VOLATILE COMPONENTS OF *Ledum palustre* var. *nipponicum* et *yesoense*

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Volatile components of Ledum palustre var. nipponicum et yesoense Nakai were investigated and found to consist of more than fifty compounds. The major compound was found to be ascaridol, which is well known to exhibit antibiotic properties.

Ledum palustre is an evergreen shrub which occurs in many varieties and produces small flowers in June. Several studies of the essential oils[1-4] of L. palustre have been reported in the last four decades. Among the components reported were p-cymene, α-terpineol, geranyl acetate, geranyl formate, camphor, borneol, bornyl acetate, β-pinene, salicylic acid, p-cresol, cuminaldehyde, cuminic alcohol, cuminyl acetate, dipentene, myrcene derivatives, ledol, and palustrol.

Apart from a report on antibiotic properties[5] of L. palustre, no useful component is known to be present in it, though leaves of L. palustre var. nipponicum et yesoense have been used in place of tea and as a medicine in some districts. This prompted us to study its essential oils by means of recent techniques. This paper presents an exhaustive study of the volatile oils isolated from different parts of this plant.

Leaves (I) of the plant were collected (May 1975) in Hokkaido, the northern island of Japan. For comparative study, the young leaves (II), the biennial leaves (III), the flowers (IV), and fresh branches (V) were collected in May 1976. The yields of the volatile oils from the five specimens are shown in Table 1. Specimen (I) (1.1 kg) was extracted with methanol at room temperature. After concentration of the solvent in vacuo to one-half its initial volume, the residue was extracted with hexane. The hexane extract was concentrated in vacuo then steam distilled. The distillate was worked up in the usual way to give the essential oil (7.3 g, yield 0.67%). The oil was separated into two fractions eluted by hexane (1.9 g, hydrocarbon fr.) and ether (4.4 g, oxygenated fr.). Each fraction was chromatographed on impregnated silica gel (15% $AgNO_3$) by successive elution with hexane and benzene-ethyl acetate. Isolation of each constituent from the fractions separated by the above chromatography was carried out by preparative gas chromatography. Characterization of the components was done by a combination of GC-MS and spectral analysis of the isolated constituents. The identities of the major constituents were confirmed by comparing their ir and pmr spectra with those of authentic samples. All the specimens for the comparative study (II-V) were steam-distilled without solvent extraction. The oils obtained were compared with that of specimen (I) using GC-MS for identification. The content of each component was calculated from the gas chromatogram using an associated computer system (Table 2).

Ascaridol (1), which was found to isomerize to isoascaridol (2)[6], 3,4-epoxy-p-menth-2-one (3), and 4-hydroxy-4-methyl-cyclohex-2-en-1-one (4) under the conditions of analytical GLC or GC-MS (150°C, 0.25 mm X 45 m, Golay column), was isolated in a pure state by preparative GLC with a short column at an operating temperature below 100°C (5 ft. CW-20M). Ascaridol, $[\alpha]_D$ 0°, was a colorless oil with the characteristic odor of the plant. Its ir and pmr spectra were identical with those reported[7] for ascaridol. Thus, ascaridol accounted for about 20 to 38% of the oils of all the specimens (I-V). Ascaridol was also transformed to ascaridol glycol (8) and 4-hydroxy-5-isopropyl-4-methyl-hexanone (9) by treatment with ferrous sulfate.[8] In addition, the structure of isoascaridol,[6,9] which has been the subject of contradictory reports,[10-13] was confirmed to be as shown by structure (2). That is, ascaridol glycol (8),[8] which was the basis for the structure of isoascaridol (2′), was found to be the isomerization product of 1,2-dihydroxy-3,4-epoxy-trans-p-menthane (7), which was obtained from isoascaridol by treatment with ferrous sulfate. The transformation of ascaridol and isoascaridol is summarized in the Scheme. Spectral data for the isomerized compounds are listed below.

