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INTRASPECIES CHEMICAL VARIABILITY OF THE ESSENTIAL OIL OF Ledum palustre

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The compositions of the essential oils of marsh tea, *Ledum palustre* L., from the southern regions of Tomsk, Transbaikalia and Amur province, and also from the southern part of the island of Sakhalin have been analyzed in detail. The results obtained indicate the sharply pronounced intraspecies chemical variability of this plant. In addition to the chemotype of march tea with myrcene as a macrocomponent which is widely distributed in the European part of the USSR, another three chemotypes with the macrocomponents limonene, sabinen, and p-cymene have been revealed.

The flora of Siberia and the Far East is distinguished by the greatest variability of representatives of the genus *Ledum* L. [1]. Marsh tea, *L. palustre*, which is widely distributed here, is represented by five varieties [2, 3]. However, hitherto the opinions of botanists in the field of the systematics of the genus have been extremely contradictory [4]. In view of this, to answer the question of the status of the species and of the intraspecies taxons, in addition to the classical morphological and microscopic features, and also information on the karyology, ecology, and geography of the plants, the results of chemical analysis and, in particular, the composition of the essential oils [5] must be taken into account.

Many plants are represented by a number of chemotypes [6] characterized by a predominating content of terpenoids of different biogenetic origins. Since marsh tea is an officinal medicinal plant [7] the expectorant and antitussive effect of which is due to its content of essential oil and ledol [8, 9], information on its composition is of great practical importance. At the same time, the few publications on the study of the essential oil of marsh tea growing in the territory of Siberia and the Far East [10-12] are far from complete.

We have studied samples of the essential oil from leafy shoots of *L. palustre* collected in the Tomsk province, Transbaikalia, the Amur province, and on the island of Sakalin, and, in these, 71 compounds have been identified by various chromatographic and spectral methods.

The main components of the essential oil of marsh tea growing in the environs of Tomsk were identified by Klokova et al. [10]. For the isolation and identification of the minor components we carried out the fractionation of combined samples of the essential oil of plants from the environs of Tomsk and the village of Suiga, Molchanovo region of Tomsk province, which are close in composition. In the samples investigated we detected 65 compounds,

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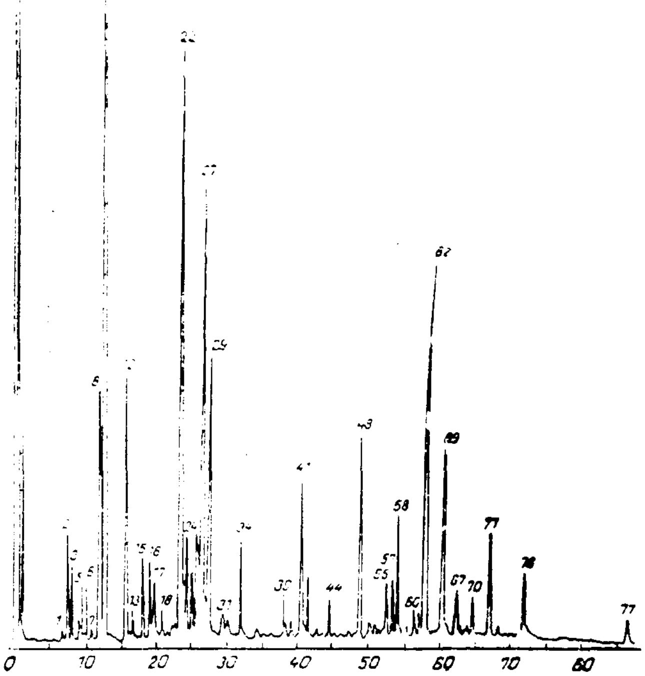


Fig. 1. Chromatogram of the combined essential oil of marsh tea from the environs of Tomsk and from the village of Suiga, Molchanovo region of Tomsk province. The interpretation of the chromatogram is given in Table 1.

of which 40 components were isolated by various chromatographic methods and were identified from their spectral characteristics, the other being identified by GLC results. A chromatogram of the combined essential oil is shown in Fig. 1. A comparison of the results obtained by capillary GLC and from the isolation of the terpenes showed that a number of components had identical retention times and their quantitative calculation was difficult. Therefore, to determine the amounts of the difficulty separable monoterpene hydrocarbons (p-cymene and α-terpinene; β-phellandrene and limonene) we selected special chromatographic conditions. The results of a calculation of the chromatograms of the samples of essential oil of L. pallustre investigated are given in Table 1.

