GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *JUNIPERUS CEDRUS* WEBB. & BERTHOL. FROM MADEIRA AND THE CANARY ISLANDS.

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ABSTRACT

The volatile leaf oils of *J. cedrus* from Madeira, Gran Canaria, La Gomera, La Palma and Tenerife were analyzed and the compositions reported and compared with *J. oxycedrus*, France. The oil of *J. cedrus* from Madeira Island was moderately high in α-pinene (24.9%), limonene (10.9%), β-phellandrene (7.3%), δ-3-carene (5.6%), (E)-caryophyllene (4.3%), with considerable amounts of diterpenes: sandaracopimara-8(14),15-diene (2.8%), abietatriene (1.4%), iso-abienol (7.2%), nezukol (0.4%), sempervirol (0.6%), trans-totarol (8.6%), and trans-ferruginol (1.2%). In contrast, *J. cedrus* from the Canary Islands was very high in α-pinene (54.6 - 66.3%), with moderate amounts of β-pinene (2.2-3.3%), myrcene (3.6-5.9%), α-phellandrene (0.5-3.0%), β-phellandrene (4.1-13.1%), with little or no δ-3-carene (0.0-0.1%), and almost no diterpenes. The major geographic trend was the divergence of *J. cedrus*, Madeira, from the populations in the Canary Islands. The level of divergence of the Madeira population from the Canary Islands populations was comparable to the divergence of *J. cedrus* from *J. oxycedrus*, implying considerable evolutionary differences. *Phytologia 92(1): 31-43 (April, 2010).*

KEY WORDS: *Juniperus cedrus, J. oxycedrus,* Cupressaceae, Madeira Island, Canary Islands, essential oil composition, α-pinene, δ-3-carene, β-phellandrene, isoabienol.

Juniperus cedrus Webb & Berthol. is endemic to Madeira and the Canary Islands (Adams, 2008) where it grows naturally on several islands (Fig. 1). Wild populations of *J. cedrus* are very rare on Madeira Island. Nobrega (pers. comm.) reported that he found 1 tree near the summit of Pico Ferreiro (1583 m) and 16 trees near the summit of Pico das Torres (1847 m), although it may grow on some nearby peaks. Fortunately, seeds from natural *J. cedrus* populations have been germinated and *J. cedrus* plants have been widely cultivated on Madeira. *Juniperus cedrus* is also generally rare in the Canary Islands.

Natural populations are found on Gran Canaria (only a few plants are known from Montaña del Cedro), La Palma (several hundred plants, near the summit of La Caldera de Taburiente), Tenerife (several hundred plants, El Teide National Park), and La Gomera (only a few plants are known from Garajonay National Park).

Adams (2000) reported (TIC values) that the leaf oils of *J. cedrus* from Tenerife contained large amounts of α-pinene (70.7%), with moderate amounts of myrcene (6.3%), limonene (4.5%), β-phellandrene (4.6%), β-pinene (4.1%), no δ-3-carene and no diterpenes except abietatriene (0.1%). Cavaleiro et al. (2002) analyzed 10 cultivated *J. cedrus* trees from Madeira and reported (FID values): 19.6 - 55.3% α-pinene, 17.3-32.7% limonene, 5.5-15.7% δ-3-carene, with moderate amounts of diterpenes (abietatriene, 0.1-0.8%; sandaracopimaradiene, 0.1-6.1%; isoabienol, 0.5-1.3%; and trans-totarol (0.4-2.2%). They commented that these differences (irrespective of TIC vs. FID quantitation), seem to indicate some geographical chemical variability between Madeira and the Canary

Islands. Pino et al. (2003) analyzed a single cultivated tree at the Agriculture Dept., Camacha, Madeira and they reported (FID values) the oil contained 36.0% α-pinene, 21.1% limonene, 14.8% δ-3-carene, 3.8% β-caryphyllene (= (E)-caryophyllene), as well as several diterpenes (abietatriene, 1.2%; iso-abienol, 0.4%, mis-identified as sclareol; sempervirol, 0.7%, mis-identified as cis-totarol; and trans-totarol, 0.6%). In general, their results agreed with Cavaleiro et al. (2002).

