Alkaloids from *Sternbergia colchiciflora*

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Twenty-one alkaloids and related compounds were found in *Sternbergia colchiciflora* (Amaryllidaceae), a hitherto not studied plant species. Twenty of them were detected by GC-MS in the crude extracts of this plant species. Ten alkaloids were isolated and their structures confirmed by NMR, MS and CD measurements. Many of the compounds found in this species, such as lycorine, tazettine, haemanthidine, are known to possess strong bioactivity. Variations in the alkaloid pattern were found during the phenological cycle of the plant. Lycorine-type compounds were dominant in the plant organs during both the flowering period and dormancy. The alkaloid pattern during both periods of leaf development and fructification was dominated by haemanthamine-type in the leaves and lycorine-type compounds in the bulbs, respectively.

Key words: Sternbergia colchiciflora, Amaryllidaceae, Alkaloids, GC-MS

Introduction

Sternbergia colchiciflora Waldst. & Kit. is an amaryllidaceous plant distributed in the Mediterranean region (Webb, 1980). Due to its small size, it has no economic or ornamental value, and there is no data on its use in folk medicine. Plants of the family Amaryllidaceae are known for their specific alkaloid composition. To our knowledge, three species of nine in the genus *Sternbergia* have been phytochemically studied and the results revealed that haemanthamine-, lycorine- and tazettine-type compounds dominate their alkaloid patterns (Evidente *et al*., 1984; Pabuççuoglu *et al*., 1989; Richomme *et al*., 1989; Tanker *et al*., 1996). In the literature, however, there are no reports on the alkaloids of *S. colchiciflora*. The Amaryllidaceae alkaloids are bioactive compounds possessing a wide range of activities, including cytotoxic, antiviral, apoptotic, acetylcholinesterase inhibitory activities (Bastida *et al*., 2006; McNulty *et al*., 2007). Analgesic and antimicrobial activities have been reported for extracts and alkaloids from *S. clusiana*, *S. sicula* and *S. lutea* (Tanker *et al*., 1996; Unver *et al*., 2005). Despite the extensive studies on the chemistry and bioactivity of Amaryllidaceae alkaloids, there are scanty data on their phenological and organ-to-organ variation (Elgorashi *et al*., 2002, 2003). In the present work we report the pattern, organ-to-organ and seasonal variation of the alkaloids in *S. colchiciflora*.

Material and Methods

Plant material

Plants of *S. colchiciflora* were collected for alkaloid isolation in March 2006, near the village of Ioglav, district of Lovech, Bulgaria. A voucher specimen (Co 3998 SOM) was deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Plants, collected in September 2005, were transplanted in pots at the greenhouse of the Faculty of Pharmacy, University of Barcelona, Spain. Fresh plant organs from three individuals per each phenological stage: developed leaves (March), fructification (May), bulb dormancy (July), and flowering (September) were sampled for GC-MS analyses.

Alkaloid extraction and isolation

Fresh plant material (*ca*. 1 kg) was crushed and extracted with 95% EtOH (3 × 5 l). After solvent evaporation under reduced pressure, the

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dry residue was dissolved in 200 ml of 2% H_2SO_4 and filtered. The acidic solution was defatted with diethyl ether (3 × 300 ml) and basified with 25% ammonia to pH 9–10. The alkaloid mixture was extracted with *n*-hexane (3 × 300 ml, extract A, 0.18 g), EtOAc (5 × 300 ml, extract B, 0.78 g), and finally with a mixture of EtOAc/MeOH (3:1, 3 × 300 ml, extract C, 2.40 g). Tazettine (**13**, 52 mg) and lycorine (**17**, 309 mg) crystallized from extracts A and B, respectively. Extracts A and B were combined and subjected to CC (3 cm × 65 cm column) on Kieselgel (100 g, mesh 45–60 μm). The alkaloids were eluted using EtOAc gradually enriched with MeOH. Fractions of 50 ml each were collected (45 in total), monitored by TLC (Dragendorff's reagent, UV light 254 nm), and combined according to their TLC profiles. Vittatine (**6**, 13 mg), 8-*O*-demethylmaritidine (**8**, 3 mg) haemanthamine (**12**, 8 mg), 11-hydroxyvittatine (**14**, 20 mg), hamayne (**15**, 11 mg), and haemanthidine (**16**, 14 mg) were isolated. Pseudolycorine (**19**, 14 mg) and ungeremine (**21**, 2 mg) were obtained from extract C by CC as described for extracts A and B. 5 mg of the three extracts dissolved in 250 μl of MeOH were subjected to GC-MS analysis for the identification of minor alkaloids.