In the essential oil of marsh tea from the southern regions of Tomsk province compounds of the p-methane series were predominant: limonene, cis- and trans-p-mentha-1(7),8(9)-dien-2-ols, and isomeric p-mentha-2,8-dien-1-ols. Since the alcohols named are formed on the photooxidation of limonene [13], the biogenetic direction of the formation of limonene is apparently dominating in this chemotype, and photochemical transformations are characteristic for the biosynthesis of the terpenoids. An analogous process has been observed in a number of sesquiterpenes: Together with a high content of δ-cadinene, the main product of its photooxidation — muurolanedienol — has been found in the essential oil.

A considerable amount of germacrone has previously been found in the essential oil of a sample of marsh tea from Suiga, but we were unable to isolate this compound. At the same time, from the still residue after fractionation, 2.5 g of β-elemenone, the peak of which on the chromatogram coincides with that of ledol, was obtained. It is possible that a small amount of β-elemenone was present in the initial oil, and this was confirmed by its presence in other samples of the oil [3, 14]. However, in our case the β-elemenone is apparently a product of the isomerization of germacrone on fractional distillation, as has been confirmed by literature information [15].

TABLE 1. Composition of the Essential Oil of Ledum palustre L. from Various Regions of Siberia and the Far East

Number of the peak in Fig. 1	Component $[\alpha]_D^t$(CHCl$_3$); mp ., °C	Quantitative amount of the component (% on the mass of the whole essential oil)					Literature
		Method of collecting the raw material – area					
		Tomsk	Suiga	Tynda	Lake Baikal	Utesnoe	
1	2	3	4	5	6	7	8
1	Tricyclene	0,1	—	2.6	2.8	0,2	14
2	α-Pinene	0,9	0.2	1,2	1.1	1,0	3,14
3	Camphene	0,6	0,1	0,8	1,1	0.6	3,14
4	Sabinene	0,3	0.2	33,2	21,9	0,5	3 14
5	β-Pinene	0,6	0.2	0,8	0,8	1,0	3,14
6	Myrcene	0,9	0,2	3,2	0.2	Tr.	3,14
7	α-Phellandene	1,4	—	—	0 5	0,4	14
