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The Leaf Essential Oils and Taxonomy of *Juniperus oxycedrus* L. subsp. *oxycedrus*, subsp. *badia* (H. Gay) Debeaux, and subsp. *macrocarpa* (Sibth. & Sm.) Ball.

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Abstract

The leaf essential oils of *Juniperus oxycedrus* L. subsp. *oxycedrus*, subsp. *badia* (H. Gay) Debeaux and subsp. *macrocarpa* (Sm.) Ball have been analyzed by GC/MS. One hundred twenty two compounds were found in these three subspecies. The leaf oils of *J. oxycedrus* were dominated by α-pinene (25-43%), and limonene (4.5-28%), with moderate amounts of β-pinene, myrcene, p-cymene, β-phellandrene and manoyl oxide, whereas the leaf oils of *J. oxycedrus* subsp. *badia* were dominated by α-pinene and germacrene D with variable amounts of manoyl oxide and moderate amounts of α-campholenal, β-bourbenene and several unknown sesquiterpenes. The oils of subsp. *badia* contained nine unknown sesquiterpenes that were only found in this taxon. The oil of subsp. *macrocarpa* was dominated by sabinene and α-pinene with moderate amounts of p-cymene, γ-terpinene and terpinen-4-ol. Chemically, the three subspecies appear to be distinct and this warrants the continued recognition of these subspecies.

Key Word Index

Juniperus oxycedrus, subsp. *oxycedrus*, subsp. *badia*, subsp. *macrocarpa*, Cupressaceae, essential oil composition, terpenes, taxonomy.

Introduction

Franco (1) reviewed the taxonomy of *Juniperus oxycedrus* L. and recognized three subspecies *Juniperus*: subsp. *oxycedrus*, "throughout all the Mediterranean region to northern Iran on dry hills and mountainous tracts to 1,900 m altitude;" subsp. *macrocarpa* (Sibth. & Sm.) Ball, "sandy or rocky tracts close to the Mediterranean Sea (also to the Atlantic in S. W. Spain)"; and subsp. *transtagana* Franco, "Pliocene sands in Western Portugal (south of the river Trejo)." In 1986, Franco (2) recognized *J. oxycedrus* subsp. *badia* (H. Gay) Debeaux but elevated subsp. *transtagana* to specific status

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(*J. navicularis* Grand). We will follow his most recent treatment.

There are several reports on the oils of the fruits of *J. oxycedrus* and (3-5) and some reports on the monoterpenes of the leaves (6-7). The most complete report on the leaf oil was of *J. oxycedrus* subsp. *macrocarpa* (5) from the island of Elaphonissos (S. Greece). The leaf oil was dominated by α-pinene (26.94%) and cedrol (13.88%) with moderate amounts of dihydro-p-cymen-8-ol (8.49%), α-terpineol (6.6%) and δ-cadinene (4.55%). There have been no comparisons of all of the subspecies of *J. oxycedrus* oils.

Experimental

Specimens used in this study: *J. oxycedrus*, subsp. *oxycedrus*: Adams 5649, 5650, 5657, 10 km W of Crysoritsi, S. Greece, 1200 m; Adams 5988, 5989, 7 km W of Lemo, N. Greece, 1100 m; Adams 7080-7082, El Penon, Spain, 720 m; Adams 7801 (JO-4), 10 km se of Jaen City, 600m, Spain; subsp. *badia*: Adams 7795-7797 (JOB-6,7,8), 18 km s of Jean City, 850 m, Spain; putative subsp. *badia*: Adams 7798-7800(JO-1,2,3), 15 km se of Jaen City, 850 m, Spain; subsp. *macrocarpa*: Adams 7205-7207, 15 km W of Tarifa on sand dunes, 30 m, Spain. Voucher specimens are deposited at SRCG herbarium, Baylor University and the JAEN herbarium.

Fresh leaves (200 g fresh wt.) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (8). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at –20°C until analyzed. The extracted leaves were oven dried (48 h, 100°C) for determination of oil yields.