Isoascaridol (2) was a colorless oil, ν_{max} 905 cm^{-1} (epoxide); δ, 0.92 (3H, d, J=7), 0.97 (3H, d, J=7), 1.34 (3H, s), 1.3-1.9 (5H), 3.09 (2H, s); m/e 168 (16%, M^+, $C_{10}H_{16}O_2$), 69 (100%). All spectral data were identical with those of an authentic sample which was prepared by the reported method.[13] 3,4-Epoxy-p-menth-2-one (3) was a colorless oil, ν_{max} 1690 (carbonyl), 842, 780 cm^{-1} (epoxide); δ, 0.98 (3H, d, J=7), 1.05 (3H, d, J=7), 1.13 (3H, d, J=7), 1.3-2.3 (6H), 3.09 (1H, s); m/e 168 (12%, M^+, $C_{10}H_{16}O_2$), 69 (100%). 4-Hydroxy-4-methyl-cyclohex-2-en-1-one (4) was a colorless oil; ν_{max} 3400 (hydroxyl), 1650 cm^{-1} (conj. carbonyl); δ, 1.46 (3H, s), 2.0-2.7 (4H), 2.9 (1H, -OH), 5.89 (1H, d, J=11), 6.79 (1H, d, J=11); m/e 126 (18%, M^+, $C_7H_{10}O_2$), 98 (100%). Contact shift by $Eu(DPM)_3$ showed that the absorptions at δ 6.79, 1.46, and 5.87 were affected in this order. 1,2-Dihydroxy-3,4-epoxy-trans-p-menthane (7) was a colorless oil; ν_{max} 3420, 855 cm^{-1}; δ, 0.98 (3H, d, J=6.5), 1.00 (3H, d, J=6.5), 1.24 (3H, s), 3.18 (1H, d, J=4.5), 3.66 (1H, d.d, J=4.5, 1). Absorption at 3.66 was observed to couple with a sec. hydroxyl group with J=1 in DMSO(d_6); m/e 186 (2%, M^+,

$C_{10}H_{18}O_3$), 78 (100%). Treatment with acetone and a catalytic amount of hydrogen chloride gave an acetonide which was identical to the acetonide of ascaridol glycol (8). Ascaridol glycol (8), ν_{max} 1120 (C-O-C), 3370 cm^{-1}(-OH); δ, 0.99 (6H, d, J=6.5), 1.0-1.3 (1H), 1.38 (3H, s), 1.5-2.4 (4H), 3.28, 3.60 (each 1H, d, J=5.8), 3.2-3.8 (2H, -OH X 2). Two sec. hydroxyl groups were observed in DMSO; m/e 186 (20%, M^+, $C_{10}H_{18}O_3$), 43 (100%), 71 (70%); Dibenzoate, mp 115-115.5°C.[8] Acetonide of (8), ν_{max} 1090 cm^{-1}(C-O-C); δ, 0.95 (6H, d, J=6.5) 1.34 (3H, s), 1.37 (3H, s), 1.54 (3H, s), 1.3-2.3 (5H), 4.15, 4.36 (each 1H, d, J=9); m/e 226 (3%, M^+, $C_{13}H_{22}O_3$), 43 (100%), 97 (86%). 4-Hydroxy-5-isopropyl-4-methyl-hexanone (9) was a colerless needles, mp 79-80°C.[8] ν_{max} 3380 (-OH), 1680 cm^{-1}(C=O); δ, 0.90, 0.95 (each 3H, d, J=7), 1.37 (3H, s), 0.9-2.8 (9H); m/e 170 (80%, M^+, $C_{10}H_{18}O_2$), 127 (100%). The mass spectrum of the deuterated compound showed an increase of four mass units. 2-Hydroxy-3,4-epoxy-p-menth-6-ene (10) was a colorless oil; ν_{max} 3420, 872 cm^{-1}; δ, 0.97, 1.01 (each 3H, d, J=6.5), 1.76 (3H, br.s), 3.32 (1H, d, J=3), 4.42 (1H, d.d, J=3, 1), 5.25 (1H, m), 2.35 (2H, br.s), 2.65 (1H, -OH). Coupling between δ 3.32 and 4.42 ppm was observed by irradiation of δ 4.42; m/e 168 (7%, M^+, $C_{10}H_{16}O_2$), 43 (100%), 135 (90%).

Table 1 Analytical samples from different parts of the plant and their contents of volatile compounds

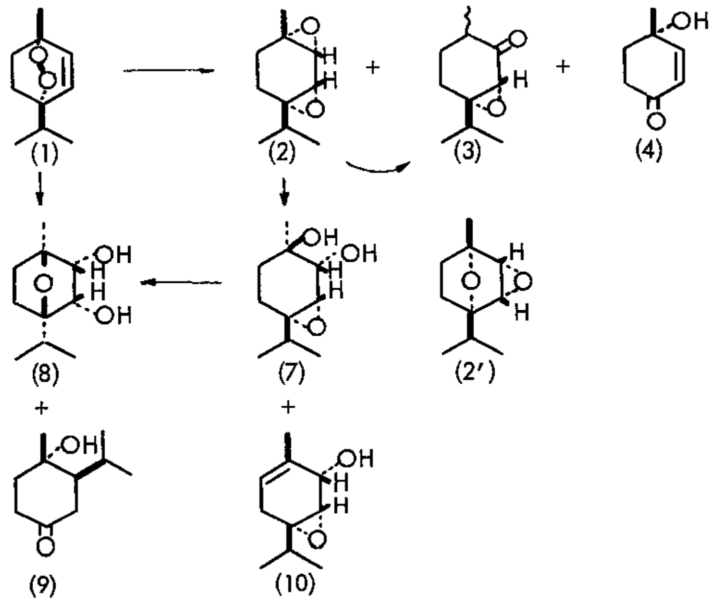
No.	Specimen	Data harvested	Essential oil (g)	Yield (%)
I	Leaf	30/May (1975)	7.3	0.67
II	Young leaf	1/June (1976)	0.05	0.72
III	Biennial leaf	1/June (1976)	0.9	0.77
IV	Flower	1/June (1976)	0.05	0.99
V	Branch	1/June (1976)	0.11	0.08

