CHEMIJA, 2004. T. 15, Nr. 1, 33–37

Volatile constituents of cultivated *Origanum vulgare* L. inflorescences and leaves

Danutë Mockutë, Asa Judþentienë and Genovaitë Bernotienë*

Institute of Chemistry,
A. Gostauto 9,
LT-2600 Vilnius, Lithuania
Fax: 370 5 2617018.
E-mail: albmal@takas.lt

Fourteen essential oils from inflorescences and leaves of cultivated *Origanum vulgare* L. were analyzed by GC and GC/MS. The main constituents in 6 inflorescence oils out of 7 and in 2 samples of 7 leaf oils were β-caryophyllene (15.4–24.9%), sabinene (6.2–19.5%) and germacrene D (11.4–14.6%) and in the 4 leaf oils - β-caryophyllene (17.2–21.3%), germacrene D (12.7–15.7%) and caryophyllene oxide (7.6–11.1%). The percentage of sabinene in the inflorescence oils exceeded 2–3 times that in the leaf oils produced from the same plant sample. An opposite corellation was found for caryophyllene oxide. The content of sesquiterpene hydrocarbons in both parts of the plants was 43.3–62.3%. The 39 constituents identified in the inflorescence oils made up 85.4–97.9%, and 41 compounds in leaf oils comprised 82.4–86.7%.

Key words: *Origanum vulgare* L., Lamiaceae, essential oil, β-caryophyllene, germacrene D, sabinene, caryophyllene oxide

INTRODUCTION

Inflorescences of some subspecies of cultivated *Origanum vulgare* L. were investigated in Italy [1]. All plants were of different origin. Four samples of *O. vulgare* L. ssp. *vulgare* produced essential oils of different chemical composition. The first main constituent was β-caryophyllene or terpinen-4-ol or thymol or p-cymene. Six oils from *O. vulgare* L. ssp. *hirtum* were of thymol or carvacrol chemotype. Two oils of *O. vulgare* L. ssp. *glandulosum* were of carvacrol type and thymol was as predominant constituent in the oil of *O. vulgare* L. ssp. *gracile*. The 7 samples of *O. vulgare* L. ssp. *virens* volatile oils contained linalool or terpineol as the first major component.

Seventy *O. vulgare* L. ssp. *vulgare* plants of different European origins were cloned in France [2]. Authors of the paper gave only the leaf oil chemical composition. The oils were distributed into 6 groups according to the main constituents. Twenty-three samples were attributed to the sabinene + β-ocimene group; 12 oils – to germacrene D + β-caryophyllene; 22 samples – to cis-ocimene hydrate, 4 ones – to terpinen-4-ol and 6 oils – to monoterpene hydrocarbons (β-ocimene) group.

The oils of inflorescences and leaves with stems from *O. vulgare* L. ssp. *vulgare* grown wild in Lithuania were of β-ocimene chemotype [3]. The content of the main constituents of oils markedly differed in separated parts of plants from 7 habitats. The percentage means of the constituents in inflorescence and leaf oils were: sabinene 13.3 and 8.3%, cis-β-ocimene 4.1 and 15.3%, trans-β-ocimene 11.0 and 8.0% (sum of β-ocimenes 15.1 and 23.3%).

The leaf oil of *O. vulgare* ssp. *virens* contained mainly terpenoids while the calli oils n-alkanes [4].

Five different parts of *O. vulgare* ssp. *hirtum* (syn. *O. heracleoticum*) plants with pink and white flowers were investigated in Italy [5]. The main constituents in oils of all investigated parts of plants with pink flowers were thymol and γ-terpinene. The amounts of the above compounds were different in oils of separated parts: bracts – 70.4% (thymol) and 11.7% (γ-terpinene), calices – 64.7 and 21.8%, basal leaves – 51.5 and 15.9%, apical leaves – 51.2 and 25.3%, corollas – 47.5 and 23.2%, respectively. Thymol and γ-terpinene were the major constituents in some parts of plants with white flowers. The basal leaf oil of these plants contained thymol methyl ether (36.2%) and p-cymene (20.4%) as the major components. γ-Terpinene (16.1%) was the third main constituent. The content of thymol in the basel leaf oil was 2.3%, while that in corollas, calices and bracts ~50%.

