Alkaloid Variability in *Leucojum aestivum* from Wild Populations

Liliya Georgieva[a], Strahil Berkov[b], Violeta Kondakova[a], Jaume Bastida[b], Francesc Viladomat[b], Atanas Atanassov[a], and Carles Codina[b,*]

[a] AgroBioInstitute, bul. Dragan Tsankov 8, 1164 Sofia, Bulgaria
[b] Departament de Productes Naturals, Biologia Vegetal i Edafologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Catalonia, Spain. Fax: +34 93 402 90 43. E-mail: carlescodina@ub.edu

* Author for correspondence and reprint requests

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Leucojum aestivum (summer snowflake) is a plant species used for the extraction of galanthamine, an acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. Extracts from bulbs collected from 18 Bulgarian populations and from shoot-clumps obtained *in vitro* from 8 different populations showed variations in their alkaloid composition. Nineteen alkaloids were detected in the studied samples by GC-MS. Typically, the alkaloid fractions of *L. aestivum* bulbs were dominated by galanthamine type compounds, but lycorine, haemanthamine and homolycorine type alkaloids were also found as dominant compounds in some of the samples. Extracts from the shoot-clumps obtained *in vitro* were found to contain galanthamine or lycorine as main alkaloids. The galanthamine content ranged from 28 to 2104 μg/g dry weight in the bulbs, and from traces to 454 μg/g dry weight in the shoot-clumps.

Key words: Leucojum aestivum, *in vitro* Cultures, GC-MS

Introduction

Leucojum aestivum L. (Amaryllidaceae), known as summer snowflake, is a threatened plant species that is currently used as a commercial source of galanthamine in Bulgaria. It is gathered from the natural habitats for industrial purposes; this causes increasing problems with depletion of the wild populations. The *in vitro* cultures of galanthamine-producing plants have also attracted attention as an alternative source for the production of this compound (Codina, 2002; Diop *et al*., 2006). Galanthamine is a reversible acetylcholinesterase inhibitor which is marketed as hydrobromide for the treatment of Alzheimer's disease (Maelicke *et al*., 2001).

The biochemical variability in plants of the family Amaryllidaceae has not been widely studied. In a recent investigation on the alkaloids of *Galanthus elwesii* plants by GC-MS we found a high level of intraspecific variability in their alkaloid patterns (Berkov *et al*., 2004). With regard to *L. aestivum*, galanthamine, lycorine and homolycorine chemotypes were found in different populations in Bulgaria (Stefanov, 1990). Reasons for this phenomenon in Amaryllidaceae species are unknown. The biochemical variability in summer snowflake could serve as a source of novel bioactive compounds, but it could also influence the technological process for the extraction of galanthamine.

At the moment, the studies on the alkaloid variability of leaves and bulbs of *L. aestivum* from different populations have been carried out only by TLC (Stefanov, 1990). More recently, the galanthamine content of shoots obtained *in vitro* of this plant has been determined by HPLC (Diop *et al*., 2006). Capillary GC-MS has been proved to be a useful and reliable method for the rapid separation and identification of compounds in complex mixtures of Amaryllidaceae alkaloids (Kreh *et al*., 1995; Berkov *et al*., 2005). In contrast to HPLC and TLC techniques, capillary GC-MS allows the simultaneous separation and identification of around 20–25 alkaloids (Kreh *et al*., 1995). A sensitive MS detector can be very useful for the unambiguous identification of compounds in trace amounts or for searching galanthamine and related alkaloid metabolites in samples obtained from a few milligrams of plant material (Berkov *et al*., 2007).

As a part of our ongoing studies on the alkaloid diversity in amaryllidaceous plants, we report here for the first time variations of the major alkaloids of *in vitro* shoot-clumps obtained from plants of 8

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wild populations of *Leucojum aestivum*. Additionally, the alkaloid pattern of 35 individual bulbs collected from 18 Bulgarian populations was analyzed by GC-MS providing valuable information for the selection of galanthamine-rich genotypes.

Material and Methods

Plant material

Plants of *Leucojum aestivum* L. (Amaryllidaceae) were collected at the flowering stage (May–June) during two years, 2004 and 2005, from 2 locations of the north-east part of Bulgaria (district of Shumen) and 16 locations of the south of the country (Table I). They were placed in soil (pots) until drying of the aerial parts (in summer), and then the bulbs were cleaned and kept in a dark and cool place. The plants collected in 2004 were planted in the winter of 2005 in a greenhouse, and their bulbs collected in summer and stored until alkaloid extraction (October 2005). Vouchers were deposited in the Herbarium of Institute of Botany (Bulgarian Academy of Sciences, Sofia).

