Chemistry of Natural Compounds, Vol. 35, No. 4, 1999

ESSENTIAL OIL AND LIPIDS FROM THE CONE BERRIES OF *Juniperus seravschanica*

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UDC 547.913;547.915

Essential oil and neutral lipids from the cone berries of Juniperus seravschanica *Kom. have been characterized for the first time. Fifty six components comprising 96.5% of the essential oil were identified;α-pinene (29.0%), β-myrcene (17.9%), germacrene B (5.9%), and cedrol (3.1%) dominated; 5 classes of neutral lipids were found with a high content of the fatty acids 22:2 (10,13), 22:1 (13), and 18:2 (9,12).*

Juniperus seravschanica Kom. (Fam. Cupressaceae Bartl.) Is an endemic evergreen tree growing in the mountains of Central Asia and Northern Afghanistan [1]. Fruits of *J. seravschanica* are cone berries with 2-3, seldom 4, seeds ripening on the second year after flowering. Cone berry extracts have diuretic, antiinflammatory,and anticholera activity and are used in rheumatism, paralysis of lower extremities, headache, and as an expectorant in bronchitis and laryngitis [1, 2].

The composition of essential oil from *Juniperus* varies to a great extent depending on the species and location of growing [3].

The essential oil obtained by steam distillation from green parts of *J. seravschanica* growing in Turkestan mountains with 1.2% yield was analyzed by the method of GLC on a packed column contained 27 components with a predominance of α-pinene, β-myrcene, limonene, cedrol, and β-pinene [3, 4]. This essential oil, especially its cedrole fraction, has a high antiseptic activity and is used without any side effects in the treatment of poorly healing wounds and ulcers and in the cosmetic, perfumery, and food industries as a bioactive and aromatizing additive.

Lipids and essential oil from cone berry of the species *J. seravschanica* have not been studied yet. Seeds of relative species *J. communis* L. accumulate to 20% lipids with a high content of 16:1 (25%), 18:2, and saturated fatty acids [5].

We studied the essential oil and lipids of ripe cone berries of *J. seravschanica*. The cone berries were crushed and subjected to hydrodistillation. The yield of essential oil, a light yellow liquid with a specific coniferous odor, was 0.69%. The results of its analysis by the method of GLC-MS in comparison with the essential oil from vegetative organs are presented in Table 1. Fifty-six components were identified in the essential oil of cone berries with a predominance of α-pinene, β-myrcene, cedrol (the data coincide with [4]), and germacrene B, but limonene was not dominant (1.7%). Thus the composition of essential oil from cone berries and green parts of *J. seravschanica* from various places was similar and differed in the content of minor components.

Their IR spectra do not show *trans*-olefinic bonds (940-990 cm^{-1}); absorption of conjugated ethylene bonds (233-235 nm) was not observed in the UV-spectrum. Methyl esters were analyzed by GLC on polar and medium polar phases. Twenty fatty acids (FA) were identified (Tables 2 and 3), which differed considerably from those extracted from the seeds of *J. communis* [5]. They were mainly unsaturated components; 46% of the FA mass consisted of unsaturated methyl esters with chain length equal to 22 carbon atoms. FA 18:2 (9,12), and 22:2 (10,13) dominated.

*Deceased.

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Published in Khimiya Prirodnykh Soedinenii, No. 4, 445-448, July-August, 1999. Original article submitted May 25, 1998.

0009-3130/99/3504-0397$22.00

TABLE 1. Composition of the Essential Oils of *Juniperus seravschanica*

Compound	Cone berries, %	Green parts [4], %	Compound	Cone berries, %	Green parts [4], %
α-Pinene	28.98	46.00	*p*-Menth-1,8-dien-4-ol	<0.01	-
Thricyclene	-	0.80	α-Terpineol	0.16	-
α-Thujene	0.73	0.80	Borneol	0.30	0.30
Isopropyl-2-metylbutyrate	0.20	-	Germacrene D	1.30	-
Camphene	0.14	-	*trans*-*p*-Menth-2-th-1,8-diol	0.15	-
Isopropyl isovalerate	0.15	-	β-Selinene	0.43	-
β-Pinene	0.99	5.30	δ-Cadinene	1.15	Traces
Sabinene	0.58	-	γ-Cadinene	0.15	Traces
β-Myrcene	17.90	22.80	β-Cadinene	-	0.30
Limonene	1.69	11.40	Calamenene	-	0.30
1,8-Cineol	0.14	-	Thujone	-	0.30
β-Phellandrene	0.14	-	Linalool	-	0.30
γ-Terpinene	0.53	-	Camphor	-	0.20
p-Cymene	0.41	1.8	Selina-3,7(11)-diene	0.48	-
Terpinolene	1.23	-	*trans*-carveol	0.16	-
α-Cedrene	-	0.50	Germacrene B	5.90	-
Hexylisobutyrate	0.12	-	(E)-Nerolidol	0.22	-
Hexylbutyrate	2.42	-	Germacrene D-4-ol	0.74	-
Hexyl-2-methylbutyrate	0.11	-	Nerol	-	Traces
δ-Elemene	1.03	-	Elemol	2.85	-
Octanol	0.77	-	Geraniol	-	0.5
Bornyl acetate	1.00	-	Cedrol	3.14	5.5
β-Elemene	0.73	-	γ-Eudesmol+T-Cadinol	0.40	-
Terpinen-4-ol	0.75	2.5	T-Muurolol	0.51	-
β-Caryophyllene	1.31	-	δ-Cadinol	0.10	-
2-Methyl-6-methylene 3,7-octadien-2-ol	0.13	-	Dimyrcene II-a	0.54	-
γ-Elemene	2.16	0.20			
Thujopsene	-	0.70	ME 16:0	0.65	-
β-Cedrene	-	0.50	α-Eudesmol	1.07	Traces
γ-Muurolene	-	0.50	α-Cadinol	2.74	-
α-Muurolene	-	1.20	Spathulenol	1.04	-
ar-Curcumene	-	1.20	Juniper camphor	0.12	-
trans-Pinocarveol	0.18	-	8,13-Abietatriene	1.27	-
trans-Verbenol	0.22	-	ME 18:2	0.36	-
α-Humulene	0.15	-	Abietal*	5.78	-

