Chem. Pharm. Bull. 24(7)1537—1543(1976)

UDC 547.94.02.05 : 581.192

Alkaloids of the Amaryllidaceae. A New Alkaloid, Sanguinine, from *Lycoris sanguinea* Maxim. var. *Kiushiana* Makino, and Pretazettine from *Lycoris radiata* Herb.

Shigeru Kobayashi,[1a] Shigeko Takeda,[1b] Hideki Ishikawa, Hiroshi Matsumoto, Masaru Kihara,[1a] Tetsuro Shingu,[1c] Atsushi Numata[1d] and Shojiro Uyeo[1e]

Faculty of Pharmaceutical Sciences, Tokushima University,[1a] Faculty of Pharmaceutical Sciences, Nagasaki University,[1b] School of Pharmacy, Kobe Gakuin University,[1c] Osaka College of Pharmacy,[1d] and Faculty of Pharmaceutical Sciences, Mukogawa Women's University[1e]

(Received October 22, 1975)

The bulbs of *Lycoris sanguinea* Maxim. var. *kiushiana* Makino (Amaryllidaceae) were found to contain a new phenolic alkaloid, sanguinine (I), as well as lycorine (II) and galanthamine (III). Sanguinine (I), $C_{16}H_{19}O_3N$, was established to be O-demethylgalanthamine. Pretazettine (VII) was isolated from the bulbs of *Lycoris radiata* Herb. (Amaryllidaceae).

This paper reports the isolation of alkaloids from the bulbs of two species of Amaryllidaceae, *Lycoris sanguinea* Maxim. var. *Kiushiana* Makino and *Lycoris radiata* Herb., and the structural assignment of a new phenolic base, named sanguinine (I), from the former plant. The former plant (Japanese name, Ookitsunenokamisori) was collected in Nagasaki Prefecture, and is different from *Lycoris sanguinea* Maxim. (Japanese name, Kitsunenokamisori) in having larger bulbs and a later flowering time.

Isolation of Alkaloids from *Lycoris sanguinea* Maxim. var. *kiushiana* Makino

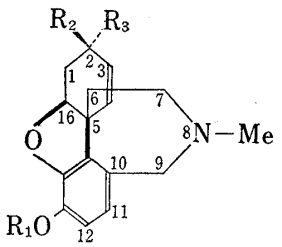
Crude basic material was extracted by the procedure of Mason, *et al.*,[2] using 1% ethanolic tartaric acid as solvent. Crystals of lycorine (II)[3] formed on treatment of the crude extract with chloroform. The base (II) was identified by its elemental analysis and by mixed melting point, and comparison of its infrared (IR) spectrum with that of an authentic sample. The chloroform solution was then concentrated, dissolved in benzene, and chromatographed on alumina. Galanthamine (III)[4] was obtained by elution with 50% ethyl acetate in benzene and sanguinine (I) by elution with 10% ethanol in chloroform, as shown in Table I. The base (III) was identified by elemental analysis and comparison of its nuclear magnetic resonance (NMR) and IR spectra with those of an authentic sample of III.

1) Location: *a) 78, Sho-machi-1-chome, Tokushima, 770, Japan; b) 1-14, Bunkyo-machi, Nagasaki, 852, Japan; c) Ikawadani, Tarumi-ku, Kobe, 673, Japan; d) 2-10-65, Kawai, Matsubara, Osaka, 580, Japan; e) 4-16, Edagawa, Nishinomiya, Hyogo, 663, Japan.*

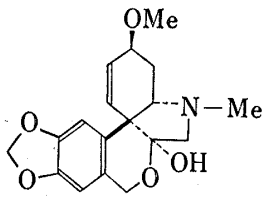
2) L.H. Mason, E.R. Pushett, and W.C. Wildman, *J. Am. Chem. Soc.*, **77**, 1253 (1955).

3) *a)* K. Morishima, *Tokyo Igakukai Zasshi*, **9**, 505 (1895), *Yakugaku Zasshi*, **16**, 131 (1896), and *Arch. Exptl. Path. Pharmakol.*, **40**, 221 (1897); *b)* H. Kondo and K. Tomimura, *Yakugaku Zasshi*, **48**, 223 (1928); *c)* L.G. Humber, H. Kondo, K. Kotera, S. Takagi, K. Takeda, W.I. Taylor, B.R. Thomas, Y. Tsuda, K. Tsukamoto, S. Uyeo, H. Yajima, and N. Yanaihara, *J. Chem. Soc.*, **1954**, 4622; *d)* K. Wiesner, W.I. Taylor, and S. Uyeo, *Chem. Ind.* (London), **1954**, 46; *e)* M. Shiro, T. Sato, and H. Koyama, *J. Chem. Soc.* (*B*), **1968**, 1544.

