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Composition of young and aged shoot essential oils of the wild *Ledum palustre* L.

Rita Butkienė[1]*,

Vaida Šakočiūtė[2],

Daiva Latvėnaitė[2],

Danutė Mockutė[1]

[1] *Institute of Chemistry, A. Goštauto 9, LT-01108 Vilnius, Lithuania*

[2] *Faculty of Chemistry, Vilnius University, Naugarduko 24, LT-03225 Vilnius, Lithuania*

Wild *Ledum palustre* L. plants were collected near Juodupė (Rokiškis region) and Šilėnai (Vilnius region) villages. Essential oils from young and aged shoots, obtained by hydrodistillation, were analysed using GC and GC/MS. The major constituents were palustrol (31.4–42.8%), ledol (23.6–30.8%), cyclocolorenones (4.0–9.3%), myrcene (1.2–8.6%) and limonene (11.0%). The essential oil from plants growing near Šilėnai village differed from that of *L. palustre* near Juodupė by a higher content of compounds with the menthane carbon skeleton (10.9–18.0% and ≤1.2%, respectively) and by the content of limonene (3.7–11.0% and not detected, respectively). All essential oils included furyl containing monoterpenoids. Young shoots biosynthesized 3–4 times larger amounts of essential oils and contained larger quantities of terpenoids than did aged shoots. The content of palustrol in the essential oils of aged shoots was higher than of young ones. The dependence of ledol quantities on the age of shoots was opposite in the localities under study. The sixty nine identified compounds comprised 98.0–99.4% of *L. palustre* essential oils.

Key words: *Ledum palustre*, Ericaceae, composition of essential oils, young and aged shoots, ledol, palustrol, cyclocolorenones, myrcene, limonene

INTRODUCTION

Ledum palustre (Ericaceae) plant preparations have been used for healing different pains, wounds, lung and other diseases from ancient times [1, 2]. Scientists have revealed that the antitussive and expectorant effect of *L. palustre* essential oils depends on the content of the sesquiterpenoid ledol [2]. The preparation of the cough medicine "Ledin" was based on the above investigations. The *L. palustre* essential oil from Siberia inhibited the growth of other plants [3]. A tick-repellent effect was observed for *L. palustre* (new name *Rhododendron tomentosum* (Stokes) H. Harmaja) essential oil from Sweden with the dominant constituents myrcene and palustrol [4].

The major constituents of *L. palustre* var. *palustre* essential oils from Northern and Central Europe were mostly palustrol, ledol and myrcene [4–13]. The next main constituent in oils of some *L. palustre* overground part samples collected in Leningrad region and in the Netherlands was cyclocolorenone and myrtenal, respectively [12, 13] beside the major compounds palustrol and ledol. The essential oils from Leningrad region did not contain compounds with the menthane carbon skeleton [12], while some oils from Siberia and Far East included larger amounts (>50%) of constituents with this carbon skeleton [14, 15]. The predominant constituent in these essential oils was limonene. Ledol was determined in 61 *L. palustre* essential oils produced by plants collected in different localities of Europe and Asia [9]. Some samples of the oil from Northern and Central Europe contained small (≤10%) amounts of ledol [9]. The above results implied that the major constituents might be other volatile compounds in the essential oils.

Essential oils were obtained from different parts of *L. palustre* (all overground parts, shoots and leaves) plants. The content of oils in young leaves [16] and shoots [17] was higher than in the corresponding aged parts. Ledol was determined in the leaf essential oil [16] and three compounds (ledol, palustrol and germacron) in shoot oil [17]. The composition of the essential oils varied in a wide range in different localities [4–17].

Only one species of the genus *Ledum L. palustre* is growing in Lithuania [18].

The variation of essential oil amounts and composition were determined in young and aged shoots of *L. palustre* plants collected in two localities.

MATERIALS AND METHODS

Ledum palustre L. plants were collected in June 2007 near A – Juodupė (Rokiškis region) and B – Šilėnai (Vilnius region) villages. Young and aged shoots were separated before drying. Essential oils were prepared by hydrodistillation (2 h) of air-dried shoots in an apparatus according to [19]. The ratio of shoots and water was 1 : 20. Essential oils were collected in 2 ml of a mixture of hexane: diethyl ether = 1 : 1. The results are represented as mean values obtained from at least two hydrodistillations of plant material. The yields of the oil of young shoots

* Corresponding author. E-mail: rita.butkiene@chi.lt

(Juodupė 2.01% and Šilėnai 1.52%) and aged shoots (Juodupė 0.45% and Šilėnai 0.55%) were obtained using 50 g of plant material and expressed in w/w% on dry weight.