The oils were analyzed on a Finnigan Ion Trap (ITD) mass spectrometer, model 800, directly coupled to a Varian 6500 gas chromatograph, using a J&W DB-5, 0.26 mm x 30 m, 0.25 μm coating thickness, fused silica capillary column, temperature programmed from 60°-240 °C at 3°C/min, with a carrier gas (He) linear velocity of 31.9 cm/sec (9). Identifications were made by library searches of our volatile oil library, LIBR(TP) (9), using the Finnigan library search routines based on fit and purity, coupled with retention time data of reference compounds.

Results and Discussion

Oil yields (calculated as oil wt./wt. of oven dried, extracted leaves) varied from 0.20% to 0.42% and were clear to very light yellow. The oils of *J. oxycedrus* from northern and southern Greece and Spain were dominated by α-pinene and limonene with moderate amounts of β-pinene, myrcene, p-cymene, β-phellandrene and manoyl oxide (Table I). There is clearly considerable geographic variation in the *J. oxycedrus* oils (cf. α-pinene, δ-3-carene and limonene in Table I). The oil of the sample of *J. oxycedrus* (7801, JO-4) from near Jaen was similar to the oil of *J. oxycedrus* from El Penon (7080-82) and is not included in Table I.

The leaf oils of *J. oxycedrus* subsp. *badia* are dominated by α-pinene and germacrene D and sometimes manoyl oxide (Table I) with moderate amounts of α-campholenal, (E)-caryophyllene, β-bourbenene and several unknown sesquiterpenes. Several compounds separate subsp. *badia* from the other taxa: n-nonanal, cis-sabinol, borneol, β-bourbenene, β-copaene, α-muurolene, unknown sesquiterpenes (KI1553, 1588, 1591,1609, 1616, 1651), germacrene B, β-oplopenone, the farnesols, and 13-epi-manoyl oxide (Table I). The presence of a series of unknown compounds that have not previously been found in *Juniperus*, testifies to the distinctiveness of subsp. *badia*. The oil of sample 7796 (JOB-7) was very similar to the oil of 7795 (JOB-6) and is not include in Table I.

The oil of *J. oxycedrus* subsp. *macrocarpa* was dominated by sabinene and α-pinene with moderate amounts of p-cymene, γ-terpinene and terpinen-4-ol (Table I). Several compounds serve to separate this taxon from the other two subspecies: (E)-β-ocimene, α-thujene, sabinene, α-terpinene, γ-terpinene, sabina ketone, cis- and trans-sabinene hydrates, terpinen-4-ol and trans-thujone (Table I). Interestingly, there were no unknown compounds found in subsp. *macrocarpa*, in contrast to subsp. *badia*. Our composite oil sample differed considerably from the oil reported from Southern Greece (5). We found no cedrol nor dihydro-p-cymen-8-ol, which were reported as major components in the Southern

Table I. Comparisons of the percentage compositions of the leaf oils of *Juniperus oxycedrus* subspecies

