The Chemical Composition of Leaf Oils of *Juniperus excelsa* M.-Bieb.

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Received: July 1989

ABSTRACT: The chemical composition of the leaf oils of *Juniperus excelsa* obtained from trees growing in northern Greece and Kew gardens have been compared. The leaf oil from native (Greek) *J. excelsa* is dominated by cedrol (28.1%), α-pinene (22.5%) and limonene (22.7%), with moderate amounts of δ-3-carene (2.3%), myrcene (1.9%) and two unidentified constituents. The oil from the cultivated juniper at Kew is also dominated by cedrol (40.0%) and α-pinene (46.2%), whereas limonene is small (1.3%). Sixty three of the 67 constituents have been identified.

KEY WORD INDEX: *Juniperus excelsa*, leaf oil, terpenes, cedrol.

INTRODUCTION: *Juniperus excelsa* M.-Bieb. (Grecian juniper) is a widely occurring tree from Northern Greece, eastward through Turkey perhaps as far as Pakistan. Southward the taxon extends into the high mountains of the Arabian peninsula (1). Kerfoot and Lavranos (1) even claim that *J. excelsa* is the east African juniper (*J. procera* Endl.).

The eastern limits of the taxon are not known with certainty. For example, Thappa, et al. (2) recently reported on the composition of the leaf oil of *J. excelsa* from Jammu, India in the western Himalayas. However, examination of his voucher specimen revealed that the foliage was much coarser than specimens of *J. excelsa* at Kew (Royal Botanic Garden, London). Examination of specimens referred to as *J. polycarpos* K. Koch from Armenia revealed a close similarity to the specimen from Jammu. In fact, *J. polycarpos* has been reduced to a subspecies of *J. excelsa* (*J. excelsa* ssp. *polycarpos* (K. Koch) Takhtadzhyan). In addition to the report on the leaf oil of *J. excelsa* (cf. ssp. *polycarpos*) from Jammu (2), Goriaev and Ignatova (3) reported that analysis of foliage of *J. excelsa* from the Crimea yielded α-pinene (60%), cedrene (6%) and cedrol (11.53%). A more recent analysis of *J. excelsa* from the Crimea (4) revealed that the oil contained α-pinene (56.2%), camphene (2.9%), β-pinene (1.2%), δ-3-carene (3.1%), limonene (10.4%), γ-terpinene (1.9%), terpinolene (0.8%), p-cymene (0.7%) and 22.8% sesquiterpenoids and unidentified. In contrast the leaf oil of *J. excelsa* (cf. ssp. *polycarpos*) from Jammu (2) was reported to possess sabinene (36.1%) and cedrol (26.8%) as the major components.

Although the issue of the taxonomic status of the taxon from Jammu is under present study (Adams and Thappa, in progress), it seems appropriate to report on *J. excelsa* leaf oil from northern Greece, where the taxon is unambiguous. In addition, the leaf oil of a plant cultivated at the Royal Botanic Gardens, Kew, London, under the name of *J. excelsa* is compared with the native *J. excelsa* of northern Greece.

EXPERIMENTAL: The cultivated specimen of *J. excelsa* was collected in July, 1987 at the Royal Botanic Gardens, Kew, England (Adams 4941; Kew Acc. #000-69.10572). The foliage was frozen after collection. The native specimens were collected from northern Greece, approx. 7km W of Lemos, NW of Mikri Prespa (lake), 1100 m elev., Oct. 5, 1988 (Adams 5983, 5984, 5985, 5987, 5989). Voucher specimens are deposited at BAYLU! herbarium.

The leaf oil was isolated by steam distillation of approximately 200 g of foliage for 2 and 24 h to determine yields (5). The oil samples were concentrated (ether trap removed) under nitrogen and stored in teflon capped vials at F20°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

Table I. Volatile oil compositions of *Juniperus excelsa*.

Compound	Percent total oil		Range
	Kew Tree	Greece Avg.	(5 trees)
tricyclene	T	0.1	(0.1-0.05)
α-thujene	T	T	
α-pinene	46.2	22.5	(31.8-10.0)
α-fenchene	0.1	0.2	(0.39-0.05)
camphene	0.1	0.5	(0.84-0.10)
sabinene	T	T	
β-pinene	0.4	0.6	(0.90-0.40)
myrcene	0.8	1.9	(2.03-1.52)
α-phellandrene	T	0.1	(0.6-0.12)
δ-3-carene	2.4	2.3	(7.08-0.05)
α-terpinene	T	0.1	(0.20-0.07)
p-cymene	T	0.4	(0.46-0.21)
limonene	1.3	22.7	(34.4-2.90)
β-phellandrene	T	T	
trans-ocimene	T	T	
γ-terpinene	0.3	0.6	(0.70-0.35)
terpinolene	0.6	0.9	(1.37-0.60)
endo-fenchol	T	0.2	(0.56-0.05)
cis-pinene hydrate	T	T	
α-campholenal	T	0.1	(0.26-0.05)
trans-pinocarveol	T	0.2	(0.30-0.05)

Compounds are listed in order of their elution from a DB5 (=SE54) column. Compounds in parenthesis are tentatively identified. Compositional values less than 0.1% are denoted as traces (T). Unidentified constituents smaller than 0.5% are not reported.