The investigation of the oils of the aerial parts of *O. vulgare* L. plants with rose-pink, pink and nearly white flowers also showed differences in the chemical composition [7]. The levels of monoterpenoids – sabinene (0.3–1.6%), 1,8-cineole (0.5–1.3%),

*Corresponding author.

cis-β-ocimene (0.2–1.4%), trans-β-ocimene (0.5–1.3%), linalool (0.3–1.4%) and terpinen-4-ol (0.5–1.5%) in the oils from plants with rose-pink and pink flowers were markedly lower than in the oils with nearly white flowers (the corresponding quantities: 4.2, 6.7, 5.2, 5.4, 3.9 and 9.2%) [6]. Only plants with white and pink flowers contained 0.2 and 0.4% of thymol (monoterpenic phenol). The essential oil of the aerial parts of oregano cultivated in Kaunas Botanical Garden contained 2.3% of phenol (thymol + carvacrol) and 1.9% of their derivatives [7]. Similar amounts of the above phenols were found in the essential oils of the aerial parts of cultivated plants in Experimental Garden of the Lithuanian Institute of Horticulture [8]. The major constituents of the above oil were sabinene, 1.8-cineole and caryophyllene oxide. The composition of essential oils of commercial oregano plants (aerial parts) bought in chemists shops [9] were similar to that of the plants grown by investigators from St.Petersburg (seeds from Moscow Botanical Garden) [6]. The levels of compounds with the caryophylane carbon skeleton in both above essential oils were close and amounted to 35.3–35.8% and 29.6–37.2%. The essential oil of commercial oregano contained more caryophyllene oxide than β-caryophyllene, while the oil of plants from Moscow seeds contained more β-caryophyllene than its oxide. The amount of caryophyllene oxide in the essential oil of plants from chemist's shops might be increased during the storage of crushed oregano plants. The content of phenol thymol in the oils of commercial plants was 1.6–1.7% [9].

Essential oils and preparations of oregano plants are used in the world [10, 11] and in Lithuania [12, 13] for healing various deseases.

The oils under study were from cultivated *O. vulgare* plants with rose-pink, pink and white inflorescences.

MATERIALS AND METHODS

The aerial parts (~35 cm) of cultivated plants (0.2–1.0 kg) from 5 different gardens (indicated here by alphabetic symbols, A–G) were collected in August 2001: sample A – Antakalnis (Vilnius city); B – Salininkai (Vilnius city); C – Šeškine (Vilnius city); D – Nemenèine (Vilnius district); E, F and G – Aleknos (Rokiškis district).

Five samples (A-E) were from plants with pink flowers, one (sample F) with rose-pink inflorescences and one (G sample) with white flowers. The seeds of cultivated plants of European origin were bought in seed shops.

All samples were collected at a full flowering stage. The plants were dried at room temperature (20–25 °C). Essential oils (in yield of 0.1–0.4%) were prepared by hydrodistillation for 2 h of 20–100 g of air-dried plants.

The analyses of the essential oils were carried out by GC and GC/MS. An HP 5890II chromatograph equipped with FID and capillary column HP-FFAP (30 m × 0.25 mm i. d., film thickness 0.25 μm) was used for quantitative analysis. The GC oven temperature was set at 70 °C for 10 min and then programmed from 70 to 210 °C at a rate of 3 °C/min, using He as the carrier gas (0.7 ml/min). The injector and detector temperatures were 200 and 250 °C, respectively. Analyses by GC/MS were performed using a chromatograph interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV) and equipped with CP-Sil 8 CB capillary column (50 m × 0.32 mm i. d., film thickness 0.25 μm). The oven temperature was held at 50 °C for 2 min, then programmed from 50 to 180 °C at the rate of 3 °C/min, kept for 1 min, then programmed from 180–250 °C at the rate of 20 °C/min and kept isothermal at 250 °C for 2 min using He as the carrier gas (2.0 ml/min). The injector and detector temperature was 250 °C.

