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Communication

Revised NMR data for Incartine: an Alkaloid from *Galanthus elwesii*

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Abstract: Phytochemical studies on *Galanthus elwesii* resulted in the isolation of five alkaloids: incartine, hordenine, hippeastrine, 8-*O*-demethylhomolycorine and lycorine. The NMR data given previously for incartine were revised and completed by two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C chemical shift correlation experiments. *In vitro* studies on the bioactivity of incartine were carried out.

Keywords: *Galanthus elwesii*; Amaryllidaceae alkaloids; incartine; NMR.

Introduction

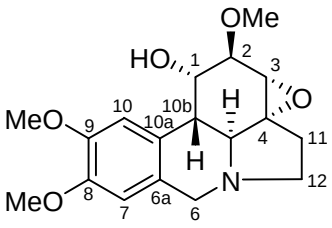
Galanthus elwesii (snow drop) is a small bulbous plant distributed throughout South-Eastern European countries and Euro-Asia [1], which has been cultivated for its elegant flowers. Earlier investigations in *Galanthus elwesii* have resulted in the isolation of a large variety of Amaryllidaceae alkaloids [2-4]. Many of these compounds have been found to exhibit among others, strong acetylcholinesterase inhibitory, cytotoxic and antiviral activities [5].

As part of our ongoing studies on the *Galanthus* species, we report on the isolation, structural elucidation of incartine (**1**) hordenine (**2**), lycorine (**3**), 8-*O*-demethylhomolycorine (**4**) and hippeastrine (**5**) from *G. elwesii* (Figure 1). Bioactivity of incartine with respect to antiretroviral, cytotoxic and acetylcholinesterase inhibitory activities, hitherto unknown, has also been studied.

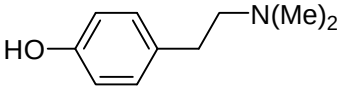
Results and Discussion

The EtOH extract from *G. elwesii* plants was fractioned as described in the Experimental Section and as a result, five alkaloids were obtained.

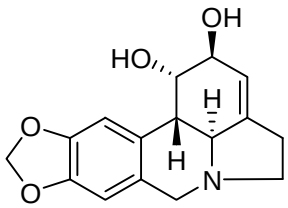
Figure 1. Chemical structures of the alkaloids isolated from *Galanthus elwesii*.



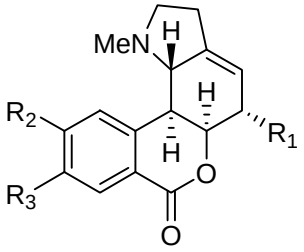
1



2



3



4: R_1= H, R_2= OMe, R_3= OH
5: R_1= OH, R_2+R_3= OCH_2O

The HRMS of compound **1**, with a parent ion at *m/z* 333.1572 (calc. mass - 333.1576), suggested the molecular formula $C_{18}H_{23}NO_5$. The complete assignment of the ^{1}H-NMR spectrum (Table 1), performed by 2D COSY and NOESY experiments, revealed a compound with a lycorine type structure whose chemical shifts were closely comparable with those of galanthine [6]. The absence of an olefinic proton in **1**, characteristic of the lycorine type compounds [5], as well as the similarity of the chemical shifts of the H-11 and H-12 protons with those of the homolycorine type C3 - C4 epoxy derivatives, galwesine and 9-*O*-demethylgalwesine [4] indicated a C3 - C4 epoxy ring. On the basis of the ^{1}H-NMR spectral data, **1** was identified as incartine, an alkaloid that has been isolated only once from the flowers of *Lycoris incarnata* [7]. Compound **1** and incartine have identical MS spectra. However, the ^{1}H-NMR spectrum of **1** was not congruent with that reported in the literature. The H-4a, H-6*α*, H-6*β*, H-11*β*, H-12*α* and H-12*β* protons of **1** are considerably shifted, with values of - 0.96, - 0.66, - 0.64, - 0.99, - 1.17, and - 0.88 ppm, respectively, as compared to those described for

incartine by Kihara *et al*. [7]. Moreover, the chemical shifts of compound **1** closely compare with those of the biosynthetically related galwesine [4]. The observed deshielding on the protons in *α* position surrounding the *N* atom of the incartine reported by Kihara *et al*. [7] as compared with **1** as well as the similarities of the ^{1}H-NMR of **1** with those of galwesine and galanthine leads us to suggest that the compound described in the literature is most probably a salt of **1**.

Table 1. ^{1}H-NMR, COSY, NOESY and ^{13}C-NMR data for **1**.

