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The Leaf Essential Oils of the Genus *Calocedrus*

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Abstract

Farjon (1) considers the genus *Calocedrus* as containing just three species, *C. decurrens* (w. N. America), *C. macrolepis* (S.W. China, N. Vietnam, N. Thailand) and *C. formosana* (Taiwan). The leaf oils were analyzed and compared to the oil of the closely related species, *Platycladus orientalis* (E., N.E. China, Korea, far eastern Russia). The oils of *C. macrolepis* and *C. formosana* were very similar, being high in α-pinene (57.2% and 67.1%, respectively) and myrcene (11.2% and 6.2%), but they differed in several smaller components. The oils of *C. decurrens* from two populations in Oregon and one disjunct population in southern California were high in δ-3-carene (15.2–20.2%), limonene (18.2–23.6%), α-pinene (8.7–15.8%), terpinolene (5.7–8.0%), α-fenchyl acetate (3.5–9.7%), with some cedrol (0.8–1.2%). No large differences in oil compositions were seen between the three *C. decurrens* populations. *Platycladus orientalis* (= *Thuja orientalis*, = *Biota orientalis*) was found to contain considerable amounts of δ-3-carene (29.8%), cedrol (22.2%), α-pinene (15.1%) and terpinolene (4.9%). Overall, the oil of *C. decurrens* is as different from *C. macrolepis* and *C. formosana* as it is from *P. orientalis*, suggesting considerable divergence between *Calocedrus* species, but not between *C. macrolepis* and *C. formosana*. These leaf oil analyses support the recognition of *C. macrolepis* var. *formosana* in Taiwan as treated in the Flora of China.

Key Word Index

Calocedrus decurrens, Calocedrus macrolepis, Calocedrus formosana, Calocedrus macrolepis var. formosana, Platycladus orientalis, Biota orientalis, Thuja orientalis, Cupressaceae, essential oil composition, α-pinene, myrcene, δ-3-carene, limonene, cedrol, geographic variation, systematics.

Introduction

In the most recent, 2005 treatment of *Calocedrus*, Farjon (1) recognizes three species: *Calocedrus* decurrens (Torr.) Florin (syn. *Libocedrus decurrens* Torr., *Heyderia decurrens* (Torr.) K. Koch, *Thuja decurrens* (Torr.) Voss, *Thuja craigiana* A. Murray, bis), the west coast of North America; *Calocedrus formosana* (Florin) Florin (syn. *Libocedrus formosana* Florin, *Libocedrus macrolepis* (Kurz) Benth. et Hook. f. var. *formosana* (Florin) Kudo, *Heyderia formosana* (Florin) H. L. Li, *Calocedrus macrolepis* Kurz var. *formosana* (Florin) W. C. Cheng et L. K. Fu), endemic to Taiwan; and *Calocedrus* macrolepis Kurz (syn. *Libocedrus macrolepis* (Kurz) Benth. et Hook. f., *Thuja macrolepis* (Kurz) Voss, *Heyderia macrolepis* (Kurz) H. L. Li), southwestern China, northern Vietnam to northern Thailand. However, the Flora of China (2) treats *C. formosana* as *C. macrolepis* var. *formosana* and from examination of the morphology the *Calocedrus* taxon from Taiwan is quite similar to *C. macrolepis* and it might be considered conspecific (i.e., *C. macrolepis* var. *formosana*).

Using DNA sequence data, Little et al. (3) showed that *C. decurrens* and *C. macrolepis* var. *formosana* form a clade that is sister to the clade of *Platycladus orientalis* (L.) Franco and *Microbiota decussata* Kom. (Figure 1). Little et al. (3) did not include *C. macrolepis* var. *macrolepis* in their analysis, so the relative merits for the recognition of *C. macrolepis* var. *formosana* as a distinct species (*C. formosana*) were not addressed.

From the synonomy above, it is clear that species of *Calocedus* have been treated as *Libocedrus*, *Heyderia* and *Thuja*. Examination of the literature included searches for all four genera, but the current names will be used in this paper for clarity. The earliest work on *Calocedrus decurrens* seems to be by Gough and Mills (4) who identified four diterpene acids. Von Rudloff (5) reported the leaf oil of *C. decurrens* from the Pacific northwest, USA, was dominated by limonene (31.3%), δ-3-carene (21.0%) and α-pinene (9.2). Fang et al. (6) reported three new mono- and diterpenes from *Calocedrus formosana* from Taiwan. Several diterpene acids and amentoflavone were isolated from the leaves of *C. formosana* by Chien et al. (7).

