Institute of Botany[1], and Institute of Organic Chemistry with Centre of Phytochemistry[2], Bulgarian Academy of Sciences, Sofia, Bulgaria

# Galanthamine distribution in Bulgarian *Galanthus* spp.

B. Sidjimova[1], S. Berkov[1] , S. Popov[2], L. Evstatieva[1]

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*Strahil Berkov, PhD, Institute of Botany, Bulgarian Academy of Sciences, 23, Acad. G. Bonchev Str., BG-1113-Sofia, Bulgaria*
*berkov@iph.bio.bas.bg*

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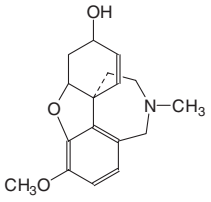
Sixteen populations of *Galanthus elwesii* Hook.fil and five populations of *Galanthus nivalis* L. growing in Bulgaria were investigated for the presence of galanthamine by TLC and GC-MS. Between 3 and 11 alkaloids were detected by TLC in the total alkaloid fractions. Galanthamine was found in 2 populations of *G. elwesii*.

## 1. Introduction

Galanthamine, an Amaryllidaceae alkalolid, is a long acting, selective, reversible and competitive acetylcholinesterase (AChE) inhibitor [1–3], which produces beneficial effects even after drug treatment has been terminated [4]. This product is marketed as a hydrobromide salt under the name Reminyl® for the treatment of Alzheimer's disease.




Galanthamine

Galanthamine was isolated for the first time from *Galanthus woronowii* Losinsk. by Proskurina and Yakovleva [5, 6] and was later found in many other of Amaryllidaceae species. On a commercial scale, galanthamine had been produced for the first time from *Galanthus nivalis* L. in Bulgaria under the name of Nivalin® [7–8].
Bubeva-Ivanova [9] found this alkaloid in *G. nivalis* L. var. *gracilis* Celak. from populations near the towns of Yambol and Koprivshtitsa (Southeast and Central Bulgaria) in 1957. Later, it was reported that *G. nivalis* plants from this region contain higher amounts of galanthamine than plants from North and Northeast Bulgaria which contain no or trace amounts of galanthamine [10].
The taxonomical status of genus *Galanthus* in Bulgaria has been changed several times since the establishment of galanthamine in *G. nivalis*. Genus *Galanthus* had been presented by a single species – *G. nivalis* s.l. up to 1964 [11, 12]. Stojanov et al. 1966 [13] divided the genus *Galanthus* into two species in the fourth edition of "Flora of Bulgaria", namely *G. elwesii* Hook.fil, and *G. nivalis* L. with several varieties and forms. Delipavlov, 1968 [14] distinguished three species of *Galanthus* in Bulgaria – *G. nivalis* L., *G. elwesii* Hook.fil. and *G. graecus* Orph. ex Boiss. According to this author, *G. nivalis* is distributed mainly in Eastern Bulgaria [15]. Recently, *G. nivalis* L. and *G. elwesii* Hook.fil. have been accepted for Bulgaria [16–18].
In this paper we report a reinvestigation on the galanthamine distribution in Bulgarian *Galanthus* species according to the latest classifications [16–18].

## 2. Investigations, results and discussion

We examined 21 populations of *Galanthus* (5 of *G. nivalis* and 16 of *G. elwesii*) for their galanthamine content by TLC and GC-MS (Table). The distribution of *G. nivalis*, which we found, coincides with those reported by Delipavlov [14] for the Black Sea Coast region. Between 3 and 11 alkaloids were detected in the total alkaloid fractions by TLC. Galanthamine was found in only 2 populations of *G. elwesii* – No 13 (Levski-021) and No 20 (Karnobat-024). The occurrence of galanthamine was confirmed by parallel GC-MS with an authentic sample. Some alkaloid fractions which do not contain galanthamine according to TLC were also examined by GC-MS. The compound was not found in them even in trace amounts.
Small differences were observed between the MS of galanthamine obtained by direct insertion [19] and the GC-MS conditions, which we used. The molecular peak of galanthamine (at *m/z* 287), which is reported in the literature [19] as a base peak (100%, relative abundance) was depressed (78%) under the GC-MS conditions. The base ion appeared at *m/z* 286 ($M^+$-H). The other characteristic fragments of galanthamine showed similar relative abundances to those reported for the direct insertion. Previously, GC-MS has been successfully applied for identification of other Amaryllidaceae alkaloids [20, 21].
Our results on the distribution of galanthamine confirms those of Ivanova and Ivanov [10] who investigated 26 populations and found galanthamine in only a few of them. On the basis of our results and the results of Ivanova and Ivanov [10] as well as on the limited distribution of *G. nivalis*, we suppose that the first commercial scale production of Nivalin® (galanthamine hydrobromide) was mainly from *G. elwesii* according to the contemporary classification of the genus *Galanthus* in Bulgaria.