Analysis of the essential oils was carried out by GC and GC/MS. The quantitative analysis was performed on a HP-FFAP capillary column (30 m × 0.25 mm × 0.25 μm) using an HP 5890II chromatograph equipped with FID. The GC oven temperature was programmed as follows: from 60 °C (isothermal for 3 min) increased to 160 °C at a rate of 5 °C/min (isothermal for 1 min), then increased to 250 °C at a rate of 10 °C/min, and the final temperature was kept for 3 min. The temperatures of the injector and the detector were 250 °C. The flow rate of carrier gas (helium) was 1 ml/min. Analyses by GC/MS were carried out with an HP 5890 gas chromatograph equipped with an HP 5971 mass selective detector and HP 7673 split/splitless injector (splitless 0.75 min) on a DB-5 capillary column (50 m × 0.32 mm × 0.25 μm). The chromatographic conditions were the same. Mass spectra in electron mode were generated at 70 eV.

The percentage composition of essential oils was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention indexes and the mass spectra with the corresponding data in the literature [20–22] and the computer mass spectra libraries (Wiley and NBS 54K).

RESULTS AND DISCUSSION

Young and aged *L. palustre* shoots were separated from wild plants collected in two localities. The amounts of young shoot essential oils (1.52–2.01%) exceeded 3–4 times those from aged parts of plants (0.45–0.55%) (Table 1). The composition of essential oils depended on the locality (Tables 1 and 2). The dominant constituent in all oils under study was palustrol (31.4–42.8%), followed by ledol (23.6–30.8%). Essential oils containing ≥17% of ledol are suitable for preparation of "Ledin" [12]. The third and fourth compounds in all *L. palustre* oils from Juodupė were myrcene (5.7–8.6%) or cyclocolorenones (5–9.3%), while essential oils of plants growing near Šilėnai village in those positions carried limonene (3.7–11.0%), myrcene (6.1%) or cyclocolorenones (4.0%). All investigated oils contained furyl monoterpenoids lepalon – 5-(3-furyl)-2-methyl-1-penten-3-one (0.6–2.3%) and lepalol – 5-(3-furyl)-2-methyl-1-penten-3-ol (1.3–2.8%). The *L. palustre* oils from Juodupė included lepalin – 5-(3-furyl)-2-methyl-1-pentene (0.1–0.4%) and 2-methyl-5-(3-furyl)-3-penten-2-ol (1.0–1.2%), besides the aforesaid furyl compounds.

A marked difference of the essential oils from plants collected in various habitats was found in the content of compounds with the menthane carbon skeleton (Table 2, Fig. 1). The *L. palustre* essential oils from Juodupė (north Lithuania) contained ≤1.2% of compounds with the above skeleton (Table 2) and their composition was close to that of oils that contained no compounds with the menthane carbon skeleton (Leningrad district) [12]. Essential oils from plants collected near Šilėnai village (~150 km from Juodupė to the south, ~20 km from Vilnius) contained a higher amount (10.9–18.0%) of constituents with the menthane carbon skeleton in comparison with oils from Juodupė (≤1.2%). In the *L. palustre* oils from Šilėnai, sesquiterpenoid shyobunon (Table 2, 0.2%) was determined, which was not found in earlier investigated oils [4–17, 22]. iso-Ascaridol (0.1–0.3%) was identified in all oils under study, but formerly was not found in *L. palustre* oils [4–17].

The expectorant and antitussive properties of *L. palustre* products depended on the content of ledol (Tables 1 and 2, Fig. 2) [2]. The quantities of ledol in the oils and in the plants might be interesting for phytotherapeutists and other users of *L. palustre* preparations (Tables 1 and 2). All young shoots included larger amounts of major compounds

Table 1. **Content (%) of essential oils (EO) and the content of ledol, palustrol, myrcene and cyclocolorenones in oils and in shoots**

Locality		EO	Ledol		Palustrol		Myrcene		Cyclocolorenones	
			in EO	in shoots	in EO	in shoots	in EO	in shoots	in EO	in shoots
Juodupė	1 year	2.01	30.8	0.62	35.2	0.71	8.6	0.17	9.3	0.19
	aged	0.45	23.9	0.11	42.8	0.19	5.7	0.03	5.0	0.02
Šilėnai	1 year	1.52	23.6	0.36	31.4	0.48	6.1	0.09	5.3	0.08
	aged	0.55	30.5	0.17	36.7	0.20	1.2	0.01	4.0	0.02