The percentage composition of the essential oils was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and indices on both columns and mass spectra with corresponding data in the literature [14–17] and computer mass spectra libraries (Wiley and NBS 54K).

RESULTS AND DISCUSSION

The chemical composition of the essential oils from cultivated *O. vulgare* L. (Tables 1 and 2) differed from that of the plants growing wild [3]. The first main constituent of the inflorescence and leaf oils of 6 samples (B–G and B'–G') was β-caryophyllene and in one garden germacrene D (A, A'), while in the oils of wild growing plants β-ocimene was the dominant constituent [3]. The samples A and A' contained low levels (≤ 25.5%) of monoterpenoids (in comparision with D–G oils) and a sesquiterpene hydrocarbons made up half of the oil content (46.2 and 51.6%). The lowest quantity of monoterpenoids was found in B and C samples of inflorescence oils (6.3 and 7.3%) and the highest percentage of sesquiterpene hydrocarbons was in B' and C' leaf oils (61.3 and 62.3%). The B and C inflorescence oils contained also more sesquiterpene hydrocarbons (56.3, 61.2%) than all other oils studied (43.3 – 51.6%). The quantity of compounds with caryophyllane skeleton (caryophyllene and caryophyllene oxide) was also higher in B and C oils (24.8 and 28.0%) than in other inflorescence oils (A, D–G: 12.9–20.4%). The content of these compounds in

the leaf oils of plants from the same locality was higher than in the flower oils, except samples A and A' (Tables 1 and 2). The most significant difference in the content of compounds with caryophyllane skeleton was noticed between F inflorescence (29.4%) and F' leaf oils (18.6%). The levels of monoterpenoids in D'-G'leaf oils (18.4–34.6%) were lower than in D–G inflorescence ones (38.3–47.3%).

The other two significant differences were noted between inflorescence and leaf oils. The content of sabinene in flowers were 2–3 times higher than in leaf oils of the plants from the same locality (Tables 1 and 2). The above regularity of sabinene in separate parts of plants was noted also in oils of wild-grown plants [3]. The second difference was the higher levels of caryophyllene oxide in leaf oils than in flower oils of the same plant samples.

The percentage of bicyclogermacrene (5.4–6.7%) and spathulenol (2.7–6.5%) in A–C and A'–C' oils was higher than in D–G and D'–G' samples (tr. – 2.1% and 0.1–3.1%, respectively).

The composition of the oils of plants from the same garden with pink and rose-pink flowers differed in some details from that of plants with white inflorescences. The relative quantity of 1,8-cineole in the oils of both parts of plants with white flowers was higher (5.6–7.8%) than in rose-pink and pink flowered plants (0.9–3.9%). The leaf oils of plants with white inflorescences contained higher levels of monoterpenoids (34.6%) than the oils of flowers of other colors (18.4–25.9%). Similar regularities were observed in a study of aerial parts of plants with different inflorescence colors in Leningrad region [6].

The dominant constituents in 6 inflorescences (B–G) and in 2 leaf (D'and G') oils were β-caryophyllene (15.4–24.9%), sabinene (6.2–19.5%) and germacrene D (11.4–14.6%). The samples A and A' contained germacrene D, β-caryophyllene, spathulenol and bicyclogermacrene as the major components. Four leaf oils (B', C', E' and F') were characterized by high levels of β-caryophyllene (17.2–21.3%), germacrene D (12.7–15.7%) and caryophyllene oxide (7.6–11.1%).