8	α-Terpinene	3,8	2,7	17,1	12,9	7,4	3 14
	P-Cymene					14,6	3,14.27,28
9	Limonene $[\alpha]_D$ —100,7° (c 1,4)	50,3 (96% of limonene and 4% of β-phellandrene)	22,2	0,9	1,6	2,9	3,10.14
	β-Phellandrene					2,2	3,14
10	cis-β-Ocinene	0,2	0,1	—	1,5	0,2	3,14
11	γ-Terpinene	0.1	—	0,7	6.3	0,9	3,14
—	1-Methyl-4-isopropylbicyclo[3.1.0]hexan-2-one	—	—	1,2	0,2	—	21,22
12	p-Cymenene	0.8	3.0	0,5	0,9	2,0	3,14
	Terpinolene			—	—	0.3	3,14
13	Linalool	0,2	0.6	—	—	—	—
	(-)-trans-Sabinene hydrate[1] $[\alpha]_D$ +36,8° (c 1,3)			1,9	1.4	0.5	14,23
—	Leibakon (5-isopropylbicyclo[3.1.0]hex-3-en-2-one	—	—	5,0	3,7	—	—
15	cis-p-Mentha-2.8-dien-1-ol $[\alpha]_D$ —66,6° (c 0,9)	0,8	0.4	0,5	0.2	0,6	13
16	trans-p-Mentha-2,8-dien-1- $[\alpha]_D$ —163,4° (c 0,9)	0,7	0,3	0,7	0,8	0,5	13,14
—	Methylchavikol	Tr.	—	—	—	—	14
17	4-Isopropenylcyclohexanone[1], $[\alpha]_D$ 0° (c 0,9)	0,4	0,6	2,5	1,7	1,3	29,30
19	Borneol	0,1	Tr.	0,4	0,6	0.9	14
20	4-Isopropylidenecyclohexa-2,5-dienone $[\alpha]_D$ +3,3° (c 2,4)	0,3	0,3	—	—	0,4	17
—	5-Isopropylbicyclo[3.1.0]hex-2-en-2-al [2]), $[\alpha]_D^{20}$ —128,1° (c 4,3)	—	—	4,1	17,1	—	20
21	Terpinen-4-ol[1],[2] $[\alpha]_D$ +5,0° (c 1,2)	0,3	0,3	7,8	7,5	1,8	3,14
22	trans-p-Mentha-1(7),8-dien 2-ol[1] $[\alpha]$ —5,0° (in the pure form)	4,8	9,1	1,8	1,8	7.5	3,13
23	Lepaloks(p-mentha-1,8(10)-diene 3,9-epoxide $[\alpha]_D$ + +33 9 (c 1,1)	0.6	0,8	—	—	1,9	—
24	Myrtenal	0,7	0.	0,8	Tr.		3.14,27
25	3,6-Dimethylbenzofuran	0.5	1,0	—		0,6	16
27	cis-p-Mentha-1(7),8-dien-2-ol[1] $[\alpha]_D^{70}$ —74,7° (in the pure form)	3,6	12,1	1,1	0,4	5,9	3,13
28	Isopiperitenone [1]) $[\alpha]_D$ —46,0° (c 1,0)	Tr.	Tr.	—	—	—	31
	Ascarid [3]), $[\alpha]_D$ 0° (c 4,1)	—	—	1,4	3,	8,7	3,23
	Isopropylphenol [2]), 62°						25
29	Carvone [1]). $[\alpha]_D$ +58,5° (c 1,1)	1,4	2,8	—	—	0,7	10 14
—	trans-carveol						10,14
31	Perillaldehyde [1]) $[\alpha]_D$ +10,9° (c 1,5)	0,2	0,5	—	—	0,6	14
31	Geraniol		0,3	1,1	0.2	1,6	10
32	Linalyl acetate		0.2	0,4	0.5	0,8	10

TABLE 1 (continued)