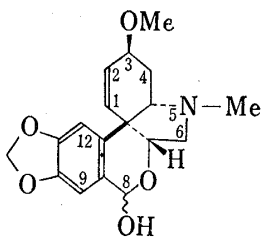
4) *a)* N.F. Proskurnina and A.P. Yakovleva, *Zhur. Obschchei Khim.*, **22**, 1899 (1952) [*C.A.*, **53**, 6959 (1953)]; *b)* S. Uyeo and S. Kobayashi, *Chem. Pharm. Bull.* (Tokyo), **1**, 139 (1953); *c)* S. Kobayashi, T. Shingu, and S. Uyeo, *Chem. Ind.* (London), **1956**, 177; *d)* J. Koizumi, S. Kobayashi, and S. Uyeo, *Chem. Pharm. Bull.* (Tokyo), **12**, 696 (1964); *e)* D.H.R. Barton and G.W. Kirby, *Proc. Chem. Soc.*, **1960**, 392 and *J. Chem. Soc.*, **1962**, 806; *f)* D.J. Williams and D. Rogers, *Proc. Chem. Soc.*, **1964**, 357.



II



VI



VII

I : $R_1=R_3=H$, $R_2=OH$
III : $R_1=Me$, $R_2=OH$, $R_3=H$
IV : $R_1=Me$, $R_2=OH$, $R_3=H$ (no double bond at C_3)
V : $R_1=Me$, $R_2=H$, $R_3=OH$

Chart 1

TABLE I. Column Chromatography of crude Alkaloids from *Lycoris sangniea* MAXIM. var. *kiushiana* MAKINO on Alumina

Fraction	Eluent	ml	Wt. of concd. eluate, mg	Product
1—24	benzene	1200	300	yellow oil
25—32	20% ethyl acetate in benzene	400	trace	oil
33—36	30% ethyl acetate in benzene	200	trace	oil
37—49	50% ethyl acetate in benzene	650	trace	oil
50—68	50% ethyl acetate in benzene	950	147	43 mg, galanthamine,
69—119	50% ethyl acetate in benzene	2550	141	brown oil
120—187	ethyl acetate	3400	87	brown oil
188—211	30% chloroform in ethyl acetate	800	42	oil
212—225	50% chloroform in ethyl acetate	1120	26	oil
226—252	chloroform	2160	41	oil
253—258	10% ethanol in chloroform	480	340	41 mg, sanguinine hydroperchlorate
259—286	20% ethanol in chloroform	2240	290	solid
287—306	50% ethanol in chloroform	2080	40	solid
307—319	ethanol	1040	31	solid

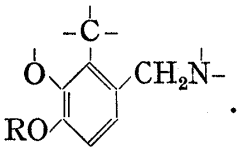
Sanguinine (I) has mp 210.5—213° (decomp.), $[\alpha]_D^{27}$ −133° (c=0.23, EtOH), and the molecular formula, $C_{16}H_{19}O_3N$, deduced by elemental analysis and its mass spectrum, m/e 273 (M^+). The base (I) gave a blue-violet color with ferric chloride reagent. The hydroperchlorate of I, $C_{16}H_{19}O_3N \cdot HClO_4$, has mp 249—251° (decomp.). The IR spectrum of I showed absorptions for hydroxyl groups at 3400 and 1040 cm^{-1} and for a double bond at 1620 cm^{-1}, but no absorption for a carbonyl group. The ultraviolet (UV) spectrum, having λ_{max}^{EtOH} 213 nm (log ε, 4.35) and 290 nm (log ε, 3.43), is quite similar to that of III, as shown in Fig. 1. In the NMR spectrum (see Table II) of deuterochloroform-slightly soluble I, two multiplets at 5.45 and 5.90 τ indicated the presence of two methine protons attached to oxygenated carbon atoms. Two singlets at 3.97 (2H) and 7.60 τ (3H) showed the presence of olefinic and N-methyl protons, respectively. A pair of doublets at 5.95 and 6.34 τ (each 1H, J=14 Hz) of an AB type indicated the presence of a benzylic methylene group attached to a nitrogen atom. A singlet at 3.45 τ

TABLE II. Chemical Shift of Galanthamine-type Alkaloids (in $CDCl_3$)(τ)