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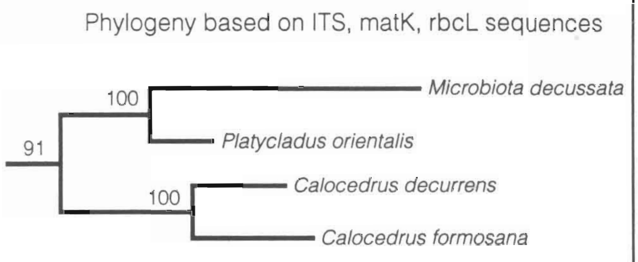

Figure 1. Single most parsimonious tree combining ITS, matK and rbcL sequence data; note that *Calocedrus decurrens* and *C. formosana* form a clade that is sister to *Platycladus orientalis* and *Microbiota decussata*; the numbers above the branch points are bootstrap percent values; adapted from (3)

The composition of the leaf oil of *C. macrolepis* var. *formosana* from Taiwan was reported (8) to contain limonene (12.4%), α-pinene (5.8%), α-cadinol (5.1%), terpinen-4-ol (3.4%), α-terpineol (2.4), δ-cadinene (3.3), caryophyllene oxide (3.3) and α-muurolol among the 37 components identified. Another analysis (7) of the composition of the oil of *C. formosana* from Taiwan reported the oil to be dominated by α-pinene (44.2%), limonene (21.6) and β-caryophyllene (8.2%).

The genus *Platycladus* is monotypic and is native to eastern and northeastern China, Korea and far eastern Russia (1). The leaf oil of *Platycladus orientalis* (L.) Franco (syn. *Thuja orientalis* L., *Thuja acuta* Moench., *Thuja decora* Salisb., *Platycladus* stricta Spach., *Biota orientalis* (L.) Endl., see (1) for taxonomic discussion) has also been reported on as *Thuja orientalis* and *Biota orientalis*.

Early analyses (10, *Biota orientalis*, 11, *Thuja orientalis*) on the leaf oil of *Platycladus orientalis* were largely qualitative, reporting the common monoterpenes such as α-pinene, β-pinene, myrcene and limonene and some common sequiterpenes such as β-caryophyllene. More recently, a large amount of cedrol (22.3%) was reported along with α-pinene (4.3%), δ-3-carene (6.1%), α-terpineol (3.75%), α-terpinyl acetate (3.8%) along with 33 other compounds from *Platycladus orientalis* cultivated in China (12). Other plants cultivated in China were reported (13) to be high in α-pinene (40%) with moderate amounts of δ-3-carene and cedrol. Fruits from *Biota orientalis* (*P. orientalis*) cultivated in India (14) had large amounts of α-pinene (67.8%) and β-phellandrene (12.3%) with a moderate amount of cedrol (2.5%). Additional reports on the oils of the leaves (15) and fruit (16) showed a similar composition. Hassanzadeh et al. (17) reported α-pinene (15.0%), sabinene (10.0%), δ-3-carene (12.0%), limonene (8.2%), cedrol (11.7%) and 11 other components in the leaf oil of *P. orientalis* cultivated in Iran.

In this study, we present detailed analyses of the leaf oils of all the *Calocedrus* taxa in order to compare the divergence between *C. macrolepis* (Yunnan) and *C. formosana* (Taiwan) and *C. decurrens*. In addition, due to the very different habitats occupied by populations of *C. decurrens* in Oregon and Southern California, we examined the oils from these different geographic areas. Because the recent DNA sequence data (2) indicated that *Platycladus orientalis* was a sister clade to *Calocedrus*, an analysis of the oil of *Platycladus orientalis* has been included in this study to provide for an outgroup comparison to *Calocedrus*.

Experimental

Specimens used in this study (species, location, collection numbers): *C. decurrens*, Rouge River, Jackson Co., OR, USA, Adams 10013-10017; Idyllwild, Riverside Co, CA, USA, Adams 10151-10153; Wasco Co., OR, USA, Adams 10295-10299; *C. macrolepis* var. *macrolepis*, Yunnan, China, Adams 10205-10209 (ex Guan Kaiyun); *C. macrolepis* var. *formosana*, Taiwan, Adams 10235 - 10239 (ex Chang-Fu Hsieh). Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU).