Table 1. **Composition (%) of inflorescence essential oils of cultivated *Origanum vulgare* L.** [a]

Component	RI	A	B	C	D	E	F	G
α-Thujene	931	t	0.4	0.5	1.7	1.1	0.5	0.4
α-Pinene	939	0.1	t	1.7	t	t	0.6	0.5
Sabinene	976	**5.8**	**8.7**	**19.5**	**14.7**	**15.7**	**14.4**	**17.1**
β-Pinene	980	0.1	0.6	0,8	1.4	1.5	1.1	0.9
Myrcene	991	0.4	–	–	t	–	0.7	1.0
α-*T*erpinene	1018	–	0.2	0.5	0.8	0.6	0.6	1.2
p-Cymene	1026	t	0.7	0.7	0.6	0.7	0.5	0.2
β-Phellandrene	1032	0.2	0.2	–	1.1	t	0.1	1.3
1,8-Cineole	1033	3.7	t	–	5.5	3.9	2.7	7.8
(Z)-β-Ocimene	1040	1.1	1.5	2.0	6,2	4.7	5.4	5.8
(E)-β-Ocimene	1050	4.3	1.7	2.8	6.4	6.1	6.3	6.9
γ-Terpinene	1062	0.8	1.3	0.5	1.5	2.3	1.7	1.1
cis-Sabinene hydrate	1068	t	–	–	0.5	0.4	0.1	0.2
Terpinolene	1088	t	t	0.1	0.6	0.2	0.4	0.2
trans-Sabinene hydrate	1096	t	–	–	0.3	–	0.1	t
Linalool	1099	0.4	1.2	0.7	1.1	1.6	0.7	1.1
Terpinen-4-ol	1177	0.6	t	0.6	1.5	0.6	1.1	0.3
α-Terpineol	1187	0.2	0.1	0.8	1.4	1.0	0.6	0.9
Thymol	1290	1.2	0.1	0.2	0.2	1.4	0.5	0.4
Carvacrol	1298	t	–	t	0.1	–	0.2	t
β-Bourbonene	1387	0.2	0.8	0.7	0.6	0.9	0.5	0.6
β-Elemene	1391	0.7	0.4	0.8	0.7	1.2	0.7	0.5
β-Caryophyllene	1418	**11,6**	**24.9**	**21.9**	**15.4**	**16.7**	**17.0**	**18.7**
β-Gurjunene	1432	1.6	1.7	1.5	1.8	1.4	0.6	0.5
α-Humulene	1454	2.5	5.3	4.7	2.5	3.7	3.3	3.2
allo-Aromadendrene	1461	1.5	1.1	t	0.3	–	0.1	t
Germacrene D	1480	**16.0**	**14.6**	**12.9**	**12.6**	**13.7**	**12.3**	**11.4**
Bicyclogermacrene	1494	6.4	5.4	5.4	0,4	0.6	2.1	1.7
α-Muurolene	1499	t	–	–	0,8	–	0.4	t
α-Farnesene	1508	–	t	0.1	6,8	4.1	5.3	4.9
β-Bisabolene	1509	5.5	5.0	4.3	0.7	2.0	1.2	1.9
γ-Cadinene	1513	1.3	t	t	0.1	t	0.1	0.2
δ-Cadinene	1524	4.3	2.0	4.0	0.6	1.4	1.6	0.7
Germacrene-D-4-ol	1574	0.3	0.1	0.3	t	0.7	1.0	1.2
Spathulenol	1576	6.5	2.7	3.0	0.1	0.9	0.1	1.3
Caryophyllene oxide	1581	1.3	3.1	2.9	2.1	3.6	1.6	1.7
epi-γ-Eudesmol	1619	2.2	0.9	0.7	0.2	0.6	0.2	0.4
epi-α-Muurolol	1642	3.6	1.5	2.4	0.3	0.4	0.9	0.5
α-Cadinol	1653	1.0	0.3	0.5	1.0	1.4	1.8	1.2
Monoterpenoids		**18.9**	**16.7**	**31.3**	**45.6**	**41.8**	**38.3**	**47.3**
Sesquiterpene hydrocarbons		**51.6**	**61.2**	**56.3**	**43.3**	**45.7**	**45.2**	**44.3**
Caryophyllane skeleton		**12.9**	**28.0**	**24.8**	**17.5**	**20.3**	**18.6**	**20.4**
Total		**85.4**	**86.5**	**97.4**	**92.6**	**95.1**	**89.1**	**97.9**

[a]t – traces (< 0.1%)
Retention index on nonpolar column.