In vitro cultures

The *in vitro* cultures were initiated from bulbs collected in 2004 (Table I). The bulbs were rinsed under a stream of water and cut in "twin-scales". The explants were treated with 70% ethanol for 30 s and sterilized with 0.1% $HgCl_2$ for 3 min. Then the explants were rinsed with distilled water (three times), placed in Petri dishes on solid B5 medium (Gamborg *et al.*, 1968) supplemented with vitamins, and kept in the darkness. The culture medium used for the initiation and maintaining of the cultures contained 30 g/l of sucrose, and it was solidified with 6 g/l of agar and adjusted to pH 5.7–7.8. Casein hydrolysate (0.5 g/l), 2,4-dichlorophenoxyacetic acid (2,4-D, 1 mg/l), adenine (2 mg/l), and glutathione (10 mg/l) were added to the starting culture medium.

The *in vitro* cultures were subcultured during four months on full Murashige and Skoog (1961) medium supplemented with 1 g/l $Ca(NO_3)_2$, 0.5 mg/l benzylaminopurine (BAP), 0.01 mg/l indolebutyric acid (IBA), and 2.93 mg/l paclobutrazol. The subcultures were done at 2.5 month intervals on the MS medium described above, but without paclobutrazol. During the subcultures, the formed shoot-clumps were cut to increase the number of explants, and the newly formed shoot-clumps were separated. The *in vitro* cultures were maintained at 23–25 °C with a 16 h light (at *ca.* 2.0 klx fluorescent cool light intensity) and 8 h dark photoperiod.

Alkaloid extraction

Both the bulbs and the *in vitro* shoot-clumps were cut and dried at 60 °C until constant weight. Dried samples (200–400 mg) were powdered in a mortar and extracted with 5 ml of methanol for 12 h. After filtration, the plant residues were rinsed with methanol (2×5 ml) and the combined methanol extract was evaporated under vacuum. The dry extract was redissolved in 3% H_2SO_4 (4 ml) and defatted with diethyl ether (3×5 ml). After basification to pH 9–10 with 25% ammonia, the alkaloids were extracted with chloroform (3×5 ml). The organic solvent was dried with anhydrous Na_2SO_4 and then evaporated. The dried alkaloid fractions were redissolved in 250 μl of methanol containing 200 μg/ml of codeine as internal standard.

GC-MS analysis

The GC-MS analyses were recorded on a Hewlett Packard 6890+ MSD 5975 instrument (Hewlett Packard, Palo Alto, CA, USA) operating in the 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}, 180–300 °C at 5 °C min^{-1}, and 10 min hold at 300 °C. Injector temperature was 250 °C. The flow rate of carrier gas (helium) was 0.8 ml min^{-1}. Split ratio was 1:20. 1 μl of the methanol solutions were injected.

Galanthamine, sanguinine, narwedine, *N*-formylnorgalanthamine, epinorgalanthamine, epigalanthamine, homolycorine, 8-*O*-demethylhomolycorine, 11-hydroxyvitattine, lycorine, haemanthamine and hordenine were identified by comparing their GC-MS data and RI (Kovach retention indexes) values with those of authentic compounds isolated in our laboratory. Methyltyramine, ungiminorine and lycoramine isomers were identified by comparing their mass spectral fragmentation pattern with standard reference spectra from NIST 05 database and literature data (Kreh *et al.*, 1995; Suau *et al.*, 1988). Codeine was used as an internal standard (IS). The mass spectra were deconvoluted by AMDIS 2.64 software (NIST).

Table I. Plant material of *L. aestivum* used for alkaloid analysis.

