

Variation in essential oil composition of *Rhododendron tomentosum* gathered in limited population (in Eastern Lithuania)

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Marsh rosemary (*Rhododendron tomentosum* L. Stokes) H. Harmaja (formerly *Ledum palustre*) plants were collected at the flowering stage in marshes near Šulnys Lake (Šalčininkai District, Eastern Lithuania) in 2004. Essential oils were prepared by hydrodistillation and analyzed by GC and GC/MS. The major oil constituents were p-cymene (6.2–20.3%), iso-ascaridol (16.7–24.1%), palustrol (1.2–31.8%) and ledol (5.2–23.4%); and their percentage changed according to the sampling sites. Despite that the plant material was gathered from the limited area, significant compositional variability was observed in the essential oils. Ninety identified (two of them tentatively) compounds comprised up to 97.9% of the oils.

Key words: *Rhododendron tomentosum* H. Harmaja, Ericaceae, essential oil, compositional variability, GC, GC/MS, p-cymene, iso-ascaridol, palustrol, ledol

INTRODUCTION

Rhododendron tomentosum (Stokes) H. Harmaja (formerly *Ledum palustre* Linnaeus: Ericaceae) is a perennial small shrub growing in marshes. Wild populations of marsh rosemary are found in Northern and Central Europe, North Asia and America. Only one species of the plant (*L. palustre* var. *palustre*) grows in Lithuania [1].

Numerous studies on the chemical composition of *Rh. tomentosum* essential oils from various European countries (such as ex-USSR, Sweden, Finland, Belarus, Netherlands, Lithuania, Estonia and Russia) [2–17], Far East and Siberia [18–24], and some Asian countries (Mongolia and China) [25–28] have been reported. Ledol, palustrol and / or myrcene were found to be the principal components of marsh rosemary oils from Central and Northern Europe [2–10]. The oil of *Rh. tomentosum* from Netherlands was rich in ledol, palustrol and myrtenal [11]. It was shown that amounts of ledol in marsh tea (more than 100 samples from the Central

zone of ex-USSR) could change (23–37%) according to the vegetation stage and growth site [12]. However, it was evaluated that the content of the main constituents (palustrol, ledol, cyclocoloronones, myrcene and limonene) depends on the age of marsh tea shoots [13]. Analysis of oils from shoots, leaves and stems collected in Estonia has shown that p-cymene, myrcene, γ -terpineol, palustrol and ledol were among the main components and their quantity depends on plant organs [14]. An unusual and rare ascaridol (about 70%) chemotype of the essential oils of leaves from Southern Finland was determined, where the typical constituents such as ledol and palustrol were found only in trace amounts [15]. During *L. palustre* (from Kostroma Region, Central Russia) essential oil analysis, two new substances – lepalol and lepalin – were isolated and identified for the first time [16]. p-Cymene, sabinene and terpinyl acetate were found as the principal components in the leaves of *Rh. tomentosum* from Southern Sweden [17].

The chemical composition of volatile oils of *L. palustre* growing in Western Siberia has varied significantly from European specimens [18, 19]. The oils were characterized by a low

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quantity of ledol (just about 4%) and appreciable amounts of allo-aromadendrene and limonene, and their derivatives [19]. Some terpenoids (among them p-mentha-1(7),8-dien-2 α -ol and p-mentha-1(7),8-dien-2 β -ol) were isolated and identified for the first time in the above study [19]. The chemical structure of a new sesquiterpenoid 7-elemol, isolated from marsh tea volatile oils from Tomsk Region (Siberia), has been elucidated [20]. Investigations of essential oils of *L. palustre* from the Sayan Mountains (Siberia) revealed that the principal compounds are p-cymene, α -pinene and germacrone [21].

Four main chemotypes of volatile oils of marsh tea from Siberia, Far East and Sakhalin Island were described with the following main components: limonene (about 48%), p-cymene (15%), sabinene (33%) and myrcene [22]. However, four compounds such as 3,6-dimethyl benzofuran, 4-isopropylidene cyclohexa-2,5-dienone, dehydroxy isocalamendiol and muuralanediol were detected for the first time in the plant material, in the *R. tomentosum* oils [22]. Even more, two new terpenoids, p-mentha-1,8(10)-diene 3,9-epoxide, which was called lepaloks, and 10-isopropyl-3-menthyl-7-methylenecyclodecanone 3,6-epoxide (3,10-epoxygermacr-4(14)-en-8-one), named as lepakson, were isolated in the study [22]. A little later, these compounds mentioned above were determined once more in the same plant essential oils from Tomsk Province [23]. Analysis of *L. palustre* from cis-Baikal Region and Amur Province has allowed to isolate and establish the structure of a new natural compound, 5-isopropylbicyclo[3,1,0]hex-3-en-2-one, which was called lebaikon [24]. In addition, two bicyclic monoterpenoids, 2-formyl-5-isopropylbicyclo[3,1,0]hex-2-ene and 4-isopropyl-1-methylbicyclo[3,1,0]hexan-2-one, have been detected for the first time in the natural material, in marsh tea volatile oils in the above study [24].