Extraction of plant organs

Fresh plant organs were extracted separately with 95% ethanol (3 × 5 ml). The combined ethanolic extracts were evaporated, dissolved in 5 ml of 2% H_2SO_4 and filtered. The acidic solutions were defatted with diethyl ether (3 × 5 ml) and basified with 25% ammonia to pH 9–10. The alkaloids were extracted with EtOAc (3 × 5 ml), and the solvent was evaporated under a stream of nitrogen. The dried alkaloid mixtures were redissolved in 250 μl of MeOH for further GC-MS analyses.

Gas chromatography-mass spectrometry

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 at 5 °C min^{-1} and a 10-min hold at 300 °C. Injector temperature was 250 °C. The flow rate of the carrier gas (helium) was 0.8 ml min^{-1}. Split ratio was 1:20. 1 μl of the solution was injected.

The spectra of co-eluting chromatographic peaks were examined and deconvoluted by use of AMDIS 2.64 (NIST, Gaithersburg, MD, USA) software before area integration. The contribution of each compound in the extracts of the plant organs is shown in Table II as percentage of the total ion current (TIC).

Alkaloid identification

The isolated alkaloids vittatine (**6**), 8-*O*-demethylmaritidine (**8**), haemanthamine (**12**), tazettine (**13**), 11-hydroxyvittatine (**14**), hamayne (**15**), haemanthidine (**16**), lycorine (**17**), pseudolycorine (**19**), and ungeremine (**21**) were identified by both direct comparison of their chromatographic and spectroscopic properties (^{1}H NMR, GC-MS, MS, CD) with those of authentic samples obtained in our laboratory from other plant sources and literature data (Bastida *et al.*, 2006). Hordenine (**3**), ismine (**4**) (Berkov *et al.*, 2007a, b), and 3-epimacronine (**18**) (Viladomat *et al.*, 1990) were identified applying GC-MS co-chromatography with previously isolated authentic standards. Tyramine (**1**) (Witte *et al.*, 1987), trisphaeridine (**5**) (Ali *et al.*, 1986), 11,12-didehydroanhydrolycorine (**11**), previously reported as 4,5-dehydroanhydrolycorine (Ghosal *et al.*, 1986), and ungiminorine (**20**) (Suau *et al.*, 1988) were tentatively identified by comparing their mass spectra with those reported in the literature. Methyltyramine (**2**), anhydrolycorine (**7**), 2,11-didehydro-2-dehydroxylycorine (**9**), and 11-deoxytazettine (**10**) were tentatively identified by comparing their mass spectral fragmentation with standard reference spectra from NIST 05 database [NIST Mass Spectral Database, PC-Version 5.0 (2005), National Institute of Standardization and Technology, Gaithersburg, MD, USA].

Kovats retention indexes (RI) of the compounds were recorded compared to standard calibration *n*-hydrocarbon mixture (C_9–C_{36}) using AMDIS 2.64 software (NIST).

Results and Discussion

Alkaloid identification

GC-MS has been proved to be a powerful tool for rapid separation and identification of Amaryllidaceae alkaloids without derivatization (Kreh *et al.*, 1995; Berkov *et al.*, 2008). Preliminary GC-MS analyses of samples from *S. colchiciflora* indicated compounds with the crinane-type structure as

well as compounds with unknown GC-mass spectra. To assign the absolute configuration of the crinane alkaloids and to identify unambiguously the compounds with unknown GC-mass spectra, the crude alkaloid extract was subjected to fractionation for isolation of individual compounds.