Voucher (SOM-Co)	Location	District	Population code	Sample code	Year of collection
Wild plant (bulbs)					
1137	Svilengrad	Haskovo	I	I	2004
1138	Biser	Haskovo	J	J_1 J_2	2004
1139	Ljubimets	Haskovo	K	K_1 K_2	2005
1143	Gradina	Burgas	L	L_1 L_2	2005
1146	Goritsa	Burgas	M	M_1 M_2	2005
1141	Vinitsa	Plovdiv	M	N_1 N_2	2005
1133	Osmar	Shumen	O	O_1 O_2	2005
1140	Kochovo	Shumen	P	P_1 P_2	2005
1146	Kosharitsa	Burgas	Q	Q_1 Q_2	2004
1134	Arkutino	Burgas	R	R_1 R_2	2004
1135	Veljiov vir	Burgas	S	S_1 S_2	2004
1145	Sozopol	Burgas	T	T_1 T_2	2004
1136	Lozenets	Burgas	U	U_1 U_2	2004
1142	Chernozem	Plovdiv	V	V_1 V_2	2005
1144	Prisad	Burgas	W	W_1 W_2	2004
1150	Jambol	Jambol	X	X_1 X_2	2005
1149	Blatets	Sliven	Y	Y_1 Y_2	2005
1148	Palauzovo	Burgas	Z	Z_1 Z_2	2005
In vitro culture (explant)					
	Svilengrad	Haskovo	I	IIV	2004
	Biser	Haskovo	J	JIV	2004
	Lubimets	Haskovo	K	KIV	2004
	Arkutino	Burgas	P	PIV	2004
	Arkutino[a]	Burgas	P	PIVS	2004
	Sozopol	Burgas	T	TIV	2004
	Lozenets	Burgas	U	UIV	2004
	Prisad	Burgas	W	WIV_1 WIV_2	2004

[a] *In vitro* culture initiated from seeds.

RI of the compounds were recorded with a standard *n*-hydrocarbon calibration mixture (C_9–C_{36}) using AMDIS 2.64 software (NIST).

For comparison of the samples, the relative alkaloid content in the alkaloid fractions was calculated using the peak area of the single compound

related to the sum of the peak areas of all compounds (100% method).

Galanthamine quantification

The calibration graph was obtained injecting solutions of 5, 10, 20, 50, 75, 100, 200, 400, 600 and 1000 μg/ml of galanthamine containing 200 μg/ml of codeine by triplicate. The ratios of the peak areas of selected ions (TIC mode) of galanthamine (*m/z* at 286) versus those of the internal standard codeine (*m/z* at 299) were plotted against the corresponding concentration of galanthamine to obtain the calibration graph.

Results and Discussion

Alkaloid identification

Nineteen compounds in the alkaloid fractions of both *L. aestivum* bulbs and shoot-clumps obtained *in vitro* showed mass spectral fragmentations typical for Amaryllidaceae alkaloids and tyramine type protoalkaloids (Table II). The compounds identified in these plant materials belong to the galanthamine (**3**–**10**), lycorine (**15**, **19**), haemanthamine (**13**, **14**) and homolycorine (**11**, **12**, **16**–**18**) type alkaloids, as well as to the tyramine type protoalkaloids (**1**, **2**). Sixteen of them were identified by comparison of their mass spectra and RI with those of authentic compounds as described in Material and Methods. Tyramine type compounds are no typical Amaryllidaceae alkaloids, but they have also been found in other plant families. Compound **1** has M^+ at *m/z* 151 which is 14 mass units less than hordenine (**2**). It was identified as *N*-methyltyramine by comparison of its mass spectrum with that of a standard compound from NIST 05 database. Compound **7** showed a mass fragmentation characteristic for galanthamine type alkaloids. Ungiminorine (**19**), an alkaloid previously isolated from *L. aestivum* (Kobayashi *et al.*, 1985), was identified by comparison of its mass spectrum with that reported in the literature (Suau *et al.*, 1988). Compounds **11** and **12** have no molecular ions but they have base ions at *m/z* 109 which is characteristic for homolycorine type alkaloids with a double bound at $\Delta^{3,4}$ and no substitution at C-2 (Kreh *et al.*, 1995). So, compounds **7**, **11**, and **12** were not possible to be identified solely by GC-MS without isolation and structure elucidation by other spectroscopic methods.