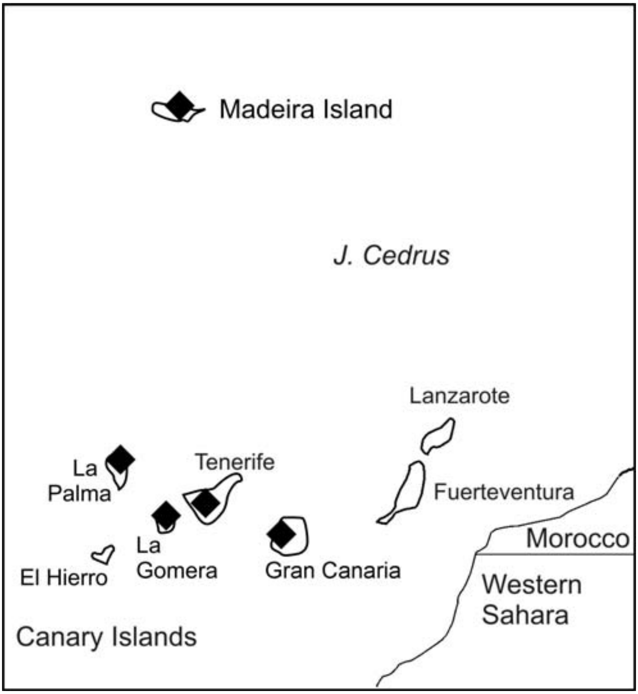

Figure 1. Distribution of *J. cedrus* from all known populations.

Thus, it appears that *J. cedrus* from Madeira may be differentiated from *J. cedrus* of the Canary Islands. The purpose of this paper is to investigate geographic variation in the volatile leaf oils of *J. cedrus* plants from throughout its range, analyzed under identical conditions.

The Madeira juniper was described as *J. oxycedrus* L. subsp. *maderensis* Menezes (Bull. Acad. Int. Georg. Bot. 18: xii. 1908), then Rivas Mart. transferred it to *Juniperus cedrus* subsp. *maderensis* (Menezes) Rivas Mart. et al. [Itinera Geobot. 15(2): 703 (2002)].

Farjon (2005) noted that leaf size and shape vary considerable in *J. cedrus*. In view of the differences in oils reported by Adams (2000, from Tenerife), and Cavaleiro et al. (2002, from Madeira), a study was undertaken to collect *J. cedrus* from all known populations on both Madeira and the Canary Islands. *Juniperus oxycedrus* from France was included in the study. The purpose of this paper is to present these results.

MATERIALS AND METHODS

Plant material - J. cedrus. Madeira Island: cultivated at Agriculture Dept., Camacha, 32° 40.374'N, 16° 50.834'W, 650 m, (= tree analyzed by Pino et al. (2003), *Adams 11496*; 32° 41.871'N, 16° 52.986'W, 1143 m, (= trees 1-5 analyzed by Cavaleiro et al. (2002), *Adams 11497-11501,* Canary Islands: Gran Canaria Island, Montaña del Cedro, 27° 57'N, 15° 44'W, 850 m, collected by Beatrice Rumeu, *Adams 11505-11507*; La Palma Island, Piedra Llana and Caldera de Taburiente, El Tiedre, 28° 45.069'N, 17° 50.150'W, 2160 m, *Adams 11509-11513*; Tenerife Island, Riscos de La Fortaleza, 28° 18.868'N, 16° 35.975'W, 2150 m, *Adams 11518-11522*; La Gomera Island, Garajonay National Park, 28° 6.544'N, 17° 13.533'W, 1339 m, *Adams 11523-11527*. *J. oxycedrus*. 4 km e of Forcalquier, France, 44° 04.06'N, 5° 59.19' E, 490 m, *Adams 9039-9041*. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh leaves (200 g.) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). 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 (48h, 100°C) for determination of oil yields.