**Table: Investigated populations of *Galanthus* spp. distributed in Bulgaria**

| No | Floristic region | Part of Bulgaria | Voucher SOM | Species | Galanthamine | Method of analysis |
|---|---|---|---|---|---|---|
| *Black Sea Coast* | | | | | | |
| 1 | Obrochiste | NE | 028 | *nivalis* | – | TLC/GC-MS |
| 2 | Obrochiste | NE | 028a | *elwesii* | – | TLC/GC-MS |
| 3 | Tsarkva | NE | 029 | *nivalis* | – | TLC/GC-MS |
| 4 | Tsarkva | NE | 029a | *elwesii* | – | TLC |
| 5 | Varna | NE | 027 | *nivalis* | – | TLC |
| 6 | Burgas | SE | 025 | *nivalis* | – | TLC |
| 7 | Primorsko | SE | 026 | *nivalis* | – | TLC/GC-MS |
| *Northeast Bulgaria* | | NE | | | | |
| 8 | Tervel (Stenata) | | 0216 | *elwesii* | – | TLC/GC-MS |
| 9 | Tervel (Dan kula) | | 0218 | *elwesii* | – | TLC/GC-MS |
| 10 | Tervel (Sujunlika) | | 0220 | *elwesii* | – | TLC/GC-MS |
| 11 | Shumen | | 0210 | *elwesii* | – | TLC/GC-MS |
| 12 | Targovoshte | | 0211 | *elwesii* | – | TLC |
| *Danubian plain* | | CN | | | | |
| 13 | Levski | | 021 | *elwesii* | 0.1 | TLC/GC-MS |
| *Forebalkan* | | CN | | | | |
| 14 | Sevlievo | | 0212 | *elwesii* | – | TLC |
| 15 | Montana | | 0213 | *elwesii* | – | TLC |
| *Stara Planina Mts* | | Central | 0224 | *elwesii* | – | TLC |
| 16 | Kozja stena | | 0224 | *elwesii* | – | TLC |
| 17 | Sliven | | 023 | *elwesii* | – | TLC |
| *Sofia region* | | SW | | | | |
| 18 | Kokaljane | | 0221 | *elwesii* | – | TLC |
| *The Rhodopes* | | Central | | | | |
| 19 | Bachkovo | | 0222 | *elwesii* | – | TLC |
| *Tundzha Hilly region* | | SE | | | | |
| 20 | Karnobat | | 024 | *elwesii* | 0.6 | TLC/GC-MS |
| 21 | Topolovgrad | | 022 | *elwesii* | – | TLC |

## 3. Experimental

### *3.1. Plant material*

Aerial parts of *Galanthus* species at flowering stage (February–March, 2002) were collected from 21 populations distributed at different floristic regions of Bulgaria. The species were determined according to Webb [16] and Petrova [18]. Voucher specimens were deposited at the herbarium of Institute of Botany – BAS (SOM), Sofia.

### *3.2. Alkaloid extraction*

Plants were dried at 60 °C, powdered and extracted with 3% $H_2SO_4$ for 2 h by shaking at room temperature. Than, the extracts were centrifuged and supernatants were separated, basified with 25% ammonia and extracted tree times with dichloroethane. The organic solvent was dried over anh. $Na_2SO_4$ and evaporated to dryness. The dry extracts were dissolved in known amounts of methanol for further analysis.

### *3.3. Alkaloid determination*

Two TLC conditions were used for the development of alkaloid fractions and identification of galanthamine: (1) chloroform/methanol/25% ammonia (11 : 1 : 0.6, v/v/v), migration distance – 160 mm, and (2) chloroform/methanol/25% ammonia (12 : 1 : 0.5, v/v/v), migration distance – 80 mm. 10 µl of the alkaloid fractions were spotted and developed on Merck aluminum sheets silica gel 60 $F_{254}$ (10 × 20 cm) together with standards. Compounds were visualized after triplicate spraying with Dragendorff's reagent. Galanthamine was quantified by TLC-densitometry. 10 min after the last spraying, the plates were scanned at 600 dpi optical resolution and images were analyzed by QuantiScan 2.1® Biosoft software. The galanthamine content of the samples was calculated from the densitogram peak areas by comparing to three standards (5, 15 and 30 µg/spot, stock solution: 1 mg/ml galanthamine hydrobromide calculated as a base) placed on the same plate.

### *3.4. Gas chromatography-mass spectrometry*

The GC-MS was recorded on a Hewlett Packard 6890+ MSD 5973 instrument 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 80–280 °C at 10 °C min and 10 min hold at 280 °C. Injector temperature was 280 °C. The flow rate of carrier gas ($He_2$) was 0.8 ml · $min^{-1}$. Split ratio was 1 : 20.

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