A total of twenty-one compounds were identified in *S. colchiciflora* (Table I) which displayed Amaryllidaceae alkaloids of the haemanthamine-, tazettine-, narciclasine-, and lycorine-types. Compounds with galanthamine-, homolycorine-, or monthanine-type skeletons were not detected. Some alkaloids were found by GC-MS as traces in a concentrated sample of the crude extract but not in samples collected during the phenological cycle (Table II). Ten alkaloids (**6**, **8**, **12**–**17**, **19** and **21**) were isolated from the crude alkaloid extract (Table I). CD analysis of the isolated crinane alkaloids showed a minimum around 245 nm and a maximum around 280 nm indicating an α configuration of their 5,10b-ethano bridge, thus showing that they belong to the haemanthamine-type (Bastida *et al*., 2006).

The GC-mass spectra of 11-hydroxyvittatine (**14**), hamayne (**15**) and haemanthidine (**16**) differed significantly from those recorded by direct inlet. Differences between GC-MS and direct inlet fragmentation were described for haemanthamine (**12**) (Kreh *et al*., 1995). GC-mass spectra and RI values of the C-3 epimers 11-hydroxyvittatine and hamayne were identical. The assignment of the substituent at position C-3 of these compounds was not possible by GC-MS, therefore they were considered as 11-hydroxyvittatine in the GC-MS analyses. Ungeremine (**21**) was not detected by GC-MS in the samples, probably because of its salt-like character and high polarity.

The alkaloid pattern of the extracts from *S. colchiciflora* suggests that they have strong biological activities such as cytotoxic (lycorine, haemanthamine, haemanthidine, tazettine), apoptotic (haemanthamine), and antiviral (lycorine) (Evidente *et al*., 1986; Bastida *et al*., 2006; McNulty *et al*., 2007).

Organ-to-organ and phenological variation of the alkaloids

Sixteen compounds with organ-to-organ and seasonal variations were detected by GC-MS during the phenological cycle (Table II, Figs. 1 and 2). In general, the alkaloid synthesis of *S. colchici-*

Table I. GC-MS data of the alkaloids and related compounds found in the extracts of *S. colchiciflora*.

Compound	RI	M^+	*m/z* (rel. int.)
Tyramine (**1**)[a, c]	1415	137(26)	120(3), 108(100), 107(90), 91(9), 77(35), 65(5), 30(96)
Methyltyramine (**2**)[a]	1458	151(40)	120(15), 108(42), 107(100), 91(26), 77(90), 65(25)
Hordenine (**3**)[b]	1468	165(1)	121(1), 107(1), 91(1), 77(4), 58(100)
Ismine (**4**)[b]	2280	257(34)	238(100), 225(6), 211(6), 196(8), 180(6), 168(8)
Trisphaeridine (**5**)[c]	2282	223(100)	222(41), 167(8), 165(9), 164(14), 138(20), 111(12)
Vittatine (**6**)[d]	2435	271(100)	254(13), 228(25), 199(95), 187(90), 157(28), 128(32)
Anhydrolycorine (**7**)[a]	2503	251(46)	250(100), 220(2), 192(13), 191(12), 165(3), 124(7)
8-*O*-Demethylmaritidine (**8**)[d]	2512	273(100)	256(22), 230(20), 201(82), 189(42), 174(23), 128(23)
2,11-Didehydro-2-dehydroxylycorine (**9**)[a]	2533	269(13)	268(27), 251(71), 250(100), 220(15), 192(20), 191(16)
11-Deoxytazettine (**10**)[a]	2541	315(21)	300(15), 250(6), 231(100), 211(16), 169(6), 141(8)
11,12-Didehydroanhydrolycorine (**11**)[c]	2605	249(63)	248(100), 190(29), 163(9), 123(12), 95(28)
Haemanthamine (**12**)[d]	2641	301(12)	272(100), 257(10), 240(16), 211(18), 199(11), 181(37)
Tazettine (**13**)[d]	2655	331(20)	316(100), 298(17), 247(100), 230(14), 181(20)
11-Hydroxyvittatine (**14**)[d]	2712	287(5)	258(100), 242(9), 212(14), 186(18), 181(21), 152(11)
Hamayne (**15**)[d]	2712	287(2)	258(100), 242(7), 212(12), 186(17), 181(13), 153(10)
Haemanthidine (**16**)[d]	2722	317(59)	284(52), 260(34), 201(78), 199(77), 181(71), 115(100)
Lycorine (**17**)[d]	2754	287(31)	268(24), 250(15), 227(85), 226(100), 211(7), 147(15)
3-Epimacronine (**18**)[b]	2821	329(26)	298(22), 245(99), 201(100), 139(31), 128(23), 70(31)
Pseudolycorine (**19**)[d]	2830	289(23)	288(16), 270(21), 229(70), 228(100), 214(10), 147(13)
Ungiminorine (**20**)[c]	2911	317(10)	316(19), 299(56), 268(100), 250(40), 242(54), 214(75)
Ungeremine (**21**)[d]	–		