Analyses - Oils from 3-6 trees from each population were analyzed and average populational values are reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 1991 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), 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 a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Table 1 shows that the oil composition of *J. cedrus* [from re-sampling the trees 1-5 of Cavaleiro et al. (2002) plus the single tree of Pino et al. (2003)] from Madeira Island was moderately high in α-pinene (24.9%), limonene (10.9%), β-phellandrene (7.3%), δ-3-carene (5.6%), (E)-caryophyllene (4.3%), with considerable amounts of diterpenes: sandaracopimara-8(14),15-diene (2.8%), abietatriene (1.4%), iso-abienol (7.2%), nezukol (0.4%), sempervirol (0.6%), trans-totarol (8.6%), and trans-ferruginol (1.2%). In contrast, *J. cedrus* from the Canary Islands (table 1) was very high in α-pinene (54.6 - 66.3%), with moderate amounts of β-pinene (2.2-3.3%), myrcene (3.6-5.9%), α-phellandrene (0.5-3.0%), β-phellandrene (4.1-13.1%), with little or no δ-3-carene (0.0-0.1%), and almost no diterpenes. The report of Cavaleiro et al. (2002) is borne out. There are considerable differences between the oils of *J. cedrus* from Madeira and the Canary Islands.

The oil of *J. oxycedrus*, France, was very high in α-pinene (62.1%), with moderate amounts of δ-3-carene (4.3%), limonene (2.8%), manool oxide (4.1%) with considerable no diterpenes.

There were also some differences in *J. cedrus* among the Canary Islands (table 1). For example, the Tenerife plants have large amounts of β-phellandrene (13.1%). However, the overall similarities among these oils are difficult to visualize. Principal coordinate analysis (PCO) based on 43 terpenoids resulted in eigenroots accounting for 60.55, 18.27, 11.90 and 9.28% of the variance among the island samples. Ordination of the samples reveals that the major trend (42% of the variance) is due to the differentiation of the Madeira plants from *J. cedrus* of the Canary Islands and *J. oxycedrus* (Fig. 2). The second axis (29%) separated *J. oxycedrus*, France, from Madeira and Canary Island plants. The third axis (14%) separated *J. cedrus* from the Canary Islands from Madeira and *J. oxycedrus* (Fig. 2).

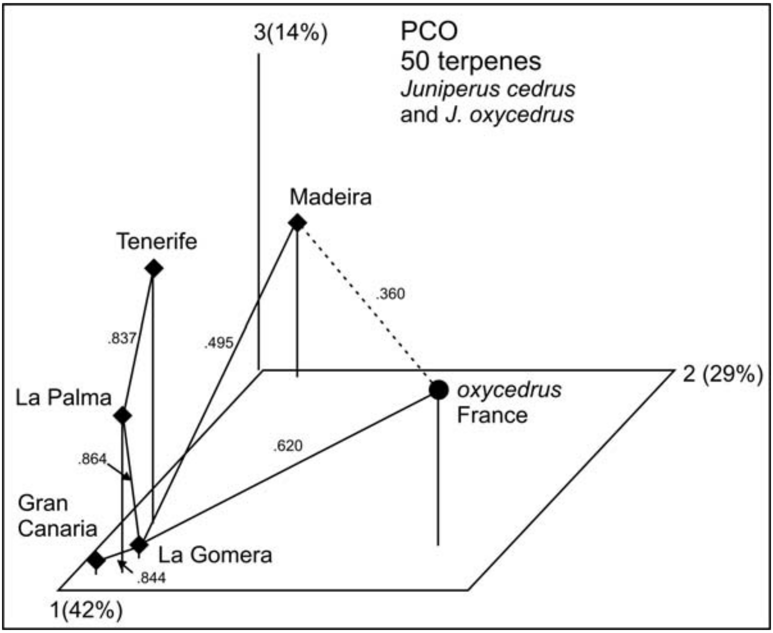

Figure 2. PCO based on 50 terpenes. Note the differentiation of *J. cedrus*, Madeira from the Canary Island populations.

To better understand the scale of geographic variation, a minimum spanning network was constructed and overlaid on a map of the islands (Fig. 3). Because the similarities were computed by the absolute difference divided by the maximum for a compound, the differences are magnified. For example, the similarity of Madeira to La Gomera (0.496) does not mean that these samples share only 49.6% of the components (see Table 1). Nevertheless, one can see that the largest geographical distance (Madeira to the Canary Islands) corresponds to the greatest amount of differentiation. And, conversely, oils of *J. cedrus* from the four Canary Islands are all very similar (0.837 - 0.864, Fig. 3).