Compound	C-11-H and C-12-H	C-3-H and C-4-H	C-9 H_2[a]	C-16-H	C-2-H	N-CH_3
Sanguinine(I)	3.45(s)	3.97(s)	5.95(d),6.34(d), J=14 Hz	5.45(m)	5.90(m)	7.60(s)
Galanthamine (III)	3.38(s)	3.98(s)	5.94(d),6.36(d), J=14 Hz	5.41(m)	5.88(m)	7.60(s)
Lycoramine(IV)	3.39(s)	—	6.00(d),6.40(d), J=14 Hz	5.64(t)	5.92(m)	7.64(s)
Epigalanthamine (V)	3.38(d),3.46(d), J=8Hz	3.79(d),6.03(d), J=10 Hz	5.93(d),6.40(d), J=15 Hz	5.28—5.50		7.66(s)

a) The signals are AB type.

(2H) similar to those of III at 3.38 τ (2H) and of lycoramine (dihydrogalanthamine) (IV)[5] at 3.39 τ (2H) revealed the presence of two aromatic *ortho* protons. These facts indicate that the aromatic ring of I resembles the benzenoid ring of III and that I has the partial formula,



. From the analytical data and the remarkable similarity between the values for I and III, shown in Table II, sanguinine is suggested to be O-demethylgalanthamine (I). Further confirmation of this structure was provided by the NMR spectrum of I in deuteropyridine. The spectrum is very similar to that of III, differing only in the regions of the aromatic and O-methyl protons, as shown in Fig. 2. Fortunately, preferential solvent shifts by deuteropyridine separated two aromatic protons as two doublets at 3.03 and 3.33 τ (each 1H, *d*, J=8 Hz), indicating a pair of *ortho* protons, C-12-H and C-11-H. The signals at 5.83 and 6.30 (each 1H, d, J=15 Hz), 3.87 (2H, s), 5.47 (1H, m),

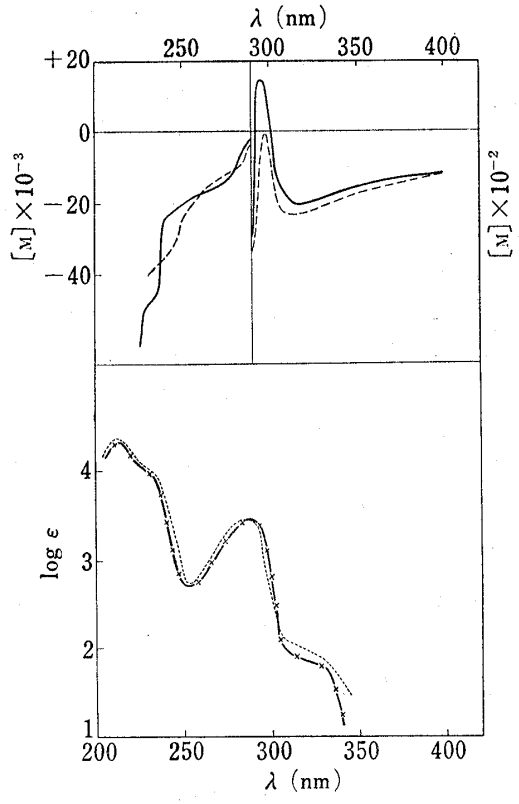


Fig. 1. Sanguinine (I); ORD (-----) and UV (—+—) Spectra in EtOH
Galanthamine (III); ORD (———) and UV (--------) Spectra in EtOH