Table 2. **Composition (%) of leaf essential oils of cultivated *Origanum vulgare* L.**[a]

Component	RI	A'	B'	C'	D'	E'	F'	G'
α-Thujene	931	t	–	t	t	0.3	–	t
α-Pinene	939	t	t	t	1.1	0.4	t	0.4
Sabinene	976	3.8	3.4	4.5	6.2	8.1	6.5	7.7
β-Pinene	980	1.4	t	–	0.3	0.4	t	0.6
Myrcene	991	–	t	t	1.1	0.5	t	2.7
α-*T*erpinene	1018	0.2	–	–	0.3	0.1	0.3	0.1
p-Cymene	1026	0.1	t	t	0.5	0.4	1.7	0.3
β-Phellandrene	1032	–	–	t	3.9	0.9	0.1	0.3
1,8-Cineole	1033	3.4	t	t	5.1	2.3	0.9	5.6
(Z)-β-Ocimene	1040	5.1	0.9	1.0	3.6	5.1	5.5	7.8
(E)-β-Ocimene	1050	3.5	0.4	0.3	2.1	2.4	2.9	6.1
γ-Terpinen	1062	0.3	t	t	1.6	2.9	0.2	0.7
cis-Sabinene hydrate	1068	0.3	–	–	0.1	0.3	t	t
Terpinolene	1088	0.2	t	t	0.1	t	–	t
Linalool	1099	0.5	0.1	t	1.1	0.2	t	0.9
allo-Ocimene	1129	3.4	0.6	t	1.9	–	–	t
Terpinen-4-ol	1177	0.3	0.2	–	0.9	0.8	0.1	1.2
α-Terpineol	1187	0.1	0.2	0.7	1.2	0.5	0.1	0.2
Thymol	1290	1.6	0.5	0.8	1.7	0.3	0.1	–
Carvacrol	1298	1.3	–	–	0.2	–	t	–
β-Bourbonene	1387	0.7	3.2	0.6	1.7	1.9	5.9	2.9
β-Elemene	1391	0.8	0.9	0.7	t	–	t	0.4
β-Caryophyllene	1418	**11.0**	**20.3**	**21.3**	**15.9**	**17.2**	**18.3**	**16.1**
β-Gurjunene	1432	1.5	1.6	1.6	1.1	0.9	1.4	0.5
α-Humulene	1454	2.3	5.3	5.0	3.1	2.8	2.3	3.1
allo-Aromadendrene	1461	1.7	t	–	t	t	0.8	t
γ-Muurolene	1477	t	1.1	1.5	t	t	0.2	0.1
Germacrene D	1480	**14.3**	**15.7**	**15.1**	**12.1**	**13.8**	**12.7**	**14.0**
Bicyclogermacrene	1494	6.0	5.5	6.7	t	1.2	0.2	2.0
α-Muurolene	1499	t	–	t	3.9	0.1	0.7	0.1
α-Farnesene	1508	–	5.5	–	4.1	0.5	0.4	2.3
β-Bisabolene	1509	2.4	2.4	4.7	3.6	4.1	1.6	1.6
γ-Cadinene	1513	1.4	0.4	0.1	t	0.1	t	t
δ-Cadinene	1524	3.7	0.4	1.2	0.9	0.6	0.1	0.6
α-Cadinene	1538	0.4	t	2.8	t	0.1	2.6	1.1
Germacrene-D-4-ol	1574	0.1	0.5	0.3	–	0.5	1.1	0.7
Spathulenol	1576	5.0	4.5	5.3	0.5	2.6	3.1	0.3
Caryophyllene oxide	1581	**2.7**	**8.9**	**7.6**	**4.7**	**8.3**	**11.1**	**5.2**
epi-γ-Eudesmol	1619	0.3	0.5	1.4	0.1	0.3	0.3	0.1
epi-α-Muurolol	1642	2.3	1.1	0.7	0.2	0.4	0.5	0.4
α-Cadinol	1653	0.4	0.4	0.4	0.8	1.1	1.2	0.6
Monoterpenoids		**25.5**	**6.3**	**7.3**	**33.0**	**25.9**	**18.4**	**34.6**
Sesquiterpene hydrocarbons		**46.2**	**62.3**	**61.3**	**46.4**	**43.3**	**47.2**	**44.8**
Caryophyllane skeleton		**13.7**	**29.2**	**28.9**	**20.6**	**25.5**	**29.4**	**21.3**
Total		**82.5**	**84.5**	**84.3**	**85.7**	**82.4**	**82.9**	**86.7**