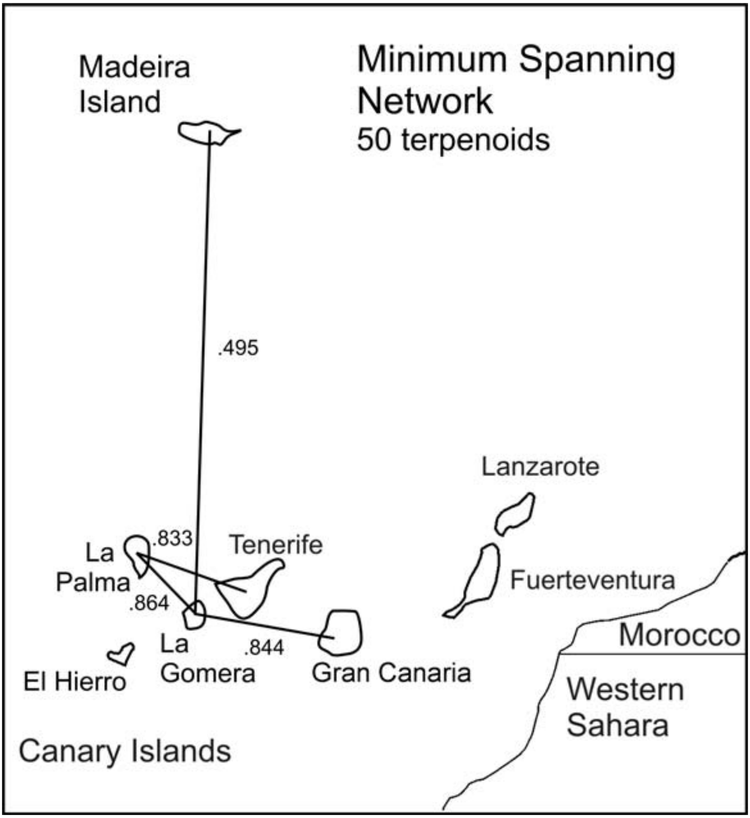

Figure 3. Minimum spanning network showing the divergence of the *J. cedrus* from Madeira from Canary Island populations.

Phylogenetically, Adams (2008) has shown that, based on nrDNA and cp trnC-trnD sequence data, *J. cedrus* is most closely related to *J. macrocarpa* (Spain) and *J. oxycedrus* (Spain) and not as related to *J. brevifolia* (Azores). It appears that *J. cedrus* may have evolved from a *J. macrocarpa/ oxycedrus* ancestor from Spain or even Morocco, as *J. oxycedrus* presently grows nearby in Morocco.

Juniperus cedrus from Madeira has been treated as a subspecies (*J. cedrus* subsp. *maderensis* (Menezes) Rivas Mart. et al.). The volatile leaf oil composition gives support for the recognition of an infraspecific taxon of *J. cedrus* from Madeira.

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Table I. Composition of the leaf oils of *J. cedrus* from Madeira (Mad) and the Canary Islands: Gran Canaria (GC), Tenerife (Ten), La Palma (LP), La Gomera (LG) and *J. oxycedrus*, France (Oxy).