Lycoramine (**5**, RI 2423) and its isomer (**8**, RI 2453), showing very similar mass spectral fragmentations but different retention times, were identified by comparison of their mass spectra with those reported in the literature (Kreh *et al.*, 1995) and with mass spectra of an authentic compound from NIST 05 database. In addition, lycoramine has 19 RI units more than galanthamine (**3**) whereas the lycoramine isomer has 30 units more than lycoramine. The same difference in RI units was reported for these compounds by Kreh *et al.* (1995). The difference in the RI values of these compounds reported in the literature and that found by us is due to the different column (HP-5 MS) used in our study. The assignment of the hydroxy group at C-3 in the β position of 11-hydroxyvitattine (**14**) was tentative by analogy to that of haemanthamine (**13**) due to the equal RI and mass spectral fragmentation of this compound and its 3α-epimer named hamayne (unpublished data). To our knowledge, compounds **1**, **2** and **4** had not been previously reported in *Leucojum aestivum* plants.

Alkaloid pattern in bulbs of wild plants

Owing to both the ontogenic and organ specific variations of alkaloids in plants (Stefanov, 1990; Elgorashi *et al.*, 2002), in this work we used only dormant bulbs, thus the plant material being in the similar ontogenic stage. The number of alkaloids detected in the samples of the different bulbs (and populations, respectively) varied greatly, from two to twelve compounds (Table II). The alkaloid patterns of the *L. aestivum* bulbs here studied were dominated by compounds coming from a *para-ortho'* (galanthamine type) and *ortho-para'* (lycorine type) oxidative coupling of *O*-methylnorbelladine (Fig. 1). Typically, the bulbs were found to contain mainly galanthamine (**3**), epinorgalanthamine (**6**), narwedine (**10**) and lycorine (**15**). Ungiminorine (**19**) was found to occur in many populations as well. Significant differences in the ratios of the individual alkaloids in the alkaloid fraction of each population were observed. Galanthamine was the dominant compound in most of the bulbs, and with the exception of the samples from the Chernozem population, galanthamine generally represented more than 50% of the total alkaloids, reaching up to 98% in the samples of the Prisad population. The percentage of lycorine also varied in a wide range, from traces up to 50% of the total alkaloid content in sample Q_1 (Kosharitsa location).

Table II. Alkaloids identified in *L. aestivum* bulbs and shoot-clumps[a].

Sample code	Galanthamine content [μg/g DW]	Methyltyramine (**1**)[1]	Hordenine (**2**)	Galanthamine (**3**)	Sanguinine (**4**)	Lycoramine (**5**)[2]	Epinor-galanthamine (**6**)	Alkaloid (galantha-mine type) (**7**)[3]	Lycoramine isomer (**8**)[4]	Epigalanthamine (**9**)	Narwedine (**10**)	Alkaloid (homo-lycorine type) (**11**)[5]	Alkaloid (homo-lycorine type) (**12**)[6]	Haemanthamine (**13**)	11-Hydroxy-vitattine (**14**)	Lycorine (**15**)	Homolycorine (**16**)	8-*O*-Demethylhomo-lycorine (**17**)	Hippeastrine (**18**)	Ungiminorine (**19**)[7]
		Retention index																		
		1456	1464	2404	2422	2423	2442	2445	2453	2454	2483	2501	2508	2633	2705	2743	2765	2817	2890	2901
I	270	tr[b]	tr	51.8												40.3				7.9
J_1	844			68.8			3.0				tr					28.1				0.1
J_2	504			65.8			1.6				2.8					29.4				
K_1	704			67.4			5.8				tr					1.0				26.4
K_2	843			66.5			4.1				tr					23.8				5.8
L_1	1148			87.3			3.2				tr					8.7				0.8
L_2	686			91.4			8.6									tr				
M_1	477			61.0			3.3									35.5				
M_2	714			57.7			3.0				tr					39.3				
N_1	1327			86.1			3.5				tr				tr	10.4				
N_2	1326			95.0			4.0				0.5					0.4				
O_1	1788			86.6	1.56		3.0				tr					7.1				1.3
O_2	1212			78.5			3.5				0.3					16.7				
P_1	2104			95.6			3.0				1.4									
P_2	940	tr	tr	67.7			2.6				tr					21.0				8.7
Q_1	399			48.3			1.8				tr					50.0				tr
R_1	805			82.9			4.5				tr					9.6				3.0
R_2	1118			96.3			3.2				0.5									
S_1	1336			99.3			0.2				0.5									
S_2	883			95.9			3.4				0.5					tr				
T_1	276	tr	tr	58.6			3.1									32.6				5.8
T_2	705			76.7			2.7			tr						10.5				10.1
U_1	1034			92.5			3.2									4.2				
U_2	424			93.4			3.3									3.3				
V_1	28			4.0		18.8		9.6	tr		1.7	7.5	7.4	34.6			2.3	3.3	10.8	
V_2	31			5.1		0.6		6.6	tr		1.5	8.0	6.7	31.5		tr	5.7	5.5	19.0	
W_1	1462	tr	tr	97.6			1.7				0.6									
W_2	1066			98.0			2.0				tr									
X_1	1190			76.7			0.9					0.5				21.9				
X_2	1062			78.3			2.3									19.4				
Y_1	339			69.5									30.5							
Y_2	409			69.3												30.8				
Z_1	895			90.9			8.4				0.8					tr				