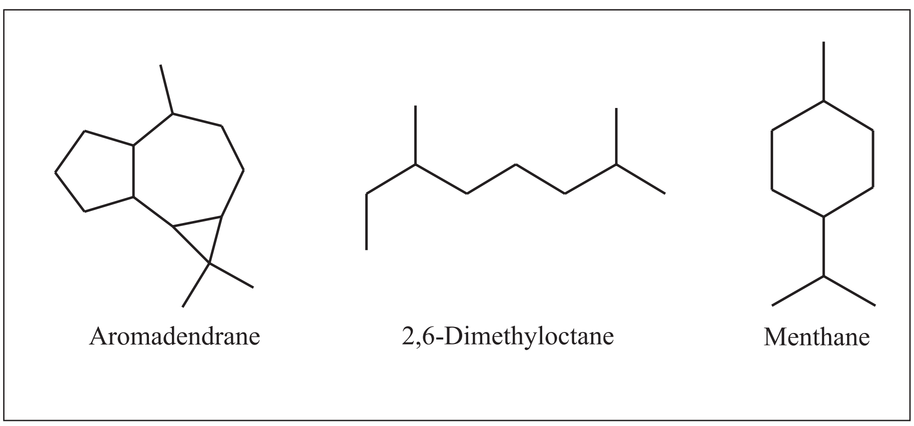

Fig. 1. Main carbon skeletons of *Ledum palustre* L. essential oil constituents

Table 2. **Composition (%) of essential oils of the wild *Ledum palustre* L. shoots**

Compounds	RI	Juodupė		Šilėnai	
		young	aged	young	aged
4-Methylene-5-hexenal	927	t	0.1	t	t
α-Pinene	939	t	t	t	t
Camphene	954	t	t	t	t
Sabinene	975	t	t	t	t
β-Pinene	979	t	t	t	t
Myrcene	990	**8.6**	**5.7**	**6.1**	1.2
(2E, 4E)-Heptadienal	1007	t	0.2	t	–
α-Terpinene	1017	0.2	0.2	0.1	–
p-Cymene	1024	t	0.3	0.1	0.1
Limonene	1029	–	–	**11.0**	3.7
(Z)-β-Ocimene	1037	t	t	t	t
(E)-β-Ocimene	1050	t	t	t	t
γ-Terpinene	1059	t	t	t	t
Terpinolene	1088	–	t	–	t
p-Cymenene	1091	–	–	0.1	t
2-Methyl-6-methylene-3,7-octadiene-2-ol	1095	0.6	2.0	0.7	0.4
p-1,3,8-Menthatriene	1110	–	–	t	t
(E)-p-Mentha-2,8-dien-1-ol	1122	–	–	1.1	1.0
Lepalin? *	1130	0.1	0.4	–	–
2-Methyl-6-methylene-1,7-octadien-3-one	1136	0.2	0.9	0.1	0.1
(Z)-p-Mentha-2,8-dien-1-ol	1137	–	–	0.9	0.8
Ipsdienol	1145	t	0.3	0.1	t
2-Methyl-6-methylene-1,7-octadien-3-ol	1157	0.4	1.6	0.4	t
Terpinen-4-ol	1177	t	t	t	t
α-Terpineol	1188	t	0.3	–	–
(E)-p-Mentha-1(7),8-dien-2-ol	1189	–	–	2.3	3.6
(E)-Piperitol	1208	–	–	0.3	0.5
iso-dihydro-Carveol	1214	–	–	0.3	0.1
(E)-Carveol	1216	–	–	1.0	0.3
2-Methyl-5-(3-furyl)-3-penten-2-ol	1225	1.0	1.2	–	–
cis-Carveol + (Z)-Ocimenone	1229	–	–	1.5	1.8
(Z)-p-Mentha-1(7),8-dien-2-ol	1230	–	–	2.7	2.2
(E)-Ocimenone	1238	–	–	–	2.2
Cumin aldehyde	1241	0.2	0.4	–	–
Carvone	1243	–	–	0.8	0.8
Lepalon	1258	1.1	2.3	0.6	0.7
Lepalol	1282	1.9	2.8	1.5	1.3
Bornyl acetate	1288	t	t	0.2	0.3
(2E, 4Z)-Decadienal	1293	–	t	t	t
iso-Ascaridol**	1303	0.1	0.1	0.3	0.2
Citronellyl acetate	1352	0.1	0.1	t	t
Geranyl acetate	1381	0.6	1.0	0.5	0.2
β-Elemene	1390	0.1	t	t	t