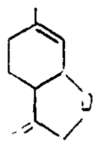
1	2	3	4	5	6	7	8
34	Bornyl acetate	0,6	0,8	0,9	1,0	2.0	3,10,14,27
39	Citronellyl acetate	0,2	1,0	Tr.	Tr.	0,1	3
41	Geranyl acetate	1,0	3,3	Tr.	Tr.	0,2	3,10,14
44	α-Gurjunene$^{1), 3)}$, $[\alpha]_D^{21}$ —171,9° (c 5,0)	1,6	0,5	—	—	1,4 —	3,14,27,28 3,14,27
45	γ-Elemene						
46	Caryophyllene	0,2	0.2	0 5	—	Tr.	3,14 27,28
	Selina-3,7(11)-diene $[\alpha]_D$ —34,7° (c 7,0)	0,2	Tr.	—	—	—	23
47	α-Humulene $[\alpha]_D$ —0,4° (c 5,0)	0,1		—	—	1,6	3,10,14,23,28
48	allo-Aromadendrene$^{1), 3)}$, $[\alpha]_D$ —22,1° (c 6,2)	1,8	3.5	0.9	0.6	0,4	10,23,28
50	$[\alpha]_D^{16}$ +24,6° (c 2,8)	0,1	0,6	—	—	—	23
51	γ-Muurolene	0,1	0,6	—	—	Tr.	3
54	α-Muurolene	0,2	0,3	—	—	Tr.	3
—	Shyobunone$^{3)}$, $[\alpha]_D^{21}$ +30,8° (c 1,7)	—	—	—	—	Tr.	32
—	Epishyobunone$^{3)}$, $[\alpha]_D^{24}$ +5,9° (c 0,7).	—	—	—	—	Tr.	32
55	Ledene$^{1)}$, $[\alpha]_D$ +23,4° (c 2,9)	0,4	2,3	—	—	—	33
57	γ-Cadinene	0,2	1,5	—	—	Tr.	23
58	δ-Cadinene$^{1)}$, $[\alpha]_D$ +18,6° (c 1,6)	0,3	6,4	—	—	Tr.	10,28
59	α-Cadinene	Tr.	0,7	—	—	—	3,28
60	Calamenene	0,2	1,1	—	—	—	3,10
61	Calacorene	0,2	1,0	—	—		3
62	Palustrol$^{1), 3)}$, $[\alpha]$ —12,4° (c 5,0)	7,2	2,0	1,8	0,7	2,3	10,14,27,28
64	Oplopenone 63—67°	0,3	0,4	—	—	—	34
65	Ledol$^{1)}$, . 104—105°, $[\alpha]_D^{20}$ —5,7° (c 1,0)	3.9	3.0	1,4	0,4		10,14,27,28
	β-Elemenone$^{1)}$, $[\alpha]_D$ +2,6° (c 0,8)			—	—	2,0	3,14
	Dehydroxyisocalamenediol $[\alpha]_D$ +26,1° (c 1,4)						18
67	Epicubenol$^{1)}$, $[\alpha]_D^{21}$ —72,4° (c 1,7)	0 2	2,4	—	—		35
	Muurolanedienol$^{1)}$, $[\alpha]_D^{21}$ + 4,2° (c 1,9)						19
70	Lepakson (3,10-epoxygermacr-4(14)-en-8-one)$^{1)}$, $[\alpha]_D$ —47,5° (c 9,0)	0,3	1,6	—	—	—	—
71	Germacrone$^{3)}$, 52—57°, $[\alpha]_D$ 0° (c 1,7)		2.0	—	—		3 14 2
75	Isocalamenediol$^{1)}$, $[\alpha]_D$ —19,4° (c 0,3)	0,2	0,1	—	—	12,6	3,36
77	Cyclocolorenone$^{1), 3)}$, $[\alpha]_D$ —419,9° (c 8,1)	1,8	0,4	—	2,9		37
	Total identified, %	95,9	94.3	97,2	97,6	91,1	

The numerical value of $[\alpha]_D^t$ and the melting points were obtained for the substances isolated from the essential oil of marsh tea: 1) from Tomsk province; 2) from Transbaikalia; 3) from the island of Sakhalin.

Among the sesquiterpenes of the essential oil from the environs of Tomsk, compounds of the aromadendrane series predominated; allo-aromadendrene, α-gurjunene, palustrol, ledol, and cyclocolorenone.

Two new terpenoids were isolated from this essential oil: p-mentha-1,8(10)-diene 3,9-epoxide, which has been called lepaloks (I) and 10-isopropyl-3-methyl-7-methylenecyclodecanone 3,6-epoxide (3,10-epoxygermacr-4(14)-en-8-one) — lepakson (VI). It was impossible to establish a definitive structure for the two terpene compounds corresponding to peaks 26 and 63 on the chromatogram, in view of their very small amount.

Four compounds that have been obtained previously by synthesis were detected for the first time in plant materials: 3,6-dimethylbenzofuran (II) [16], 4-isopropylidenecyclohexa-2,5-dienone (III) [17], dehydroxyisocalamenediol (IV) [18], and muurolanedienol (V) [19]. In addition, six components were detected in *Ledum* essential oil for the first time. These were linalool, 4-isopropylcyclohexanone, (—)-cis-p-mentha-2,8-dien-1-ol, isopiperitone, epicubenol, and oplopenone.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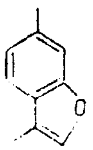
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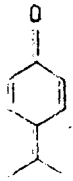

