# THE TAXNOMIC AFFINITY OF A JUNIPER POPULATION FROM COLONIA PACHECO, MEXICO

**Robert P. Adams**
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA, email Robert_Adams@baylor.edu

## ABSTRACT

The taxonomic affinity of junipers from Colonia Pacheco was examined using volatile leaf oils, nrDNA SNPs, morphology, and ecology. The leaf oil was compared with oils from *J. scopulorum* (Durango, CO), *J. blancoi*, *J. b.* var. *huehuentensis* and *J. b.* var. *mucronata*. The juniper population at Colonia Pacheco appears to be the northern-most known population of *J. blancoi* with its leaf oil containing a few components characteristic of *J. scopulorum* suggesting gene exchange in the Pleistocene. *Phytologia 93(1): 132-145 (April 1, 2011).*

**KEY WORDS:** *Juniperus scopulorum, J. blancoi, J. b.* var. *huehuentensis, J. b.* var. *mucronata,* leaf terpenoids, geographic variation, nrDNA, SNPs, Pleistocene refugia.

---

In a previous study, Adams (2011) reported that the leaf volatile oil of putative *Juniperus scopulorum* Sarg. from Colonia Pacheco, Mexico was very divergent from typical *J. scopulorum* in the Rocky Mountains. In an effort to further analyze the affinities of this divergent population, the composition of the volatile leaf oils of *J. scopulorum,* Durango, CO, *J. blancoi* Mart., El Oro, Mexico, *J. blancoi* Mart. var. *huehuentensis* R. P. Adams et al., Cerro Huehuento, Durango, Mexico and *J. blancoi var. mucronata* (R. P. Adams) Farjon, w. of Maicoba, Chihuahua/ Sonora border, Mexico were analyzed and compared. The volatile leaf oils of *J. blancoi* and *J. scopulorum* were reported in Adams et al. (2006) and Adams (2011), respectively.

## MATERIALS AND METHODS

Specimens used in this study were: *J. blancoi*: *Adams 6849-6851 & 6903-6904*, 2580 m, 7 km s of Carmona (s of El Oro) Mexico, Mexico; *J. blancoi* var. *huehuentensis Adams 10247-10251*, 3227 m, Cerro Huehuento, Durango, Mexico; *J. blancoi* var. *mucronata*, *Adams 8453-8463*, 1180 m, 19 km w of Maicoba, Chihuahua/ Sonora border, Mexico; *J. scopulorum*, *Adams 2010-2024*, 2012 m, Durango, CO, USA; putative *J. scopulorum*, *Adams 2501-2510*, 2120 m, Colonia Pacheco, Chihuahua, Mexico. In addition, DNA was extracted from a 1978 herbarium specimen (*Adams 2512*, Colonia Pacheco, Mexico). Voucher specimens are deposited at BAYLU, Baylor University.

Isolation of Oils - See Adams (2011). Oils from 10-15 trees of each of the taxa were analyzed and average values 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 5 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.

Terpenoids Data Analysis - Terpenoids (as per cent total oil) were coded and Gower or Manhattan metric (Adams, 1975; Gower, 1971) were computed among all populations using equal character weighting. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

DNA was extracted from the *Adams 2512* herbarium specimen (1978) by use of a Qiagen mini-plant kit as per manufacturer's instructions. Degraded DNA was obtained that ranged from 2600 - 100 bp (mode 300 bp).

ITS (nrDNA) amplification was performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E or K (final concentration: 50 mM KCl, 50 mM Tris-HCl [pH 8.3], 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$, according to the buffer used) 1.8 µM each primer (see Adams, Bartel and Price, [2009] for buffer enhancers used).

Primers (5'-3'):
ITS: ITSA-42F GAT TGA ATG ATC CGG TGA AGT
ITSB+57R ATT TTC ATG CTG GGC TCT

However, the sequences were messy after about 350- 400 bp. Additional internal primers were sequenced by 'walking' along sequenced data:
ITS463F CTG TGT TAA GGA TGG GTG CA Tm 59.6ºC
ITS650F GCG CAC CTT AGA AAT CCA Tm 57.4ºC
ITS739F AAC GGA TAT CTC GGC TCT, Tm 52ºC

The PCR reactions were purified by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and cleaned using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2006).

