Isolation and Structure Elucidation of a Novel Alkaloid, Incarline, 
a Supposed Biosynthetic Intermediate, from Flowers of *Lycoris incarnata*

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A novel alkaloid incarline (1), a supposed biosynthetic intermediate from galanthine (2) to narcissidine (3), was isolated from flowers of *Lycoris incarnata* (Amaryllidaceae) together with the known alkaloids galanthine (2), ungiminorine (4), ungiminorine N-oxide (5), galanthamide (6), galanthamine N-oxide (7), lycoramine (8), sanguinine (9), lycorine (10), and O-demethyllycoramine (11). 1-Palmitoyl-2-linoleoylphosphatidylethanolamine (12) and 1-palmitoyl-2-linoleoylphosphatidylmethanol sodium salt (13) were also identified in the flower.

Keywords: incarline; Amaryllidaceae alkaloid; biosynthetic intermediate; *Lycoris incarnata*; flower; phospholipid

The Amaryllidaceae alkaloids have been studied extensively because of the variety of their structures and biological activities and also from the biosynthetic viewpoint. However, flowers of Amaryllidaceae plants have not attracted much attention from phytochemists. In a previous paper, we reported the isolation of a new alkaloid, hippeastrine N-oxide, together with known alkaloids from flowers of *Lycoris radiata* Herb. Recently, we have isolated a novel alkaloid, incarline (1), which was supposed to be a biosynthetic intermediate from galanthine (2) to narcissidine (3), from flowers of *Lycoris incarnata*. This paper describes in detail the isolation and the structural elucidation of incarline (1), together with nine known alkaloids, galanthine (2), ungiminorine (4), ungiminorine N-oxide (5), galanthamine (6), galanthamine N-oxide (7), lycoramine (8), sanguinine (9), lycorine (10), and O-demethyllycoramine (11), from fresh flowers of this plant. 1-Palmitoyl-2-linoleoylphosphatidylethanolamine (12) and 1-palmitoyl-2-linoleoylphosphatidylmethanol sodium salt (13) were also isolated.

Crude extract of fresh flowers of *Lycoris incarnata* obtained by the modified method of Ghosal et al. was subjected to column and preparative thin layer chromatographies (PTLC), as described in Experimental to give compounds 1, 2, 4, 12 and 13.

The new compound, incarline, 

$\text{C}_{18}\text{H}_{23}\text{NO}_5$ was isolated as colorless prisms, mp $183-185^\circ\text{C}$. The infrared (IR) spectrum showed hydroxy group absorption at 3433 cm$^{-1}$, but no absorption due to a carbonyl group. The proton nuclear magnetic resonance ($^1\text{H-NMR}$)
spectrum revealed the presence of two para-oriented aromatic protons (δ 6.97 and 6.95), two aromatic methoxy groups (δ 3.92 and 3.90), one aliphatic methoxy group (δ 3.57), and benzyl protons (δ 4.71 and 4.31, each doublet, J = 13.0 Hz), but no N-methyl signal. These data suggested that the compound has a lycorine-type skeleton. This suggestion was supported by a two-dimensional 1H-1H shift correlation spectroscopy (2D-COSY) experiment, which indicated the presence of the sequence H/H/H/H (see also below). These findings and the molecular formula (C18H22NO5) indicate that the structure of incartine should be very similar to that of narcissidine (3),6 galanthine N-oxide (14),7 or the 3,3a-epoxy derivative of galanthine (2). Since the 1H-NMR spectrum of incartine did not show an olefinic proton signal, the compound was considered to be an α- or β-3,3a-epoxy derivative (1 or 15) of galanthine (2).

In order to elucidate the structure and the stereochemistry of incartine, a decoupling experiment and the nuclear magnetic double resonance (NMDR) analysis were carried out. The completely assigned chemical shifts, the coupling patterns (see Experimental), and the nuclear Overhauser effect (NOE) enhancements (Fig. 1) suggested the relative configuration of the 3,3a-epoxy ring of incartine to be α. The conformation of α,3,3a-epoxy-galanthine (1) inspected from a Dreiding model was compared with that of the 3a,3,3a-glycol derivative (16) reported by Toda et al.8 (see Fig. 2). It was reported8 that the C-ring of 16 took a distorted boat conformation with trans diaxial orientation of H-2 and H-3 (J = 8.3 Hz). On the contrary, a small coupling (J = 1.1 Hz) of the corresponding hydrogens in incartine suggested the dihedral angle between them to be ca. 90°. A long-range coupling (J = 0.8 Hz) between H-1 and H-3 was observed in this case. These observations show that the C-ring takes a distorted chair conformation in the α,3,3a-epoxy compound. The β-orientation of the epoxy ring, such as in 15, would give a different coupling pattern. From these findings, incartine was concluded to be galanthine α,3,3a-epoxy (1).