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
859	(E)-2-hexenal	0.4	0.2	0.5	0.7	1.5	0.2
921	tricyclene	0.1	0.1	0.2	0.1	0.1	0.1
924	α-thujene	0.1	t	t	t	t	t
932	**α-pinene***	**24.9**	**60.9**	**54.6**	**66.3**	**65.3**	**62.1**
945	α-fenchene	0.3	0.2	t	t	t	0.2
946	camphene*	0.1	0.3	0.4	0.4	0.4	0.4
953	thuja-2,4(10)-diene	-	-	-	-	-	0.1
969	sabinene	0.1	t	0.3	t	0.2	0.4
974	1-octen-3-ol	0.3	0.1	t	0.3	0.3	t
974	**β-pinene***	**0.7**	**3.3**	**3.1**	**3.4**	**2.2**	**1.7**
988	**myrcene***	**1.2**	**5.3**	**5.9**	**4.5**	**3.6**	**2.2**
1001	δ-2-carene	0.2	0.1	0.2	t	0.1	0.1
1002	**α-phellandrene***	**-**	**1.1**	**3.0**	**0.5**	**0.5**	**0.1**
1008	**δ-3-carene***	**5.6**	**-**	**-**	**0.1**	**0.1**	**4.3**
1020	p-cymene*	0.1	0.5	0.8	0.2	0.3	0.3
1024	**limonene***	**10.9**	**3.0**	**1.5**	**2.0**	**3.0**	**2.8**
1025	**β-phellandrene***	**7.3**	**6.1**	**13.1**	**4.1**	**4.6**	**0.8**
1044	(E)-β-ocimene	0.1	-	-	-	0.1	t
1054	γ-terpinene	0.1	-	t	t	-	0.1
1063	n-octanol*	-	0.1	0.1	0.3	-	-
1086	terpinolene*	0.7	0.4	1.2	0.5	0.4	0.6
1095	linalool	-	-	-	-	-	0.1
1100	n-nonanal*	t	0.3	0.2	0.2	0.2	t
1110	**1-octen-3-yl acetate***	**0.5**	**-**	**0.1**	**0.2**	**t**	**t**
1118	cis-p-menth-2-en-1-ol	-	-	0.2	0.1	-	-
1120	3-octanyl acetate	t	-	-	-	t	t
1122	α-campholenal*	-	0.1	t	0.1	-	0.6
1135	trans-pinocarveol	-	0.1	0.1	0.1	-	0.4
1141	camphor	t	0.2	0.1	0.2	t	-
1165	borneol	-	0.2	t	0.2	t	-
1174	terpinen-4-ol	0.1	0.1	t	0.2	t	0.1
1178	naphthalene	0.1	0.1	0.1	0.1	0.2	0.1
1179	p-cymen-8-ol	t	t	t	0.1	-	t

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
1186	α-terpineol*	0.1	0.3	0.3	0.3	0.2	0.4
1195	myrtenal	-	-	t	t	-	0.1
1232	**thymol, methyl ether***	**-**	**0.3**	**t**	**t**	**0.1**	**-**
1241	**carvacrol, methyl ether***		**0.6**	**-**	**-**	**-**	**-**
1287	bornyl acetate*	0.4	0.1	0.7	0.9	0.1	0.4
1292	(E,Z)-2,4-decadienal	t	-	t	0.1	t	t
1298	**carvacrol***	**0.3**	**-**	**-**	**-**	**-**	**-**
1309	**p-vinyl guiacol***	**0.1**	**-**	**-**	**-**	**-**	**-**
1315	(E,E)-2,4-decadienal	0.1	0.1	0.1	0.1	t	-
1324	myrtenyl acetate	-	-	0.1	t	-	-
1346	**α-terpinyl acetate***	**1.4**	**0.3**	**3.5**	**0.3**	**0.3**	**t**
1373	**α-ylangene***	**0.1**	**-**	**-**	**-**	**-**	**-**
1379	geranyl acetate	-	-	0.1	t	t	-
1387	β-cubebene	0.1	t	0.1	t	t	-
1387	β-bourbenone	-	-	-	-	-	0.2
1400	**β-longipinene***	**0.5**	**-**	**-**	**-**	**-**	**-**
1411	**cis-α-bergamotene***	**0.1**	**-**	**-**	**-**	**-**	**-**
1417	**(E)-caryophyllene***	**4.3**	**0.5**	**2.7**	**2.0**	**1.0**	**0.3**
1429	**cis-thujopsene***	**1.0**	**-**	**-**	**-**	**-**	**-**
1448	cis-muurola-3,5-diene	t	-	t	t	t	-
1452	α-humulene*	1.0	0.2	0.7	0.6	0.3	0.2
1469	n-dodecanol	-	-	0.1	0.1	-	-
1475	γ-gurjunene	0.2	-	-	-	-	-
1478	γ-muurolene	-	-	-	-	-	0.1
1480	**germacrene D***	**0.7**	**0.1**	**0.8**	**0.3**	**t**	**1.5**
1493	trans-muurola-4(14),5-diene	-	-	t	0.1	-	-
1493	**epi-cubebol***	**0.7**	**0.1**	**t**	**0.1**	**0.3**	**-**
1495	2-tridecanone	-	-	-	-	-	0.1
1500	α-muurolene	0.1	-	-	-	-	-
1505	E,E-α-farnesene	-	-	t	0.1	-	-
1513	**γ-cadinene***	**0.4**	**-**	**-**	**-**	**-**	**0.5**
1513	cubebol*	0.4	0.3	0.1	0.3	0.8	-
1522	δ-cadinene*	0.6	0.2	0.2	0.4	0.5	0.3
1533	trans-cadina-1,4-diene	-	-	t	t	-	-
1548	elemol	-	t	t	0.1	-	-

