Available online at www.sciencedirect.com
SCIENCE DIRECT®

Phytochemistry 65 (2004) 579–586

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Intraspecific variability in the alkaloid metabolism of *Galanthus elwesii*

Strahil Berkov[a,*], Borjana Sidjimova[a], Luba Evstatieva[a], Simeon Popov[b]

[a] *Institute of Botany, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev Str., 1113 Sofia, Bulgaria*
[b] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 9 Acad. G. Bonchev Str., 1113 Sofia, Bulgaria*

Received 10 October 2003; received in revised form 13 December 2003

Abstract

Alkaloid pattern of individuals from 16 Bulgarian *Galanthus elwesii* populations was investigated by GC/MS and TLC. Twenty-one Amaryllidaceae alkaloids were detected and 14 of them were identified. Crinane type alkaloids, haemanthamine or crinine, dominated alkaloid metabolism in most of the populations. With exception of one population, where the separate individuals showed variable alkaloid profiles (dominated by crinine or haemanthamine) the individuals of the rest of populations have identical and characteristic alkaloid profiles. Some populations showed remarkable differences in respect to their alkaloid pattern—type of biosynthesis, main alkaloids and number of alkaloids. Populations dominated by galanthamine type alkaloids were found as well. These data demonstrate that like the morphological features, the alkaloid metabolism of *G. elwesii* is also variable.

Keywords: *Galanthus elwesii*; Amaryllidaceae; GC/MS; TLC; Amaryllidaceae alkaloids

1. Introduction

Galanthus elwesii Hook. fil., (snowdrop) is a bulbous monocotyledon plant belonging to the family Amaryllidaceae. It is distributed in the Balkan peninsula, the Aegean Islands, Ukraine and Turkey (Davis, 1999). The plant exhibits a great morphological variability and as a result, it has been associated with a number of other species that have glaucous leaves and two marks on the inner periant segments, namely the closely related *G. gracilis* Čelak., *G. graecus* Orph. ex Boiss., and *G. maximus* Velen. (Davis, 1999). According to the recent classifications, *G. elwesii* is presented with a number of subspecies. Webb (1980) describes two subspecies: subsp. *elwesii* and subsp. *minor* in the European flora, concretely for Bulgaria and North Aegean region, where as Anchev (1992) found three subspecies for Bulgaria, subsp. *elwesii*, subsp. *minor* and subsp. *maximus*. In addition, Zeybek (1988) described four other subspecies in Turkey: subsp. *tuebitaki*, subsp. *melihae*, subsp. *yayintaschii*, and subsp. *akmanii*. Typical plants of these subspecies are very distinct, but intermediates occur throughout the aerial parts and the populations can be either one of the extreme types or of an intermediate type (Davis, 1999).

Contrary to the number of reports and monographs on the morphological variability in genus *Galanthus*, including *G. elwesii*, there are scanty data about their biochemical variability. *Galanthus* species biosynthesize pharmacologically active alkaloids so called "Amaryllidaceae alkaloids". More than 20 alkaloids have been found in *G. elwesii* including the acetylcholinesterase inhibitor galanthamine (Latvala et al., 1995). Earlier phytochemical investigations are based on kilogram biomass extraction of the plant material, usually collected from a single population. In such a case, valuable information about alkaloid variability in the species is omitted.

In the recent years, GC/MS has been proved to be a powerful method for rapid separation and identification of the components in complex alkaloid mixtures (Wink et al., 1983; Witte et al., 1987; Berkov et al., 2003). There are only few reports on GC/MS of underivatized alkaloids from Amaryllidaceae plants, which demonstrated that the method could be successfully applied for this alkaloid group (Kreh et al., 1995; Tram et al.,

---

[*] Corresponding author. Tel.: +359-99472383; fax: +359-2719032.
E-mail address: berkov@iph.bio.bas.bg (S. Berkov).

0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2003.12.013

2002). Of particular significance is the observation that minor changes in stereochemistry of these alkaloids are frequently sufficient to cause appreciable differences in the mass spectra of many of the stereoisomers (Duffield et al., 1965; Wildman and Brown, 1968).

To the best of our knowledge, there are no reports on the inter- and intraspecific variability of the secondary metabolism in genus *Galanthus*. As the morphological features of *G. elwesii* are very variable and the taxa are difficult to be distinguished, aim of our work was to investigate the alkaloid metabolism in different populations of this species growing in Bulgaria. Thus, on the one hand, the significance of the *Galanthus* alkaloids as a chemotaxonomical tool will be estimated, and on the other hand, the distribution of pharmacologically active compounds will be recorded.