## RESULTS AND DISCUSSION

The oils are of these taxa are very similar (Table 1). The oils from Colonia Pacheco and *J. scopulorum* share three unique components p-cymen-8-ol, methyl eugenol and safrole (Table 1). Colonia Pacheco (CM) uniquely shares one component with all the varieties of *J. blancoi*: hexanoic acid, 4-methyl, methyl ester. Colonia Pacheco shares two components with one or more *J. blancoi* varieties (but not *J. scopulorum*): 2-heptyl acetate and myrtenol.

Principal Coordinates Ordination (PCO) reveals the overall similarities in the oils (Figure 1). The minimum spanning network connects the Colonia Pacheco (CM, table 1) to *J. scopulorum* from Durango, CO (0.656). However, *J. scopulorum* (Durango, CO) is about as similar to *J. blancoi var. huehuentensis* (0.646). The oil from the Colonia Pacheco juniper is much less similar to *J. b.* var. *huehuentensis,* the next most similar node (dashed line, Fig. 1).

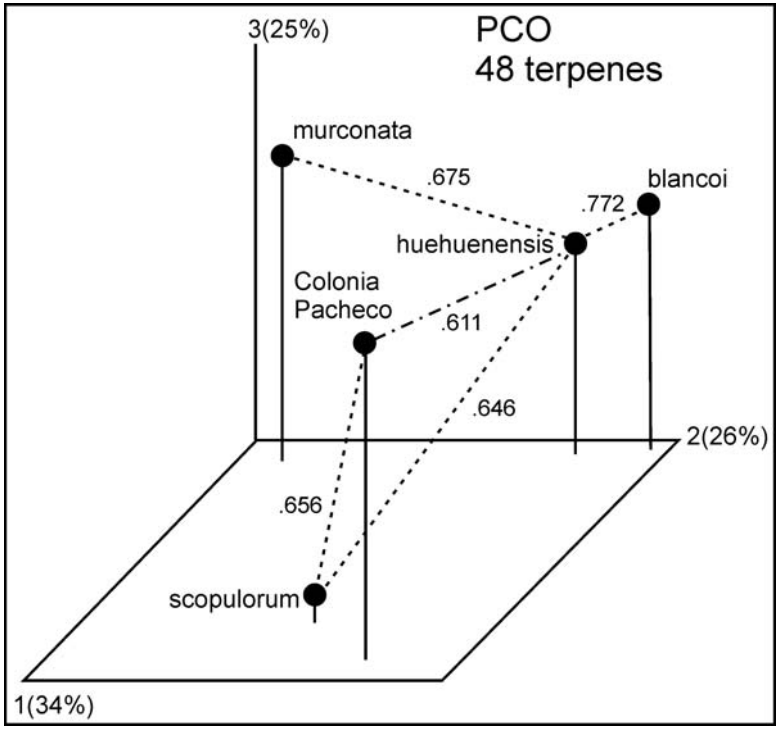


Figure 1. PCO based on 48 terpenes with a minimum spanning network superimposed (dotted line). The dashed line shows the second nearest link from Colonia Pacheco to *J. blancoi* var. *huehuentensis* (0.611).

Mapping the minimum spanning network (Fig. 2) gives a spatial perspective to the similarities. Notice that *J. b.* var. *mucronata* is less than 200 km from Colonia Pacheco and that the nearest known population of *J. b.* var. *huehuentensis* (Cerro Mohinora, Chi.) is about 350 km.

