Alkaloidal Constituents of *Leucojum autumnale* L. (Amaryllidaceae)

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Two new alkaloids, 3-*O*-acetylnarcissidine (4) and 3-*O*-acetylnarcissidine *N*-oxide (5), were isolated from *Leucojum autumnale* L. (Amaryllidaceae) together with the known alkaloids narcissidine (3) and lycorine (6).

Key words *Leucojum autumnale*; Amaryllidaceae; 3-*O*-acetylnarcissidine; 3-*O*-acetylnarcissidine *N*-oxide; narcissidine

Many kinds of alkaloids have been isolated from Amaryllidaceae plants, and some have interesting biological activities.[1] For example, galanthamine and its *n*-butyl carbamate derivative were found to be centrally active competitive cholinesterase inhibitors[2] and are undergoing clinical evaluation for the treatment of Alzheimer's disease.[3] In the previous papers,[4,5] we reported the isolation of a novel alkaloid incartine (**1**), a supposed biosynthetic intermediate in the pathway from galanthine (**2**) to narcissidine (**3**), together with galanthamine from flowers of *Lycoris incarnata*. We now report the isolation and structural elucidation of two new alkaloids, 3-*O*-acetylnarcissidine (**4**) and 3-*O*-acetylnarcissidine *N*-oxide (**5**), together with the known alkaloids narcissidine (**3**) and lycorine (**6**) from *Leucojum autumnale* L.

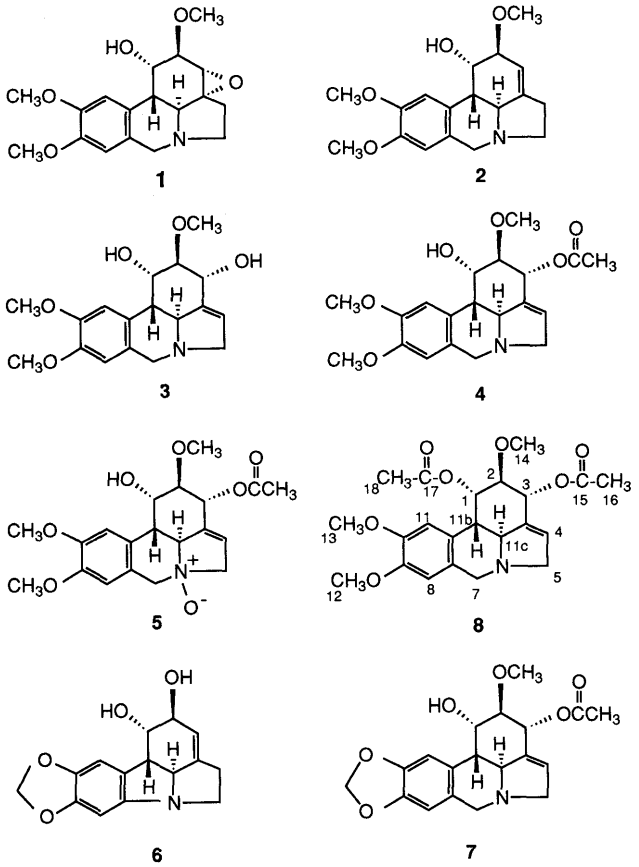

Chart 1

Crude basic material extracted from fresh bulbs of *L. autumnale* by the modified method of Wildman and Bailey[6] was subjected to preparative thin layer chromatography (PTLC) as described in Experimental, to give compounds **3**—**6**.