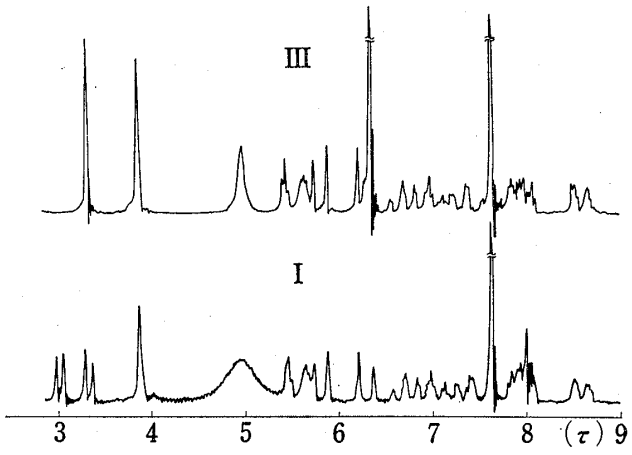


Fig. 2. NMR Spectra of Sanguinine (I) and Galanthamine (III) in Pyridine-d_5

5) *a*) H. Kondo, K. Tomimura, and S. Ishiwata, *Yakugaku Zasshi*, **52**, 433 (1932); *b*) H. Kondo and S. Ishiwata, *Chem. Ber.*, **70**, 2427 (1937); *c*) S. Uyeo and J. Koizumi, *Chem. Pharm. Bull.* (Tokyo), **1**, 202 (1953); *d*) N. Hazama, H. Irie, T. Mizutani, T. Shingu, M. Takeda, S. Uyeo, and A. Yoshitake, *J. Chem. Soc.* (*C*), **1968**, 2947; *e*) Y. Misaka, T. Mizutani, M. Sekido, and S. Uyeo, *ibid.*, **1968**, 2954.

and 5.68 τ (1H, m) were assigned to C-9H_2, C-3-H, and C-4-H, C-16-H, and C-2-H, respectively, as in the case of the NMR spectrum in deuterochloroform.

The rotation, optical rotatory dispersion (ORD) curve of I, and the signals of aromatic and olefinic protons (see Table II) of I were similar to those of III, respectively, but different from those[6] of epigalanthamine (V).[7] These facts indicate that the configuration of the hydroxyl group in C-2 of I is the same as that of III.

The mass spectrum of I also supported structure I for sanguinine. The fragmentation pattern of I can be explained in the same way as in the case of III,[8] as shown in Fig. 3 and Chart 2.

To confirm this assignment, the base (I) was methylated with diazomethane in dimethyl sulfoxide. The methylated product was found to be identical with an authentic sample of III by direct comparison. The base (I) is the second example of a phenolic base in galanthamine-type alkaloids, the first example being chlidanthine.[9]

Isolation of Pretazettine from *Lycoris radiata* Herb.[10]

In studies on alkaloids isolated from *Sprekelia formosissima* L. (Herb.) and *Ismene calithina* (Nichols), Wildman and Bailey[11] reported that tazettine (VI)[12] may be an artifact formed by rearrangement of pretazettine (isotazettine) (VII)[13] under a variety of basic conditions. To confirm this, we tried to isolate VII from the bulbs of *Lycoris radiata* Herb. (Japanese name, Higanbana), collected in our Faculty Plantation.[1a]

We extracted crude basic material from fresh bulbs of this plant by the method of Wildman and Bailey.[11] The crude base was submitted to column chromatography and preparative thin-layer chromatography (TLC) on silica gel and an amorphous base (VIII), $[\alpha]_D^{25}$ +189.8° (c=0.64, $CHCl_3$), was obtained in 0.0022% yield (from the fresh bulbs). The base (VIII) was crystallized as its hydrochloride, mp 223—224° (decomp.), and picrate, mp 204—205° (decomp.).

The chemical and physical properties of VIII and its hydrochloride are in good agreement with those reported by Wildman and Bailey[11] for VII and its hydrochloride, respectively: in the NMR spectrum of VIII, the shift values of two aromatic protons (3.17 and 3.26 τ) and those of benzylic methine (3.93 τ), methylenedioxyl (4.11 τ) and O- and N-methyl (6.59 and 7.52 τ, respectively) are identical with those of VII reported in the literature.[11,13c] The rotation and the ORD are also quite similar to those reported.[11] The picrate of VIII was also identical with that[14] of VII isolated from *Narcissus tazetta* L. by Furusawa, *et al.* Treatment of VIII with sodium carbonate solution at pH 10 gave basic material quantitatively. The base appeared identical with an authentic sample of VI by comparison of the TLC and IR spectra and by the mixed melting point test. From these facts the base (VIII) was confirmed to be pretazettine (VII). Previously VI was isolated in 0.0016% yield[15] from the bulbs of this plant under basic conditions.