Table I (cont). Volatile oil compositions of *Juniperus excelsa*.

Compound	Percent total oil Kew Tree	Greece Avg.	Range (5 trees)
trans-pinene hydrate	T	—	
camphor	T	0.5	(1.23-0.05)
terpinen-4-ol	T	0.2	(0.25-0.05)
borneol	T	T	
δ-terpineol	T	T	
naphthalene	T	T	
p-cymen-8-ol	T	T	
α-terpineol	0.2	T	
myrtenol	T	T	
verbenone	T	0.1	(0.23-0.05)
trans-carveol	T	0.1	(0.26-0.05)
endo-fenchyl acetate	T	0.3	(0.86-0.05)
piperitone	T	T	
bornyl acetate	0.2	0.4	(0.93-0.11)
carvacrol	T	T	
RT1172	—	3.3	(6.65-0.05)
β-cubebene	—	0.1	(0.15-0.05)
α-cedrene	T	T	
RT1424	0.5	1.7	(2.06-1.31)
β-cedrene	0.3	0.9	(1.51-0.62)
thujopsene	0.2	0.4	(0.47-0.27)
α-cadinene	T	0.2	(0.32-0.05)
α-humulene	T	0.2	(0.22-0.12)
cis-β-farnesene	T	0.2	(0.25-0.14)
β-acoradiene	—	0.1	(0.21-0.07)
β-cadinene	T	0.4	(0.62-0.18)
γ-muurolene	0.1	T	
germacrene D	0.9	0.9	(2.06-0.55)
valencene	—	0.5	(0.82-0.05)
α-muurolene	0.1	0.2	(0.21-0.05)
α-alaskene	T	0.3	(0.40-0.07)
γ-cadinene	0.2	0.8	(1.30-0.05)
(1S)-cis-calamene	T	T	
δ-cadinene	0.3	0.7	(0.84-0.37)
trans-γ-bisabolene	T	0.2	(0.23-0.22)
RT1854	1.8	2.0	(3.09-1.75)
cedrol	40.0	28.1	(40.51-25.67)
cubenol	T	0.6	(0.97-0.05)
T-cadinol	0.2	T	
T-muurolol	T	T	
torreyol (= δ-cadinol)	T	T	
α-cadinol	0.4	T	
RT2033	—	0.6	(0.86-0.10)
(cis-abietal)	T	0.1	(0.25-0.05)

Compounds are listed in order of their elution from a DB5 (=SE54) column. Compounds in parenthesis are tentatively identified. Compositional values less than 0.1% are denoted as traces (T). Unidentified constituents smaller than 0.5% are not reported.

(6), LIBR(TP) using the Finnigan library search routines and standardized retention times (6,7).

RESULTS AND DISCUSSION: The oil of *J. excelsa* is very complex with 67 constituents reported in Table I. The oil from native trees is dominated by cedrol (28.1%), α-pinene (22.5%) and limonene (22.7%), with moderate amounts of δ-3-carene (2.3%), myrcene (1.9%) and two unidentified constituents (RT1172, 3.3%; RT1854, 2.0%). Yields from 2h and 24h distillations were 0.78% and 1.85% (dry wt. basis). The oil from the cultivated juniper at Kew is also dominated by cedrol (40.0%) and α-pinene (46.2%); limonene is small (1.3%).

Both the native and cultivated *J. excelsa* differ from the *J. excelsa* oil from Jammu (2) in having large amounts of α-pinene and only a trace of sabinene, whereas the Jammu sample had a small amount of α-pinene (Adams and Thappa, in progress) and 36.1% sabinene (2). In fact, about the only major component in common is cedrol. The hydrocarbon composition of the leaf oil of *J. excelsa* trees from Greece agrees in general with the Crimea analysis (4) in that they reported high α-pinene (56.2%) and a moderate amount of limonene (10.4%) and all the other monoterpenes found in the Grecian samples.

Examination of variation within the *J. excelsa* population sampled in northern Greece reveals that there is some polymorphism with regard to δ-3-carene (range 7.08 to 0.05%) and limonene (34.4 to 2.90%). The range of cedrol found was from 40.51% to 25.67%. Although a-pinene did not range as high in the natural population (only 5 trees sampled) as seen in the Kew juniper (table 1), the values for limonene and cedrol in the *J. excelsa* cultivated at Kew do not seem out of the natural range for *J. excelsa*.

Mass spectral data for the 4 unidentified constituents [ITMS, m/z (rel. int.)]: RT1172, ?[M]+, 41(100), 55(56), 67(45), 83(52), 95(23), 110(4), 136(11); RT1424, 204[M]+(11), 41(100), 55(30), 69(42), 79(33), 91(48), 105(18), 119(26), 133(25), 147(11), 161(73), sesquiterpene; RT1854, ?[M]+, 43(100), 55(25), 67(31), 79(28), 95(40), 109(28), 121(22), 135(11), 149(48), 161(27), 179(7), 189(13), 207(13); RT2033, ?[M]+, 41(88), 57(100), 67(30), 79(51), 85(72), 91(17), 105(4), 136(10).

ACKNOWLEDGMENTS: This research supported by funds from Baylor University, CSFAA.

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