Compound **3**, mp 195—196 °C, $[\alpha]_D$ −22.5° ($CHCl_3$), $C_{18}H_{23}NO_5$, was isolated as colorless pillars. Its infrared (IR) spectrum showed absorption due to a hydroxyl group at 3410 cm^{-1} but no absorption due to a carbonyl group. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed the presence of two *para*-oriented aromatic protons (δ 6.88 and 6.68), one aliphatic methoxy group (δ 3.44), two aromatic methoxy groups (δ 3.86 and 3.82), and benzyl protons (δ 4.09 and 3.54, each doublet, J= 13 Hz), but no *N*-methyl gorup (Table I). These data suggested that compound **3** is narcissidine, a lycorine-type alkaloid. The physical and spectral data of compound **3** were identical with those of narcissidine isolated from the *Narcissus* hybrid "Deanna Durbin."[7]

The new compound **4**, mp 191—192.5 °C, $[\alpha]_D$ −154.2° ($CHCl_3$), $C_{20}H_{25}NO_6$, was isolated as colorless cubes. The IR spectrum showed absorptions due to a hydroxyl group at 3400 cm^{-1} and a carbonyl group at 1730 cm^{-1}. The ^{1}H-NMR spectrum was similar to that of narcissidine (**3**), except for the presence of a signal due to an acetyl group (δ2.03) and the down-field shift of the H-3 signal (δ5.90), as shown in Table I. From these findings, the new compound **4** was assigned as 3-*O*-acetylnarcissidine. This assignment for **4** was supported by the similarity of the

TABLE I. ^{1}H-NMR Spectral Data for Compounds **3**—**5**

	3[a]	4[a]	5[b]
H-1	4.66 (m)	4.70 (br s)	4.76 (br s)
H-2	4.18—3.36 (m)	4.30—3.50 (m)	3.70 (dd, J=3, 2 Hz)
OCH_3-2	3.44 (s)	3.50 (s)	3.51 (s)
H-3	4.66 (m)	5.90 (br s)	5.67 (br s)
$COCH_3$-3	—	2.03 (s)	2.03 (s)
H-4	5.56 (br s)	5.71 (br s)	6.01 (dd, J=3.5, 2 Hz)
H-5β	4.18—3.36 (m)	4.30—3.50 (m)	4.87 (ddd, J=17, 2.5, 2 Hz)
H-5α	4.18—3.36 (m)	4.30—3.50 (m)	4.60 (ddd, J=17, 2, 1 Hz)
H-7β	4.09 (d, J=13 Hz)	4.19 (d, J=13 Hz)	4.52 (d, J=13 Hz)
H-7α	3.54 (d, J=13 Hz)	3.66 (d, J=13 Hz)	4.06 (d, J=13 Hz)
H-8	6.68 (s)	6.74 (s)	7.02 (s)
OCH_3-9	3.82 (s)	3.85 (s)	3.86 (s)
OCH_3-10	3.86 (s)	3.88 (s)	3.88 (s)
H-11	6.88 (s)	6.94(s)	7.13 (s)
H-11b	2.70 (d, J=11 Hz)	2.85 (d, J=11 Hz)	2.90 (ddd, J=12.5, 2, 1 Hz)
H-11c	4.18—3.36 (m)	4.30—3.50 (m)	4.39 (d, J=12.5 Hz)

a) In $CDCl_3$. *b*) In CD_3OD.

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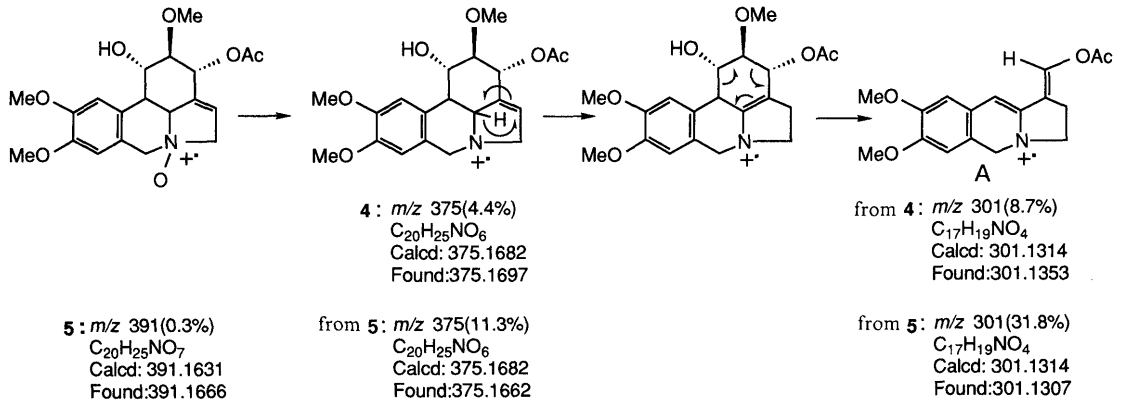

