Comparative Analysis of the Composition of Flower Volatiles from *Lamium* L. Species and *Lamiastrum galeobdolon* Heist. ex Fabr.

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Z. Naturforsch. **58 c**, 779–782 (2003); received March 26/May 26, 2003

The volatiles of fresh flowers from nine natural populations of four *Lamium* species and *Lamiastrum galeobdolon* were analyzed by GC/MS. 49 compounds, 43 of them new for *Lamium* and *Lamiastrum*, were identified. The studied samples showed similarity of the volatile profiles and dependence of the oil-composition on the collection site. Significant amounts of squalene were found in all samples. The presence of homological series of straight chain alkanes from C_{12} to C_{31} was shown. Phenethyl alcohol was found only in *L. maculatum* f. *alba*.

Key words: Lamium, *Lamiastrum*, Volatiles

Introduction

In Bulgaria occur seven of the known about 40 *Lamium* species (*L. album* L., *L. amplexicaule* L., *L. maculatum* L., *L. moschatum* L., *L. purpureum* L., *L. bifidum* L., *L. garganicum* L.) and the sole *Lamiastrum galeobdolon* Heist. ex Fabr. (= *Galeobdolon luteum* Huds.) (Asenov, 1989). *Lamium* plants are used in official and folk medicine as blood tonic, uterytonic, astringent, antispasmodic and anti-inflammatory agents, and for prostrate, skin and respiratory disorders (Bremness, 1995). Until now, the composition of the essential oils from *L. purpureum* (Kurihara and Kikuchi, 1976) and aerial parts of *L. garganicum* (Roussis *et al.*, 1996) and *L. maculatum* (El-Sattar *et al.*, 1993) were studied. 1-Octen-3-ol, phenethyl alcohol, *o*-, *m*- and *p*-cresols, guajakol and eugenol were reported as main components in *L. purpureum* and respectively, 1,8-cineol (47.55% of total volatiles), citronelal (25.12%) and isoeugenol (11.81%) in *L. garganicum* and hexahydrofarnesylacetone (22%) in *L. maculatum*.

In this paper, we report the results of the GC/MS comparative analysis of the volatiles from fresh flowers of the perennials *L. album*, *L. garganicum* (two populations), *L. maculatum* (three populations) and *Lamiastrum galeobdolon* and the annual species *L. purpureum* (two populations).

Experimental

Plant material

Flowers from nine populations of *Lamium* and *Lamiastrum* species were collected in April and May 2002 as follows: *L. album* at Vlado Trichkov (No 1); *L. purpureum* at Sofia (No 2) and Vlado Trichkov (No 3); *L. garganicum* at the Losen mountain (No 4) and Christo Danovo (No 5); *L. maculatum* s. str. at Ljaskovetz (No 6), the Losen mountain (No 7) and f. *alba* at Ljaskovetz (No 8); *Lamiastrum galeobdolon* at Christo Danovo (No 9). Voucher specimens were identified by Dr. L. Evstatieva and deposited in the herbarium of the Institute of Botany (SOM), Bulgarian Academy of Sciences, Sofia.

Isolation of volatiles

Fresh flowers of *Lamium album* (No 1: 9.7 g), *L. purpureum* (No 2: 1.5 g; No 3: 1.9 g) *L. garganicum* (No 4: 10.5 g; No 5: 12.8 g), *L. maculatum* (No 6: 5.0 g; No 7: 9.8 g; No 8: 1.8 g), and *Lamiastrum galeobdolon* (No 9: 10.3 g) were subjected to a 4 h distillation-extraction in a Likerson-Nickerson apparatus. The volatiles were collected in diethyl ether and dried over anhydrous sodium sulfate [yields in % of fresh wt: 0.02% (No 1), 0.1% (No 2), 0.06% (No 3), 0.03% (No 4), 0.01%

0939–5075/2003/1100–0779 $ 06.00

(No 5), 0.02% (No 6), 0.01% (No 7), 0.06% (No 8), 0.01% (No 9)].

GC/MS

The analysis was performed with a Hewlett-Packard Gas Chromatograph 5890 Series II Plus linked to Hewlett-Packard 5972 mass spectrometer system (Hewlett-Packard, Palo, Alto, CA, USA) equipped with a HP5-MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, Wilmington, DE, USA). The temperature was programmed from 40 °C to 280 °C at a rate of 6 °C/min. The ion source was set at 250 °C and ionization voltage at 70 eV. Helium was used as carrier gas.

