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Variation in the Chemical Composition of the Leaf Oil of *Juniperus foetidissima* Willd.

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ABSTRACT: The major components of the volatile leaf oil of *J. foetidissima* of Greek origin are sabinene (19.6%), α-thujone (18.6%), terpinen-4-ol (17.6%) and γ-terpinene (6.5%). Minor compounds are α-terpinene (4.3%), β-thujone (3.5%), cedrol (3.2%), myrcene (2.7%), and α-pinene (2.6%). Polychemism for high and low α-thujone was found (39.9 to 0.2%). Fifty two of 56 constituents are identified.

KEY WORD INDEX: Cupressaceae, *Juniperus foetidissima*, leaf essential oil, variation, α-thujone.

INTRODUCTION: *Juniperus foetidissima* is a small shrub/tree (to 4 m) found from Greece into Asia Minor and the USSR: Caucasus, Crimea and especially in Azerbaijan (1). Analyses of the volatile oils from the wood (2,3) and fruit (4) have been reported. The monoterpene hydrocarbons from leaf oil from Turkey have been reported (5) and shown to be rather dissimilar to an analysis from the Crimea (6).

A limited sampling of *J. foetidissima* in Greece revealed that chemical polymorphisms were present. This paper reports on these variations in leaf oil composition and compares these results (as far as possible) with the reports from Turkey and the Crimea.

EXPERIMENTAL: Foilage specimens were collected on Mt. Parnassus, 1,760 m elev., in central Greece (Adams 5645, 5646, Aug. 3, 1987) and in the far northwestern Greece, approx. 7 km W of Lemos, just NW of Mikri Prespa (lake), 1100 m elev. (Adams 5982, 5986, Oct. 5, 1988). Voucher specimens are on deposit at the herbarium (BAYLU!).

The foliage was kept cool and frozen within 2 to 4 days after collection. The leaf oil was isolated by steam distillation of approximately 200 g of foilage for 2 and 24 h to determine yields (7). The oil samples were concentrated (ether trap removed) under nitrogen, weighed, diluted to 10% concentration (ether) and stored in teflon-capped vials at −20°C until analyzed.

Mass spectra were recorded with a Finnigan Ion Trap mass spectrometer (ITMS), model 800, directly coupled to a Varian 6500 gas chromatograph, using a J & W DB5(=SE54), 0.26 mm id x 30 m, 0.25 micron coating thickness, fused silica capillary column. The GC/ITMS was operated under the following conditions: injector temperature: 220°C; transfer line: 240°C; oven temperature programmed: 60°C to 240°C @ 3°C/min; carrier gas: He @ 31.9 cm/sec or 1.017 ml/min (@ 210°C); injection: 0.1 μl (10% soln.), split 1:20, 500 ng/on column. Tuning values for the ITMS were 100, 100, 100, 100 using cedrol as a tuning standard.

Internal standards (n-octane and n-eicosane) were added to each sample to aid in the standardization of retention times. Identifications were made by library searches of our volatile oil library (8), LIBR(TP) using the Finnigan library search routines and standardized retention times (8,9).

RESULTS AND DISCUSSION: Yields were 1.1% and 3.0% (dry wt. basis) for 2 and 24 h distillations, respectively. The leaf oil of *J. foetidissima* is moderately complex for juniper oils with 56 constituents found in this study (Table I). Of course, fraction cutting and increased sensitivity would presumably result in the detection of many more trace constituents.

The major components are sabinene (19.6%), α-thujone (18.6%), terpinen-4-ol (17.6%) and γ-terpinene (6.5%). Minor compounds are α-terpinene (4.3%), β-thujone (3.5%), cedrol (3.2%), myrcene (2.7%), and α-pinene (2.6%). The plants sampled were extremely polychemic for α-thujone and β-thujone. This polychemism is inversely correlated with α-pinene, sabinene, myrcene, α-terpinene, γ-terpinene and terpinen-4-ol (Table I).

It is interesting that both high and low α-thujone plants were found in central and northwestern Greece. Clearly additional field work will be needed to supplement this preliminary report on the extent of this polychemism.

Comparisons with analyses of the monoterpene hydrocarbons of *J. foetidissima* oils from Turkey (5) and the Crimea, USSR (6) are shown in Table I. *Juniperus foetidissima* from Turkey did not have nearly as much sabinene and had more limonene, but otherwise agreed with *J. foetidissima* from Greece. The analysis from the Crimea, USSR *J. foetidissima* (6) showed some agreement with the sample from Greece but had a much higher amount of limonene (21.2% vs. 0.8%). However, thujone (α+β?) content was rather similar (13.4%, Crimea vs. 18.6%, Greece). Unfortunately, 38.6% of the oil (the sesquiterpenoids) was not accounted for in the Russian study (6).

