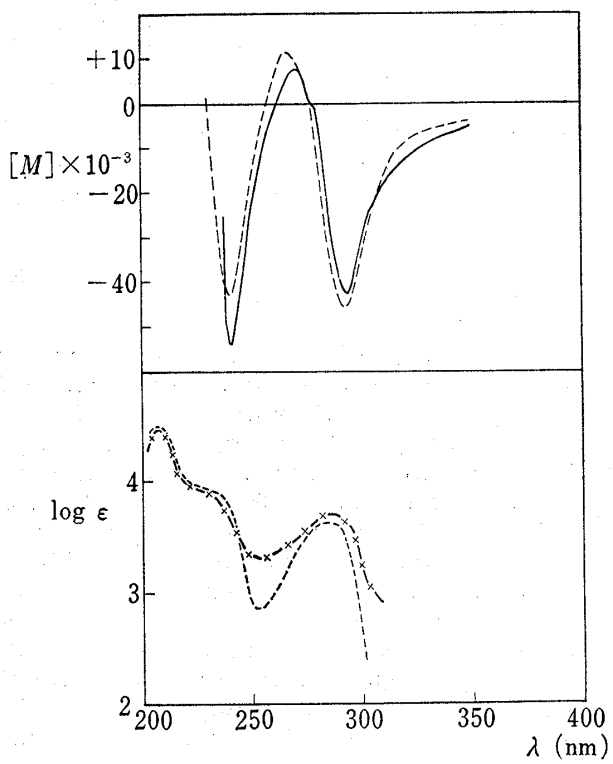


Fig. 1. Carinatine (II); ORD (------) and UV (—+—) Spectra in EtOH, Galanthine (IV); ORD (———) and UV (--------) Spectra in EtOH

TABLE I. Chemical Shifts of Carinatine (II) and Galanthine (IV) (in $CDCl_3$, δ)

Compound	C-11-H	C-8-H	C-3-H	C-1-H	C-7 H_2	C-9-OCH_3	C-2-OCH_3	C-10-OCH_3
Carinatine (II)	6.83(s)	6.57(s)	5.56(br, s)	4.51(s)	4.11(d) 3.48(d) J=14 Hz	3.80(s)	3.43(s)	—
Galanthine (IV)	6.84(s)	6.61(s)	5.58(br s)	4.63(s)	4.14(d) 3.48(d) J=14 Hz	3.83(s)	3.48(s)	3.87(s)

7) *a*) T.H. Kinstle, W.C. Wildman, and C.L. Brown, *Tetrahedron Lett.*, **1966**, 4659; *b*) T. Ibuka, H. Irie, A. Kato, S. Uyeo, K. Kotera, and Y. Nakagawa, *ibid.*, **1966**, 4745.
8) H. Inouye, Y. Kanaya, and Y. Murata, *Chem. Pharm. Bull.* (Tokyo), **7**, 573 (1959).

two O-methyl protons, C–9–OCH_3 and C–2–OCH_3, was achieved by study of nuclear magnetic double resonance (NMDR): monitoring the line of C–11–H gave an NOE (intramolecular nuclear Overhauser effects) peak at δ 4.51, since irradiation at δ 4.51 (C–1–H) gave a 16% NOE increment in the signal of C–11–H. Monitoring the line of C-8–H led us to find the AB type signals at δ 4.11 and 3.48 (each d, J=14 Hz, C–7 H_2) and an NOE peak at δ 3.80. Irradiation at δ 3.80 (C–9–OCH_3) gave a 12% NOE increment in the signal of C–8–H. From these physical and spectral data and specially the remarkable similarity between the chemical shifts for II and those for IV (see Table I), carinatine was assigned O-demethylgalanthine (II) having a phenolic hydroxyl group in the position of C–10.

To confirm this assignment, II was methylated with diazomethane in dimethyl sulfoxide (DMSO). The methylated product was found to be identical with an authentic sample of IV by direct comparison.