2. Results

2.1. GC/MS of the alkaloid fractions

More than 25 compounds in the alkaloid fractions showed mass spectral fragmentation characteristic for the Amaryllidaceae alkaloids. 21 compounds are presented in Table 1, 14 of them have been identified. Under GC conditions used, underivatized alkaloids were well separated. They were of galanthamine, lycorine, lycorinine, crinane, phenanthridine and tazettine types (Fig. 2).

2.2. Interpopulation variability in alkaloid metabolism

The populations analyzed showed remarkable differences in respect to their alkaloid pattern- type of biosynthesis, main alkaloid and number of alkaloids. TLC analysis of the alkaloid fractions showed 3–11 alkaloids for the different populations, with 1–3 of them as major components (Fig. 3). Many populations such as 023, 0211, 0210, and 0212 with one major alkaloid (haemanthamine) showed similar TLC profiles, whereas other populations as 021, 022, 024, and 029 (with 1–3 major alkaloids), displayed different and characteristic TLC profiles. TLC was impossible to be applied in 5 out of 16 populations, which were not included in this study due to the very low alkaloid accumulation in the plants (Fig. 3, plate 3, lanes x_2, x_3, x_4).

The main alkaloids in the populations are listed in Table 2. Haemanthamine (**9**) dominates in the most of studied populations. Two populations (0216 and 022) accumulated mainly crinine (**7**), which is biosynthetically related to haemanthamine, both of crinane type. Other two populations (021 and 024) accumulated galanthamine as a main alkaloid.

The more sensitive GC/MS analysis revealed different biosynthetic pathways in the populations. Four typical alkaloid patterns are presented in Table 1. Plants of population 024 synthesize alkaloids of three types: mainly galanthamine type alkaloids (more than 58% of the alkaloid fraction, seven compounds) crinane type (<5%, two compounds) and phenanthridine type (<1%, one compound). The alkaloid production of this population is dominated by galanthamine (**2**, 54%).

In contrast to the above-mentioned population, the plants from population 021 synthesize alkaloids of six types: galanthamine (39%, two compounds), lycorenine (20%, four compounds), crinane (<18%, three compounds), tazzettine (8%, one compound), phenantridine (1%, one compound) and lycorine (<1%, one compound) alkaloids. Galanthamine is dominant compound again (**2**, 36%), but in contrast to 024, the relative concentrations of the other alkaloid types in the spectrum are much higher. A lycorine type alkaloid **12** was detected (as a trace) in the alkaloid fraction of this population only.

Two alkaloid types were detected in the plants of population 022—mainly crinane alkaloids (75%, four compounds) as well as phenanthridine alkaloid trispheridine (**1**, 1%). Crinine (41%) dominates in this population followed by haemanthamine (27%). In contrast to the previous two populations, 022 exhibited a simpler spectrum (five compounds).

Like population 022, crinane type biosynthesis (79%) also prevails in the plants from population 022A and haemanthamine (**9**) is accumulated as a main alkaloid (42%). Lycorenine type alkaloids (**11** and **16**, 8%) were also detected. Five alkaloids were found in this population similarly to population 022.

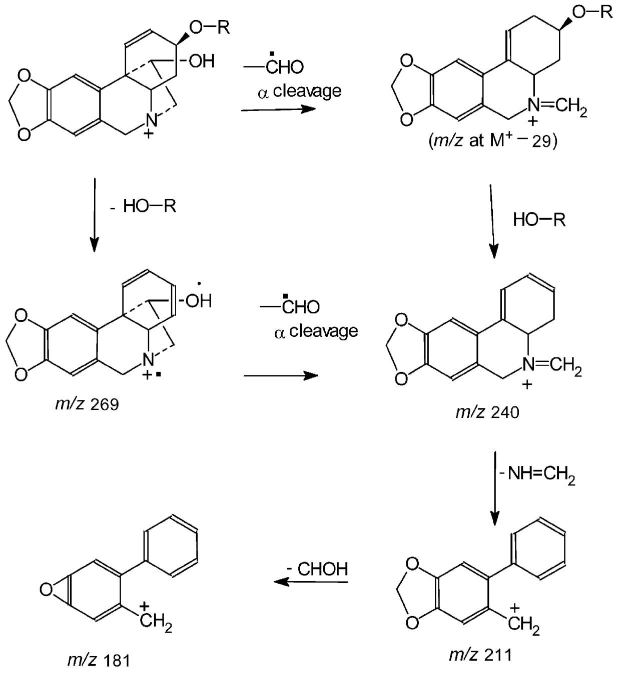

Fig. 1. Presumed mass spectral fragmentation of alkaloids G1, G6 and G7 after Duffield et al. (1965).