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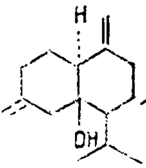

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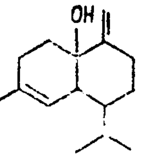

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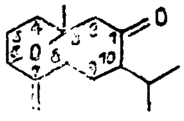

VI

The main component of the essential oil of *L. palustre* from Transbaikalia was sabinene (~33%), the amount of which in the other essential oils was only slight (as a rule, less than 1%). Among the macrocomponents were found p-cymene, terpenen-4-ol, and 2-formyl-5-isopropylbicyclo[3.1.0]hex-2-ene (VIII). Among the monoterpenoids found in the essential oils in small amount a ketone was isolated which, from its spectral characteristics, was identified as 4-isopropyl-1-methylbicyclo[3.1.0]hexan-2-one (IX). Compounds (VIII) and (IX) have previously been obtained synthetically [20-22], and this is the first time that they have been detected in natural materials. A new compound 5-isopropylbicyclo[3.1.0]hex-5-en-2-one (VII), which we have called lebaikon has been isolated from the fraction of weakly acid monoterpenoids.

A distinguishing feature of the marsh tea growing in Transbaikalia is the considerable amount of bicyclic monoterpenoids. Among the sesquiterpenoids in the essential oil of *L. palustre* from the environs of Lake Baikal, cyclocolorenone predominates, the amount of ledol being slight; in a sample from the Amur province the amounts of ledol and palustrol, were higher, and cyclocolorenone was absent.

The essential oil of marsh tea growing on the island of Sakhalin differed in its composition from the other samples. The main component was p-cymene, which agrees with the results of Japanese workers [11], and, of the oxygen-containing monoterpenoids, ascaridole. Their biogenetic precursors are apparently the α- and γ-terpinenes that are present in considerable amounts in the essential oil investigated. The presence of ascaridole has been reported previously for the regionally close varieties *L. palustre* var. *nipponicum et yesoense* [3] and *L. hypoleucum* [23]. The amount of epimeric p-mentha-1(7),8-dien-2-ols was considerably lower than in the Tomsk samples.

Among the sesquiterpene compounds, cyclocolorenone predominated, which agrees with the presence in the essential oil of gurjunene and allo-aromadendrene, while the amount of ledol and palustrol was considerably lower. The composition of the sesquiterpene hydrocarbons is interesting, the main component being α-humulene. The essential oil of marsh tea from the island of Sakhalin contained a series of germacrane derivatives — germacrone, shyobunone, and epishyobunone, which have been found previously in *L. palustre* var. *nipponicum et yesoense*. In spite of the closeness of the qualitative composition of the sample investigated to the essential oil of the Japanese variety, differences were observed in the amounts of a number of the main components: α- and γ-terpenenes and cyclocolorenone.

Thus, the presence of four main chemotypes of marsh tea on the territory of Siberia and the Far East has been established, with predominance of the following macrocomponents: limonene (southern regions of Tomsk province), p-cymene (Sayan Mountains [12], Sakhalin), sabinene (Transbaikalia), and myrcene (north-eastern regions of Tomsk province). The results obtained indicate a highly pronounced intraspecies chemical variability of *L. palustre*, which is the result either of a systematic inhomogeneity of the species [24] or of the influence of ecogeographic factors [12].

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument using CCl_4 solutions, and UR spectra on a Specord UV-VIS for solutions in ethanol. NMR spectra were recorded on Varian A-56/60 and Bruker WP 200 SY (^1H – 200.13; ^{13}C – 50.32 MHz) instruments for solutions in CCl_4 and $CDCl_3$, with HMDS as internal standard. Mass spectra were taken on a Finnigan 8200 spectrophotometer with a glass inlet system. Specific rotations were measured on Zeiss and Polaromat A polarimeters for solutions in $CHCl_3$. Melting points of crystalline substances were determined on a Kofler stage.