Air dried leaves (200 g) were water distilled for 2 h using a circulatory Clevenger-type apparatus (18). 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 an HP5971 MSD mass spectrometer, directly coupled to an HP 5890 gas chromatograph, using a J&W DB-5, 0.26 mm x 30 m, 0.25 µm coating thickness, fused silica capillary column (see 19 for operating details). Identifications were made by library searches of our volatile oil library (19), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using the HP Chemstation software.

Results and Discussion

The compositions of the leaf oils are given in Table I. One is immediately impressed that the oils of *C. macrolepis* and *C. formosana* were very similar, being high in α-pinene (57.2% and 67.1%), myrcene (11.2% and 6.2%) and β-caryophyllene (3.7% and 3.7%). They differed in limonene (0.6% and 13.9%) and several minor components (borneol, p-mentha-1,5-dien-8-ol, bornyl acetate, p-vinyl guaiacol, β-elemene, *cis*-muurola-4(14),5-diene, epi-cubebol, germacrene A, hedycaryol, 1-epi-cubenol, α-cadinol, etc.). The present analysis of *C. formosana* leaf oil from Taiwan is in good general agreement with Cheng et al. (9), who reported the oil to be dominated by α-pinene (44.2%), limonene (21.6%), myrcene (8.9%) and β-caryophyllene (8.2%). Interestingly, just like our analysis, Cheng et al. (9) also found no δ-3-carene in their *C. formosana* oil.

The oils of *C. decurrens*, from two populations in Oregon and one disjunct population in southern California, were high in δ-3-carene (15.2–20.2%), limonene (18.2–23.6%), α-pinene (8.7–15.8%), myrcene (6.2–8.2%), terpinolene (5.7–8.0%), α-fenchyl acetate (3.5–9.7%), with some cedrol (0.8–1.2%). This is similar to the von Rudloff (5) report on the leaf oil of *C. decurrens* from the Pacific northwest, USA [limonene (31.3%), δ-3-carene (21.9%) and α-pinene (9.2%)]. Even though the disjunct poulation of *C. decurrens* in southern California has probably been isolated from the more contiguous northern populatons (from central California Sierra through Oregon) since the Pleistocene (20), there were no large differences in oil compositions between the three *C. decurrens* populations

Table I. Comparisons of the percent total oil for leaf oils for *Calocedrus decurrens*, *Calocedrus macrolepis*, *Calocedrus formosana* and *Platycladus orientalis* (= *Biota orientalis*)