Table II (continued).

Sample code	Galanthamine content [μg/g DW]	Methyltyramine (**1**)[1]	Hordenine (**2**)	Galanthamine (**3**)	Sanguinine (**4**)	Lycoramine (**5**)[2]	Epinor-galanthamine (**6**)	Alkaloid (galantha-mine type) (**7**)[3]	Lycoramine isomer (**8**)[4]	Epigalanthamine (**9**)	Narwedine (**10**)	Alkaloid (homo-lycorine type) (**11**)[5]	Alkaloid (homo-lycorine type) (**12**)[6]	Haemanthamine (**13**)	11-Hydroxy-vittatine (**14**)	Lycorine (**15**)	Homolycorine (**16**)	8-*O*-Demethylhomo-lycorine (**17**)	Hippeastrine (**18**)	Ungiminorine (**19**)[7]
		Retention index																		
		1456	1464	2404	2422	2423	2442	2445	2453	2454	2483	2501	2508	2633	2705	2743	2765	2817	2890	2901
Z_2	352			73.6			11.7				tr					14.7				
IIV	169			32.9												77.1				
JIV	350			100			tr													
KIV	25			100												tr				
PIV	454			100												tr				
PIVS	335			100																
SIV	143			100																
TIV	tr			tr																
UIV	346	tr		84.9												15.9				
WIV_1	82	tr		41.2												58.9				
WIV_2	57			100			tr				tr				tr	tr				

[a] The area of the GC-MS peaks depends not only on the concentration of the corresponding compounds but also on the intensity of their mass spectral fragmentation; so the data given in the table do not correspond to a real quantification but can be used for comparison of the samples, which was the objective of this work.

[b] Traces.

Identification, MS (rel. int.):

[1] NIST 05: M^+ 151 (50), 120 (15), 91 (25), 89 (12), 77 (80), 65 (23), 63 (18), 55 (19), 53 (28), 51 (38).

[2] Kreh *et al.* (1995); NIST 05: M^+ 289 (61), 288 (100), 274 (3), 232 (8), 213 (8), 202 (14), 187 (14), 174 (11), 128 (12), 115 (19), 103 (7).

[3] M^+ 287 (73), 286 (100), 272 (3), 230 (8), 218 (17), 202, (29), 187 (18), 174 (11), 159 (15), 128 (14), 115 (23), 103 (6).

[4] Kreh *et al.* (1995); NIST 05: M^+ 289 (60), 288 (100), 274 (3), 232 (9), 213 (8), 202 (16), 187 (16), 174 (11), 128 (13), 115 (20), 103 (5).

[5] M^+ (-), 301 (0.5), 191 (5), 110 (10), 109 (100), 108 (17), 94 (3), 82 (2).

[6] M^+ (-), 201 (0.5), 177 (6), 110 (8), 109 (100), 108 (15), 94 (3), 82 (2).

[7] Suau *et al.* (1988); M^+ 317 (9), 316 (19), 299 (56), 268 (100), 250 (39), 242 (54), 226 (20), 225 (20), 224 (26), 214 (68), 212 (75), 173 (15), 154 (17), 147 (31), 135 (23), 128 (10), 119 (20), 115 (11).