Chart 2

^{1}H-NMR spectrum to that of 3-*O*-acetylungiminorine (**7**) isolated from *Leucojum asetivum* L.[8] and by the observation of mass fragment A, $C_{17}H_{19}NO_4$, characteristic of lycorine-type alkaloids,[8,9] indicating the presence of a 3-*O*-acetyl group (Chart 2). Finally, the structure of **4** was confirmed by the preparation of diacetylnarcissidine (**8**) from both **3** and **4**.

The new compound **5**, mp 150—152 °C, $[\alpha]_D$ −102.3° (EtOH), was isolated as colorless pillars. The IR spectrum revealed absorptions due to a hydroxyl group at 3400 cm^{-1} and a carbonyl group at 1725 cm^{-1}. The mass spectrum (MS) indicated the molecular formula $C_{20}H_{25}NO_7$ for **5** with one more oxygen than in **4**, and its characteristic fragment pattern was similar to that of 3-*O*-acetylnarcissidine (**4**), except for the molecular ion peak (Chart 2). The ^{1}H-NMR spectrum was also similar to that of **4**, as shown in Table I. However, the signals for protons (H-5, H-7, and H-11c) attached to *N*-bearing carbon atoms in **5** were deshielded by *ca.* 0.3—0.8 ppm in comparison with those in **4**. These data indicated that the compound **5** is the *N*-oxide of 3-*O*-acetylnarcissidine. This structure for **5** was also supported by a comparison of the ^{13}C-NMR spectra of **5** and **8** (see Experimental). All carbon signals of **5** were assigned with the aid of two-dimensional ^{1}H–^{13}C shift correlation spectroscopy (2D-COSY). The chemical shifts of **5** were similar to those of **8** except for the deshielded shifts of C-5, C-7, and C-11c, indicating **5** to be an *N*-oxide derivative. The structure **5** was confirmed by reduction with sulfur dioxide to afford 3-*O*-acetylnarcissidine (**4**).

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are given as uncorrected values. The spectrophotometers used were a Perkin-Elmer 1720 infrared Fourier-transform spectrophotometer for IR spectra, a JEOL JMS-D 300 for MS, a Union PM-201 for optical rotations, and JEOL JNM-FX 200 and Bruker AM-400 spectrometers for ^{1}H- and ^{13}C-NMR spectra, with tetramethylsilane as an internal standard. The plates used for PTLC were coated with silica gel (Kieselgel PF_{254}, Merck). The following solvent systems were used: 1) $CHCl_3$–ether–MeOH (4 : 1 : 2); 2) $CHCl_3$–MeOH (5 : 1). UV light, I_2 vapor, and Dragendorff's reagent were used to locate compounds.

Extraction and Separation of Alkaloids Following the modified method of Wildman and Bailey,[6] fresh bulbs (9.2 kg) of *L. autumnale* collected in our Faculty plot were ground in 11 l of EtOH in a mixer. The mixture was kept at room temperature overnight and then filtered. The filtrate was concentrated *in vacuo*. H_2O (130 ml) and ether (250 ml) were added to the residue. The aqueous layer separated from the mixture was made acidic (pH 4.0) with 25% tartaric acid and washed with ether (100 ml × 3). The aqueous layer was made basic (pH 7.2) with NH_4OH and extracted with $CHCl_3$ (100 ml × 5). The extract was evaporated *in vacuo* to give a sticky oily residue. $CHCl_3$ (50 ml) was added to the residue and crude lycorine (**6**) was obtained as a powder (259 mg) by filtration of the mixture. The $CHCl_3$ solution separated from lycorine (**6**) was evaporated *in vacuo* to give an oil (1.500 g). This was subjected to PTLC (solvent 1) to give three fractions (*Rf* 0.65—0.93, fraction (fr.) I, 190 mg; *Rf* 0.54—0.65, fr.II, 95 mg; *Rf* 0.42—0.54, fr. III, 226 mg).