KI	Compound	*Calocedrus decurrens* s OR	n OR	s CA	*macrolep.* Yunnan	*formos.* Taiwan	*Platy. orient.*
802	hexanal	0.2	0.1	t	0.2	0.1	-
855	**(E)-2-hexenal**	-	-	-	0.5	0.4	-
890	**unknown, 105, 91, 79 120(M⁺)**	1.7	1.7	2.7	-	-	-
871	hexanol	-	-	-	0.1	t	-
926	tricyclene	t	t	t	0.1	t	t
931	α-thujene	t	t	t	0.2	t	0.2
939	**α-pinene**	8.7	9.6	15.8	67.1	57.2	15.1
953	α-fenchene	0.3	0.3	0.4	t	t	1.8
953	camphene	0.4	0.4	0.5	1.0	0.4	-
967	verbenene	0.3	2.3	0.3	-	-	-
976	sabinene	0.4	0.5	0.3	t	0.4	0.9
979	**1-octen-3-ol**	-	-	-	2.5	0.5	-
980	β-pinene	0.8	1.3	1.1	2.5	2.8	1.1
984	3-octanone	-	-	-	t	-	-
991	myrcene	7.5	6.2	8.2	11.2	6.2	2.9
1102	δ-2-carene	-	-	-	-	-	0.5
1005	α-phellandrene	t	t	t	-	t	0.5
1011	**δ-3-carene**	16.8	20.2	15.2	-	-	29.8
1018	α-terpinene	t	t	0.5	0.1	0.1	t
1026	p-cymene	t	t	t	t	t	t
1026	sylvestrene	-	-	-	-	-	0.4
1031	**limonene**	23.6	19.1	18.2	0.6	13.9	t
1031	β-phellandrene	t	t	t	1.3	t	5.5
1050	(E)-β-ocimene	-	-	-	0.1	-	t
1062	γ-terpinene	0.6	0.4	0.5	0.1	0.1	0.2
1068	*cis*-sabinene hydrate	0.2	0.2	0.2	-	-	t
1087	fenchone	t	t	t	-	-	-
1088	**terpinolene**	8.0	5.7	7.1	0.6	1.0	4.9
1097	*trans*-sabinene hydrate	t	-	-	-	-	t
1098	linalool	1.4	0.2	1.0	0.2	t	-
1101	**nonanal**	-	-	-	0.1	t	-
1117	**α-fenchol**	-	-	-	0.1	t	-
1121	*cis*-p-menth-2-en-1-ol	0.5	t	t	-	-	0.1
1121	**aromatic, 82, 91, 135, 150(M⁺)**	1.2	1.2	0.6	-	-	-
1125	**α-campholenal**	-	-	-	0.1	0.1	-
1139	***trans*-pinocarveol**	-	-	-	0.1	0.1	-
1140	*trans*-p-menth-2-en-1-ol	0.2	t	t	-	-	0.1
1143	**camphor**	0.4	1.0	0.6	-	-	-
1145	***trans*-verbenol**	-	-	-	0.1	0.1	-
1148	**camphene hydrate**	0.3	0.6	0.5	-	-	-
1165	borneol	0.1	0.2	0.5	0.2	-	-
1170	p-mentha-1,5-dien-8-ol	-	-	-	-	0.1	-
1177	terpinen-4-ol	1.8	1.4	0.9	0.1	0.2	0.3
1182	**43, 95, 137, 180(M⁺)**	0.8	0.6	0.3	-	-	-
1189	α-terpineol	0.5	0.5	0.8	0.2	0.1	-
1194	(Z)-4-decenal	0.2	1.0	-	-	-	-
1202	**95,84,121,139,180(M⁺)**	0.8	0.9	0.6	-	-	-
1204	verbenone	-	-	-	-	0.1	t
1205	**94, 79, 150, 180(M⁺)**	3.8	2.8	2.9	-	-	-
1205	*trans*-piperitol	-	-	-	-	-	t
1220	**α-fenchyl acetate**	3.5	4.0	9.7	-	0.1	-
1235	**methyl thymol**	0.1	0.6	0.1	-	-	-
1235	***trans*-chrysanthenyl acetate**	0.3	0.7	0.1	-	-	-
1240	**108, 93, 117, 182(M⁺)**	0.6	0.3	0.6	-	-	-
1242	**carvone**	0.3	0.3	0.3	-	-	-
1252	**piperitone**	0.6	0.8	0.4	-	-	-
1254	linalyl acetate	-	-	-	-	-	t
1259	**(Z)-4-decenol**	1.0	1.4	t	-	-	-
1285	bornyl acetate	0.5	0.5	0.4	-	0.3	0.3
1295	methyl myrtenate	2.3	1.5	4.3	-	0.1	-