Table 1
Alkaloids of *G. elwesii* presented as a% of total ion current of GC-MS chromatograms

				Population #				MS Ref.
Alkaloids	RT [min^{-1}]	M$^+$	*m/z* (rel. int.)	024	021	022	022A	
Trispheridine (**1**)[a]	15.09	223(100)	222(39), 167(10), 165(10), 164(16), 138(22)	0.23	1.12	1.12		Ali et al., 1986
Galanthamine (**2**)[b]	16.85	287(82)	286(100), 244(24), 230(12), 216(35), 174(31)	53.8	36.44			Kreh et al., 1995
Buphanisine (**3**)[c]	17.04	285(100)	270(32), 254(33), 230(18), 215(82), 201(22), 187(10), 185(18), 172(18), 157(20), 115(31)			<1		Viladomat et al., 1995
Nor-galanthamine (**4**)[b]	17.31	273(98)	272(100), 230(33), 202(27), 174(12)	1.68				Kreh et al., 1995
Nor-lycoramine (**5**)[b]	17.53	275(77)	274(100), 202(11), 188(17), 178(10)	<1				Kreh et al., 1995
Narwedine (**6**)[b]	17.78	285(84)	284(100), 242(18), 216(20), 199(18), 174(31)	2.59	2.88			Kreh et al., 1995
Crinine (**7**)[c]		271(100)	270(14), 254(10), 228(23), 214(12), 199(65), 187(57), 173(18), 115(22)		<1	40.84	9.22	Viladomat et al., 1995
Marithidine (**8**)[c]	18.37	287(92)	270(15), 268(17), 258(10), 244(33), 215(100), 203(56), 196(12), 167(8), 128(25), 115(29), 91(11), 77(13)			6.80	28.2	Youssef and Frahm, 1998
Haemanthamine (**9**)[c]	19.86	301(15)	272(100), 240(18), 257(20), 211(16), 181(23)		17.24	27.32	41.48	Kreh et al., 1995
Tazettine (**10**)[d]	20.01	331(30)	316(14), 298(22), 260(5), 247(100), 227(13), 211(12), 201(14), 181(12), 152(10), 141(9), 128(10), 115(16)		8.28			Duffield et al., 1965
Galwesine (**11**)[e]	20.71	361(10)	206(3), 178(9), 155(91), 140(100)				2.54	Latvala et al., 1995
Lycorine (**12**)[f]	21.13	287(27)	270(20), 243(18), 223(20), 2 4(14), 199(18), 185(29), 141(8), 128(8), 115(10)		<1			Likhitwitayawuid et al., 1993
N-Formylgalanthamine (**13**)[b]	21.85	301(100)	272(2), 243(6), 230(8), 225(15), 211(16), 128(11), 115(10)	<1				Bastida et al., 1987
O-Methylleucotamine (**14**)[b]	22.88	373(24)	272(22), 286(2), 171(13), 270(100), 216(18), 165(8)	<1				Latvala et al., 1995
G1 (**15**)[e]	18.34	329(100)	286(20), 269(65), 252 (34), 240(43), 225(33), 224(45), 211(28), 210(45), 181(55)		<1			—
G2 (**16**)[e]	19.17	?	264(1), 253(1), 206(1), 192(1), 164(3), 136(2), 109(100), 94(3), 82(3)				5.60	—
G3 (**17**)[e]	21.30	?	281(1), 270(1), 206(1), 178(2), 109(100), 94(3), 82(3)		3.89			—
G4 (**18**)[e]	22.01	?	300(1), 192(1), 164(3), 136(2), 109(100), 94(3), 82(3)		10.70			—
G5 (**19**)[c]	23.46	?	314(1), 206(2), 178(4), 150(2), 125(100), 96(35), 82(3)		5.48			—
G6 (**20**)[c]	25.29	373(100)	344(8), 300(1), 286(22), 269(67), 252(22), 240(40), 225(27), 224(35), 211(20), 210(30), 181(37),	3.91	<1			—
G7 (**21**)[c]	26.01	429(100)	400(16), 300(6), 269(87), 252(8), 240(37), 225(37), 224(45), 211(28), 210(11), 181(51)	<1				—

[a] Alkaloid types: phenanthridine.
[b] Galanthamine.
[c] Crinane.
[d] Tazettine.
[e] Lycorenine.
[f] Lycorine.

2.3. Intrapopulation variability of the alkaloid metabolism

The alkaloid pattern of the individuals within the populations was studied by TLC only. With an exception of population 022A (Fig. 4), the individuals of all other populations showed identical TLC profiles, characteristic for each population. Rarely, separate individuals have TLC profiles, which are different to those of the rest plants within the population (Fig. 3, plate 1, lane 7). The difference between the individuals was in the alkaloid concentrations mainly.