Identification: [a] NIST05 database; [b] authentic standard, co-chromatography; [c] literature data; [d] isolated from the plant.

Table II. Alkaloids (% of TIC) found in *S. colchiciflora* during the phenological cycle. The results are presented as the mean of three individuals.

Compound	I			II			III				IV	
	Roots	Bulbs	Flowers	Roots	Bulbs	Leaves	Roots	Bulbs	Leaves	Seeds	Bulbs	Leaves
Hordenine (**3**)	0.1											
Ismine (**4**)		0.1	0.9			0.1						
Trisphaeridine (**5**)	0.3	0.8	1.8		0.3	0.8		2.2	11.8	3.4	4.2	
Vittatine (**6**)	2.5	3.1	15.2		3.0	72.1		2.4	58.9		0.4	
Anhydrolycorine (**7**)	0.8	19.0	7.2	4.3	14.3	0.5		26.7	1.3	20.5	45.2	
8-*O*-Demethylmaritidine (**8**)						1.4						
2,11-Didehydro-2-dehydroxylycorine (**9**)		3.1			1.4			2.0			2.5	
11-Deoxytazettine (**10**)		1.5			0.2			0.7	0.3		0.8	
11,12-Didehydroanhydrolycorine (**11**)	0.7	8.8	2.6	5.4	5.4	0.7	traces	45.3	21.1	76.1	42.0	traces
Haemanthamine (**12**)	0.6	6.3	1.7		1.6	0.6		4.5	1.8			
Tazettine (**13**)	22.1	5.3	26.6	4.8	0.6	13.6		0.2	0.5			
11-Hydroxyvittatine (**14**)	0.3	3.0										
Haemanthidine (**16**)	4.2		5.5									
Lycorine (**17**)	68.4	48.5	38.9	85.4	71.9	9.8		16.0	1.5		4.7	
3-Epimacronine (**18**)						0.3						
Pseudolycorine (**19**)	0.1	0.4	0.5									

Phenological stages: I, flowering; II, developed leaves; III, fructification; IV, bulb dormancy.

flora was dominated by lycorine-type compounds coming from *ortho-para'* oxidative coupling of *O*-methylnorbelladine. Exceptions were found in the alkaloid patterns of the leaves, during leaf development and fructification. The number of alkaloids decreased from the flowering period up to the period of dormancy. Thus, the flowering plants contained the highest number of alkaloids from lycorine, haemanthamine, tazettine, narciclasine, and tyramine types. Roots, bulbs and flowers (*S. colchiciflora* does not develop leaves during the flowering stage) were dominated by lycorine (**17**). A relatively high portion of tazettine- and haemanthamine-type alkaloids was found in both flowers and roots.

A tendency to accumulate alkaloids coming from different oxidative coupling processes in both underground and aerial parts was observed. During the early phenological stages (flowering and leaf development), the alkaloid patterns of bulbs and roots were dominated by lycorine-type alkaloids, mainly lycorine (**17**). In contrast, the aerial parts (leaves) accumulated mainly vittatine (**6**, 72.1%), tazettine (**13**, 13.6%), and other alkaloids coming from the *para-para'* oxidative coupling (haemanthamine and tazettine types). During fructification, the bulbs and leaves accumulated lycorine- and haemanthamine-type alkaloids, respectively (Fig. 1 and Table II). The portion of lycorine in the alkaloid mixtures of bulbs and seeds was significantly decreased while the lycorine derivatives **7** and **11** were dominant. Vittatine (**6**) was the main alkaloid in the leaves

Fig. 1. Distribution of alkaloids during the phenological cycle. Phenological stages: I, flowering; II, developed leaves; III, fructification; IV, bulb dormancy.