Fuganti et al.5 suggested that galanthine (2) is probably transformed to narcissidine (3) via the α,3,3a-epoxy (1), since they found that galanthine (2) was converted to narcissidine (3) in Sempre avanti daffodil with loss of pro-S hydrogen from C-4 of the lycorine skeleton. Toda et al.8 gave chemical support to this elimination step. However, the proposed intermediate, the α-epoxide (1), has never been isolated or synthesized. Therefore, this paper is the first to report isolation of the proposed epoxy intermediate.

Compound 5, [α]D −58.9° (EtOH), was isolated as a pale yellow oil. The mass (MS) spectrum showed the molecular formula C17H16NO5, suggesting the presence of one more oxygen atom than in unguminorine (4). The 1H-NMR spectrum of 5 showed a similarity to that of 4 except for shielding of the protons at C-5, C-7 and C-11c. This alkalioid was identical with unguminorine N-oxide (5) isolated from Pancratium maritimum (Amaryllidaceae).9

Compounds 2, 4, 6–10 and 11 were identified as galanthine, unguminorine, galanthamine, galanthamine N-oxide, lycoramine, sanguinine, lycorine, and O-de-
linoleoylphosphatidylethanolamine.\(^1,2\) The FAB-MS of \(13\) showed the molecular ion peak of \(m/z\) 709 (M+1) for \(C_{38}H_{69}NO_{13}P\) and the \(\text{^1}H\)-NMR spectrum indicated the presence of a methoxy group. These findings suggested that compound \(13\) is 1-palmitoyl-2-linoleoylphosphatidylethanolamine sodium salt. Compound \(13\) may be an artifact derived from a corresponding phosphatidylcholine or a phosphatidylethanolamine during the extraction of the flower with MeOH–CHCl\(_3\).

**Experimental**

All melting points are given as uncorrected values. The spectrophotometers used were a Perkin-Elmer-1700 infrared Fourier-transform spectrometer for IR spectra, a JEOL JMS-D 300 for MS, a Union PM-201 for optical rotations, and JEOL JNM-FX 200, JEOL JNM-GSX 400 and Bruker AM-400 spectrometers for \(^1H\)-NMR spectra with tetramethylsilane as an internal standard. The plates used for TLC were coated with silica gel (Kieselgel PF\(_{254}\), Merck) and aluminum oxide (PL Merck). The following solvent systems were used: 1) CHCl\(_3\)–MeOH (5:1); 2) CHCl\(_3\)-MeOH-H\(_2\)O (60:35:10); 3) CHCl\(_3\)-AcEt- MeOH-H\(_2\)O (70:30:10:2); 4) CHCl\(_3\)-MeOH-NH\(_2\)OH-H\(_2\)O (70:26:2:2); 5) CHCl\(_3\)-MeOH (1:10). UV light, \(V_2\) vapor, Dragendorff’s reagent and molybdenum blue reagent\(^103\) were used for location of compounds.

**Preparation**

Following the modified method of Ghosal et al.,\(^2\) fresh flowers (5.3 kg) of *Lycoris incarnata* collected in our Faculty plot were ground in 8.741 of CHCl\(_3\)-MeOH (2:1) in a mixer. The extract was warmed at 60°C for 1 h, then 0.1 m EDTA (87 ml) was added to reduce the phosphatase activity and the mixture was kept at room temperature overnight, then filtered to give two layers. The CHCl\(_3\) and MeOH–H\(_2\)O layers were concentrated in vacuo to afford sticky extracts, 18.5 and 98 g, respectively.

