TERPENOIDS OF THE ESSENTIAL OIL OF *Ledum hypoleucum*

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UDC 581.192:547.913(582.912+571.6)

The chemical composition of the terpenoids of the essential oil of *Ledum hypoleucum* growing in the Far East has been studied. It differs from *L. palustre* by a larger amount of compounds of the bisabolane series in the essential oil. Among the monoterpenoids, p-cymene, β-phellandrene, and ascaridole predominate. The main components of the sesquiterpenoids are ar-curcumene and ar-turmerone, now found for the first time in *Ledum* essential oils.

Continuing a study of plants of the genus *Ledum*, we have investigated the terpenoids of the essential oil of *Ledum hypoleucum* Kom. This plant is widespread in the Far East (Maritime Territory, Khabarovsk krai, the island of Sakhalin) and its reserves are fairly considerable [1]. It is used for medicinal purposes by the people of the Far East [2, 3] but it is not used by official medicine because of the absence of chemical and pharmacological information, and this forms the basis for its further investigation.

In the terpene part, 21 monoterpene and 12 sesquiterpene compounds were identified by chromatographic and spectral methods (Table 1). A comparative analysis of the terpenoids of *L. hypoleucum* and of *L. palustre* L. var *palustre* [4] indicated considerable differences in the compositions of these compounds in the species studied. The main components of the monoterpene hydrocarbons of *L. hypoleucum* are p-cymene and β-phellandrene, while in the essential oil of *L. palustre* limonene predominates. In the sesquiterpene hydrocarbons of *L. hypoleucum* ar-curcumene predominates, while in the case of *L. palustre* the main component is allo-aromadendrene.

Ledol, which is a chemical marker of the *Ledum* genus and is responsible for its antitussive action [11], was detected in insignificant amount in the essential oil of *L. hypoleucum*. The antiinflammatory activity of the essential oil and of the terpenoid fraction of *L. hypoleucum* that has been reported [12] is apparently explained by the considerable amount of compounds of the bisabolene series, which possess antiinflammatory properties [13, 14].

The authors thank N. S. Pavlov of the Far Eastern Scientific Center, Academy of Sciences of the USSR, for the collection and supply of the plant raw material.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in CCl_4. PMR spectra were recorded on Varian A-56/60 and Bruker SY-200 instruments using solutions in CCl_4 and $CDCl_3$, with HMDS as internal standard. Mass spectra were taken on a MS 902 instrument with a glass inlet system (120°C) at 70 eV. Specific rotations were measured on a Zeiss instrument using solutions in chloroform.

The raw material was collected in the environs of the village of Mukhen, Khabarovsk krai in June-July, 1981. The essential oil for analysis was obtained by the steam distillation of leafy shoots of *L. hypoleucum* (the yield of essential oil averaged 1.48%), and was separated into fractions of acids, phenols, and terpenoids by the method of Liberti and Cartoni [15]. The terpenoid fraction, averaging 99.3% of the essential oil, was separated by chromatography on air-dry KSK silica gel (0.140-0.315 mm) at a ratio of substance to sorbent of 1:10. Petroleum ether eluted hydrocarbons (18.50 g), and diethyl ether eluted oxygen-containing compounds (9.70 g).

Tomsk State Medical Institute. Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 104-107, January-February, 1987. Original article submitted July 14, 1986.

0009-3130/87/2301-0087$12.50

TABLE 1. Composition of the Terpenoids of the Essential Oil of *L. hypoleucum*

Monoterpene hydrocarbons (50.5%)		Sesquiterpene hydrocarbons (7.0%)		Oxygen-containing compounds (40.5%)	
name of the component	amount in the fraction, %	name of the component	amount in the fraction, %	name of the component	amount in the fraction, %
Tricyclene	Tr.	Caryophyllene	0,7	Sabinene hydrate	0,2
Santene	1,9	Selina-3,7(11)-diene	20,3	Borneol	0,2
α-Pinene	5,8	α-Humulene	4,4	Terpineol-4	5,2
Camphene	2,4	allo-Aromadendrene	3,3	α-Terpineol	0,2
β-Pinene	4,6	ar-Curcumene	37,8	Ascaridole	48,4
Myrcene	1,3	α-Selinene	15,5	Geraniol	0,1
Δ^3-Carene	1,9	α-Muurolene	1,2	Bornyl acetate	4,7
β-Phellandrene	22,9	β-Bisabolene	5,6	Citronellyl acetate	0,5
Limonene	3,6	γ-Cadinene	1,3	Geranyl acetate	3,2
γ-Terpinene	3,7	δ-Cadinene	3,9	Ledol	Tr.
Terpinolene	9,4			ar-Turmerone	31,4
p-Cymene	39,3				
Sum of unidentified Components	3,3	Sum of unidentified components	6,2	Sum of the unidentified components	5,8

Fractional Distillation of the Hydrocarbons. The hydrocarbon fraction (18.50 g) was subjected to vacuum distillation into monoterpene hydrocarbons (13.24 g; bp 90-120°C/10 mm Hg) and sesquiterpene hydrocarbons (1.85 g — still residue).