Fraction I (190 mg) was purified by PTLC (solvent 2) to give 3-*O*-acetylnarcissidine (**4**) (*Rf* 0.59—0.66, 87 mg). Fraction II (95 mg) was subjected to PTLC (solvent 2) to afford 3-*O*-acetylnarcissidine *N*-oxide (**5**) (*Rf* 0.34—0.47, 47 mg). Fraction III (226 mg) was subjected to PTLC (solvent 2) to give crude crystals (*Rf* 0.18—0.33, 121 mg), which were purified by PTLC with EtOH to afford narcissidine (**3**) (*Rf* 0.14—0.32, 45 mg).

Narcissidine (3) The crude base (45 mg) of **3** was recrystallized from acetone to give colorless pillars (22 mg), mp 195—196 °C (dec.) (lit.[7] mp 201—203 °C (dec.)). $[\alpha]_D^{22}$ −22.5° (c=0.58, $CHCl_3$) (lit.[7] $[\alpha]_D^{23}$ −31.0° (c=1.5, $CHCl_3$), lit.[9] $[\alpha]_D^{28}$ −28.3° (c=0.32, $CHCl_3$)). The ^{1}H-NMR spectral data are shown in Table I.

3-*O*-Acetylnarcissidine (4) The crude base (87 mg) of **4** was recrystallized from benzene–$CHCl_3$ to give colorless cubes (10 mg), mp 191—192.5 °C. $[\alpha]_D^{25}$ −154.2° (c=0.25, $CHCl_3$). IR (KBr): 3400 (OH), 1730 cm^{-1} (C=O). UV λ_{max}^{MeOH} nm (log ε): 281 (3.62), 225 (4.02) (shoulder). EI-MS *m/z* (%): 375 (M^+, 4.4), 316 (15.2), 315 (42.5), 314 (7.7), 301 (8.7), 284 (100), 266 (37.4), 258 (6.2), 241 (5.1), 230 (6.3), 228 (14.1). High-MS *m/z* (M^+): Calcd for $C_{20}H_{25}NO_6$: 375.1682. Found: 375.1697. The ^{1}H-NMR spectral data are shown in Table I.

3-*O*-Acetylnarcissidine *N*-Oxide (5) The crude base (47 mg) of **5** was recrystallized from acetone–MeOH to afford colorless pillars (16 mg), mp 150—152 °C (dec.). $[\alpha]_D^{22}$ −102.3° (c=0.11, EtOH). IR (KBr): 3400 (OH), 1725 cm^{-1} (C=O). UV λ_{max}^{MeOH} nm (log ε): 233 (3.89), 281 (3.59). High-MS *m/z* (M^+): Calcd for $C_{20}H_{25}NO_7$: 391.1631. Found: 391.1666. ^{13}C-NMR (CD_3OD) δ: 20.97 (q, C-16), 42.65 (d, C-11b), 56.84 (q, C-12 or 13), 56.90 (q, C-13 or 12), 58.74 (q, C-14), 68.46 (d, C-1), 69.70 (d, C-3), 69.86 (t, C-7), 81.56 (d, C-2), 81.88 (t, C-5), 85.75 (d, C-11c), 110.28 (d, C-11), 114.23 (d, C-8), 123.02 (d, C-4), 125.28 (s, C-7a or 11a), 129.82 (s, C-11a or 7a), 134.15 (s, C-3a), 149.50 (s, C-9 or 10), 151.21 (s, C-10 or 9), 171.70 (s, C-15). The ^{1}H-NMR spectral data are shown in Table I.