**Treatment of the CHCl\(_3\) Extract**

The CHCl\(_3\) extract was subjected to column chromatography using HCl-washed Florisil (25x5.5 cm). Elution was carried out successively with benzene (1.651), CHCl\(_3\)-MeOH (95:5, 2.851), fraction (fr.) 1, 1.627 g, CHCl\(_3\)-MeOH (9:1, 1.75 l), fr. 2, 1.451 g, fr. 3H, 305 mg), and CHCl\(_3\)-MeOH (1:1, 3.491 l, fr. IV, 360 mg).

Fraction I (1.627 g) was subjected to column chromatography on SiO\(_2\). Elution was carried out successively with CHCl\(_3\)-MeOH (10:1), CHCl\(_3\)-MeOH (5:1), and MeOH. The MeOH fraction gave an oil, which was subjected to PTLC (SiO\(_2\), solvent 2) to afford irinocarone (1) (RT 0.53–0.62, 14 mg).

Fraction II (488 mg) was subjected to PTLC (SiO\(_2\), solvent 1) to afford an oil (RT 0.28–0.36, 45.4 mg). This crude material was further purified by PTLC (SiO\(_2\), solvent 4) to give 1-palmitoyl-2-linoleoylphosphatidylethanolamine (12) (RT 0.68–0.74, 7.7 mg).

Fraction III (305 mg) was subjected to PTLC (SiO\(_2\), solvent 1) to give two fractions (RT 0.38–0.41, fr. III-A, 17.2 mg; RT 0.13–0.23, fr. III-B, 52.9 mg). Purification of fr. III-A and fr. III-B by PTLC (Al\(_2\)O\(_3\), solvent 3 and 1) gave ungirinomine (4) (RT 0.65–0.75, 7.2 mg) and ungirinomine N-oxide (5) (RT 0.61–0.65, 2.9 mg), respectively.

Fraction IV (360 mg) was subjected to PTLC (SiO\(_2\), solvent 4) to afford two fractions (RT 0.80–0.92, fr. IV-A, 33 mg, RT 0.45–0.55, fr. IV-B, 139 mg). Fraction IV-A was subjected to PTLC (SiO\(_2\), solvent 1) to give galanathine (6) (RT 0.19–0.27, 5 mg) and lycoramine (8), (RT 0.37–0.44, 8.5 mg). Fraction IV-B was subjected to PTLC (SiO\(_2\), solvent 4) to afford an oil (RT 0.59–0.69, 75.1 mg), which was further separated by PTLC (Al\(_2\)O\(_3\), solvent 1) to give galanathine N-oxide (7) (RT 0.78–0.81, 1.6 mg) and 1-palmitoyl-2-linoleoylphosphatidylethanolamine sodium salt (13) (RT 0.02–0.08, 47 mg).

**Treatment of the MeOH–H\(_2\)O Extract**

The MeOH–H\(_2\)O extract (98 g) was successively triturated with hot hexane, benzene, CHCl\(_3\), and CHCl\(_3\)-MeOH (1:1) (fr. V, 6,454 g). The insoluble material was an oil (fr. VII, 70 mg, which was soluble in MeOH-CHCl\(_3\)).

Fraction V (6,454 g) was subjected to flash chromatography on SiO\(_2\) with solvent 4 to give two fractions (550 ml, fr. V-A, 165.8 mg; 500 ml, fr. V-B, 119.8 mg). Fraction V-A was subjected to PTLC (SiO\(_2\), solvent 2) to give three fractions (RT 0.55–0.63, fr. V-A-1, 34.7 mg; RT 0.66–0.74, fr. V-A-2, 76 mg; RT 0.86–0.89, fr. V-A-3, 2.1 mg of galanathine (2)). Fraction V-A-1 was purified by PTLC (Al\(_2\)O\(_3\), solvent 3) to give ungirinone (9) (RT 0.59–0.69, 3.9 mg). Fraction V-A-2 was triturated with MeOH to afford lycorine (10) (53.3 mg). Fraction V-B was subjected to PTLC (SiO\(_2\), solvent 2) to give an oil (21.3 mg), which was further purified by PTLC (Al\(_2\)O\(_3\), solvent 1) to afford *O*-methyllycorine (11) (4.9 mg).