In contrast to that, the individuals from population 022A showed instability in the alkaloid profiles. The plants from this population differ in the main alkaloid, crinine or haemanthamine as well as in the ratio between the major alkaloids (Fig. 4).

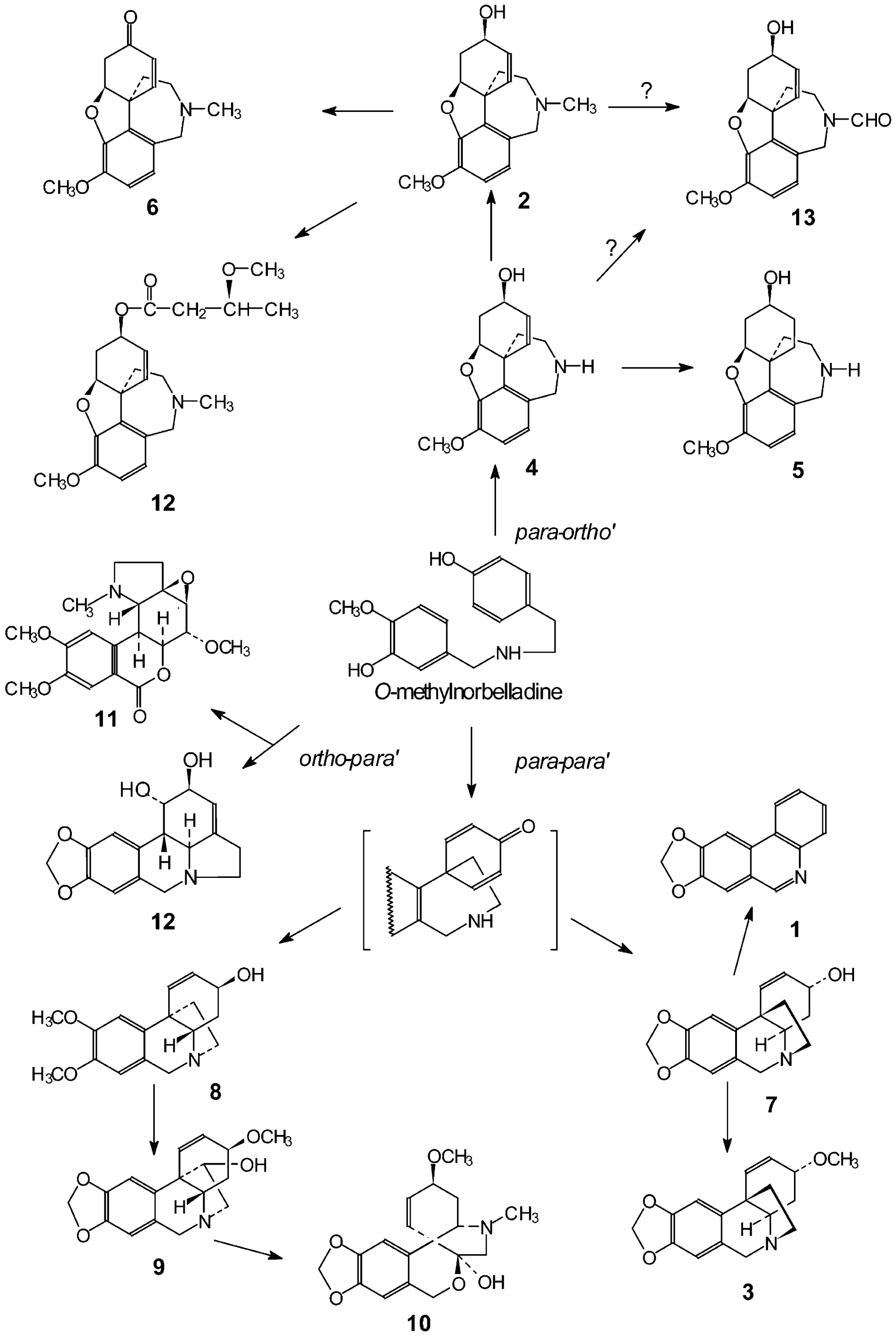

Fig. 2. Biosynthetic pathways of Amaryllidaceae alkaloids after Bastida and Viladomat (2002). The numbers of the alkaloids are identical with those in Table 1.

3. Discussion

For the needs of alkaloid analysis in the individuals, a TLC method (described in the experimental section) was adapted for small-sized alkaloid-containing plants such as *Galanthus* species. It showed sufficient sensitivity and reproducibility (1–3 μg/spot galanthamine, corresponding to 0.09% alkaloids in the dry biomass; CV-20%) for investigations on the alkaloid pattern of individuals in the most of populations (Figs. 3 and 4). Main advantage of the method is that a high number of samples could be analyzed in a short time (50–80 samples per day), which is of interest for studies on the alkaloid metabolism within and between populations or for selection of interesting genotypes.