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
1559	germacrene B	-	0.1	0.1	0.1	0.2	t
1561	(E)-nerolidol*	0.1	0.9	0.2	0.2	0.2	-
1582	caryophyllene oxide*	2.3	3.1	1.3	2.0	1.6	0.3
1594	ethyl dodecanoate	-	t	0.2	0.1	-	-
1594	**salvial-4(14)-en-1-one**	**-**	**-**	**-**	**-**	**-**	**0.2**
1600	hexadecane	-	-	-	0.1	-	0.2
1600	cedrol	0.1	t	t	0.1	t	-
1608	humulene epoxide II*	0.6	0.8	0.3	0.2	0.2	0.6
1627	1-epi-cubenol*	0.5	0.6	0.1	0.3	0.6	-
1638	epi-α-cadinol*	t	-	-	-	-	0.7
1638	epi-α-muurolol	t	-	-	-	-	0.2
1639	caryophyllla-4(12),8(13)-dien-5-α-ol	-	0.1	t	0.1	-	-
1649	β-eudesmol	-	t	t	0.1	-	0.2
1652	α-eudesmol	-	t	-	t	-	-
1652	**α-cadinol***	**t**	**t**	**0.2**	**t**	**-**	**0.8**
1667	**(6Z)-pentadecen-2-one***	**-**	**-**	**-**	**-**	**-**	**0.9**
1685	germacra-4(15),5,10(14)-trien-1-al	0.3	0.1	0.1	0.1	t	0.9
1700	heptadecane	0.1	t	t	0.1	-	0.2
1713	(2E,6Z)-farnesal	-	-	-	-	-	0.3
1740	(2E,6E)-farnesal	-	-	-	-	-	0.3
1800	octadecane	t	-	t	t	-	0.1
1900	nonadecane	t	-	t	t	-	t
1929	**C_{20}, 41, 55, 257, 270 (isopimaridiene?)***	**0.8**	**-**	**-**	**-**	**-**	**-**
1968	**sandaracopimara-8(14),15-diene***	**2.8**	**-**	**-**	**-**	**-**	**0.1**
1987	**manool oxide***	**-**	**-**	**-**	**-**	**-**	**4.1**
2009	**epi-13-manool oxide**	**-**	**-**	**-**	**-**	**-**	**0.2**
2022	abieta-8,12-diene	-	-	-	-	-	0.1
2055	abietatriene	1.4	0.8	t	0.6	2.7	1.0
2087	**abietadiene***	**0.1**	**-**	**-**	**-**	**-**	**1.0**
2105	**isoabienol***	**7.2**	**-**	**-**	**-**	**-**	**-**
2132	**nezukol***	**0.4**	**-**	**-**	**-**	**-**	**-**
2184	**sandaracopimarinal***	**-**	**-**	**-**	**-**	**-**	**0.3**
2189	**1-docosene**	-	-	-	-	-	0.2
2200	**docosane**	**-**	**-**	**-**	**-**	**-**	**0.2**

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
2200	**C_{20}-OH, 41,135, 270,288***	**0.9**	**-**	**-**	**-**	**-**	**-**
2218	**(E)-phytol acetate**	**-**	**-**	**-**	**-**	**-**	**0.2**
2282	**sempervirol***	**0.6**	**-**	**-**	**-**	**-**	**-**
2300	tricosane	-	-	0.1	0.1	-	0.3
2314	**trans-totarol***	**8.6**	**0.5**	**-**	**0.1**	**2.3**	**-**
2331	trans-ferruginol	1.2	0.4	-	-	0.9	-

AI = Arithmetic Index on DB-5 column. *Used in numerical analyses. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.