(58.9%). During the period of dormancy, the alkaloid patterns of the bulbs were dominated by compounds **7** and **11**. Traces of **11** were found in the senile (dry and yellow) leaves. It is interesting to note that the alkaloids **7** and **11** are potent inhibitors of ascorbic acid synthesis, inhibiting the cell division in plants and fungi (Evidente *et al.*, 1986). Their accumulation in the seeds and dormant bulbs could be associated with a possible defense role.

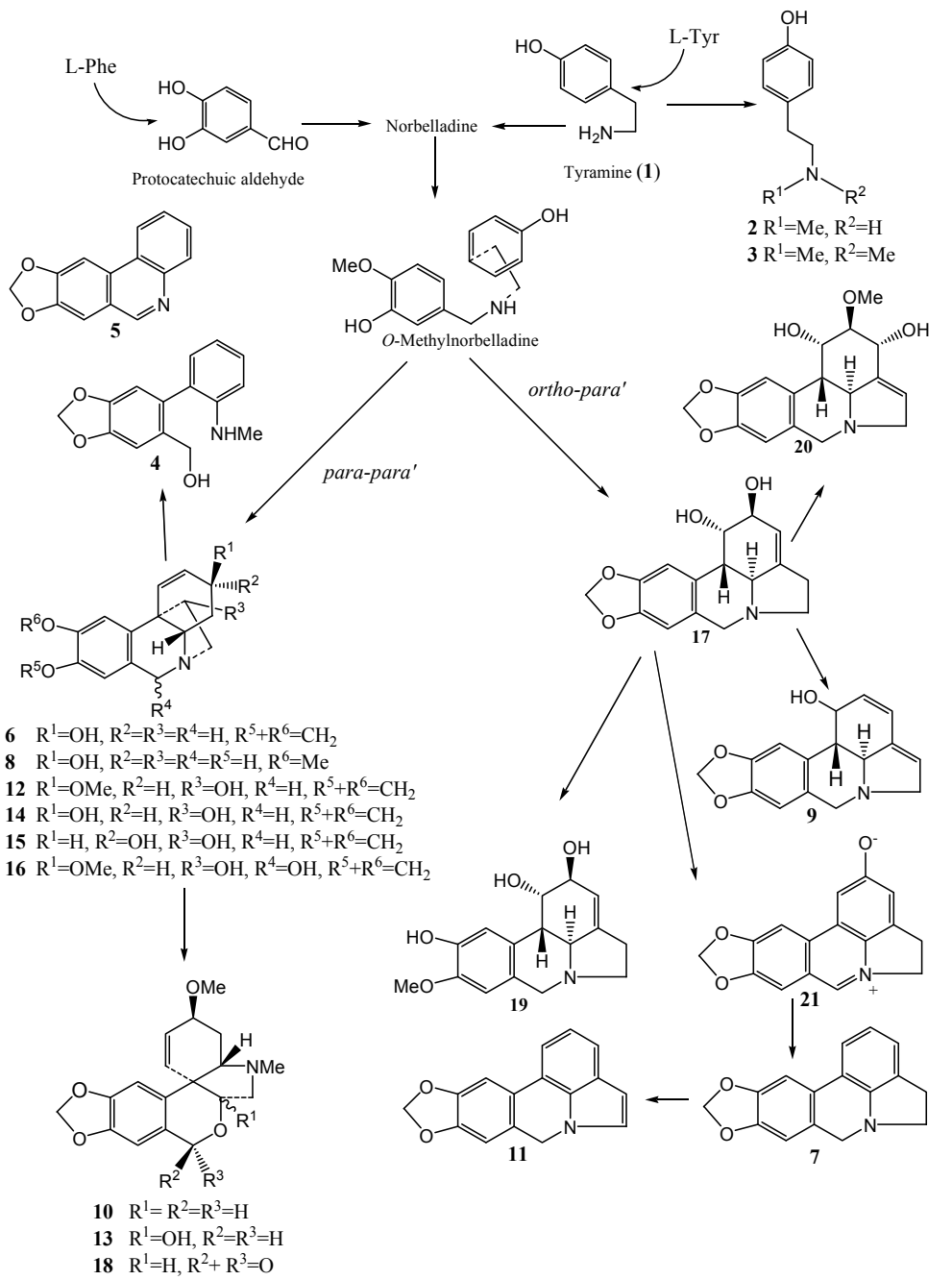

Fig. 2. Biosynthetic relationship of the alkaloids identified in *Sternbergia colchiciflora.*

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

Bastida J., Lavilla R., and Viladomat F. (2006), Chemical and biological aspects of *Narcissus* alkaloids. In: The Alkaloids, Vol. 63 (Cordell G. A., ed.). Elsevier Scientific Publishing, Amsterdam, pp. 87–179.