		ssp. *oxycedrus*			ssp. *badia*			ssp. *badia?*		ssp. *macrocarpa*
RI	**Compound**	**OxNG**	**OxSG**	**OxES**	**OxBa1**	**OxBa2**	**OxBa3**	**OxBa4**	**OxBa5**	**OxMa**
854	(E)-2-hexenal	0.5	1.5	0.1	0.1	-	-	-	-	-
926	tricyclene	0.2	t	0.1	0.1	t	t	t	t	0.1
931	**α-thujene**	**t**	**t**	**0.1**	-	**t**	-	**t**	**t**	**2.8**
939	α-pinene	25.3	42.7	41.3	24.9	9.0	28.1	5.1	17.0	22.6
953	α-fenchene	t	0.9	-	-	-	-	-	-	0.1
953	camphene	0.4	0.3	0.2	0.2	0.1	0.2	t	0.3	0.1
957	thuja-2,4(10)-diene	0.4	0.1	0.1	0.2	0.1	-	-	-	0.1
976	**sabinene**	**0.3**	**0.5**	**0.6**	**0.1**	**0.1**	**0.2**	**0.1**	**0.1**	**26.5**
978	1-octen-3-ol	-	-	-	0.3	0.4	1.2	0.6	0.2	-
980	β-pinene	3.1	4.3	1.7	0.4	0.4	1.1	0.5	0.6	0.8
991	myrcene	3.8	3.4	4.7	0.4	0.4	1.0	0.6	0.8	2.9
1001	δ-2-carene	0.4	0.4	0.3	0.1	t	t	t	t	0.4
1005	α-phellandrene	1.9	0.8	8.2	0.2	0.1	0.5	t	0.4	0.4
1011	δ-3-carene	t	13.7	t	0.1	t	t	t	6.9	0.5
1018	**α-terpinene**	**t**	-	**0.2**	-	-	-	-	-	**1.8**
1026	**p-cymene**	**2.2**	**1.5**	**6.2**	**0.2**	**0.6**	**0.7**	**t**	**0.5**	**3.4**
1031	**limonene**	**27.7**	**17.1**	**4.5**	**0.2**	**0.1**	**0.1**	**0.2**	**0.1**	**2.5**
1031	β-phellandrene	1.6	2.0	5.0	0.2	0.6	2.2	0.2	1.5	2.5
1050	(E)-β-ocimene	-	-	-	-	-	-	-	-	0.4
1062	**γ-terpinene**	**0.2**	**0.1**	**0.4**	-	-	-	**t**	**t**	**3.0**
1068	cis-sabinene hydrate	t	-	-	-	-	-	-	-	1.7
1070	n-octanol	-	-	-	-	-	0.1	t	-	-
1086	p-mentha-2,4(8)-diene	-	-	-	-	-	-	-	t	-
1088	terpinolene	1.3	1.4	2.9	1.1	0.4	0.9	0.5	1.3	1.2
1097	trans-sabinene hydrate	t	-	-	-	-	-	-	-	1.7
1098	**linalool**	**0.3**	-	**t**	-	**0.3**	**0.4**	**0.5**	**0.1**	-
1102	**n-nonanal**	-	-	-	**0.2**	**0.2**	**0.1**	**0.1**	**t**	-
1114	**trans-thujone (=β-thujone)**	-	-	-	-	-	-	-	-	**0.1**
1121	**cis-p-menth-2-en-1-ol**	**0.3**	-	**0.1**	-	-	-	-	**t**	**0.7**
1125	α-campholenal	1.9	0.3	0.5	4.8	3.2	0.8	0.6	0.3	0.7
1139	trans-pinocarveol	1.0	0.3	0.5	1.8	1.9	0.3	0.2	0.2	1.2
1140	trans-sabinol	0.4	t	-	0.5	0.6	-	t	-	-
1140	cis-verbenol	-	-	t	-	-	-	-	-	0.2
1143	**cis-sabinol***	**2.1**	**0.4**	-	**1.0**	**2.0**	**0.4**	**0.3**	**0.1**	-
1143	**trans-verbenol**	-	-	**0.5**	-	-	-	-	-	**1.3**
1156	**sabina ketone**	-	-	-	-	-	-	-	-	**0.5**
1159	p-mentha-1,5-dien-8-ol	-	0.2	0.3	-	-	-	-	0.2	t
1160	trans-pinocamphone	0.1	-	-	0.4	0.4	-	-	-	-
1162	pinocarvone	0.5	t	-	0.6	0.6	t	0.1	-	0.1
1165	**borneol**	-	-	-	**t**	**0.8**	**2.2**	**1.0**	**0.6**	-
1166	δ-terpineol	0.5	0.2	-	1.2	0.7	-	-	-	0.3
1173	cis-pinocamphone	0.2	-	-	0.1	t	-	-	-	-
1177	**terpinen-4-ol**	**0.3**	**0.4**	**1.5**	**0.2**	**0.3**	**0.4**	**0.3**	**0.7**	**7.3**
1183	p-cymen-8-ol	0.2	0.3	0.4	0.2	0.3	t	t	0.1	0.7
1189	α-terpineol	0.2	0.2	5.0	0.3	0.7	1.9	0.7	0.9	1.4
1193	myrtenal	0.6	-	t	0.7	0.8	t	0.2	-	0.4
1194	myrtenol	-	-	-	0.4	0.5	t	t	-	-
1204	verbenone	0.3	0.2	t	0.4	0.5	-	t	-	0.4
1217	trans-carveol	0.7	0.2	0.2	0.7	0.8	-	t	-	0.3