Döpke[9] isolated goleptine, $C_{17}H_{21}NO_4$ [mp 141°, $[\alpha]_D$ −99° (chloroform); its picrate, mp 174°] from Narcissus hybrid "Golden Sceptre". It was assigned O-demethylgalanthine, partial formula VII,[10] by conversion of VII to IV, because the position of the phenolic hydroxyl group was unknown. Carinatine was not identical with goleptine, since its optical rotation and the melting point of the picrate of II were different from those of VII, respectively. Goleptine seems to be the structural isomer of II.

Experimental

All melting points are given as uncorrected values. The spectrophotometers used were a Hitachi, EPI-G2 model for IR spectra, a Shimadzu, UV-200 model for UV spectra, a Hitachi, RMU-6C model for mass spectra, a Yanagimoto, OR-50 model for optical rotations, a JASCO model ORD/UV-5 for ORD spectra, and a JEOL, JNM-PS-100 or a Hitachi, R-22 model for NMR spectra using TMS as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Isolation of Alkaloids from *Z. carinata* Herb.——Following the method of Wildman and Bailey,[3] fresh bulbs (6.4 kg) of this plant were ground in 99% EtOH in a mixer. The insoluble material was extracted three times with 9.6 liter of 99% EtOH. The ethanolic extract was evaporated to approximately 1.5 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 four times with 700 ml-portions of $CHCl_3$. The extract was evaporated *in vacuo* to give crude alkaloids (5.40 g). The crude alkaloids gave 16 mg of $CHCl_3$-insoluble material and $CHCl_3$-soluble one (5.38 g) when mixed with $CHCl_3$ (50 ml). Furthermore, the above aqueous solution (pH 8) was made more strongly basic (pH 10) with conc. NH_4OH and extracted four times with 600 ml-portions of $CHCl_3$. Treatment of the extract by the manner as described above gave $CHCl_3$-insoluble material (143 mg, total 159 mg, 0.0025 % yield) and $CHCl_3$-soluble one (1.32 g, total 6.70 g, 0.1047% yield).

The $CHCl_3$-insoluble material gave III (127 mg) after recrystallization from EtOH. The $CHCl_3$-soluble material (5.38 g) was submitted to preparative TLC using SiO_2–[$CHCl_3$–MeOH–Et_2NH (92: 3: 5)] to give three fractions, *Rf* 0.47—0.56, *Rf* 0.37—0.44, and *Rf* 0.19—0.30: elution of the first fraction with MeOH–$CHCl_3$ (1: 1) afforded crude bases (2.73 g), which were submitted to preparative TLC using SiO_2–[$CHCl_3$–MeOH (5: 1)] to give two fractions, *Rf* 0.57—0.68 and *Rf* 0.43—0.50. The former fraction gave crude IV (1.59 g) and the latter one crude V (463 mg). Elution of the second fraction (*Rf* 0.37—0.44) with $CHCl_3$–MeOH (1: 1) gave 293 mg (0.0046% yield) of amorphous I. Elution of the third fraction (*Rf* 0.19—0.30) with the same solvent gave 1.25 g of crude bases. The $CHCl_3$ solution of the bases was extracted with 5% NaOH. The basic aqueous solution was made acidic with HCl, then basic with Na_2CO_3, and extracted with $CHCl_3$. The extract gave a crude phenolic base (467 mg), which was purified by preparative TLC using SiO_2–[$CHCl_3$–MeOH (5: 1)] to give amorphous II (346 mg, 0.0054% yield).

Lycorine (III)——This base melted at 259—264° (dec.), undepressed on admixture with an authentic sample of III [lit.[2] mp 280° (dec.)]. The IR spectrum of this compound was also identical with that of III. *Anal.* Calcd. for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96; N, 4.88. Found: C, 66.77; H, 5.95; N, 4.76.