To the best of our knowledge, GC/MS was applied for the first time for studies on the alkaloid metabolism of *G. elwesii* populations. Four alkaloids: trispheridine (**1**), norlycoramine (**5**), crinine (**7**), and marithidine (**8**) are reported for the first time for *G. elwesii*. Alkaloids **3**, **5**, **12**, **13**, and **14** were present in trace amounts in the alkaloid mixtures and under these circumstances it seems that the only method for their identification is GC/MS. Compounds G1–G7, with mass spectral fragmentation characteristic for the Amaryllidaceae alkaloids, were left unidentified because of the absence of

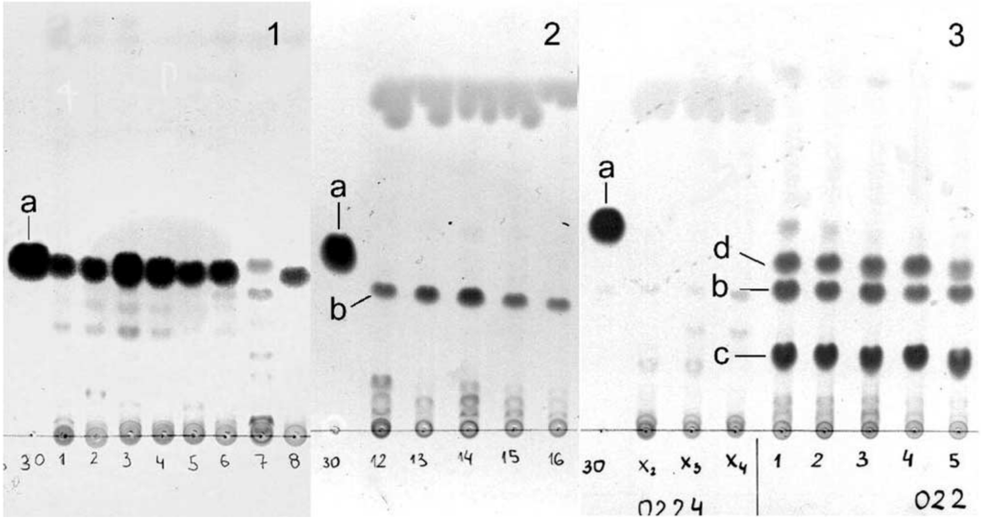

Fig. 3. TLC profiles of *G. elwesii* individuals from population 024 (plate 1), 0210 (plate 2), 0224 (plate 3; lanes x_2, x_3 and x_4) and 022 (plate 3; lanes 1–5). a) galanthamine (standard), b) heamanthamine, c) crinine and d) unknown alkaloid.

Table 2
Main alkaloids in the alkaloid fractions of *G. elwesii*

Floristic region	Part of Bulgaria	Voucher # SOM	Galanthamine	Haemanthamine	Crinine	Unknown alkaloid
Black Sea Coast	NE					
Obrochiste		028a		+ +	+	+
Tsarkva		029a		+ +	+	+
Northeast Bulgaria	NE					
Tervel (Stenata)		0216			+ +	
Shumen		0210		+ +	+	
Targovoshte		0211		+ +		
Danubian plain	CN					
Levski		021	+ +	+		+
Forebalkan	CN					
Sevlievo		0212		+ +		
Stara Planina Mts	Central					
Sliven		023		+ +		
Tundzha Hilly region	SE					
Karnobat		024	+ +			
Topolovgrad		022		+	+ +	+
Topolovgrad (rekata)		022A		+ +	+	+

Legend: + +—main alkaloid; +—accompanying alkaloid.

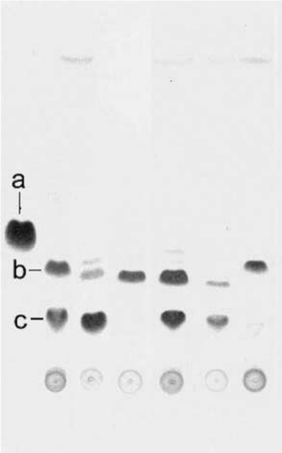

Fig. 4. TLC profiles of individuals from population 022A. a) galanthamine (standard), b) heamanthamine, and c) crinine.

similar spectra in the available literature or database. Four alkaloids G2–G5 showed mass spectral fragmentation typical for lycorenine type alkaloids with a $\Delta^{3,4}$ double bound- no molecular ion peak and very low intensities of all fragments besides the base peak at m/z 109 or at m/z 125 respectively. The M$^+$ ions of this type of alkaloids can not be determined by electron impact mass detector (Kreh et al., 1995). Alkaloid G5 showed the base peak at m/z 125 as well as an abundant peak at m/z 96, which are indicative for a hydroxyl group at C-2. The rest abundant peaks at m/z 162 and at m/z 190 are characteristic for hippeastrine (Hesse and Bernhard, 1975), but due to the very low M$^+$ it can not be identified unambiguously.