p-Cymene was a predominant compound in the *R. tomentosum* oils from Mongolia [25], while myrcene, β -thujene, p-cymene, ascaridol and myrtenal were the main constituents in the oils from China [26–28].

The literature survey has shown a significant compositional variability in *Rh. tomentosum* oils of various origins (from Europe to Asia), while the aim of the present study is to show the compositional variation in the essential oils of the plants gathered at a narrow growing area in Lithuania.

EXPERIMENTAL

Rhododendron tomentosum L. plants were collected at the flowering stage (June 2004, the same sampling day) in Kernavė marshes near Šulnys Lake (Šalčininkai District, Eastern Lithuania) in the limited area (distances between sampling sites 1–1.5 km). Voucher specimens have been deposited in the Herbarium of the Institute of Botany (BILAS), numbers 68886–68888. The essential oils were prepared by hydrodistillation (2 h) of air-dried all aerial parts (up to 20 g for each portion) in a Clevenger-type apparatus according to the European Pharmacopoeia. At least two repetitions of oil

isolation were performed. The ratio of plant material and water was 1 : 20. A yellow-greyish grease-like mass with a characteristic oil taste and odor was obtained.

GC (FID)

Quantitative analysis of the essential oils was carried out by GC on a capillary column DB-Wax (polyethylene glycol 30 m \times 0.25 mm i. d., film thickness 0.25 μ m) using a chromatograph Clarus 500 (Perkin-Elmer) equipped with FID. The GC oven temperature was programmed as follows: from 60 °C (isothermal for 2 min) increased to 160 °C (isothermal for 2 min) at a rate of 7 °C/min, then increased to 250 °C at a rate of 10 °C/min, and the final temperature was kept for 2 min. Injector and detector temperatures were 250 °C. The flow rate of carrier gas (hydrogen) was 1 mL/min. At least two repetitions ($n \geq 2$) per analysis were performed.

GC/MS

Analyses were carried out by a HP 5890 gas chromatograph equipped with a HP 5971 mass selective detector and a HP 7673 split/splitless (splitless 0.75 min) injector on a capillary column DB-5 (5%-phenyl)-methylpolysiloxane; 50 m \times 0.32 mm, film thickness 0.25 μ m). GC oven temperature was programmed as follows: from 60 °C (isothermal for 3 min) increased to 160 °C (isothermal for 1 min) at a rate of 5 °C/min, then increased to 250 °C at a rate of 10 °C/min, and the final temperature was kept for 3 min. Injector and detector temperatures were 250 °C. Mass spectra in electron mode were generated at 70 eV, 0.97 scans / second, mass ranges 35–650 m/z. The flow rate of carrier gas (helium) was 1 mL/min. The percentage of the composition of the essential oils was computed from GC peak areas without correction factors. Qualitative analysis was based on comparison of retention times, indexes and mass spectra with the corresponding data in literature [29], co-injection of some terpenes references and computer mass spectra libraries (Wiley and NBS 54K).

RESULTS AND DISCUSSION

The composition of *Rh. tomentosum* essential oils was presented in the Table. Ninety identified (one of them tentatively and one as unknown) compounds comprised up to 97.9% of the total oil content. Despite that the plant material was gathered from the limited area, variability in the composition of the oils was observed. The content of the principal oil constituents such as p-cymene (6.2–20.3%), iso-ascaridol (16.7–24.1%), palustrol (1.2–31.8%) and ledol (5.2–23.4%) changed according to the sampling sites. Composition variability was also observed according to the different compound classes. Especially significant changes were noticed in the content of monoterpene fraction (from 9.0 to 31.1%) and oxygenated sesquiterpenes (including shyobunone isomers) (from 15.9 to 57.9%). However, the content of compounds with aromadendrene skeleton varied from 10.5 to 59.7%, while constituents with menthane skeleton from 8.2 to 30.1%.