Galanthine (IV)——This crude IV (1.59 g) was recrystallized from ethyl acetate to give IV (775 mg, 0.012%) as white needles, mp 128—130.5° [lit., mp 132—134° and 160—162,[4a] 134—136°,[4b] 166—167°[4c]]. $[\alpha]_D^{22}$ −94.75° (c=0.686, chloroform), [reported,[4c] $[\alpha]_D^{22}$ −85°], $[\alpha]_D^{22}$ −81.0° (c=0.642, ethanol), [reported,

9) W. Döpke, *Naturwissenschaften*,, **50**, 645 (1963).
10) W. Döpke, *Arch. Pharmaz.*, **297**, 39 (1964).

$[\alpha]_D^{27}$ −81.6°,[4b] $[\alpha]_D^{21}$ −81.0°[4c]]. The IR spectra (in Nujol and chroloform) of this compound were identical with those[11] of authentic samples of IV, respectively. The mass spectrum was shown in Fig. 2. *Anal.* Calcd. for $C_{18}H_{23}NO_4$: C, 68.12; H, 7.30; N, 4.41. Found: C, 67.82; H, 7.29; N, 4.23.

Galanthine Picrate——Crude picrate prepared from IV (11 mg) and picric acid (8 mg) in MeOH was crystallized from MeOH to give the picrate (10 mg) of IV as yellow needles, mp 198—199.5° (dec.) [lit., mp 188—190° (dec.),[4b] mp 199—200° (dec.)[4c]]. *Anal.* Calcd. for $C_{18}H_{23}NO_4 \cdot C_6H_3N_3O_7 \cdot 1/2H_2O$: C, 51.90; H, 4.90; N, 10.09. Found: C, 51.87; H, 4.75; N, 9.88.

Galanthine Hydroperchlorate——Crude hydroperchlorate obtained from IV was recrystallized from EtOH as white needles, mp 195—197.5° (dec.) [lit., mp 199—201°,[4a] mp 218° (dec.)[4b,c]]. *Anal.* Calcd. for $C_{18}H_{23}NO_4 \cdot HClO_4 \cdot 1/2H_2O$: C, 50.67; H, 5.90; N, 3.28. Found: C, 50.98; H, 5.67; N, 3.07.

Haemanthamine (V)——Crude V (463 mg) was recrystallized from EtOH as white prisms (290 mg, 0.0045%), mp 196.5—197° [lit., mp 200—201°,[5a] mp 203—203.5°[5b]]. $[\alpha]_D^{22}$ +49.3° (c=0.670, chloroform) [reported,[5a] $[\alpha]_D^{22}$ +33°], $[\alpha]_D^{27}$ +22.7° (c=0.706, methanol) [reported,[5b] $[\alpha]_D^{25}$ +19.66°]. NMR ($CDCl_3$) δ: 6.81 (1H, s, C-10-H), 6.46 (1H, s, C-7-H), 6.41 (1H, d, J=14 Hz, C-1-H), 6.33 (1H, d d, J=4, 14 Hz, C-2-H), 5.88 (2H, s,

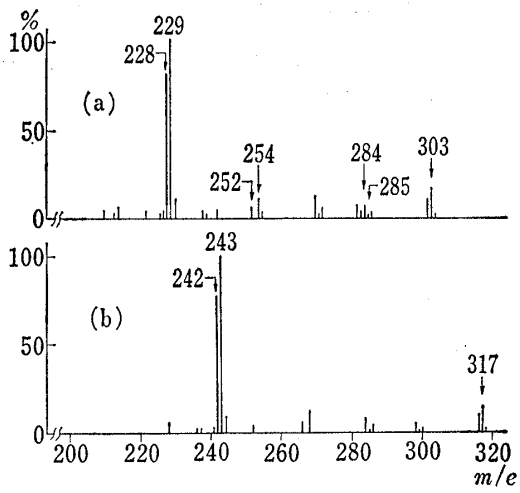


Fig. 2. Mass Spectra of II (a) and IV (b)