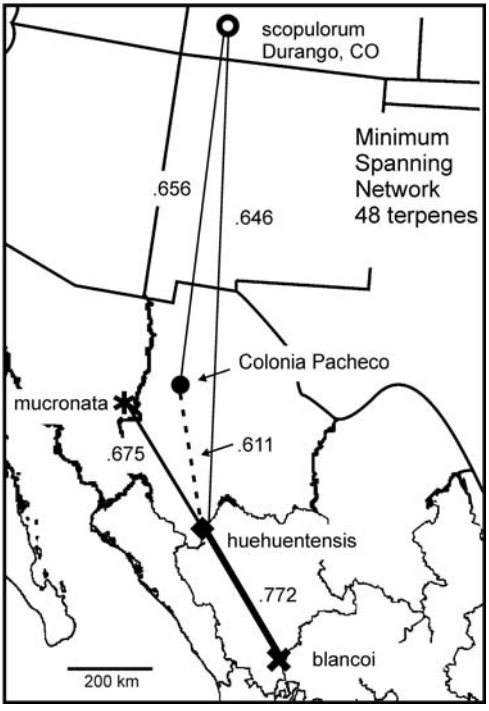


Figure 2. Minimum spanning network based on 48 terpenes. The heavy lines indicate greater similarity. The dashed line is the second nearest link to Colonia Pacheco (0.611 to *J. b.* var. *huehuentensis*).

However, the oils at Colonia Pacheco are much more similar to *J. scopulorum* (Durango, CO, 0.656) than to *J. b.* var. *huehuentensis* (0.611, dashed line in Fig. 2).

The most robust analysis of DNA sequences for the smooth-leaf margined junipers of Mexico revealed (Fig. 3) that three species

are resolved: *J. blancoi* (and it varieties), *J. scopulorum* and *J. virginiana* (Adams, 2009).

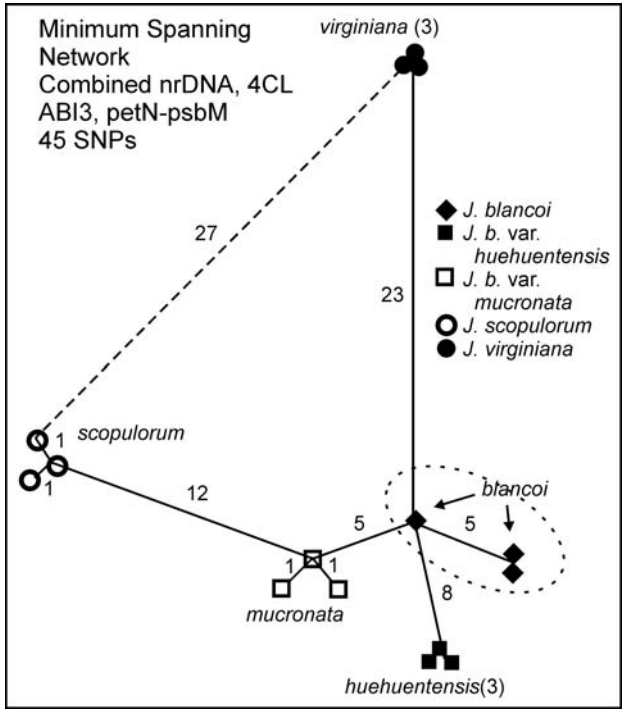


Figure 3. Minimum spanning network based on 45 SNPs (from Adams, 2009).

Sequencing the degraded DNA for nrDNA from the 1978 specimen from Colonia Pacheco proved to be somewhat difficult and additional internal primers were necessary. Analysis of the SNPs (2 single mutations in 2512 were ignored) revealed that the sequence differs by only 1 SNP from *J. blancoi* (El Oro) but differed by 4 SNPs from *J. scopulorum* (Fig. 4, dashed line). The nrDNA data shows