Fraction VI (55 g) was subjected to flash chromatography on SiO\(_2\) with solvent 4. The solid obtained from the first fraction (1000 ml) was washed with MeOH to afford lycorine (10) (114 mg). The MeOH solution gave an oil (208 mg), which was subjected to PTLC (SiO\(_2\), solvent 2) to give an amorphous solid (68.6 mg). This was further purified by PTLC (Al\(_2\)O\(_3\), solvent 5) to afford ungirinomine (4) (RT 0.38–0.44, 2.6 mg) and lycoramine (8) (RT 0.78–0.82, 5.6 mg).

**Irinocarone** (1) Colorless prisms (from MeOH), mp 183–185°C. IR (KBr): 3433 (OH), 1615, 1516, 1456, 1326, 1290, 1085 cm\(^{-1}\). High MS m/z: [M\(^+\)]* \(2070.392(100)\). High MS m/z: [M\(^+\)]* \(2070.392(100)\).

**Galantamine** (2) Pale yellow oil, [\(\text{[\alpha]}\] \(20^\circ\) 65.9° (c 0.046, CHCl\(_3\)) (lit.\(^1\) 1977, 58.4°). High MS m/z: [M\(^+\)]* \(333.190(100)\). High MS m/z: [M\(^+\)]* \(333.157(100)\).

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mp 257–258°C.

O-Demethyllycoramone (11) Pale yellow oil, \( [\alpha]_D^20^0 = -103.1^0 \) (c = 0.21, EtOH) (lit. \( [\alpha]_D^24^0 = -111.9^0 \) (c = 0.59, EtOH)). High MS m/z [M]+:
Caled for C\(_16\)H\(_{12}\)NO\(_3\): 275.1522. Found: 275.1513. \( ^1\)H-NMR (CDCl\(_3\), 200 MHz) \( \delta \): 6.64 and 6.52 (each 1H, d, \( J = 8 \) Hz, H-11 and 12), 4.36 (1H, br s, H-17), 4.11 (1H, m, H-2), 3.98 and 3.60 (each 1H, d, \( J = 15 \) Hz, CH\(_2\)-9), 2.37 (3H, s, NCH\(_3\)).

1-Palmitoyl-2-linoleoylphosphatidylethanolamine (12) Pale yellow oil.
El-MS m/z (%): 757 (12), 337 (65), 313 (80), 263 (48), 239 (25). FAB-MS m/z: 716 [M + H] (thioglycerol), IR (KBr): 2925, 2854, 1741, 1652, 1446, 1385, 1074, 1045 cm\(^{-1}\). \( ^1\)H-NMR (CDCl\(_3\), 200 MHz) \( \delta \): 5.35 (4H, m, \( -\text{CH} = \text{CH} \times 2 \)), 4.88–3.87 (4H, m, OC\(_3\)H\(_7\)-CH\(_2\)-x), 3.59–3.04 (4H, m, OCH\(_2\)CH\(_2\)N), 2.76 (2H, t, \( J = 6 \) Hz, =C-CH\(_2\)-C=), 2.05 (4H, m, COCH\(_3\)CH\(_2\)-x), 1.58 (4H, br s, COCH\(_3\)CH\(_2\)-x), 0.88 (6H, m, CH\(_3\)).

1-Palmitoyl-2-linoleoylphosphatidylmethanol Sodium Salt (13) Pale yellow oil.
El-MS m/z (%): 575 (58), 337 (31), 313 (82), 263 (39), 239 (32). FAB-MS m/z: 709 [M + H] (thioglycerol); 731 [M + Na] (thioglycerol + Na). IR (KBr): 2926, 2855, 1742, 1466, 1234, 1073, 1050 cm\(^{-1}\). \( ^1\)H-NMR (CDCl\(_3\), 200 MHz) \( \delta \): 5.34 (4H, m, \( -\text{CH} = \text{CH} \times 2 \)), 4.37–3.88 (4H, m, OCH\(_2\)CH\(_2\)-x), 3.57 and 3.51 (3H, each s, OCH\(_3\) ), 2.27 (2H, t, \( J = 6 \) Hz, =C-CH\(_2\)-C=), 2.30 (4H, m, COCH\(_3\)CH\(_2\)-x), 2.05 (4H, m, =C-CH\(_2\)-x), 1.59 (4H, br s, COCH\(_3\)CH\(_2\)-x), 0.89 (6H, m, CH\(_3\)).

References and Notes
7) This compound has not previously been reported.