Table I. Continued

		ssp. *oxycedrus*			ssp. *badia*			ssp. *badia?*		ssp. *macrocarpa*
RI	Compound	OxNG	OxSG	OxES	OxBa1	OxBa2	OxBa3	OxBa4	OxBa5	OxMa
1220	endo-fenchyl acetate	-	-	-	-	-	-	t	-	-
1228	citronellol	-	-	-	-	-	-	-	-	0.2
1235	thymol, methyl ether	-	-	-	-	0.1	0.3	-	-	t
1239	cumin aldehyde	t	-	-	-	0.1	-	-	-	t
1242	carvone	0.7	0.2	-	-	0.2	-	-	-	t
1244	methyl carvacrol	-	-	-	-	0.1	-	-	t	-
1252	piperitone	-	-	t	-	-	-	-	-	0.2
1261	(E)-2-decenal	0.1	t	-	-	-	-	-	-	-
1273	p-menth-1-en-7-al	0.1	t	-	-	0.2	-	-	-	0.1
1285	bornyl acetate	1.2	0.5	-	0.1	3.7	0.3	4.7	t	-
1287	p-cymen-7-ol	-	-	-	-	-	-	-	-	0.1
1297	trans-pinocarvyl acetate	-	-	-	-	0.2	-	-	-	-
1298	carvacrol	-	-	t	-	-	-	-	-	-
1337	trans-carvyl acetate	-	-	-	-	0.1	-	-	-	-
1350	α-terpinyl acetate	-	-	-	-	2.5	8.3	0.1	-	-
1351	α-cubebene	-	-	t	-	-	-	0.1	0.3	-
1376	α-copaene	0.4	0.2	-	-	-	-	0.2	-	-
1384	**β-bourbenene**	**0.1**	**-**	**0.2**	**2.3**	**2.9**	**0.7**	**1.7**	**2.2**	**t**
1390	β-cubebene	-	-	-	-	-	-	0.2	-	-
1391	β-elemene	-	-	-	-	-	-	t	-	-
1398	β-longipinene	-	-	-	-	-	-	0.2	-	-
1418	(E)-caryophyllene	2.3	0.2	0.2	0.4	0.9	1.5	3.6	2.7	0.1
1428	**β-copaene**	**-**	**-**	**-**	**0.2**	**0.3**	**0.2**	**0.3**	**0.3**	**-**
1454	α-humulene	1.2	t	t	0.4	0.9	1.6	3.5	1.9	t
1460	cis-muurola-4(14),5-diene	-	-	-	-	-	-	0.1	-	-
1477	γ-muurolene	-	-	-	0.4	1.0	0.9	-	1.8	-
1480	**germacrene D**	**0.9**	**0.3**	**1.0**	**3.4**	**7.9**	**14.1**	**24.5**	**15.9**	**0.5**
1483	ar-curcumene	2.2	0.5	-	-	-	-	-	-	-
1494	2-tridecanone	-	-	-	2.1	1.0	-	-	-	0.1
1495	β-alaskene*	0.1	-	-	-	-	-	-	-	-
1499	**α-muurolene**	**0.4**	**-**	**-**	**0.3**	**0.5**	**0.4**	**1.5**	**0.5**	**t**
1513	α-alaskene	0.6	t	-	-	-	-	-	-	-
1513	γ-cadinene	0.8	0.4	0.5	0.2	0.9	2.7	2.0	4.6	t
1524	δ-cadinene	1.1	0.5	t	0.5	1.2	0.8	4.6	2.0	0.3
1532	trans-cadina-1,4-diene	-	-	-	-	-	-	0.2	-	-
1538	α-cadinene	-	-	-	-	-	-	0.2	0.3	-
1542	α-calacorene	0.9	0.1	-	-	-	-	0.2	0.2	-
1553	**sesquiterpene**	**-**	**-**	**-**	**1.1**	**2.5**	**1.6**	**2.8**	**2.1**	**-**
1556	**germacrene B**	**-**	**-**	**-**	**t**	**0.7**	**0.3**	**0.6**	**0.3**	**-**
1563	β-calacorene	t	-	-	-	-	-	-	-	-
1564	(E)-nerolidol	-	-	3.3	-	-	-	-	-	-
1574	germacrene D-4-ol	-	-	-	-	-	-	1.3	-	0.1
1578	ar-tumerol	0.3	-	-	-	-	-	-	-	-
1581	caryophyllene oxide	1.7	-	0.2	1.0	2.2	1.2	2.4	1.8	0.4
1588	**sesquiterpene alcohol**	**-**	**-**	**-**	**0.2**	**0.9**	**0.6**	**1.5**	**1.6**	**-**
1591	**sesquiterpene alcohol**	**-**	**-**	**-**	**0.4**	**1.7**	**0.6**	**1.3**	**1.1**	**-**
1596	cedrol	0.9	t	-	-	-	-	-	-	-
1606	humulene epoxide II	0.6	-	-	0.2	0.5	0.5	0.7	0.3	0.2
1606	**β-oplopenone**	**-**	**-**	**-**	**0.2**	**0.4**	**t**	**0.5**	**0.4**	**-**