*Tentative identification from MS data alone.

A part of the crushed cone berries was extracted by *n*-hexane to obtain 6.2% of neutral lipids with a weak coniferous odor. According to data obtained by TLC in systems 1-3, the lipids were represented by free fatty acids, phytosterols, triacylglycerols, esters of fatty acids, hydrocarbons, and some groups of essential oil components. By alkaline hydrolysis, the common FA were liberated from the lipids and were transformed into methyl esters. The methyl esters were purified by CC, and the purity was controlled by TLC in systems 1 and 2.

TABLE 2. Composition of Neutral Lipid Fatty Acids of *Juniperus seravschanica* (%, GLC)

Fatty acid	Content	Fatty acid	Content
11:0	0.3	20:0	0.7
12:0	0.3	20:1(11)	Traces
13:0	0.4	20:2(11,14)	4.5
14:0	0.4	20:3(8,11,14)*	3.3
16:0	9.2	22:0	4.7
16:1	0.9	22:1(13)	10.9
17:0	0.3	22:2(10,13)	27.5
18:0	0.3	22:3(10,13,16)	7.7
18:1(9)	6.6	24:0	5.6
18:2(9,12)	15.2	$\Sigma_{sat.}$	22.4
18:3(9,12,15)	1.0	$\Sigma_{unsat.}$	77.6

*Possible structure.

TABLE 3. GLC Data of the High-Molecular-Weight Unsaturated Fatty Acids of *Juniperus seravschanica*

Fatty acid	$r_{18:0}$[1]		Effective chain-length	
	Calculated	Reference [7]	Calculated	Reference [8]
20:3(8,11,14)	2.87	2.76	21.51	21,53(20:3,n-6)[2]
20:3(11,14,17)	-	3.10	-	21,60(20:3,n-3)
20:2(11,14)	2.50	2.45	21.00	21,15(20:2,n-6)
22:3(10,13,16)	5.0	5.0	23.61	-
22:2(10,13)	4.0	4.2	22.75	-

1 - Retention time relative to ME 18:0 on 17% PEGS.

2 - n is the number of carbon atoms from the CH_3 end of the chain to the first double bond of the fatty acid.

EXPERIMENTAL

The UV spectrum was taken on a Hitachi EPS-3T instrument in hexane. The IR spectrum was taken on a Perkin-Elmer 2000 (Sweden) IR Fourier spectrometer in film.

GLC analysis of fatty acid methyl esters was done on a Chrom-4 device (Czech Republic) with flame-ionizing detector (FID) in the isothermic regimen, on a stainless-steel column (2.5 m× 4 mm) packed with Chromaton N-AW-DMCS (0.160-0.200 mm) with 17% PEGS, carrier gas He, and thermostat temperature 198°C; on a Varian Star 3400 CX device with FID, temperature kept at 80°C for 1 min and programmed to 250°C (10°C/min), kept at 250°C for 30 min, quartz column 4 m×320 mk with DB-1 and layer thickness 1 m, carrier gas He (3.0 atm), air pressure 4 atm, injector temperature 200°C, and detector temperature 300°C. The methyl esters of fatty acids were identified by the values of the separating factors [6, 7], the effective chain length [7, 8], and by the data of [9].

The essential oil was analyzed by GLC-MS using a Hewlett-Packard GCD system [10].

TLC was carried out on "Silufol UV-254" plates (Czech Republic) in the following developing solvents: 1) C_6H_{14}-$(C_2H_5)_2O$-ice CH_3COOH (70:30:1); C_6H_{14}-$(C_2H_5)_2O$: 2) (99:1), and 3) (7:8). Chromatograms were developed by iodine vapor and a 50% methanolic solution of H_2SO_4 with subsequent heating. For ME column chromatography, silica gel L 200/250 (Czech Republic) and the eluting system C_6H_{14}-$(C_2H_5)_2O$ (98:2) were used.

The essential oil was obtained by hydrodistillation for 3 h using a Clevenger type apparatus. Percentage yields of the oil were calculated on a moisture-free basis. Neutral lipids were obtained by three fold hexane extraction of crushed cone berries.

Cone berries of *J. seravschanica* were collected in February 1996 in West Tian Shan in the Chatkal mountains, at a height of 1800 m above sea level.

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