In comparison to the rest of the samples, those of the Chernozem population differed significantly regarding both the alkaloid skeleton types and alkaloid profile (Table II). Thus, compounds coming from a *para-para'* oxidative coupling of *O*-methylnorbelladine (haemanthamine type alkaloids) were found to occur together with alkaloids coming from *ortho-para'* and *para-ortho'* oxidative couplings. In addition to lycorine type alkaloids, the products of the *ortho-para'* oxidative coupling were also accumulated as homolycorine derivatives in those plants. The main alkaloid was haemanthamine (**13**), whereas lycorine type alkaloids (lycorine) were detected only as traces. Instead of galanthamine, plants of this population accumulated a higher amount of its 4,4a-dihydroderivative, lycoramine (**5**), in the bulbs. The ratio between galanthamine, haemanthamine and homolycorine type alkaloids was almost equivalent. In contrast to the rest of the samples, the percentage and amount of galanthamine found in the alkaloid fractions of the bulbs from the Chernozem population were significantly lower. The galanthamine content of the bulbs varied in a wide range, from 28 to 2104 μg/g dry weight (DW).

In a previous study, we found a population of *L. aestivum* in the west-north region of Bulgaria which contained over 50% of epinorgalanthamine in the alkaloid fraction of the bulbs at the flowering stage (Berkov *et al*., 2005). Thus, up to the moment, galanthamine, lycorine, haemanthamine and epinorgalanthamine have been found to occur as the main alkaloids in *L. aestivum* bulbs. The occurrence of different alkaloid biosynthetic pathways in summer snowflake explains the chemotypes found in this plant species. Such a biochemical deviation in the alkaloid profiles has also been reported for another amaryllidaceous plant, *Galanthus elwesii* (Berkov *et al*., 2004), as well as for plants of other families, like Fabaceae (*Genista lobelia*; Kirch *et al*., 1995) and Asteraceae (*Senecio jacobaea*; Macel *et al*., 2004).

Alkaloid pattern in the in vitro obtained plantlets

The six alkaloids identified in shoot-clumps of *L. aestivum* obtained *in vitro* are shown in Table II. Galanthamine and lycorine were found to be the main alkaloids, whereas the rest of the compounds occurred in trace amounts. It is noteworthy that some of the shoot-clumps obtained *in vitro* produced mainly galanthamine, whereas others accumulated lycorine as main compound. Also, the two *in vitro* strains initiated from bulbs of the Prisad location showed a remarkable difference in their alkaloid pattern, one of them producing mainly galanthamine (approx. 100% of the total alkaloids, sample WIV_2) and the other producing mainly lycorine (59% of the total alkaloid fraction, sample WIV_1). Some shoot-clumps showed an alkaloid pattern similar to that of the wild plants, like those obtained from the Svilengrad and Veljiov vir locations, whereas the plantlets obtained from the Biser population did not show such a correlation. The content of galanthamine was found to range from traces to 454 μg/g DW in the shoot-clumps obtained *in vitro*. The accumulation of this compound in both the intact plants and the shoot-clumps obtained *in vitro* is in accordance with previously reported data for summer snowflake (Stefanov, 1990; Diop *et al*., 2006).

The results obtained in the present work are in agreement with those reported recently by Diop *et al*. (2006), who studied the variation in galanthamine accumulation in explants of *L. aestivum* grown on culture media with different combinations of growth regulators. These authors found a remarkable variation in the content of this alkaloid among the explants they obtained, but they did not determine the levels of other alkaloids from this plant species. Our results also showed a variation in the galanthamine content in the *in vitro* shoot-clumps initiated from different populations of *L. aestivum* but grown on the same culture medium (only one combination of growth regulators). Furthermore, the results of the GC-MS analysis showed, as mentioned above, not only a variation in the galanthamine content but also in the alkaloid profile among the *in vitro* cultures initiated from different genotypes, and even among different strains. Despite galanthamine, the alkaloid pattern is also an important feature which can be used to optimize the composition of the culture medium aiming to the manipulation of the biosynthetic pathway of the plants and also for the selection of *in vitro*-obtained explants with specific alkaloid profiles.

In former studies, it was found that the production of galanthamine in *L. aestivum* plants can be influenced by genetic factors, growth conditions and/or chemical composition of the soil (Stefanov, 1990; Poulev *et al*., 1993; Gorinova *et al*., 1993). However, the reasons for this variation in the alkaloid profile of wild summer snowflake and of