Identification of compounds

The GC/MS identification was based on the interpretation of the mass spectral fragmentation followed by comparisons of the spectra obtained with those of authentic samples. Computer searches in HP Mass Spectral Library NIST98 Wiley were also applied. If possible reference compounds were co-chromatographed to confirm GC retention times. When no suitable authentic samples and/or spectra for comparison were available, no identification was proposed.

Results and Discussion

The results are summarized in Table I. 49 compounds were identified. To the best of our knowledge, 43 of them are reported here for the first time for *Lamium* and *Lamiastrum*. Similarity of the volatile profile of all samples was shown. Qualitative and quantitative differences in the oil-composition isolated from plants collected at different locations were observed.

All studied samples contained significant amounts of hydrocarbons with C_{12} to C_{31} carbon atoms almost exclusively with straight chains and fully saturated. As expected, hydrocarbons with odd numbers of carbon atoms prevailed over those with even numbers. The ratio of odd/even hydrocarbons was higher for the *Lamium* species (1:1 to 3:1) than for *Lamiastrum* (6:1). Changes of this ratio for samples from different locations were also found. Two mono unsaturated hydrocarbons were present in four of the samples (Nos 3, 9, 6, 8).

Aldehydes often possess allelochemical functions in plants serving as attractants, repellents, pheromones, etc. Aldehydes were found in traces in most of the studied samples. Nonanal and decanal are characteristic of the so-called "green odor" taking part in higher plant-insect relationships (Wang *et al.*, 1999) even in low concentrations. That's why some allelopathic functions could be expected. Benzene acetaldehyde was found only in *Lamiastrum galeobdolon*.

Free fatty acids are often found in volatiles from plants and algae. They serve as energetic sources and some times as defensive compounds. Free fatty acids can be produced by hydrolysis of lipids through isolation. In the present study, the volatiles were isolated by distillation of flowers, which excludes the formation of acids. Most of the identified acids were fully saturated and with straight chains corresponding to the hydrocarbons in the same plants. Lower fatty acids were found only in one population of *L. maculatum* (No. 6). Hexadecanoic acid (No 6: 11.4%; No 4: 7.4%; No 9: 7.3%) and octadecanoic acid (No 7: 4.5%; No 9: 3.2%; No 1: 2.6%) appeared in high concentrations only in some samples. Lower concentrations of fatty acids with odd number of carbon atoms as pentadecanoic and heptadecanoic acids were also found. Unsaturated fatty acids were found in all *Lamium* samples but not in *Lamiastrum galeobdolon*.

Recently, significant amounts of fatty acid esters mostly with methanol/ethanol, were found in some plants and algae. Their origin remains unknown as they could be artifacts formed by pre-esterification of natural lipids with the alcohols used in the isolation procedures. In this study, no alcohols were used, which proves the natural origin of the identified esters. Isopropyl myristate was present in most of the samples, but in high concentration only in one sample of *L. purpureum* (No 2: 3.9%). Contrary to other plants and algae, here the esters of higher alcohols than methyl/ethyl esters are predominant.

Terpenoids are often found in volatiles from plants and animals. The plants from the genera *Lamium* and *Lamiastrum* are non-essential oil bearing plants. However, terpenoids were found in all of the studied samples. Surprisingly, in all studied plants from different localities unusual high concentrations of squalene up to 19% (*L. purpureum*, No 3) were found, which might be

Table I. Volatile compounds from flowers of *Lamium* species (No 1–8) and *Lamiastrum galeobdolon* (No 9) analyzed by GC/MS (% of total volatiles)*.