Chromatograms were recorded on a Chrom-41 instrument with a flame-ionization detector. Columns: glass capillary 50 m long filled with polymethylsiloxane; glass, 25 m; and quartz, 25 m, with the phase PMS. The pressure of the carrier gas (nitrogen) was 0.5 atm. For recording chromatograms of the essential oils we used programming of the temperature from 50 to 240°C at the rate of 5°C per minute, and the calculation was carried out on an Iskra-226 computer. The fractions of essential oils were analyzed in the isothermal regime. The GLC of the monoterpene hydrocarbons was conducted at a temperature of 60-80°C; that of the oxygen-containing monoterpenes at 100°C; that of the sesquiterpene hydrocarbons and acetates at 120°C; and that of the oxygen-containing sesquiterpenes and still residue at 140°C.

Preparative GLC was performed on a Pye-105 instrument with a 6 mm × 5 m column containing SE-30 (10%) on Chromaton N (0.315-0.250 mm); rate of flow of the carrier gas (nitrogen), 100 ml/min; column temperature 115°C.

The raw material for the investigations was collected: in the environs of Tomsk (Lake Peschanoe) and in the village of Suiga, Molchanovo region of Tomsk province, in July, 1987; in Transbaikalia (Baikal region of the Buryat ASSR, shores of Lake Baikal), and Amur province (environs of Tynda) in July 1981; and on the island of Sakhalin (environs of the village of Utesnoe) in July, 1982. With respect to its morphological characteristics, the marsh tea from South Sakhalin was similar to its broad-leaved form (var. *dilatum* Wahlb.); from Transbaikalia – to the narrow-leaved form (var. *angustum* E. Busch); and from the Tomsk province it corresponded to the ordinary variety (var. *palustre*). However, from microscopic characteristics and the shape of the leaves all the samples mentioned were assigned to *L. palustre* var. *palustre*.

The samples of essential oil were obtained from leafy shoots of marsh tea by steam distillation. The results of a determination of the quantitative amount and physicochemical contents of the essential oils, carried out by generally adopted procedures [25] are given below:

Site of collection of the raw material	Essential oil, % on the mass of the air-dry raw material	n_D^{20}	d_D^{20}	$[\alpha]_D^{20}$, deg CHCl$_3$)
Environs of Tomsk	1,75	1,4856	0,9732	–27,3(*c* 2,4)
„ Suiga	1,87	1.4848	0,8871	–31,0(*c* 2.6)
„ L. Baikal	1.68	1,4895	0,9135	+24.0(*c* 4,3)
„ Tynda	1.33	1,4878	0,9135	–20.0(*c* 7,1)
„ Utesnoe	1,54	1,4953	0,9337	–65,8(*c* 13.4)

After the separation of the acids and phenols by the method of Liberti and Cartoni [26], the terpenoid fraction of the essential oil under investigation was separated into hydrocarbon and oxygen-containing fractions by chromatography on silica gel (1:10). Petroleum and diethyl esters were used as eluents. The fractions obtained were separated with the aid of vacuum distillation into monoterpenes (90-120°C/10 mm Hg) and sesquiterpenes (120-130°C/4 mm Hg).

The sesquiterpene hydrocarbons were separated by column chromatography on silica gel (0.075-0.160 mm) in a ratio of 1:50 and on silica gel impregnated with 20% of $AgNO_3$. The eluents were petroleum ether and 5% of diethyl ether in petroleum ether.

On analysis of the oxygen-containing mono- and sesquiterpenoids by gradient elution (first, petroleum, ether, then 5, 10, 20, and 50% of diethyl ether in petroleum ether, and finally 100% diethyl ether), individual substances or mixtures of substances were isolated successfully, and these were subjected to further separation. Chromatography was conducted on silica gel columns in ratios of the mixture of substances to the sorbent of 1:40 and 1:80 or in columns of silica gel impregnated with $AgNO_3$. Separation was monitored by GLC and TLC on Silufol plates, the substances being visualized in UV light and by spraying with concentrated H_2SO_4.