Table 2. **Continued**

Compounds	RI	Juodupė		Šilėnai	
		young	aged	young	aged
α-Gurjunene	1409	0.8	0.2	0.6	0.2
(E)-Caryophyllene	1419	0.2	t	t	t
β-Copaene	1432	0.1	t	t	t
γ-Elemene	1436	t	t	t	t
Calarene	1437	0.1	0.1	t	t
α-Guaiene	1439	t	0.2	t	t
Aromadendrene	1441	0.3	0.2	t	t
α-Humulene	1454	0.3	t	t	0.2
Allo-aromadendrene	1460	2.7	2.3	1.6	2.1
cis-Cadina-1(6),4-diene	1463	0.1	0.2	t	0.1
(E)-9-epi-Caryophyllene	1466	0.2	0.2	t	t
Shyobunon	1488	–	–	0.2	0.2
Ledene	1491	0.6	0.8	0.3	0.1
δ-Cadinene	1523	0.3	0.1	0.2	0.7
Palustrol	1568	**35.2**	**42.8**	**31.4**	**36.7**
Caryophyllene oxide	1583	t	t	t	t
Globulol + Viridiflorol	1590	0.6	1.1	0.8	1.0
Ledol	1602	**30.8**	**23.9**	**23.6**	**30.5**
epi-α-Muurolol	1642	1.1	0.9	1.2	0.2
α-Cadinol	1654	0.8	0.6	0.5	0.1
Isobicyclogermacral	1734	0.4	0.1	0.2	t
Isocalamendiol	1758	t	–	0.7	0.2
Cyclocolorenone + epi-Cyclocolorenone	1770	**9.3**	**5.0**	**5.3**	**4.0**
Total		99.3	98.4	99.4	98.0
Monoterpene hydrocarbons		8.8	6.2	17.5	5.1
Oxygenated monoterpenes		6.3	13.2	15.5	16.7
Sesquiterpene hydrocarbons		5.4	3.6	2.4	3.4
Oxygenated sesquiterpenes		78.3	74.4	64.0	72.9
Aromadendrane skeleton		79.6	76.1	63.1	74.5
Aliphatic monoterpenoids		10.5	11.5	8.0	2.0
Menthane skeleton		0.4	1.2	18.0	10.9
Furyl compounds		4.1	6.6	2.1	2.0

* Mass spectrum, m/z (%) : 148(M^+,100) 133(68) 119(63) 105(85) 91(76) 79(55) 77(19).

** According to [20].

than did aged shoots (Table 1). The same regularity was determined for myrcene, cyclocolorenones and limonene. An opposite dependence was found for palustrol in the oils (Table 1). The essential oil of aged and young shoots contained, respectively, 36.7–42.8% and 31.4–35.2% of palustrol. Such regularity for palustrol was noted only in some localities of Tomsk region [17]. The oil of young shoots from Juodupė contained more ledol (30.8%) than aged shoots (23.9%), while young shoots from Šilėnai village contained smaller amounts (23.6%) of this compound than did aged shoots (30.5%) (Tables 1 and 2). In formerly investigated *L. palustre* essential oils from various habitats, the percentage of ledol during plant vegetation was different [16, 17].

The largest part of *L. palustre* essential oils under study comprised oxygenated sesquiterpenoids (64.0–78.3%) and compounds with the aromadendrane carbon skeleton (63.1–79.6%) (Table 2, Fig. 1). The main compounds ledol, palustrol and cyclocolorenones have the aromadendrane skeleton (Figs. 1 and 2). *L. palustre* plants biosynthesized the total of nine constituents with this skeleton.

All identified constituents comprised 98.0–99.4% of the *L. palustre* essential oils.