Position	H δ (*J* in Hz)	COSY	NOESY	HMQC	HMBC
1	4.46 *br s*	H-2	H-2, H-10, H-10b, 2-OCH_3	67.7 *d*	C-2, C-3, C4a
2	3.85 overlapped	H-1	H-1, H-3, H-4a, 2-OCH_3	78.2 *d*	C-1, C-4, C-10b, 2-OCH_3
3	3.48 *brd d* (1.0)	-	H-2, H-11*β*, 2-OCH_3	59.0 *d*	C-1, C-2
4				66.9 *s*	
4a	2.56 *d* (11.2)	H-10b	H-2, H-10b	61.3 *d*	C-3, C-4, C-6, C-10a
6*α*	3.65 *dd* (14.0, 1.0)	H-6*β*	H-6*β*, H-7, H-12*α*	56.7 *t*	C-4a, C-6a, C-7, C-12
6*β*	4.07 *d* (14.0)	H-6*α*	H-6*α*,H-7, H-12*β*	56.7 *t*	C-4a, C-6a, C-7
6a				128.5 *s*	
7	6.57 *s*		H-6*α*, H-6*β*, 8-OCH_3	110.7 *d*	C-6, C-8, C-9,C-10a, 8-OMe
8				147.8 *s*	
9				147.9 *s*	
10	6.78 *s*		H-1, 9-OCH_3	107.6 *d*	C-6a, C-8, C-9, C-10b
10a				127.9 s	
10b	2.82 *d* (11.2)	H-4a	H-1, H-4a, 2-OCH_3	40.1 *d*	C-4a, C-10a
11*α*	2.00 *ddd* (14.5, 6.5, 2.0)	H-11*β*, H-12*α*, H-12*β*	H-11*β*, H-12*α*	30.1 *t*	C-3, C-4, C-12
11*β*	2.39 *ddd* (14.5, 10.3, 7.5)	H-11*α*, H-12*α*, H-12*β*	H-3, H-11*α*, H-12*β*	30.1 *t*	C-4, C-4a, C-12
12*α*	2.59 *ddd* (10.3, 9.0, 6.5)	H-11α, H-11*β*, H-12*β*	H-6*α*, H-11*α*, H-12*β*	53.0 *t*	C-4, C-6
12*β*	3.20 *ddd* (9.0, 7.5, 2.0)	H-11α, H-11*β*, H-12*α*	H-6*β*, H-11*β*, H-12*α*	53.0 *t*	C-4, C-4a, C-11
2-OMe	3.54 *s*		H-1, H-2, H-3, H-10b	59.2 *q*	C-2
8-OMe	3.80 *s*		H-7	56.1 *q*	C-8
9-OMe	3.84 *s*		H-10	56.2 *q*	C-9

The ^{13}C-NMR data of **1**, reported here for the first time (Table 1), are in agreement with the proposed structure. The heteronuclear chemical shift correlation experiments (HMBC, HMQC) were performed in order to assign all the signals of the ^{13}C-NMR spectrum and to confirm the assignments made for the ^{1}H-NMR spectrum. Thus, the skeleton of compound **1** contains 18 carbon atoms with the following characteristic signals: (1) three signals at *δ* 59.2, 56.1, 56.2 for the methoxyl groups at C-2, C-8 and C-9, respectively, (2) three triplets at *δ* 56.7, 30.1 and 53.0, corresponding to the methylene carbons C-6, C-11 and C-12, (3) two doublets at *δ* 110.7 and 107.6, assigned to the aromatic carbons C-7 and C-10, and five doublets at *δ* 67.7, 78.2, 59.0, 61.3, and 40.1, corresponding to the carbons C-1, C-2, C-3, C-4a and C-10b, and (4) five singlets at *δ* 66.9, 128.5, 147.8, 147.9 and 127.9, assigned to the quaternary carbons C-4, C-6a, C-8, C-9 and C-10a. The ^{13}C-NMR spectrum of compound **1** is in agreement with that reported for galanthine, with the exception of carbons C-3 and C-4 due to the

deshielding effect of the double bond $\Delta^{3,4}$ in galanthine [6]. Later examination of its ^{1}H-NMR revealed that compound **1** is unstable and degrades after several months. Compounds **2-5** were identified by comparison of their spectroscopic data with those reported in the literature, as described in the Experimental Section.