Table 2 Compounds identified in the five specimens (I–V) and their contents as determined by GLC

Constituent	I	II	III	IV	V
α-Pinene	2.07	0.04	0.30	+	0.04
Camphene	1.08	0.07	+	+	0.12
β-Pinene	0.66	+	{0.87	{+	{+
Sabinene	3.42	0.29			
Myrcene	0.51	+	0.04	+	0.02
α-Terpinene	0.65	0.05	0.26	0.20	0.77
Limonene	3.63	0.10	0.18	+	1.16
β-Phellandrene	0.79	0.09	0.57	+	+
cis-β-Ocimene	0.46	+	+	+	+
γ-Terpinene	{12.55	{3.18	{14.82	{1.32	{1.52
p-Cymene					
Terpinolene	0.18	+	+	+	+
p-Isopropenyl toluene	+	+	+	+	+
Terpineol-1	0.09	1.08	0.76	0.42	0.53
Terpinene-4-ol	1.49	3.14	3.98	3.93	2.30
Myrtenal	0.09	0.30	0.35	0.16	0.10
Bornyl acetate	3.78	1.98	1.09	0.86	6.16
Limonene-4-ol	0.21	0.15	0.29	0.15	0.14
α-Terpineol	0.21	0.30	0.52	0.26	+
3,4-Epoxy-p-menth-2-one*	2.53	3.88	5.79	3.94	2.28
Citronellyl acetate	1.32	0.86	1.60	1.97	0.60
trans-p-Menthadiene-1(7),8(9)-ol-2	0.27	0.99	1.03	3.43	0.49
p-Cymene-8-ol	0.29	1.51	1.56	0.05	0.29
Geranyl acetate	0.90	+	+	+	0.86
Isoascaridol*	15.91	18.27	14.15	24.25	15.03
cis-p-Menthadiene-1(7),8(9)-ol-2	0.90	+	+	0.47	1.03
4-Hydroxy-4-methyl-cyclohex-2-en-1-one*	5.52	13.85	4.44	9.91	1.83
Acetophenone	0.28	+	1.66	0.81	0.72

cont.

Constituent	I	II	III	IV	V
α-Copaene	0.10	1.40	0.82	0.06	+
α-Gurjunene	0.68	0.14	0.10	0.03	0.05
β-Elemene	+	+	+	+	+
Caryophyllene	0.78	1.21	1.59	1.85	0.63
γ-Elemene	0.10	0.18	0.09	0.49	2.22
Alloaromadendrene	1.57	1.61	3.22	0.16	1.06
β-Farnesene	0.46	0.75	1.22	0.48	1.18
α-Humulene	1.62	0.31	0.38	0.24	0.97
γ-Muurolene	0.20	+	+	0.35	2.40
β-Selinene	0.64	2.28	2.19	+	0.42
α-Muurolene				0.55	0.42
δ-Cadinene	2.56	+	0.24	1.08	4.71
Selina-4(14),7(11)-diene	0.11	+	+	+	-
Selina-3,7(11)-diene		+	+	+	0.33
α-Cadinene	0.50	1.36	1.98	1.07	0.50
Calamenene	0.11	-	-	+	0.21
α-Calacorene	0.17	-	-	0.02	0.21
Epishobunone	0.25	+	0.36	2.90	1.37
Isoshobunone	0.60	+	0.43	3.07	1.95
Shobunone	1.43	0.35	0.55		
α-Humulene-epoxide-II	0.39	0.15	0.61	0.25	+
β-Elemenone	0.34	1.80	0.93	0.41	0.73
T-Cadinol	0.35	0.11	0.09	0.42	1.39
Germacrone	1.12	0.46	0.03	0.17	0.79
δ-Cadinol					
α-Cadinol	1.16	0.05	+	1.50	2.68
Isocalamenediol	3.32	1.88	6.06	3.97	2.19
Total (identified)	78.35	64.12	75.15	71.20	62.40
Monoterpenes HC	26.00	3.82	17.04	1.52	3.63
Oxy	33.51	45.99	35.56	49.80	31.64
(ascaridol *)	(23.96)	(36.00)	(24.38)	(38.10)	(19.14)
Sesquiterpenes HC	9.60	9.24	11.83	6.38	15.31
Oxy	8.96	4.80	9.06	12.69	11.10



Scheme: The transformation of ascaridol (1) and isoascaridol (2)

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