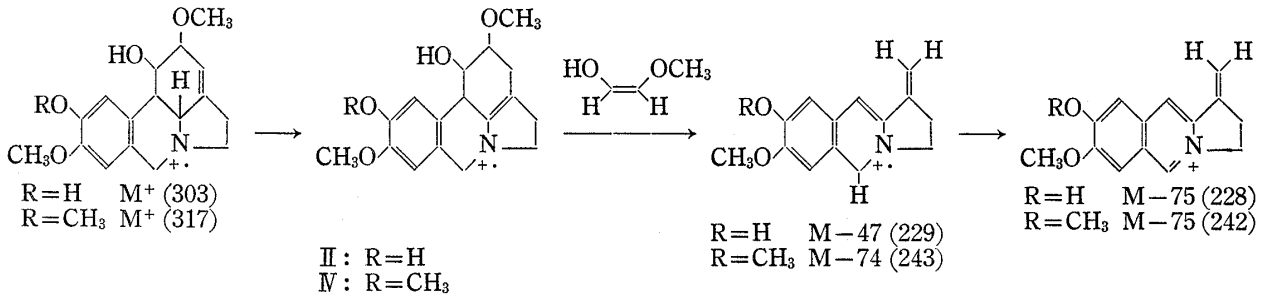


Chart 2

OCH_2O), 4.31 and 3.66 (each 1H, d, J=17 Hz, AB type of C-6 H_2), 3.97 (center) (1H, m, C-11-H), 3.83 (center) (1H, m, C-3-H), 3.50—3.13 (3H, m, C-4a-H and C-12 H_2), 3.34 (3H, s, OCH_3), 2.38 (1H, b s, OH), 2.09 (center) (2H, m, C-4 H_2). *Anal.* Calcd. for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.85; H, 6.42; N, 4.49.

Pretazettine (I) Picrate——This picrate (5 mg) was prepared from I (10 mg) by the method of Furusawa, *et al.*[11] and melted at 204—205° (dec.) [lit.,[11] mp 218—225° (dec.)]. This picrat was shown to be identical with that of an authentic sample of I by direct comparison. *Anal.* Calcd. for $C_{18}H_{21}NO_5 \cdot C_6H_3N_3O_7$: C, 51.43; H, 4.32; N, 10.00. Found: C, 50.99; H, 4.28; N, 9.74.

Pretazettine (I) Hydrochloride——This hydrochloride, mp 223—223.5° (dec.) (from EtOH) was shown to be identical with that of an authentic sample of I by direct comparison [lit.,[11] mp 234—236° (dec.)]. *Anal.* Calcd. for $C_{18}H_{21}NO_5 \cdot HCl$: C, 58.77; H, 6.03; N, 3.81. Found: C, 58.76; H, 6.18; N, 3.65.

Carinatine (II)——In the mass spectrum, the additional characteristic fragment[7] occurred at m/e 284 (M^+-19), 285 (M^+-18), 252 (M^+-51), and 254 (M^+-49).

Carinatine (II) Picrate——This picrate (7 mg), prepared from II (13 mg) and picric acid (10 mg) in MeOH, melted at 195—197° (dec.) after recrystallization from MeOH. *Anal.* Calcd. for $C_{17}H_{21}NO_4 \cdot C_6H_3N_3O_7$: C, 51.88; H, 4.54; N, 10.52. Found: C, 51.53; H, 4.50; N, 10.21.

Conversion of II to IV——A mixture of II (12 mg), DMSO (1 ml), and ethereal diazomethane solution (2 ml) (from N-methyl-N-nitroso-*p*-toluenesulfonamide) was stood at room temperature for three days. The solvent was evaporated off under reduced pressure and further an ethereal diazomethane solution was added to the residue. The mixture was stood at room temperature for one day. After evaporation of the solvent and addition of H_2O, the aqueous solution was made basic (pH 10) with Na_2CO_3 and extracted with

11) Comparison of the IR spectra of our sample with those of Wildman's sample (IV) was carried out by W.C. Wildman.

$CHCl_3$. The extract was washed with H_2O and dried, and the solvent was evaporated off. The residue was triturated with ethyl acetate to give IV (2 mg) as white needles, mp 127—128.5°, $[\alpha]_D^{22}$ −76.6° (c=0.209, ethanol). This compound was shown to be identical with an authentic sample of IV by direct comparison.

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