Table. Chemical composition of essential oils of *Rhododendron tomentosum* growing in Eastern Lithuania

Compounds	RI _{DB-5}	N1	N2	N3
α-Thujene	930	0.1	0.1	
α-Pinene	939	2.2	1.1	0.3
Camphene	954	1.1	0.7	0.1
Hexanoic acid	973	0.1		tr
Sabinene	975	0.3	0.3	0.3
β-Pinene	979	1.3	1.1	0.5
Myrcene	990	0.1	0.2	1.0
α-Terpinene	1017	3.8	2.5	0.2
p-Cymene	1024	20.3	15.3	6.2
Limonene	1029		0.9	tr
β-Phellandrene	1030	tr	tr	0.1
(E)-β-Ocimene	1050	0.1	0.1	
γ-Terpinene	1059	1.1	0.9	0.1
p-Cymenene	1091	0.6	0.4	0.2
Linalool	1096	0.2	0.3	0.2
trans-Sabinene hydrate	1098	0.2	0.2	0.3
p-1,3,8-Menthatriene	1110	0.1	0.1	tr
2-Isopropyl-5-methyl-(2Z)-hexenal	1114	1.2	0.3	0.1
dehydro-Sabina ketone	1120		0.1	0.2
α-Campholenal	1126	0.2	0.3	
Lepaline	1130			tr
cis-p-Menth-2,8-dien-1-ol	1137	0.2		tr
trans-Pinocarveol	1139	1.3	0.8	0.6
trans-Verbenol	1144	tr	tr	
Sabina ketone+Pinene oxide	1159	0.4	0.3	0.4
Pinocarvone	1164	0.7	0.3	0.2
Borneol	1169	0.7	0.6	0.4
Terpinen-4-ol	1177	1.4	1.1	0.4
p-Cymen-8-ol	1182	0.9	0.9	0.5
cis-Pinocarveol	1184	0.3		
α-Terpineol	1188	0.3	0.2	0.2
Myrtenal+Myrtenol	1195	1.4	1.0	0.8
Piperitol+Safranal	1196	0.2	tr	
(2Z)-Octenol acetate	1198			0.3
trans-Carveol	1216	0.3		
Citronellol	1225	tr		0.6
cis-p-Mentha-1(7),8-dien-2-ol	1230	0.6		
cis-Ascaridol	1237	0.5	0.6	0.4
Cuminaldehyde	1241	0.5	tr	
Carvone	1243	0.3	tr	
Geraniol	1252	0.2	1.1	1.6
cis-Piperitone epoxide	1254	0.6	1.2	
trans-Piperitone epoxide	1256	1.2	0.3	0.1
trans-Ascaridole glycol	1269			0.3
Nonanoic acid	1270	0.1	0.1	
5-(3-Furyl)-2-methyl-1-penten-3-ol=lepalol	1282			0.8
p-Mentha-1-en-7-al	1275	0.2		
Bornyl acetate	1288	4.6	2.6	0.4
Thymol+p-Cymen-7-ol	1290	0.3	0.2	0.3
Carvacrol	1299	0.7		tr
iso-Ascaridol*	1303	20.5	24.1	16.7
Citronellyl acetate	1352	0.1	0.6	0.2
Eugenol	1359	0.1		
Geranyl acetate	1381	0.5	2.1	0.8
β-Longipinene	1400			0.1