A wide range of bioactivities have been reported for compounds **2-5** [5, 8]. Alkaloids with the lycorine type skeleton possess significant antiviral and cytotoxic activities. Compound **3** is the most studied alkaloid of this group as it possesses one of the strongest inhibitory properties such as antiviral and cytotoxic among others [6, 9, 10]. However, incartine showed no remarkable activity against human immunodeficiency virus (HIV) in a human cell culture system or was cytotoxic at the concentrations tested (CC_{50} 42.9 μM/mL). Incartine (IC_{50} 102 μM/mL) and hordenine (IC_{50} 473 μM/mL) inhibited electric eel acetylcholinesterase *in vitro* considerably less than the positive control galanthamine (5.14 μM/mL).

Experimental

General

^{1}H-, ^{13}C-NMR, COSY, NOESY, HMQC and HMBC spectra were recorded in an Inova 500 MHz using $CDCl_3$ as a solvent and TMS as an internal standard. Chemical shifts were reported in δ units (ppm) and coupling constants (*J*) in Hz. Mass spectra were recorded on a CG-MS Hewlett Packard 6890+ MSD 5975, (Hewlett Packard, Palo Alto, CA, USA) operating in EI mode at 70 eV. A HP-5 MS column (30 m × 0.25 mm × 0.25 μm) was used. The temperature program was: 100-180°C at 15°C·min^{-1}, 1 min hold at 180°C, 180-300°C at 5°C·min^{-1} and 1 min hold at 300°C. Injector temperature was 280°C. The flow rate of carrier gas (Helium) was 0.8 mL·min^{-1}. Split ratio was 1:20.

Plant material

Whole plants of *Galanthus elwesii* Hook fil. (Amaryllidaceae) were collected in the flowering stage, in March 2003, from a wild population near the village of Obrochishte, district of Varna, Bulgaria. The plant material was identified by Dr. Ljuba Evstatieva - Institute of Botany, Bulgarian Academy of Sciences (BAS). A voucher specimen was deposited at the Institute of Botany (BAS), with No. SOM-162923.

Extraction and isolation

Fresh *G. elwesii* plants (2.9 kg) were extracted with 95% ethanol (5 x 10 L, 72 h each). The solvent was evaporated under vacuum and the residue was dissolved in 2% sulfuric acid (300 mL) and defatted with Et_2O (5 x 300 mL). Then the acidic soln. was made alkaline with 25% ammonia to pH 9-10, and the alkaloids were extracted with $CHCl_3$ (5 x 500 mL). 2.55 g of crude alkaloid mixture (0.088% referred to fresh weight) were obtained after evaporation of the organic solvent. The crude alkaloid mixture was subjected to column chromatography (5 x 50 cm column) on silica gel (250 g, 40-60 μm, SDS France) using EtOAc gradually enriched with MeOH (100:0, 400 mL; 90:10, 300 mL; 75:25, 300

mL; 50:50, 1000 mL; 20:80, 500 mL and 0:100, 1200 mL). Fractions of 50 mL were collected and monitored by TLC (silica gel, EtOAc/MeOH/25% ammonia 12:3:0.1; Dragendorff's reagent). Incartine (**1**, 32 mg) was isolated as a brown solid by CC on silica gel of fraction 24 (40-60 μm; 1 x 30 cm; EtOAc/MeOH 8:2; 5 mL fractions) from sub-fractions 10-12. Hordenine (**2**, 65 mg) was obtained after crystallization in EtOAc from fractions 39-77. Lycorine (**3**, 44 mg) crystallized directly from the crude alkaloid mixture. 8-*O*-Demethylhomolycorine (**4,** 134 mg) and hippeastrine (**5**, 158 mg) were purified by pre-crystallization in MeOH from fractions 31-34 and 25-27, respectively. Hordenine (**2**) was identified by comparison of its spectroscopic data with those already reported [11]. Lycorine (**3**) [12], 8-O-demethylhomolycorine (**4**) [13] and hippeastrine (**5**) [14] were identified by direct comparison of their chromatographic and spectroscopic properties (TLC, CG-MS, MS, ^{1}H-NMR) with those of authentic samples obtained in our laboratory from other plant sources.

Bioactivity assays

Acetylcholinesterase (AChE) inhibitory activity of incartine and hordenine were measured exactly as described by López *et al*. [15]. Galanthamine was used as a positive control. Antiretroviral activity and cytotoxicity were evaluated in human MT-4 cells based on viability of cells that have been infected or not infected with HIV-1 following exposure to various concentrations of the test compound according to the procedure previously described by López *el al*. [16]. AZT and nevirapine were used as positive controls.

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Sample Availability: Samples of compounds **2 - 5** and reference NMR spectra of **1**-**5** are available from the authors.