Table I. Volatile leaf oil composition of *Juniperus foetidissima*

	Percent total oil						
	C. Greece		N.W. Greece				
Compound	5645*	5646*	5982*	5986*	Avg.	Turkey	Crimea
2-hexanol	T	0.2	0.1	0.4	0.2		
α-thujene	0.3	1.4	2.0	1.3	1.3	2.0	1.5
α-pinene	0.5	1.6	2.0	6.1	2.6	2.8	4.6
α-fenchene	T	T	T	—	T	0.1	
camphene	T	T	T	T	T	0.2	0.1
sabinene	7.0	28.9	16.7	25.9	19.6	4.5	16.6
β-pinene	—	—	—	—	—	0.4	0.4
myrcene	1.1	3.6	2.7	3.5	2.7	1.8	0.7
α-phellandrene	0.1	0.2	0.1	0.2	0.2	0.3	0.7
δ-3-carene	T	T	—	—	T		0.7
α-terpinene	3.1	6.1	2.4	5.7	4.3	1.4	
p-cymene	0.7	0.3	0.3	0.5	0.5	1.0	1.1
limonene	0.5	1.0	0.6	0.9	0.8	5.2	21.2

Table I (cont.). Volatile leaf oil composition of *Juniperus foetidissima*

	Percent total oil						
	C. Greece		N.W. Greece				
Compound	5645*	5646*	5982*	5986*	Avg.	Turkey	Crimea
β-phellandrene	0.5	1.0	0.2	1.0	0.6	0.5	
1,8-cineole	—	—	0.5	T	0.2		
pentyl isobutyrate	—	T	—	0.2	0.1		
γ-terpinene	4.7	8.6	3.7	8.8	6.5	3.2	5.3
trans-sabinene hydrate	1.7	2.6	1.8	1.2	1.8		
cis-linalool oxide	—	—	0.3	—	0.1		
terpinolene	1.3	2.7	1.2	2.4	1.9	0.9	2.3
cis-sabinene hydrate	1.8	2.0	2.1	1.6	1.9		
linalool	T	1.2	2.8	T	1.0		
α-thujone	39.9	0.2	31.7	2.5	18.6		13.4
β-thujone	5.6	0.1	6.9	1.2	3.5		
cis-p-menth-2-en-1-ol	1.1	1.6	0.8	1.2	1.2		
trans-p-menth-2-en-1-ol	1.7	1.0	1.0	1.0	1.2		
RT749	0.6	T	0.1	T	0.2		
β-pinene oxide	0.4	0.1	0.3	0.2	0.2		
terpinen-4-ol	17.0	24.8	9.6	18.9	17.6		
p-cymen-8-ol	0.2	T	0.2	T	0.1		
α-terpineol	0.7	0.9	0.5	0.8	0.7		
cis-piperitol	0.3	0.3	0.3	0.3	0.3		
trans-piperitol	0.3	0.5	0.3	0.4	0.4		
citronellol	—	—	0.2	T	0.1		
methyl citronellate	—	—	0.3	T	0.1		
bornyl acetate	—	0.3	—	—	0.1		
cis-sabinyl acetate	0.1	T	3.5	T	0.9		
RT1193	0.5	T	T	0.3	0.2		
caryophyllene	0.3	T	—	T	0.1		
α-humulene	0.1	0.1	—	—	0.1		
α-muurolene	—	0.1	T	T	0.1		
γ-cadinene	—	0.2	T	T	0.1		
δ-cadinene	—	0.4	0.3	0.2	0.3		
elemol	—	0.2	—	—	0.1		
4-hydroxygermacrene D	—	—	0.2	T	0.1		
caryophyllene oxide	—	T	—	—	T		
cedrol	4.4	0.2	T	8.3	3.2		
β-oplopenone	0.2	1.2	0.2	0.5	0.5		
τ-cadinol	—	0.5	0.3	0.5	0.3		
τ-muurolol	—	0.5	0.2	0.5	0.3		
torreyol (= δ-cadinol)	—	0.1	0.1	T	0.1		
α-cadinol	—	1.4	0.8	1.7	1.0		
manoyl oxide	0.3	0.4	0.1	T	0.2		
RT2759	0.9	0.7	T	T	0.4		
abietatriene	T	0.4	T	0.3	0.2		
RT2890	T	0.6	0.5	0.5	0.4		
trans-totarol	0.4	0.6	T	0.4	0.4		

Legend: Compounds are listed in order of their elution from a DB5 (=SE54) column. Compositional values less than 0.1% are denoted as traces (T). Unidentified constituents smaller than 0.5% are not reported. The analysis from Turkey used air dried leaves (5). The status of the foliage from the Crimea, USSR (6) is not known.
* = Accession No.

Another analysis of *J. foetidissima* leaves from the USSR (1) reported an oil yield of 2.26% and merely noted that the major components were α-pinene and cedrol. Since neither of these compounds are major constituents in *J. foetidissima* from Greece, it is possible that a different taxon was sampled in one of the studies (1) from the USSR. Clearly, a study utilizing large sample sizes and geographical diversity is needed to resolve if the chemotypes are chemical races and if the differences in oil composition are due to infra- or intra-specific variation.

Mass spectra of unknowns [ITMS, m/z(rel. int.)]: *RT749*, 154?$[M]^+$(0), 41(100), 55(52), 67(39), 81(39), 95(56), 107(18), 121(33), 136(13), a terpene alcohol?;
RT1193, 164$[M]^+$(13), 43(21), 51(10), 65(9), 77(12), 91(39), 105(9), 121(2), 134(4), 149(100), aromatic ether;
RT2759 290$[M]^+$(0), 43(93), 55(50), 67(51), 81(40), 95(35), 109(23), 123(20), 137(15), 149(11), 163(7), 177(25), 191(16), 257(100), 275(21), isomer of manoyl oxide;
RT2890 272?$[M]^+$(90), 41(100), 55(40), 69(29), 81(38), 91(44), 105(48), 117(17), 136(38), 148(24), 159(12), 173(13), 187(18), 201(11), 229(87), 257(37), isomer of phyllocladene.

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