Berkov S., Codina C., Viladomat F., and Bastida J. (2007a), Alkaloids from *Galanthus nivalis*. Phytochemistry **68**, 1791–1798.

Berkov S., Reyes-Chilpa R., Codina C., Viladomat F., and Bastida J. (2007b), A revised NMR data of incartine: an alkaloid from *Galanthus elwesii.* Molecules **12**, 1430–1435.

Berkov S., Bastida J., Sidjimova B., Viladomat F., and Codina C. (2008), Phytochemical differentiation of *Galanthus nivalis* and *Galanthus elwesii* (Amaryllidaceae); a case study. Biochem. Syst. Ecol. **36**, 638–645.

Elgorashi E., Drewes S., and van Staden J. (2002), Organ to organ and seasonal variation in alkaloids from *Crinum macowanii*. Fitoterapia **73**, 490–495.

Elgorashi E., Drewes S., Morris C., and van Staden J. (2003), Variation among three *Crinum* species in alkaloid content. Biochem. Syst. Ecol. **31**, 601–615.

Evidente A., Iasiello I., and Randazzo G. (1984), Isolation of sternbergine, a new alkaloid from bulbs of *Sternbergia lutea*. J. Nat. Prod. **47**, 1003–1008.

Evidente A., Arrigoni O., Liso R., Calabrese G., and Randazzo G. (1986), Further experiments on structure-activity relationship among the lycorine alkaloids. Phytochemistry **25**, 2739–2743.

Ghosal S., Kumar Y., Chakrabarti D., Lal J., and Singh S. (1986), Parasitism of *Imperata cylindrica* on *Pancratium biflorum* and the concomitant chemical changes in the host species. Phytochemistry **25**, 1097–1102.

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

McNulty J., Nair J., Codina C., Bastida J., Pandey S., Gerasimoff J., and Griffin C. (2007), Selective apoptosis-inducing activity of crinum-type Amarylidaceae alkaloids. Phytochemistry **68**, 1068–1074.

Pabuççuoglu V., Richomme P., Guzler T., Freyer A. J., and Shamma M. (1989), Four new crinine-type alkaloids from *Sternbergia* species. J. Nat. Prod. **52**, 785–791.

Richomme P., Pabuççuoglu V., Tekant G., Alan F., and Shamma M. (1989), Siculinine: A lycorine-type alkaloid from *Sternbergia sicula*. J. Nat. Prod. **52**, 1150–1152.

Suau R., Gómez A., Rico R., Vázquez-Tato M., Castedo L., and Riguera R. (1988), Alkaloid *N*-oxides of Amaryllidaceae. Phytochemistry **27**, 3285–3287.

Tanker M., Citoglu G., Gumuhel B., and Hener B. (1996), Alkaloids of *Sternbergia clusiana* and their analgetic effects. Int. J. Pharmacog. **34**, 194–197.

Unver N., Kaya I., and Ozturk T. (2005), Antimicrobial activity of *Sternbergia sicula* and *Sternbergia lutea.* Fitoterapia **76**, 226–229.

Viladomat F., Bastida J., Tribó G., Codina C., and Rubiralta M. (1990), Alkaloids from *Narcissus bicolor*. Phytochemistry **29**, 1307–1310.

Webb D. A. (1980), Genus *Sternbergia*. In: Flora Europaea, Vol. 5 (Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M., and Webb D. A., eds.). Cambridge University Press, Cambridge, p. 76.

Witte L., Muler K., and Alfermann H.-A. (1987), Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas-liquid-chromatography-mass-spectrometry. Planta Med. **53**, 192–197.