Alkaloids G1, G6, and G7 are biosynthetically related. They exhibit similar mass spectral fragmentation differing in the M$^+$ only. Their fragmentation resembles the reported in the literature mass spectrum of crinamine: prominent peaks at m/z 269 (base ion), 240, 225, 224, 211, and 210 (Duffield et al., 1965). Crinamine differs to haemanthamine (**9**), found in the studied samples, in the configuration of the methoxyl group at C-3 only. These compounds have distinct EIMS spectra (Duffield et al., 1965). The mechanism of electron impact mass spectral fragmentation of crinamine and haemanthamine can be characterized by favorable expulsion of the substituent at C-3, yielding the ion at m/z 269, as well as by α-cleavage of the hydroxylated ethylene bridge. Contrary to crinamine, which looses the hydroxylated ethylene bridge from the fragment ion at m/z 269, the loss of 29 mass units from the molecular ion (M$^+-{}^{\bullet}$CHO) is a characteristic process for haemanthamine, especially under GC/MS conditions (Duffield et al., 1965; Kreh et al., 1995). The presence of such a peak in G1, G6, and G7 as well as the lack of crinamine in the alkaloid fraction suggests that they are haemanthamine derivatives. In a case that the hydroxyl group of haemanthamine is substituted, as in narcimarkine, a prominent peak at m/z 272 appears (Döpke and Sewerin, 1981). Such a peak is absent in G1, G6, and G7. Further more, all these compounds have prominent peaks at m/z M$^+$-29, 269, and 240 (loss of 29 mass units from the fragment ion with m/z 269), as well as at m/z 252 (loss of 17 mass units, hydroxyl group from the ion at m/z 269), indicating substitution at C-3 and free hydroxyl group at C-11. Presumed mass spectral fragmentation of G1, G6, and G7 is presented in Fig. 1.

The M$^+$ of G1 (m/z 329) is with 28 mass units higher than this of haemanthamine (or crinamine), suggesting the presence of an additional carbonyl group. However, peaks at m/z 286 (M$^+$-43) and at m/z 269 (M$^+$-60) clearly indicate an acetoxy group, which can be situated at C-3 only. Consequently, G1 is an ester of acetic acid. Instead of 60 mass units as in G1, the C-3 substituents in G6 and G7 have 104 and 160 mass units respectively. 3-Hydroxybutyric acid is a possible substituent at C-3 in compound G6 as it has 104 mass units and this acid is found as a substituent in the molecule of *O*-methylleucotamine (**14**) as well. For unambiguous identification of G1, G6, and G7 however, they must be isolated in pure state and subjected to other spectroscopic analysis.

Although not all of the compounds in the alkaloid fractions were identified, GC/MS data give us an opportunity to estimate the main pathways of alkaloid metabolism in *G. elwesii*. At the present stage of our investigations, we can not establish any correlation between the morphology and metabolism of this species. Also, there was no correlation between the vegetation areas of the populations and the main accumulated alkaloids (Table 2). We found significant differences between the populations on the relatively small geographic area of Bulgaria. These differences are not only in the presence or absence of a particular metabolite but also in the type of the alkaloid metabolism. The differentiation appeared at the first stage of alkaloid biosynthesis—the oxidative coupling of norbelladine or related alkaloids, which led to domination of separate skeletons of the Amaryllidaceae alkaloids in the different populations (Fig. 2). Thus, the products of *para–ortho'* oxidative phenolic coupling dominate in population 024, whereas the products of *para–para'* oxidative coupling dominate in populations 022 and 022A. Contrary to population 022A, which accumulates crinane type alkaloid with α-configuration of the ethylene bridge (haemanthamine), population 022 accumulates a product with β-configuration of the ethylene bridge (crinine). It have to be noted that population 022 and 022A are adjacent.

In contrast to the above-mentioned populations, plants from population 021 biosynthesize alkaloids which are products of the all oxidative couplings: *para–ortho'*, *para–para'* and *ortho–para'*. As a whole, the alkaloid metabolism in the most populations of *G. elwesii* was dominated by the products of *para–para'* oxidative coupling, mainly crinane type alkaloids.