Table I. Continued

		ssp. *oxycedrus*			ssp. *badia*			ssp. *badia?*		ssp. *macrocarpa*
RI	Compound	OxNG	OxSG	OxES	OxBa1	OxBa2	OxBa3	OxBa4	OxBa5	OxMa
1609	**sesquiterpene alcohol**	-	-	-	**1.2**	**2.4**	**1.4**	**3.1**	**2.5**	-
1616	**sesquiterpene alcohol**	-	-	-	**0.6**	**0.9**	**0.4**	**0.5**	**0.4**	-
1626	**sesquiterpene alcohol**	-	-	-	**0.1**	**0.2**	**t**	**0.4**	**0.7**	-
1640	epi-α-cadinol (=T-cadinol)	0.2	t	t	-	0.8	1.8	3.7	2.3	t
1646	**sesquiterpene alcohol**	-	-	-	**0.4**	**0.3**	**0.6**	**1.6**	**0.8**	-
1649	β-eudesmol	-	-	-	-	-	0.4	0.4	0.7	-
1651	**sesquiterpene alcohol**	-	-	-	**1.1**	**2.3**	**2.7**	**3.0**	**4.5**	-
1653	α-cadinol	-	-	t	t	-	0.7	5.0	t	0.3
1659	cis-calamen-10-ol	t	-	-	-	-	-	-	-	-
1670	**sesquiterpene alcohol**	-	-	-	**10.4**	**4.6**	**4.2**	**0.3**	**0.5**	-
1674	cadalene	0.1	t	-	-	-	-	-	-	-
1685	eudesma-4(15),7-dien-1-β-ol	-	-	-	0.8	1.7	1.1	3.3	2.6	-
1698	2-pentadecanone	-	-	-	0.6	t	t	0.1	-	-
1700	heptadecane	-	-	-	-	0.1	0.2	-	0.1	-
1713	**(Z,Z)-farnesol**	-	-	**0.3**	**0.3**	**0.4**	**0.5**	**0.3**	**0.4**	-
1722	**(E,E)-farnesol**	-	-	**0.9**	**0.6**	**0.9**	**0.6**	**1.0**	**0.8**	**0.2**
1742	**(E,Z)-farnesol**	-	-	**0.4**	**0.4**	**0.6**	**0.4**	**0.4**	**0.4**	-
1961	sandaracopimara-8(14), 15-diene	t	-	-	t	0.3	-	0.5	0.3	t
1989	**manoyl oxide**	**2.5**	**1.7**	**5.3**	**21.0**	**11.2**	**0.6**	**0.2**	**2.9**	**2.6**
2010	**epi-13-manoyl oxide**	-	-	**t**	**0.4**	**0.6**	**0.4**	**0.3**	**0.3**	-
2054	abietatriene	1.0	t	t	1.9	3.6	1.7	0.9	0.3	0.3
2080	abietadiene	1.2	t	0.7	1.4	2.5	1.5	0.2	0.6	0.7
2302	abietal	0.1	-	-	-	-	-	-	-	-