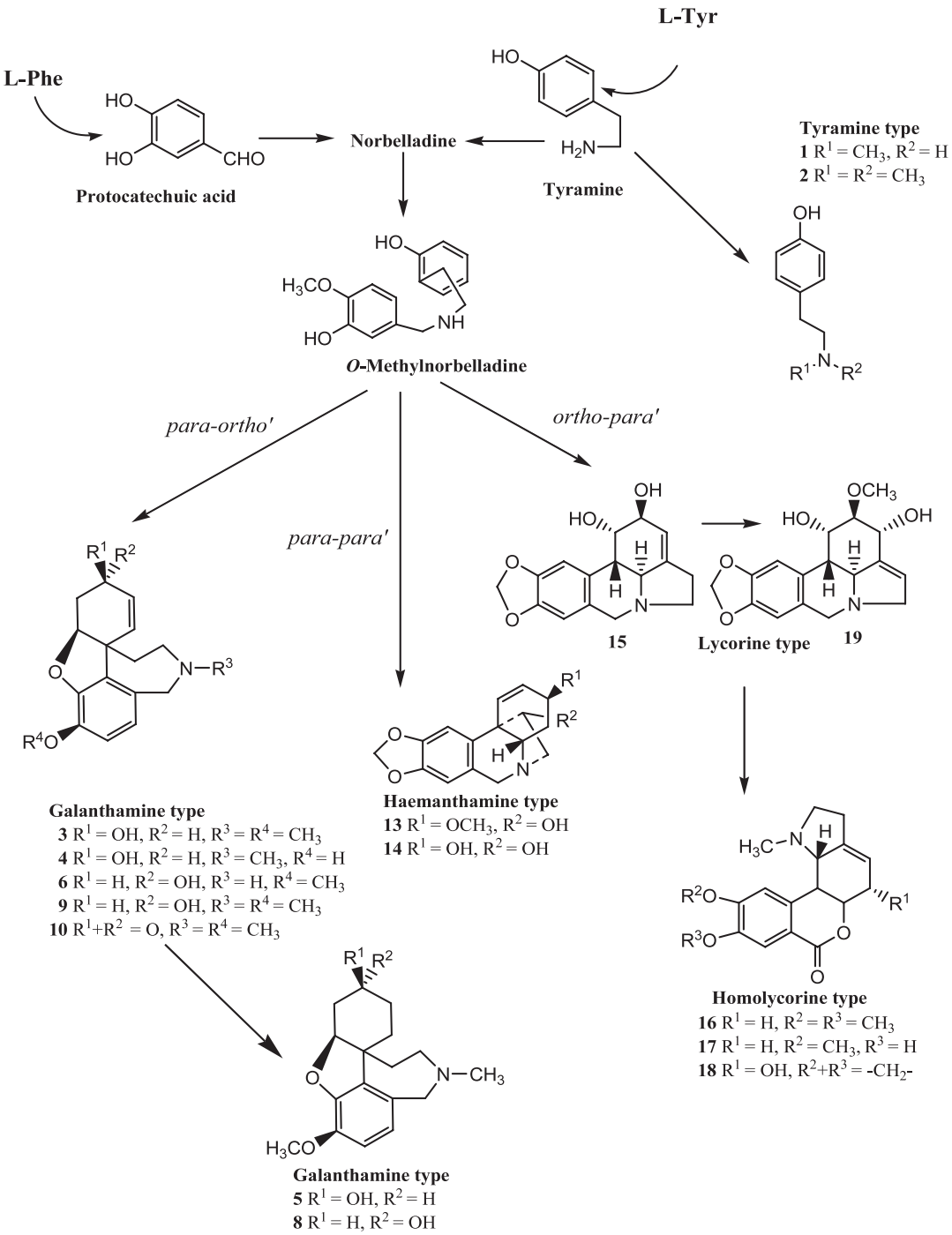

Fig. 1. Biosynthetic relationships of the alkaloids identified in *L. aestivum* bulbs and *in vitro* cultures.

shoot-clumps obtained *in vitro* are unknown. Due to the importance of summer snowflake as a commercial source of galanthamine, there is a need for further investigations, on the one hand on the factors determining the alkaloid variations in both the *in vitro* cultures and intact plants, and on the other hand on the variations of the alkaloid patterns within and among wild populations. The variations in both the alkaloid content and alkaloid profile of the *in vitro* shoot-clumps (cultivated under the same conditions) and wild plants suggest that the genetic factor plays an important role in the alkaloid biosynthesis in this plant species. In this respect, an intensive selection, both in wild plants and *in vitro* cultures, is necessary to obtain galanthamine-rich cultivars for the extraction of this pharmaceutically interesting substance.

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