6) G.G. DeAngelis and W.C. Wildman (*Tetrahedron Letters*, **1969**, 729) compared the ORD of III and V.
7) *a*) H. Kondo, S. Ishiwata, and S. Okayama, *Yakugaku Zasshi*, **53**, 807 (1933); *b*) H.M. Fales, L.D. Giuffrida, and W.C. Wildman, *J. Am. Chem. Soc.*, **78**, 4145 (1956).
8) R. Razakov, V.N. Bochkarev, Kh. A. Abduazimov, N.S. Vul' fson, and S. Yu. Yunusov, *Khim. Prir. Soedin.*, **5**, 280 (1969) [*C.A.*, **72**, 43932*j* (1970)].
9) W. Dopke and H. Dalmer, *Naturwiss.*, **52**, 61 (1965).
10) The alkaloids of this plant have been reviewed by J.W. Cooks and J.D. London, and W.C. Wildman, in R.H.F. Manske "The Alkaloids," Vol. II, VI, and XI, Academic Press, Inc., New York., 1952, p. 331, 1960, p. 289, and 1968, p. 307, respectively.
11) W.C. Wildman and D.T. Bailey, *J. Org. Chem.*, **33**, 3749 (1968).
12) *a*) T. Ikeda, W.I. Taylor, Y. Tsuda, S. Uyeo, and H. Yajima, *J. Chem. Soc.*, **1956**, 4749; *b*) T. Sato and H. Koyama, *ibid.* (*B*), **1971**, 1070.
13) *a*) N.F. Proskurnina, *Zhur. Obshchei Khim.*, **27**, 3365 (1957); *b*) W.C. Wildman and D.T. Bailey, *J. Am. Chem. Soc.*, **91**, 150 (1969); *c*) W. Dopke and P.W. Jeffs, *Tetrahedron Letters*, **1968**, 1307.
14) The sample was provided by H. Irie, one of the authors of "Isolation of Pretazettine from *Narcissus tazetta* L." (E. Furusawa, S. Furusawa, S. Tani, H. Irie, K. Kitamura, and W.C. Wildman, *Chem. Pharm. Bull.* (Tokyo), **24**, 336 (1976).)
15) S. Uyeo, "Jitsuken-kagaku-kooza," Vol. 22, 449 (1966).

These above results indicate that in this plant also, VI is an artifact formed by rearrangement of VII, as reported by Wildman and Bailey.[11]

Experimental[16]

Isolation of Alkaloids from *Lycoris sanguinea* MAXIM. var. *kiushiana* MAKINO——Fresh bulbs of this plant were cut into samll pieces and dried. The dried pieces (3.43 kg) were ground in a mortar and extracted at 55° with 40 liter of 1% ethanolic tartaric acid solution. The extract was concentrated and diluted with H_2O (1 liter). The aqueous solution was divided in three equal parts. Each part was washed five times with 150 ml-portions of $CHCl_3$. Then each aqueous solution was made basic with solid Na_2CO_3 and extracted fifteen times with 150 ml-portions of $CHCl_3$. Each $CHCl_3$ solution was extracted eight times with 30 ml-portions of 8% HCl and the aqueous solution was made basic with solid Na_2CO_3. The basic aqueous solution was extracted sixteen times with 100 ml-portions of $CHCl_3$. The $CHCl_3$ extracts of each part were combined and concentrated to *ca.* 300 ml and 616 mg of insoluble basic material were removed by filtration. The filtrate was dried and concentrated to a constant weight of 2.025 g (total crude alkaloids, 2.641 g, 0.077%). The crude base (2.025 g) was dissolved in benzene (32 ml) containing $CHCl_3$ (2 ml) and chromatographed on Al_2O_3 (180 g, Wako), as shown in Table I.

Lycorine (II)——The $CHCl_3$-insoluble basic material (616 mg) was triturated with hot EtOH to give a crude brown solid (470 mg), mp 243—250° (decomp.). The solid was dissolved in 8% HCl to give pale brown crystals (480 mg). Recrystallization from H_2O gave lycorine hydrochloride as white prisms, mp 205—207° (decomp.) [lit. 208° (decomp.),[3a] 207° (decomp.)[3b]]. *Anal.* Calcd. for $C_{16}H_{17}O_4N \cdot HCl \cdot H_2O$: C, 56.22; H, 5.90; N, 4.10. Found: C, 56.64; H, 5.95; N, 4.31.

The crude free base (II) derived from the salt was recrystallized from EtOH to afford II as white prisms, mp 256—262° (decomp.) [lit.[3b] 280° (decomp.)]. *Anal.* Calcd. for $C_{16}H_{17}O_4N$: C, 66.88; H, 5.96; N, 4.88. Found: C, 66.66; H, 5.94; N, 4.98.