Reduction of 3-*O*-Acetylnarcissidine *N*-Oxide (5) Sulfur dioxide was introduced into a solution of **5** (4 mg) in MeOH (1 ml) until **5** disappeared on TLC. The mixture was evaporated *in vacuo* and $CHCl_3$ (5 ml) and 1% $NaHCO_3$ (1 ml) were added. The aqueous layer was extracted with $CHCl_3$ (2 ml × 2). The combined $CHCl_3$ solution was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was subjected to PTLC with $CHCl_3$–MeOH (10 : 1) to give **4** as pale brown cubes (0.7 mg) (from acetone), mp 178—180 °C (dec.). This compound was shown to be identical with a sample of natural **4** by mixed melting point test and by comparison of their IR spectra.

Lycorine (6) A part of the crude **6** was recrystallized from MeOH to give colorless pillars, mp 225—227 °C. This prodcut was shown to be identical with an authentic sample of **6** (lit.[6] mp 238—241 °C) by comparisons of their MS and IR spectra.

Diacetylnarcissidine (8) From Narcissidine (**3**): A mixture of **3**

(6.8 mg), acetic anhydride (0.1 ml), and dry pyridine (0.15 ml) was heated in a sealed tube at 100 °C for 1.6 h. Then MeOH (0.2 ml) was added and the mixture was evaporated. The residue was subjected to PTLC with $CHCl_3$–acetone–MeOH (5:5:1) to give **8** as colorless needles (1 mg) (from acetone), mp 148—151 °C (lit.[10] mp 170—172 °C). ^{1}H-NMR ($CDCl_3$) δ: 6.93 (1H, s, H-8), 6.57 (1H, s, H-11), 5.99 (1H, br s, H-4), 5.93 (1H, br s, H-1), 5.69 (1H, br s, H-3), 4.96 (1H, d, J=16 Hz, H-5β), 4.71 (1H, d, J=15 Hz, H-7β), 4.60 (1H, m, H-11c), 4.10—3.40 (2H, m, H-7α and H-5α), 3.89 and 3.82 (each 3H, s, OCH_3-9 and 10), 3.73 (1H, dd, J=3, 2 Hz, H-2), 3.55 (3H, s, OCH_3-2), 3.21 (1H, d, J=10 Hz, H-11b), 2.11 and 2.07 (each 3H, s, $COCH_3 \times 2$). ^{13}C-NMR ($CDCl_3$) δ: 20.86 (q, C-16 or 18), 20.93 (q, C-18 or 16), 38.87 (d, C-11b), 56.23 (q, C-12 or 13), 56.30 (q, C-13 or 12), 58.89 (q, C-14), 66.94 (d, C-1 or 3), 67.60 (d, C-3 or 1), 53.36 (t, C-7), 77.12 (d, C-2), 61.62 (t, C-5), 64.93 (d, C-11c), 106.85 (d, C-11), 111.78 (d, C-8), 121.67 (d, C-4), 123.55 (s, C-7a or 11a), 126.43 (s, C-11a or 7a), 133.96 (s, C-3a), 148.85 (s, C-9 or 10), 150.40 (s, C-10 or 9), 169.25 (s, C-15 or 17), 170.22 (s, C-17 or 15). IR (KBr): 1735 cm^{-1}(C=O). High-MS m/z (M^+): Calcd for $C_{22}H_{27}NO_7$: 417.1785. Found: 417.1740.

From 3-*O*-Acetylnarcissidine (**4**): A mixture of **4** (5.4 mg), acetic anhydride (0.1 ml), and dry pyridine (0.1 ml) was heated in a sealed tube at 70 °C for 1.5 h. Work-up in the same way as above gave **8** as pale brown needles (1 mg), mp 148—151 °C. High-MS m/z (M^+): Calcd for $C_{22}H_{27}NO_7$: 417.1786. Found: 417.1786. This compound was shown to be identical with a sample of **8** prepared from **3** as above by the mixed melting point test and by comparisons of their ^{1}H-NMR spectra and MS.

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