J. oxycedrus, (N. Greece, OxNG), *J. oxycedrus*, (S. Greece, OxSG), *J. oxycedrus*, (El Penon, Spain, OxES), *J. oxycedrus* subsp. *badia* (Jaen, Spain, OxBa1 = 7795, OxBa2 = 7797, OxBa3 = 7798), putative *J. oxycedrus* subsp. *badia* (Jaen, Spain, OxBa4 = 7799, OxBa5 = 7800), and *J. oxycedrus* subsp. *macrocarpa* (Tarifa, Spain, OxMa). Components that separate the subspecies are highlighted in boldface. RI = Retention Index on DB-5 (=SE54) column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Greek oil (5). It appears that detailed analysis of geographic variation is needed in subsp. *macrocarpa*.

The putative subsp. *badia* plants (Table I), share all the unknown sesquiterpenes and the distinguishing compounds and these plants are definitely subsp. *badia*, not just large trees of *J. oxycedrus* subsp. *oxycedrus*. Based on the leaf oil compositions, the three *J. oxycedrus* subspecies appear to be distinct and merit continued recognition.

Mass spectra for unidentified constituents: [ITMS, 240°C, m/z (rel. int.):

RI 1553, M^+220?, sesquiterpene alcohol?, 41(100), 55(35), 67(27), 79(46), 91(50), 105(36), 123(40), 131(39), 145(20), 159(17), 177(3), 187(4), 202(3), 205(3); RI 1588, M^+220 (9), sesquiterpene alcohol, 41(100), 55(31), 67(33), 79(41), 93(55), 107(36), 117(28), 131(19), 149(35), 159(40), 177(20), 187(10) 202(13), 220(9); RI 1591, M^+220, sesquiterpene alcohol, 41(100), 55(31), 67(40), 81(96), 93(23), 107(17), 123(100), 137(10), 149(48), 159(7), 177(23), 203(4), 220(1); RI 1609, M^+220?, sesquiterpene alcohol, 41(100), 55(32), 67(31), 79(45), 91(46), 105(41), 119(22), 131(40), 145(23), 159(30), 177(10), 187(10), 205(6); RI 1616, M^+220?, sesquiterpene alcohol, 41(100), 55(15), 69(58), 81(18), 95(13), 109(18), 123(18), 133(4), 149(5), 161(12), 179(8), 204(2); RI 1626, M^+220?, sesquiterpene alcohol, 41(100), 55(35), 67(33), 81(43), 93(35), 105(36), 119(61), 134(10), 147(6), 161(51), 179(10), 189(6), 204(27); RI 1646, M^+220, sesquiterpene alcohol, 41(100), 55(37), 67(67), 79(47), 91(43), 105(32), 123(48), 131(18), 147(7), 161(37), 177(26), 187(10), 205(13), 220(2); RI 1651, M^+220, sesquiterpene alcohol, 41(100), 55(20), 67(33), 81(35), 91(50), 105(33), 117(34), 131(27), 149(13), 159(56), 177(19), 187(10), 202(13), 220(17); RI 1670, M^+?, sesquiterpene alcohol?, 41(100), 54(29), 67(35), 81(34), 96(19), 109(8), 125(7), 138(4), 166(2), 183(1), 206(1).

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