The factors (genetic or environmental), regulating the biosynthetic pathways of alkaloid biosynthesis in *G. elwesii* are still unknown, but their establishment is important. In a case of genetic determination of the biosynthesis, it could contribute to a more reliable classification of the species and subspecies taxa. In a case of environmental determination, it could be used for studies on the control of alkaloid production. It is well known that galanthamine (**2**), an alkaloid with acetylcholinesterase inhibitory properties, is used in modern medicine for the treatment of nervous system disorders, including Alzheimer's disease (Maelicke et al., 2001). A biotechnological approach has been considered as an alternative method for its production. In this respect, populations with alkaloid metabolism dominated by galanthamine derivatives, like population 024, could be of interest for further investigations. In addition, norgalanthamine (**4**), marithidine (**8**), haemanthamine (**9**), tazettine (**10**), and lycorine (**12**), found in the studied populations are also of pharmacological interest (Bastida and Viladomat, 2002).

In conclusion, native populations of *G. elwesii* have variable alkaloid metabolism and appear to be an interesting source of bioactive molecules. Further experiments on the establishment of factors regulating alkaloid biosynthesis in *G. elwesii* are in progress.

4. Experimental

4.1. Plant material

Aerial parts of 50 individuals per population from *Galanthus elwesii* at flowering stage (February–March) were collected from 16 populations distributed at different floristic regions of Bulgaria in 2002. The species was determined according to Petrova (1999). No subspecies differentiation of the collected plants was done because many plants of intermediate types were observed within the populations. Voucher specimens were deposited at the herbarium of Institute of Botany-BAS (SOM), Sofia (voucher numbers are listed in Table 2).

4.2. Sample preparation

Plants were dried at 60 °C and powdered. The plant samples (50 mg per plant) were extracted with 3 ml 3% H_2SO_4 (2 h shaking at room temperature). Than, the extracts were centrifuged and supernatants (2 ml) were separated, basified with 25% NH_4OH (1 ml) to pH 10–11 and applied on Extrelut 3® Merck columns. The retained alkaloids were eluted with CH_2Cl_2 (20 ml). The organic solvent was dried over anhydrous Na_2SO_4 and evaporated to dryness under a stream of air. The dry extracts were dissolved in 100 μl of methanol for further TLC analysis. 50 individuals per each population were analyzed. Reproducibility of the extraction procedure was estimated by the coefficient of variation (CV%) based on five 50 mg samples prepared from the dry biomass of one population.

For GC/MS analysis, the alkaloid fractions of the populations were obtained from the excess leaf biomass (0.1–1 g, left after analysis of the individuals). Plant materials were extracted with 10 ml 3% H_2SO_4 for 2 h and centrifuged. Supernatants were basified with 25% aq. NH_4OH to pH 10–11 and than extracted with CH_2Cl_2 (3×10 ml). The organic solvent was dried over anhydrous Na_2SO_4 and evaporated to dryness. The dry extracts were dissolved in MeOH (4% concentration). 1 μl of the solutions was used for GC/MS analysis.

4.3. Thin layer chromatography (TLC)

Two TLC systems were used for analysis of the alkaloid fractions: system 1, $CHCl_3$/MeOH/25% aq. NH_4OH (11:1:0.6, v/v.), migration distance-160 mm and system 2, $CHCl_3$/MeOH/25% aq. NH_4OH (12:1:0.5, v/v.), migration distance- 80 mm. 10 μl of the alkaloid fractions were spotted on Merck sheets silica gel 60 F_{254} plates (10×20 cm, 0.2 mm layer) together with standards and developed. Compounds were visualized after triplicate spraying with Dragendorff's reagent.

4.4. Preparative TLC

The identification of the main alkaloids on the TLC plates was done after their preliminary isolation by preparative TLC. The alkaloid fractions, obtained from 5–10 g dry leaves were developed on Merck silica gel 60 F_{254} sheets (10×20 cm, 2 mm layer) using system 1 as a mobile phase. The compounds, visible under UV_{254} nm were eluted from the layer with methanol and subjected to GC/MS analysis for identification.

4.5. Gas chromatography/mass spectrometry (GC/MS)

The GC/MS were recorded on a Hewlett Packard 6890+ MSD 5973 (Hewlett Packard, Palo Alto, CA, USA) 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° min^{-1} and 10 min hold at 280 °C. Injector temperature was 280 °C. The flow rate of carrier gas (He) was 0.8 ml min^{-1}. Split ratio was 1:20. The identification of the alkaloids was confirmed by comparing the mass spectral data with those of authentic compounds from database NIST 98 (a Hewlett Packard Mass Spectral Library, Palo Alto, CA, USA), or with data obtained from the literature.

Acknowledgements

The authors thank Dr. Borjana Damjanova and Mrs. Daniela Andonova for performing the GC/MS analysis. This work was supported by the Bulgarian Ministry of Environment and Waters (project 3228/264).