[a] Retention index on nonpolar column, t – traces (< 0.1%).

Low contents of carvacrol were found in two inflorescence (Table 1) and in two leaf oils (Table 2). The percentage of thymol was 0.1–1.7% in 13 out of 14 oils. One sample (G') did not contain phenols.

The chemical composition of the oils under study was similar to that of some formerly investigated 70 cloned plants in France [2]. The leaf oils of more than half of the samples contained β-caryophyllene, germacrene D, sabinene and caryophyllene oxide as major constituents (Table 2). The large part of the cloned plants had low quantities of phenols (< 1.5%) [2]. Carvacrol was found only in some oils in France (up to 0.95%). The main compounds, β-caryophyllene (13.7–31.3%) and germacrene D (9.6–19.2%), and low amounts (0–0.4%) of phenols were found in the oils of aerial parts from Leningrad region [6].

The 39 identified constituents made up 85.4–97.9% in the inflorescence oils. The inflorescence oil A, beside the constituents listed in Table 1, contained 3.7% of terpenyl acetate, which increased the total sum from 85.4 to 89.1%. The 41 identified compounds in the leaf oils made up 82.4–86.7%. The amount of sesquiterpene hydrocarbons in both parts of the plants was 43.3–62.3%.

The constituents with caryophyllane carbon skeleton, β-caryophyllene and caryophyllene oxide, are bioactive [10, 18, 19]. The main constituent of the essential oils studied, β-caryophyllene, showed fungistatic and bacteriostatic activities [10]. The above compound exhibited cytotoxicity against two human carcinoma cell lines [18]. The cytotoxicity of β-caryophyllene enhanced indole and indole-3-carbinol. β-Caryophyllene is approved by the U.S. Food and Drug Administration for food use [18].

CONCLUSIONS

The main constituents of the essential oils under study were β-caryophyllene, sabinene, germacrene D and caryophyllene oxide. The percentage of sabinene in the inflorescence oils exceeded 2–3 times that

in the leaf oils produced from the same plant sample. An opposite correlation was found for caryophyllene oxide.

Received 24 November 2003
Accepted 04 December 2003

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D. Mockutë, A. Judþentienë, G. Bernotienë

KULTIVUOJAMO PAPRASTOJO RAUDONËLIO (*Origanum vulgare* L.) ÞIEDØ IR LAPØ LAKIEJI JUNGINIAI

S a n t r a u k a

Þydintys kultûriniai raudonëliai (*Origanum vulgare* L.) buvo surinkti 7 soduose. Þiedø ir lapø eteriniuose aliejuose vyrauja β-kariofilenas (≤24,9%), sabinenas (≤19,5%), germakrenas D (≤14,6%) ir kariofileno oksidas (≤24,9%). Kiekvieno augalø pavyzdþio þieduose randama kur kas daugiau sabineno negu jø lapuose, o lapai sukaupia daugiau kariofileno oksido negu þiedai. Seskviterpeniniai junginiai sudaro didþiàjà dalá lakiøjø komponentø tiek þieduose, tiek lapuose (43,3–62,3%). Trisdeðimt devyni junginiai, identifikuoti þiedø eteriniuose aliejuose, sudaro 85,4–97,0% ir keturiasdeðimt vienas junginys, identifikuotas lapø eteriniuose aliejuose, – 82,4–86,7%.