Galanthamine (III)——The crude material (III) (147 mg) was triturated with ether to give 67 mg of crystals, mp 123—127°, which after recrystallization from ether–acetone melted at 127—128° (lit. mp 126—127°,[4a] 127—129°[4b]). *Anal.* Calcd. for $C_{17}H_{21}O_3N$: C, 71.05; H, 7.37; N, 4.87. Found: C, 71.01; H, 7.15; N, 4.57. $[\alpha]_D^{27}$ −118° (c=0.24, EtOH) [reported $[\alpha]_D$ −118.8°,[4a] $[\alpha]_D^{32}$ −121.4°[4b]]. ORD (c=0.142, EtOH) $[M]^{27}$ (nm): −1155° (400), −1727° (350), −2057° (325) (trough); (c=0.017), −302° (300), 0° (299), +1445° (295) (peak), 0° (292); (c=0.002), −20592° (250). NMR ($CDCl_3$) τ: 8.42 [1H, m, C-6-H (higher)], 8.18—7.50 [3H, m, C-1-H (higher), OH, and C-6-H (lower)], 7.60 (3H, s, NCH_3), 7.32 [1H, m, C-1-H (lower)], 6.86 (2H, m, C-7H_2), 6.19 (3H, s, OCH_3). NMR (pyridine-d_5) τ: 8.59 [1H, m, C-6-H (higher)], 8.14—7.80 [2H, m, C-1-H (higher) and C-6-H (lower)], 7.66 (3H, s, NCH_3), 7.34 [1H, m, C-1-H (lower)], 7.06 [1H, m, C-7-H (higher)], 6.72 [1H, m, C-7-H (lower)], 6.34 (3H, s, OCH_3), 6.29 and 5.82 (each 1H, d, J=15 Hz, AB type of C-9H_2), 5.63 (1H, m, C-2-H), 5.44 (1H, m, C-16-H), 3.87 (2H, s, C-3-H and C-4-H), 3.33 (2H, s, C-11-H and C-12-H).

Galanthamine hydrobromide formed instantaneously when 47% HBr was added to a solution of III in acetone. The product was recrystallized from EtOH–H_2O (10: 1) to give white prisms, mp 250—252° (decomp.). *Anal.* Calcd. for $C_{17}H_{21}O_3N \cdot HBr$: C, 55.44; H, 6.02; N, 3.80. Found: C, 55.26; H, 5.96; N, 3.86.

Sanguinine (I)——A mixture of crude material (340 mg), EtOH, and, 6% $HClO_4$ was concentrated under reduced pressure to give sanguinine hydroperchlorate (41.5 mg) as white prisms, mp 249—251° (decomp.) (from MeOH–acetone). *Anal.* Calcd. for $C_{16}H_{19}O_3N \cdot HClO_4$: C, 51.49; H, 5.39; N, 3.75. Found: C, 51.89; H, 5.07; N, 3.39.

The free base (I) was obtained as follows: an aqueous solution of the salt was made basic with Na_2CO_3 and extracted with $CHCl_3$. The extract was dried over K_2CO_3 and concentrated to give I, mp 210.5—213° (decomp.), as white cubes after recrystallization from benzene–EtOH (10: 1). *Anal.* Calcd. for $C_{16}H_{19}O_3N$: C, 70.31; H, 7.01; N, 5.13. Found: C, 69.96; H, 6.95; N, 4.95. NMR (pyridine-d_5) τ: 6.71 [1H, m, C-7-H

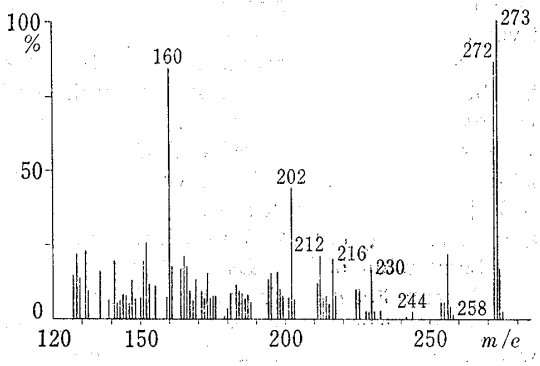


Fig. 3. Mass Spectrum of I

16) All melting points are given as uncorrected values. The spectrophotometers used were a Hitachi, EPI-G2 model for IR spectra, a Hitachi, EPS-2 model for UV spectra, a Hitachi, RMU-6C model for mass 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. The assignment of the signals was achieved by study of nuclear magnetic double resonance.