References

Ali, A., El Sayed, H., Abdallah, O., Steglich, W., 1986. Oxocrinine and other alkaloids from *Crinum americanum*. Phytochemistry 25, 2399–2401.

Ancev, M., 1992. Genus *Galanthus*. In: Kozuharov, S. (Ed.), Guidebook to the Higher Plants in Bulgaria. Nauka i Izkustvo, Sofia, p. 91.

Bastida, J., Viladomat, F., Llabres, J., Codina, C., Felitz, M., Rubiralta, M., 1987. Alkaloids from *Narcissus confusus*. Phytochemistry 26, 1519–1524.

Bastida, J., Viladomat, F., 2002. Alkaloids of *Narcissus*. In: Hanks, G. (Ed.), Medicinal and Aromatic Plants—Industrial Profiles: The Genus *Narcissus*. Taylor and Francis, London and New York, pp. 141–214.

Berkov, S., Pavlov, A., Kovatcheva, P., Stanimirova, P., Philipov, S., 2003. Alkaloid spectrum in diploid and tetraploid hairy root cultures of *Datura stramonium*. Z. Naturforschung 58c, 42–46.

Davis, A., 1999. The genus *Galanthus*. Timber Press, Portland Oregon. pp. 140–152.

Döpke, W., Sewerin, E., 1981. Zur Struktur des Narcimarkins. Z. f. Chem. 21, 71.

Duffield, A., Aplin, R., Budzikiewicz, H., Djerassi, C., Murphy, C., Wildman, W., 1965. Mass spectrometry and stereochemical problems. LXXXII. A study of the fragmentation of some Amaryllidaceae alkaloids. J. Am. Chem. Soc. 87, 4902–4917.

Hesse, M., Bernhard, H., 1975. Amaryllidaceae alkaloids. In: Budzikiewicz, H. (Ed.), Progress in Mass Spectrometry, Vol. 3. Verlag Chemie, Weinheim, pp. 164–184.

Kreh, M., Matusch, R., Witte, L., 1995. Capillary gas chromatography-mass spectrometry of Amaryllidaceae alkaloids. Phytochemistry 38, 773–776.

Latvala, A., Önür, M., Gözler, T., Linden, A., Kivçak, B., Hesse, M., 1995. Alkaloids of *Galanthus elwesii*. Phytochemistry 39, 1229–1240.

Likhitwitayawuid, K., Angerhofer, C., Chai, H., Pezzuto, J., Cordell, G., 1993. Cytotoxic and antimalarial alkaloids from the bulbs of Crinum amabile. J. Nat. Prod. 56, 1331–1338.

Maelicke, A., Samochocki, M., Jostock, R., Fehrenbacher, A., Ludwig, J., Albuquerque, E.X., Zerlin, M., 2001. Allosteric sensitization of nicotinic receptors by galanthamine, a new treatment strategy for Alzheimer's disease. Biological Psychiatry 49, 279–288.

Petrova, A., 1999. Genus *Galanthus*. In: Petrova, A., Ancev, M., Palamarev, E. (Eds.), How to Determine the Plants in the Nature. Excursion Guidebook. Prosveta, Sofia, p. 631.

Tram, N., Mitova, M., Bankova, V., Handjieva, N., Popov, S., 2002. GS-MS of *Crinum latifolium* L. alkaloids. Z. Naturforschung 57c, 239–242.

Viladomat, F., Codina, C., Bastida, J., Mathee, S., Campbell, W., 1995. Further alkaloids from *Brunsvigia josephinae*. Phytochemistry 40, 961–965.

Webb, D.A., 1980. Genus *Galanthus*. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, Vol. 5. Cambridge University Press, Cambridge, p. 77.

Wildman, W., Brown, C., 1968. Mass spectra of 5,11β-methanomorphanthridine alkaloids. The structure of pancracine. J. Am. Chem. Soc. 90, 6439–6446.

Wink, M., Witte, L., Hartmann, T., Theuring, T., Volz, V., 1983. Accumulation of quinolizidine alkaloids in plants and cell suspention cultures: genera *Lupinus*, *Cytisus*, *Baptista*, *Genista*, *Laburnum* and *Sophora*. Planta Medica 48, 253–257.

Witte, L., Muller, K., Arfmann, H.-A., 1987. Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas–liquid-chromatography–mass-spectrometry. Planta Medica 53, 192–197.

Youssef, D., Frahm, A., 1998. Alkaloids of the flowers of *Pancratium maritimum*. Planta Medica 64, 669–670.

Zeybek, N., 1988. Taxonomic investigations on Turkish snowdrops. Turk. J. Bot 12, 89–102.