The fraction of terpenoids of the combined essential oil of L. palustre from Tomsk province (~250 g) was further fractionated in a column with a nichrome spiral having an efficiency of 40 t.t. at temperatures of from 60 to 92°C and pressures of from 44 to 5 m Hg. Fractions with a volume of 3 ml were collected. All the fractions obtained were subjected to GLC, and those similar in composition were combined. The fractions containing the minor components of the mono- and sequiterpenoids in large amount were chromatographed on silica gel L in a similar way to that described above.

In order to determine trans-carveol in the marsh tea essential oil from the village of Utesnoe (South Sakhalin), the peak of which on the chromatogram was superposed on the peaks of ascaridole and carvone, 0.53 g of this essential oil was acetylated with the pyridine and acetic anhydride. This gave 0.50 g of a mixture of acetate of trans- and cis-p-mentha-1(7)-8-dien-2-ols and trans-carveol, the peaks of which were well separated on the chromatogram. The amount of trans-carveol in the essential oil was calculated from the ratio of the areas of the peaks of the acetates.

The terpenoids were identified by GLC, IR, UV, PMR, and ^{13}C NMR spectroscopy and mass spectrometry and by comparison with literature information.

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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Melandrium

I. POLYPODIN B 22-ACETATE FROM Melandrium turkestanicum

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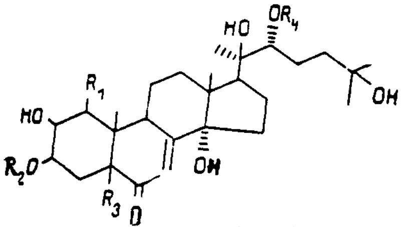
Known ecdysteroids — ecdysterone, ecdysterone 22-acetate, polypodin B, integristerone A, and sileneosides A and D — and a new phytoecdysteroid — polypodin B 22-acetate — have been found in the epigeal parts of the plant *Melandrium turkestanicum* (Rgl.) Vved. (family Caryophyllaceae).

Extending the range of plants of the family Caryophyllaceae containing ecdysteroids that have been studied [1], we have investigated *Melandrium turkestanicum* (Rgl.) Vved., not infrequently growing on the rocky slopes in the central zone of the mountains of the Pamir-Alai and the western Tien-Shan. From the epigeal organs of this plant we isolated known ecdysteroids: ecdysterone (I), ecdysterone 22-acetate (II), polypodin B (III), integristerone A (IV), and sileneosides A (V) and D (VI). In addition to these compounds, we isolated a new ecdysterone (VII), with the composition $C_{29}H_{46}O_9$.



I. $R_1=R_2=R_3=R_4=H$
II. $R_1=R_2=R_3=H$; $R_4=Ac$
III. $R_1=R_2=R_4=H$; $R_3=OH$
IV. $R_1=OH$; $R_2=R_3=R_4=H$
V. $R_1=R_2=R_3=H$; $R_4=\alpha$-*D*-Gal
VI. $R_1=R_3=R_4=H$; $R_2=\alpha$-*D*-Gal
VII. $R_1=R_2=H$; $R_3=OH$; $R_4=Ac$

The IR spectra of substance (VII) had absorption bands due to hydroxy groups (3400-3500 cm^{-1}) and to an α,β-saturated keto grouping (1685 cm^{-1}). In addition to them, absorption at 1735 and 1260 cm^{-1} and the presence of a three-proton singlet at 2.04 ppm in the PMR spectrum (Table 1) showed the presence of one acetyl group in this compound.

The enzymatic hydrolysis of the acetate (VII) by the combined enzymes isolated from bakers' yeast [2], and also alkaline saponification, led to polypodin B (III) [3, 4]. The peak of an ion with m/z 379 (C-20—C-22 cleavage) observed in the mass spectrum of ecdysterone (VII), and its derivatives with m/z 361, 343, and 325, permitted the assumption that the acetyl group was located in the side chain and not in the steroid nucleus [3, 5-7].

On comparing the PMR spectrum of polypodin B (III) and the acetate (VII), an appreciable difference was observed only in the position of the resonance lines of the proton at C-22,

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