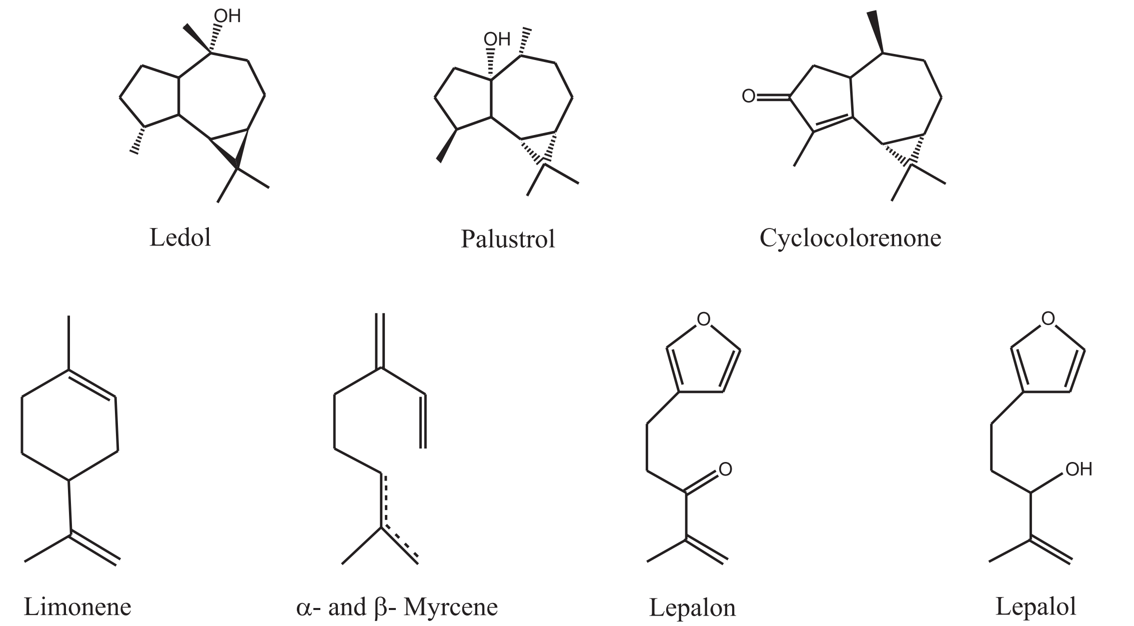

Fig. 2. Characteristic compounds of the essential oils studied

CONCLUSIONS

Young shoots of *L. palustre* plants biosynthesized 3–4 times more of essential oils than did aged ones. The dominant constituents of the oils were palustrol (31.4–42.8%) and ledol (23.6–30.8%). The content of the main compounds in young shoots was higher than in aged ones. However, the content of palustrol was higher in aged shoot essential oils. The content of ledol in the oils of *L. palustre* shoots depended not only on age but also on the locality. Considerable differences between the oils from two localities were found in the content of compounds with the menthane carbon skeleton (≤1.2% from Juodupė and 10.9–18.0% from Šilėnai village).

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JAUNŲ IR BRANDŽIŲ LAUKINIO *LEDUM PALUSTRE* L. ŠAKELIŲ ETERINIŲ ALIEJŲ SUDĖTIS

Santrauka

Laukiniai *Ledum palustre* L. augalai rinkti 2007 m. birželį netoli Juodupės (Rokiškio rajonas) ir Šilėnų kaimo (Vilniaus rajonas). Iš jaunų ir brandžių šakelių hidrodistiliacijos būdu gauti eteriniai aliejai analizuoti dujų chromatografijos ir dujų chromatografijos / masių spektrometrijos metodais. Pagrindiniai komponentai buvo palustrolis (31,4–42,8%), ledolis (23,6–30,8%), ciklokolorenonai (4,0–9,3%), mircenas (1,2–8,6%) ir limonenas (11,0%). Netoli Šilėnų kaimo augančių augalų eteriniuose aliejuose rasta daugiau mentano skeletą turinčių junginių (10,9–18,0%; ≤1,2%) ir limoneno (3,7–11,0%; nenustatyta), lyginant su šalia Juodupės rinktų augalų aliejais. Visuose eteriniuose aliejuose rasta furilo turinčių monoterpenoidų. Jaunose šakelėse biosintezuojasi 3–4 kartus daugiau eterinių aliejų ir terpenoidų juose yra daugiau, nei brandžiose šakelėse. Palustrolio kiekis eteriniuose brandžių šakelių aliejuose buvo didesnis, nei jaunų. Ledolio kiekio priklausomybė nuo šakelių amžiaus buvo skirtinga tirtose vietovėse. Identifikuoti šešiasdešimt devyni junginiai sudaro 98,0–99,4% gailio eterinių aliejų.