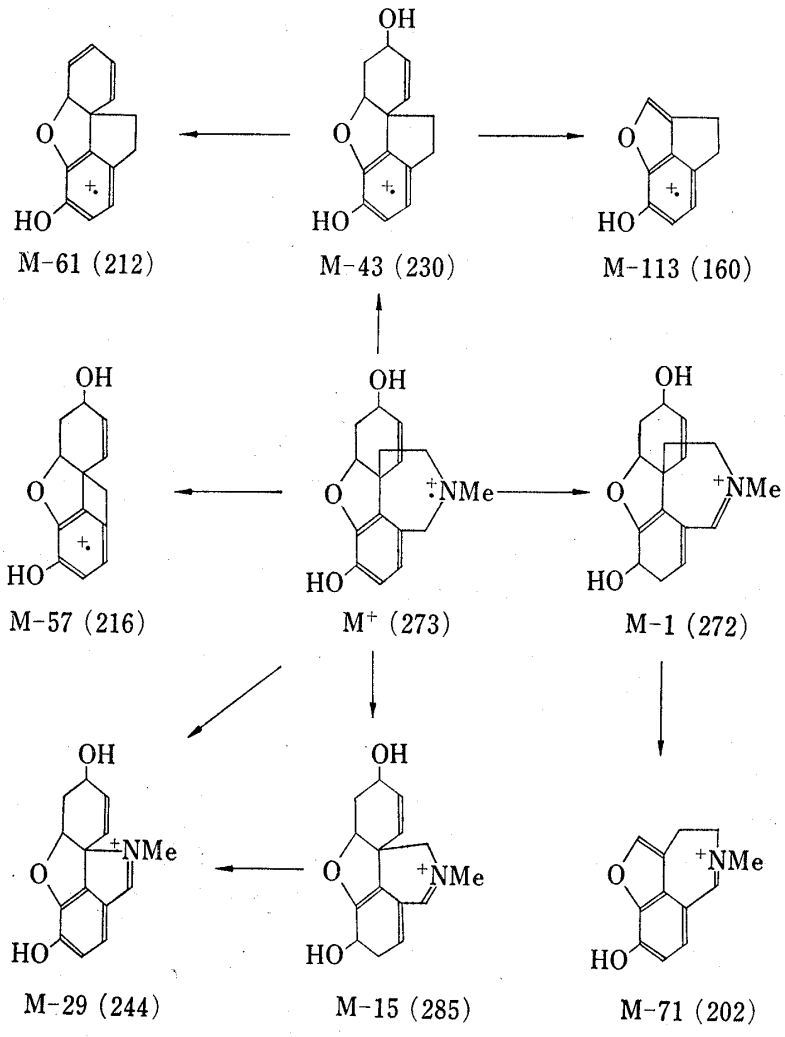


Chart 2

(lower)], 7.04 [1H, m, C-7-H (higher)], 7.39 [1H, m, C-1-H (lower)], 7.66 (3H, s, NCH_3), 7.78—8.14 [2H, m, C-1-H (higher) and C-6-H (lower)], 8.60 [1H, m, C-6-H (higher)]. ORD (c=0.14, EtOH). $[M]^{27}$ (nm): −1312° (400), −2080° (350), −2566° (316) (trough); (c=0.035), −782° (300); (c=0.085), 0° (296); (c=0.005), −24517° (250). The mass spectrum and the fragmentation pattern are showed in Fig. 3 and Chart 2, respectively.

An alternative method was used to isolate I directly: the dried bulbs (1 kg) of this plant were treated by the method described above to obtain a $CHCl_3$-soluble base (275 mg). A solution of the base in $CHCl_3$ was extracted with 7% NaOH. The aqueous solution was made acidic with concentrated HCl, made basic with Na_2CO_3, and extracted with $CHCl_3$. The extract was washed with H_2O, dried, and evaporated, leaving 92 mg of brown oil, which gave a positive $FeCl_3$ test. The oil was submitted to preparative TLC using SiO_2–[MeOH–acetone (3: 2)]. Elution of material of Rf 0.48—0.28 with MeOH–$CHCl_3$–acetone (1: 1: 1) gave I (14 mg, 0.0014%) as white plates (from acetone), mp 210.5—213° (decomp.).

Conversion of I to III——A mixture of I (6 mg), DMSO (1 ml), and ethereal diazomethane solution (2 ml) (from 300 mg of N-methyl-N-nitroso-*p*-toluenesulfonamide) was stood at room temperature overnight. The solvent was evaporated off under reduced pressure and the same ethereal diazomethane solution (2 ml) was added to the residue. The mixture was stood at room temperature for two days. After evaporation of the ether and addition of H_2O, the aqueous solution was extracted with ether. The extract was washed with H_2O and dried, and the solvent was evaporated off. The residue was triturated with ether to give III as white needles, mp 121—124°. This compound was shown to be identical with an authentic sample of III by comparison of their IR spectra and by the mixed melting point test.