Table I. continued

KI	Compound	*Calocedrus decurrens* s OR	n OR	s CA	*macrolep.* Yunnan	*formos.* Taiwan	*Platy. orient.*
1309	p-vinyl guaiacol	-	-	-	0.1	-	-
1314	(E,E)-2,4-decadienal	-	0.3	0.1	-	0.1	-
1327	myrtenyl acetate	0.2	0.5	-	-	-	-
1330	**43,92,119,134,152(M+)**	2.8	1.9	0.1	-	-	-
1339	acetate, 93,43,121,136,(196?)	0.3	0.4	0.1	-	-	0.8
1348	**α-cubebene**	-	-	-	0.1	0.2	-
1350	**α-terpinyl acetate**	3.6	2.9	0.2	-	-	2.0
1376	α-copaene	-	-	-	-	0.1	-
1379	geranyl acetate	-	-	-	-	t	-
1390	β-elemene	-	-	-	-	0.4	0.1
1394	methyl perillate	0.2	0.2	0.1	-	-	-
1411	α-cedrene	-	-	-	-	-	0.2
1414	2-epi-β-funebrene	-	-	-	-	-	0.2
1418	β-caryophyllene	0.1	0.2	t	3.7	3.7	0.7
1419	β-cedrene	-	-	-	-	-	0.8
1429	*cis*-thujopsene	0.1	0.1	t	-	-	0.1
1454	**α-humulene**	-	-	-	0.2	0.3	1.0
1461	*cis*-muurola-4(14),5-diene	-	-	-	-	0.2	-
1480	**germacrene D**	-	-	-	1.0	2.3	0.4
1493	epi-cubebol	-	-	-	-	0.2	-
1499	**α-muurolene**	-	-	-	0.4	0.3	-
1509	germacrene A	-	-	-	-	0.2	-
1513	γ-cadinene	-	-	-	-	-	t
1524	δ-cadinene	-	-	-	0.2	0.5	0.2
1549	hedycaryol	-	-	0.2	-	0.2	1.0
1559	germacrene B	-	-	-	-	-	0.1
1574	germacrene D-4-ol	-	-	-	-	-	0.3
1581	**caryophyllene oxide**	-	-	-	1.4	1.2	0.2
1589	allo-cedrol	-	-	-	-	-	1.8
1596	**cedrol**	0.8	1.1	1.2	-	-	22.1
1606	humulene epoxide II	-	-	-	-	-	0.3
1627	1-epi-cubenol	-	-	-	0.1	-	-
1630	α-acorenol	t	t	t	-	-	0.4
1637	β-acorenol	-	-	-	-	-	0.3
1640	epi-α-cadinol	-	-	-	-	0.3	0.1
1640	epi-α-muurolol	-	-	-	0.3	-	0.1
1649	β-eudesmol	-	-	-	-	0.1	0.1
1652	α-eudesmol	t	t	0.1	-	-	0.2
1653	α-cadinol	t	t	0.1	-	0.6	0.2
1759	benzyl benzoate	-	-	-	-	0.1	-
1767	**cedryl acetate**	-	-	-	-	-	0.5
1960	**sandaracopimara-8(14),15-diene**	-	-	-	-	-	0.1
1960	hexadecanoic acid	-	-	-	2.2	-	-
1998	**epi-13-manoyl oxide**	0.1	t	0.1	-	-	0.1
2054	**abietatriene**	0.1	t	0.1	-	-	t
2116	diterpene,191,43,69,81,95,(290)	-	-	-	-	2.2	-
2133	**nezukol**	-	-	-	-	-	0.3
2185	sandaracopimarinal	t	t	t	-	-	-
2275	dehydro abietal	t	t	t	-	-	-
2314	***trans*-totarol**	-	-	-	-	-	0.3

s OR = southern Oregon; n OR = northern Oregon; s CA = southern California; compounds are in boldface that separate the taxa; KI = Kovat's 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

(Table I). The leaf oil of *Platycladus orientalis* (= *Thuja orientalis*, = *Biota orientalis*) was found to contain considerable amounts of δ-3-carene (29.8%), cedrol (22.2%), α-pinene (15.1%) and terpinolene (4.9%) similar to the report from plants cultivated in Iran (17). It might be noted that an analysis of the oil of "*Thuja orientalis*" cultivated in Vienna (21) showing it to have large amounts of thujones (62%) and fenchone (12.2%), appears to be in error due to misidentification of the plants. It seems more likely that Chizzola et al. (21) analyzed *Thuja occidentalis* leaves, which are known to be high in thujones and fenchone (22). It is interesting that the oil of *C. decurrens* seems about as similar to *Platycladus orientalis* as to *C. macrolepis* (Table I). Note the shared components such as δ-3-carene, α-terpinyl acetate, cedrol, epi-13-manoyl oxide and abietatriene (Table I). However, the oil of *P. orientalis* is unique (in this comparison) for several compounds such as cedryl acetate, sandaracopimara-8(14),15-diene, nezukol and *trans*-totarol (Table I).

Overall, the oil of *C. decurrens* is about as different from *C. macrolepis* and *C. formosana*, as it is from *Platycladus orientalis*, suggesting considerable divergence between these *Calocedrus* species. This is not surprising because *C. macrolepis* and *C. formosana* occur on the same land mass of southeast Asia, whereas, *C. decurrens* grows on the North American continent. These analyses of leaf oils lend support to the *Calocedrus* taxonomy treatment in the *Flora of China* that recognized *C. formosana* from Taiwan as a variety of *C. macrolepis* (*C. macrolepis* var. *formosana*). Analyses of DNA sequences in our lab are being conducted to further address these relationships.

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