Compound	*L. album* (%)	*L. purpureum* (%)		*L. garganicum* (%)		*L. maculatum* (%)			*Lamiastrum galeobdolon* (%)
	1	2	3	4	5	6	7	8	9
Hydrocarbons									
Dodecane		1.1	< 0.1	< 0.1		< 0.1			
Heptadecane		1.3							
Octadecane		1.1							
Eicosane		1.3	0.8		0.2	0.2	< 0.1	< 0.1	
Heneicosane	0.6		0.5	0.5	0.8				
Docosane	0.4	2.3	1.5	0.5	0.7			0.6	0.9
Tricosane	2.4		5.9	1.1		7.2			12.3
Tetracosane	1.2	3.6	4.3	1.1	1.5	2.4	2.4	2.1	2.3
Pentacosane	2.3	6.5	13.9	1.9	4.1		8.2	7.4	6.7
Hexacosane	1.0	2.0	2.7	1.2	2.6	1.5	1.6	1.6	1.1
Heptacosane	1.6	4.4	15.4	1.8	4.4	4.5	3.4	4.1	3.7
Octacosane	1.4	1.8	2.2	1.1	1.1		1.1	0.9	
Nonacosane	1.5	1.8	6.9	1.3	1.5	2.7	1.2	1.7	2.9
Triacontane	1.6		1.3	0.9		1.4	0.6	0.5	0.6
Hentriacontane						1.7			
Tetradecene						< 0.1			
12-Pentacosene			1.7			0.9		< 0.1	1.2
Aldehydes									
Nonanal	< 0.1	< 0.1	< 0.1	< 0.1		< 0.1		< 0.1	< 0.1
Decanal	< 0.1	< 0.1	< 0.1	< 0.1		< 0.1			< 0.1
Benzene acetaldehyde									< 0.1
Acids									
2-Ethyl hexanoic acid						< 0.1			
Octanoic acid						< 0.1			< 0.1
Nonanoic acid						< 0.1			
Tetradecanoic acid	0.5		0.9	0.6	1.2			1.1	1.4
Pentadecanoic acid	0.5		0.7						
Hexadecanoic acid				7.4		11.4			7.3
Esters									
Isopropyl myristate	< 0.1	3.9		0.5	0.4			0.6	2.8
Glycerol-tricaprylate						0.9			
Tetradecanoic acid tetradecyl ester						0.8			
Hexadecanoic acid, butyl ester			0.6						
9,12-Octadecadienoic acid methyl ester				0.5					
Terpenoids									
α-Terpineol					0.3				< 0.1
β-Caryophyllene					0.3	0.3	1.6		< 0.1
α-Humulene					0.2	< 0.1	0.8	< 0.1?	
Caryophyllene oxide					0.2	< 0.1			
Hexahydrofarnesyl acetone	0.2		1.0			1.2	1.9		
Squalene	13.5	5.2	18.7	8.6	2.5	9.5	11.9	11.3	4.6
Others									
Phenethyl alcohol								32.0	
Benzene-2,4-dichloro-1-methyl		2.1							
t-Butyl-phenol									< 0.1
2,6-Di-*t*-butyl-4-dimethyl-amino-methylphenol					0.5				
1-Octadecanol									1.3
Hexadecane amide	0.2			0.3					
Octadecane amide	0.3		0.8						
Heptadecanoic acid	0.3			0.3		0.3			0.5
Octadecanoic acid	2.6						4.5		3.2
Hexadecenoic acid				0.8			2.1		
9-Octadecenoic acid	2.0	2.9	2.1	3.0		2.1	6.0	4.5	
9,12-Octadecadienoic acid				1.0	1.3			1.0	

* The ion current generated depends on the characteristics of the compound and is not a true quantitation.

characteristic for both genera. The squalene concentrations strongly depended on the location of the collection site. This triterpene is precursor of steroids and triterpenes and is found in low concentrations in all eucaryots. Besides its biosynthetic role squalene possesses significant biological activity as bactericidal, anti-tumor and immunostimulant (Harborne and Baxter, 1993). It is a flower attractant for pollinating insects and repellent against ants (Dutton *et al.*, 2002). Hexahydrofarnesyl acetone was found in *L. maculatum* (No 7: 1.9%; No 6: 1.2%), *L. purpureum* (No 3: 1%) and *L. album* (No 1: 0.2%) plants but not from all location sites. Particularly in *L. maculatum* and *L. garganicum* were found also other terpenoids. For example, β-caryophyllene and α-humulene appeared in highest concentrations in *L. maculatum* (No 7: 1.6% and 0.8%).

One chlorine-containing compound, benzene-2,4-dichloro-1-methyl, with expected antimicrobial activity was found in *L. purpureum* (No 2: 2.1%).

Surprisingly, high concentrations of phenethyl alcohol were found only in *L. maculatum* f. *alba* (No 8: 32%). Phenethyl alcohol has antimicrobial activity (Harborne and Baxter, 1993) and is important for plant-insect relationships. Previously it was found also in *L. purpureum* (Kurihara and Kikuchi, 1976). That's why its taxonomic importance for forma *alba* stays unclear.

Some taxonomic problems concerning *Lamium* and *Lamiastrum* exist. In spite of the fact that some pecularities were observed, for example, no mono- and sesquiterpenes in *L. purpureum* and *L. maculatum*, highest ratio of hydrocarbons with odd/even number of carbon atoms and no unsaturated fatty acids in *Lamiastrum galeobdolon*, they are unsufficient for taxonomical purposes. It must be noted, that the high content of squalene seems to be the most important characteristic feature of the oil-composition from *Lamium* and *Lamiastrum* plants.

Acknowledgement

This Project B-1001 was supported by the National Foundation for Scientific research (Bulgaria).

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