Isolation of Pretazettine (VII) from *Lycoris radiata* Herb.——Following the method of Wildman and Bailey,[11] dormant fresh bulbs (3.0 kg) of this plant were ground in 99% EtOH in a mixer. The insoluble material was extracted three times with 4.5 liter of 99% EtOH. The ethanolic solution 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 the neutral and acidic material. The aqueous acidic solution was made

basic (pH 8) with concentrated NH_4OH and extracted five times with 120 ml-portions of $CHCl_3$. The extract was evaporated *in vacuo* to give crude alkaloids (3.46 g). The crude base gave 0.39 g of $CHCl_3$-insoluble crystals when dissolved in $CHCl_3$ (50 ml). A sample (34 mg) of the crystals was recrystallized from EtOH to give 15 mg of II as white prisms, mp 258.5—264° (decomp.). The $CHCl_3$-soluble material was submitted to column chromatography using SiO_2–($CHCl_3$–MeOH). Crude solutions of most of the other alkaloids were obtained by elution with $CHCl_3$ and 3% MeOH in $CHCl_3$. Elution with $CHCl_3$–MeOH (1: 1) gave 0.7 g of crude base contained mainly VII. This solution was divided in half. One half was submitted to preparative TLC using SiO_2–[$CHCl_3$–MeOH (10: 1)]. Elution of material of *Rf* 0.0—0.06 with MeOH–$CHCl_3$ (1: 1) gave crude VII (110 mg). This crude VII (110 mg) was submitted to preparative TLC using SiO_2–[$CHCl_3$–MeOH–Et_2NH (92: 3: 5)]. Elution of material of *Rf* 0.48—0.52 gave 32.3 mg (0.0022% yield) of amorphous VII. NMR ($CDCl_3$) τ: 4.14 and 4.50 (each 1H, d, J=10.5 Hz, AB type, C-2-H and C-1-H, respectively), 5.68 (1H, q, J_{6a-6h}=8 Hz, J_{6a-6l}=11 Hz, C-6a-H), 5.84 (1H, m, C-3-H), 7.03 [1H, q, J_{6l-6a}=11 Hz, J_{6l-6h}=10 Hz, C-6-H (lower)], 7.07 (1H, m, C-4a-H), 7.53 [1H, m, C-4-H (lower)], 8.29 [1H, m, J_{4h-4l}=14 Hz, J_{4h-3}= 10 Hz, J_{4h-4a}=2 Hz, C-4-H (higher)]. Mass Spectrum *m/e*: 331 (M^+).

Pretazettine Picrate——To a solution of VII (13.8 mg) in 0.1% HCl (3 ml) was added an aqueous solution of picric acid (11.2 mg) and the mixture was evaporated to dryness under reduced pressure. The residue was crystallized from acetone to give the picrate (7.9 mg) as yellow cubes, mp 204—205° (decomp.). This material was identical with an authentic sample of pretazettine picrate provided by Irie.[14] *Anal.* Calcd. for $C_{18}H_{21}O_5N \cdot C_6H_3O_7N_3$: C, 51.43; H, 4.32; N, 10.00. Found: C, 51.33; H, 4.30; N, 9.71.

Pretazettine Hydrochloride——VII (7.5 mg) was dissolved in 0.1% HCl (3 ml) and the solution was evaporated to dryness under reduced pressure. The residue was crystallized from EtOH to give pretazettine hydrochloride (4.5 mg) as white prisms, mp 223—224° (decomp.) [lit.[11] mp 224—225° (decomp.)]. *Anal.* Calcd. for $C_{18}H_{21}O_5N \cdot HCl$: C, 58.77; H, 6.03; N, 3.81. Found: C, 58.49; H, 6.10; N, 3.30.

Conversion of VII to VI——A solution of VII (6.5 mg) in H_2O (10 ml) was made basic (pH 10) with Na_2CO_3 and stirred at room temperature for 1 hr. The reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ was washed with H_2O, dried, and evaporated off under reduced pressure. The crude VI (5.6 mg) obtained was recrystallized from EtOH to give prisms (1.0 mg), mp 200—203° (decomp.) [lit.[5a] mp 208—209° (decomp.)]. This material was shown to be identical with an authentic sample of VI by the mixed melting point test and by comparison of the IR spectrum.

Acknowledgement The authors wish to express their thanks to Assistant Professor H. Irie, Kyoto University, for valuable information and for kindly providing a sample of pretazettine picrate. They also thank Dr. A. Kato, Kyoto University, for mass spectral data.