Analysis of the Monoterpenes. The analytical GLC of the monoterpene hydrocarbons was carried out on a Chrom 4 instrument with a glass capillary column 52 m long filled with XE-60 at a column temperature of 70°C. The components were identified by the method of additives, quantitative determination being performed by the method of simple normalization. The results of the analysis are given in Table 1.

Identification of the Sesquiterpene Hydrocarbons. The sesquiterpene hydrocarbon fraction (1.85 g) was chromatographed on silica gel L (0.075-0.160 mm) at a ratio of substance to sorbent of 1:70. Petroleum ether eluted four fractions successively. The first (0.04 g) contained residues of monoterpenes and was not investigated. From the second (0.28 g), by chromatography on silica gel impregnated with 20% $AgNO_3$, petroleum ether with the addition of 0.10% of diethyl ether yielded 0.18 g of selina-3,7(11)-diene, 0.06 g of allo-aromadendrene, and 0.04 g of α-selinene. Chromatography of the third fraction (0.77 g) under analogous conditions by gradient elution yielded selina-3,7(11)-diene (0.14 g), α-selinene (0.11 g), γ-cadinene (0.02 g), and β-bisabolene (0.09 g). According to GLC and PMR spectroscopy, the fourth fraction (0.46 g) consisted of ar-curcumene $[\alpha]_D^{19}$ +24.6° (c 2.84; chloroform). The spectral characteristics of the compounds isolated agreed with those for authentic samples. The analytical GLC of the sesquiterpene hydrocarbons was performed on a Chrom-5 instrument with a flame-ionization detector using a glass capillary column 0.2 mm in diameter and 50 m long with the stationary phase OV-101, the temperature of the analysis being raised from 80 to 190°C at the rate of 3°C per minute. The GLC results are given in Table 1.

Identification of the Oxygen-Containing Terpenoids. The oxygen-containing compounds (9.7 g) were subjected to preliminary separation by column chromatography on silica gel into four fractions (I-IV). The weakly polar components — oxides, alcohol acetates — were concentrated into fraction I (6.40 g); terpenoids the R_f values of which were close to terpineol-4 into fraction II (1.33 g); components chromatographically close to geraniol into fraction III (1.24 g); and polar compounds into fraction IV (0.74 g).

The rechromatography of fraction I (3.09 g) on silica gel (0.1) with gradient elution yielded 0.63 g of ar-tumerone ($[\alpha]_D^{19}$ +36.4° (c 3.85; chloroform)), 0.7 g of a mixture of geranyl acetate, bornyl acetate, citronellyl acetate, and ar-tumerone (according to PMR), 0.73 g of ascaridole ($[\alpha]_D^{19}$ −4.9° (c 4.10; chloroform)), and 0.11 g of terpineol-4.

The rechromatography of fraction II (1.33 g) on silica gel (0.1) in a ratio of 1:40 yielded 0.41 g of terpineol-4 and 0.6 g of a mixture of more polar components which was combined with fraction III (1.24 g) and chromatographed on silica gel impregnated with 20% of $AgNO_3$. From this fraction 0.1 g of ledol, 0.03 g of borneol, and 0.07 g of geraniol were obtained.

Under similar conditions, fraction IV (0.74 g) yielded 0.35 g of sabinene hydrate. The spectral characteristics of the compounds isolated were identical with those for authentic samples. The results of the GLC of the fraction of oxygen-containing compounds performed under the same conditions as for the sesquiterpene hydrocarbons are given in Table 1.

SUMMARY

1. The essential oil of *Ledum hypoleucum* differs considerably in its chemical composition from the essential oil of *L. palustre* by a predominating amount of compounds of the bisabolane series.

2. The main component of the monoterpenoids of the essential oil of *Ledum hypoleucum* are p-cymene, β-phellandrene, and ascaridole.

3. Among the sesquiterpenoids ar-curcumene and ar-turmerone predominate, this being the first time that they have been found in essential oils of the *Ledum* genus.

4. The very small amount of ledol does not justify the recommendation of *L. hypoleucum* as an antitussive agent together with *L. palustre*.

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