Table. Continued

Compounds	RI _{DB-5}	N1	N2	N3
α-Gurjunene	1409		0.1	
β-Caryophyllene	1419	tr	0.1	0.1
Aromadendrene	1441			0.1
Geranyl acetone	1455	0.1	0.1	
(E)-β-Farnesene	1456		tr	tr
allo-Aromadendrene	1460	2.3	1.3	1.2
9-epi-β-Caryophyllene	1466			0.3
Shyobunone isomer I	1488	0.5	0.3	tr
Ledene	1491	tr	0.2	0.2
Shyobunone isomer II	1495	tr	0.5	tr
α-Muurolene	1500	0.3	0.2	tr
Shyobunone isomer III	1510	0.9	0.5	tr
δ-Cadinene	1523	0.8	0.4	0.1
(Z)-Nerolidol	1532	0.4	0.3	
α-Calacorene	1545	0.1		tr
Shyobunone isomer IV**	1560	1.3	0.6	
Palustrol	1568	1.2	11.9	31.8
Caryophyllene oxide	1583		0.2	0.4
Globulol	1590	tr	0.5	0.3
Viridiflorol	1592	1.7		0.8
Ledol	1602	5.2	11.1	23.4
β-Oplopenone	1607	1.5		
Caryophylla-4(12),8(13)-dien-5α-ol	1640	2.5	1.9	0.8
epi-α-Muurolool	1642	0.3	0.3	
α-Cadinol	1654	0.8	0.5	0.3
14-hydroxy-(Z)-Caryophyllene	1667	0.2	tr	tr
Unknown	1680	4.4	2.4	0.1
Germacre-4(15),5,10(14)-trien-1α-ol	1686	0.1	0.2	0.1
Isocalamenediol	1758	0.8	0.6	tr
Cyclocolorenone + epi-Cyclocolorenone	1770	0.1	0.7	2.0
Total		97.6	97.9	97.9
Monoterpene hydrocarbons		31.1	23.7	9.0
Oxygenated monoterpenes		40.8	39.2	26.8
Sesquiterpene hydrocarbons		5.2	3.3	4.5
Oxygenated sesquiterpenes		15.9	29.4	57.9
Aromadendrene skeleton		10.5	25.7	59.7
Menthane skeleton		30.1	21.6	8.2

RI – Retention index, tr – traces (<0.05%).

Unknown: M⁺ 236, (m/z): 43(100), 93(65), 69(60), 55(45), 109(36), 107(33), 137(24), 151(16), 178(10), 236(7).

* Probably it is an ascaridol, not iso-ascaridol.

** Tentatively identified.

p-Cymene (20.3%) and iso-ascaridol (20.5%) were found as the major constituents in oil N1. The oil with such a composition could be described as p-cymene + iso-ascaridol chemotype or it could be a mixture of two different chemical profiles of the oils, one of them of p-cymene and another one of iso-ascaridol type. It should be mentioned that ascaridol is a heat sensitive compound, which undergoes a partial thermal isomerization to iso-ascaridol during GC analyses. So, actually the compound named as iso-ascaridol has to be an ascaridol. Wild rosemary oils with the predominant compound p-cymene were determined in some countries such as Finland, Mongolia and China [15, 25, 26–28]. Ascaridol type oils are rare and atypical found only in Finland [15], while

oils with ascaridole (26.8%) and p-cymene (17.6%) were determined in the plants from Northeast China [28].

Sample N3 was characterized by palustrol (31.8%), ledol (23.4%) and iso-ascaridol (16.7%). It could be described as palustrol + ledol + iso-ascaridol type, or as a mixture of two chemotypes: palustrol + ledol and iso-ascaridol. The marsh rosemary oils of ledol and palustrol chemotype are quite common (see Introduction).

Analysis of sample N2 evaluated such representative constituents: iso-ascaridol (24.1%), p-cymene (15.3%), palustrol (11.9%) and ledol (11.1%). It is evident that such chemical composition of the oil is because of the combination of the previous two oils containing various chemical profiles.

It could be concluded that marsh rosemary (*Rh. tomentosum*) plants can synthesize essential oils of various chemical profiles and this ability can assert even in plants grown under the same climatic conditions (in limited population, gathered in a distance up to 1.5 km). The oils could be attributed to p-cymene, palustrol + ledol and / or iso-ascaridol chemotypes.

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PELKINIO GAILIO (*RHODODENDRON TOMENTOSUM*), SURINKTO NEDIDELIAME PLOTE RYTŲ LIETUVOJE, ETERINIŲ ALIEJŲ ĮVAIROVĖ

Santrauka

Pelkinio gailio antžeminė dalis surinkta 2004 m. žydėjimo metu pelkėse prie Šulnio ežero (Rytų Lietuva, Šalčininkų r.). Eteriniai aliejai, išgauti hidrodistiliacijos būdu, buvo išanalizuoti dujų chromatografijos ir dujų chromatografijos-masių spektrometrijos metodais. Identifikuota 90 cheminių junginių, kurie sudarė iki 97,9 % viso aliejaus. Nustatyti šie pagrindiniai komponentai: p-cimenas (6,2–20,3 %), izo-askaridolis (16,7–24,1 %), palustrolis (1,2–31,8 %) ir ledolis (5,2–23,4 %), kurių kiekiai keitėsi priklausomai nuo augavietės. Nepaisant to, kad augalinė žaliava buvo rinkta nedideliame plote, buvo pastebėta didelė eterinių aliejų sudėties įvairovė.