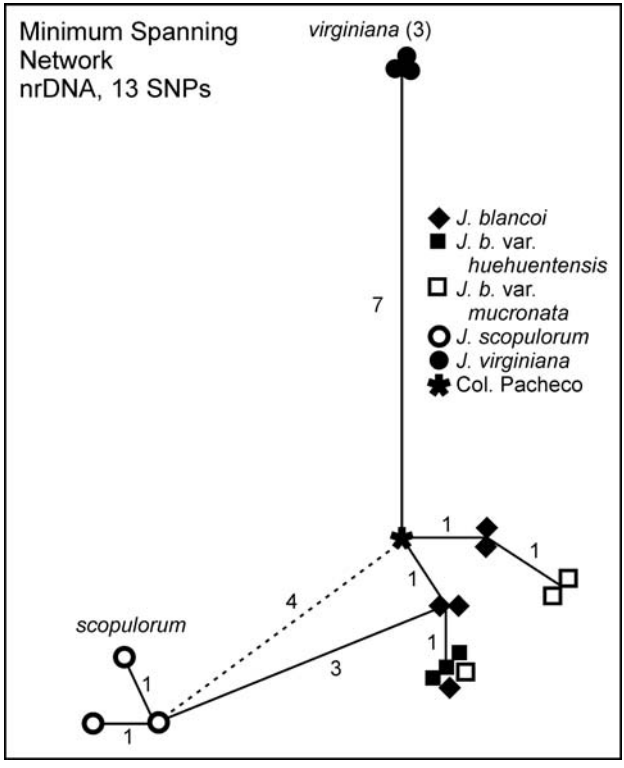


Figure 4. Minimum spanning network based on 13 SNPs from nrDNA. The dashed line shows the nearest link from the Col. Pacheco juniper to *J. scopulorum* is 4 SNPs.

the juniper at Colonia Pacheco is most closely related to *J. blancoi* (El Oro, Mexico).

The junipers at Colonia Pacheco grow along a running stream which is a common habitat for *J. blancoi* (var. *blancoi*). About half of the female cones are single-seeded (and ovoid) and the rest have two seeds and are bilobed (reniform). Bilobed cones are characteristic of *J. blancoi* (Adams, 2008). However, it is common for *J. scopulorum* to

have a few or some two-seeded, bilobed cones on female trees. Therefore, the cone shape is not definitive in classifying the junipers at Colonia Pacheco. The scale leaf-tips of the junipers at Colonia Pacheco are acute as found in *J. blancoi* var. *blancoi* and *J. b.* var. *huehuentensis*. The leaf-tips are not obtuse, as usually found in *J. scopulorum,* nor mucronate as found in *J. b*. var. *mucronata* (Adams, 2008).

Considering the terpenoids, nrDNA, morphology, distribution and ecology, the juniper at Colonia Pacheco seems more likely to be the northern-most population of *J. blancoi* than a relict of *J. scopulorum* from the Pleistocene. But gene flow between *J. blancoi* and *J. scopulorum* seems likely during the Pleistocene, making interpretation of isolated populations difficult. A few specimens of putative *J. scopulorum* have been collected from northwestern Sonora (van Devender, ASU, ARIZ). Analysis of DNA from these specimens (Adams, in progress), may show that typical *J. scopulorum* is present in Mexico.

## ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for lab assistance. Thanks to Tom Zanoni for the use of his *J. blancoi* oils from El Salto, Mexico and assistance on trips to Colonia Pacheco and El Oro and many other trips and David Riskind for arranging a trip to the Serranias del Burro. A special thanks to Socorro Gonzales Elizondo for sharing both field data and specimens of *J. b.* var. *huehuentensis* from Cerro Mohinora and other data.

## LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 1991. Cedarwood oil - Analysis and properties. pp. 159-173. in: Modern Methods of Plant Analysis, New Series: Oil and Waxes. H.-F. Linskens and J. F. Jackson, eds. Springler- Verlag, Berlin.

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 2nd ed. Allured Publ., Carol Stream, IL.

Adams, R. P. 2008. The junipers of the world: The genus *Juniperus*. 2nd ed. Trafford Publ., Victoria, BC.

Adams, R. P. 2009. Variation among the smooth-leaf margined *Juniperus* of Mexico: Analysis of nrDNA, 4CL, ABI13 and petN-psbM SNPs. Phytologia 91: 571-580.

Adams, R. P. 2011. Infraspecific terpenoid variation in *Juniperus scopulorum:* Pleistocene refugia and Post-Pleistocene recolonization. Phytologia 93: 3-12.

Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the new world. Phytologia 91: 160-185.

Adams, R. P., M. S. Gonzalez Elizondo, M G. Elizondo and E. Slinkman. 2006. DNA fingerprinting and terpenoid analysis of *Juniperus blancoi* var. *huehuentensis* (Cupressaceae), a new subalpine variety from Durango, Mexico. Biochem. Syst. Ecol. 34: 205-211.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Veldman, D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

Table 1. Comparisons of the leaf essential oils for *J. scopulorum* (SC), Durango, CO, USA, putative *J. scopulorum* (CM), Colonia Pacheco, Mexico, *J. blancoi* (BL), *J. blancoi* var.. *huehuentensis* (BH) and *J. blancoi* var. *mucronata* (BM), and. Components that tend to separate the species are highlighted in boldface.

| KI | Compound | SC | CM | BM | BH | BL |
|---|---|---|---|---|---|---|
| 899 | 2-ethyl-2-pentanol* | 0.1 | 0.5 | 0.4 | 0.3 | 0.4 |
| 926 | tricyclene | t | - | t | - | - |
| 931 | α-thujene | 1.1 | 1.1 | 1.5 | 1.2 | 1.6 |
| **939** | **α-pinene** | **4.7** | **1.9** | **2.2** | **1.6** | **1.8** |
| 953 | α-fenchene | t | t | t | t | t |
| 953 | camphene | 0.1 | 0.1 | 0.1 | t | t |
| 976 | sabinene | 46.3 | 46.0 | 52.6 | 45.5 | 48.3 |
| 980 | β-pinene | t | t | t | t | t |
| 991 | myrcene | 1.3 | 1.7 | 2.9 | 2.0 | 2.4 |
| **996** | **hexanoic acid, 4-methyl, methyl ester*** | **-** | **1.1** | **0.7** | **0.2** | **0.2** |
| **1001** | **δ-2-carene** | **t** | **0.2** | **0.4** | **0.4** | **0.2** |
| 1005 | α-phellandrene | 0.1 | t | 0.1 | 0.2 | 0.2 |
| 1011 | δ-3-carene | 0.1 | 0.1 | 0.1 | - | t |
| 1018 | α-terpinene | 1.1 | 0.7 | 1.2 | 1.9 | 1.8 |
| 1026 | p-cymene | 0.5 | 1.3 | 0.3 | 0.2 | 0.1 |
| **1031** | **limonene** | **5.6** | **1.2** | **1.3** | **1.1** | **1.6** |
| 1031 | β-phellandrene | 0.8 | 0.8 | 1.0 | 1.1 | 0.4 |
| 1034 | 2-heptyl acetate | - | 0.1 | - | - | 0.1 |

| KI | Compound | SC | CM | BM | BH | BL |
|---|---|---|---|---|---|---|
| 1050 | (E)-β-ocimene | 0.1 | 0.1 | 0.5 | 0.2 | 0.2 |
| 1062 | γ-terpinene | 1.9 | 1.0 | 2.0 | 3.3 | 2.9 |
| 1068 | cis-sabinene hydrate | 1.4 | 1.4 | 1.5 | 1.0 | 1.1 |
| **1067** | **cis-linalool oxide (furanoid)** | **t** | **-** | **-** | **-** | **-** |
| 1088 | terpinolene | 1.0 | 0.6 | 1.1 | 1.3 | 1.1 |
| 1091 | 2-nonanone | 0.3 | 2.6 | 0.9 | 0.1 | 1.9 |
| 1097 | trans-sabinene hydrate | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 1098 | linalool | 1.3 | 2.6 | 1.5 | 0.1 | 0.8 |
| 1102 | n-nonanal | t | t | 0.1 | 0.2 | 0.3 |
| 1114 | trans-thujone(=β-thujone) | 0.1 | t | 0.1 | 0.1 | t |
| 1121 | cis-p-menth-2-en-1-ol | 0.4 | 0.5 | 0.4 | 0.5 | 0.5 |
| 1140 | trans-p-menth-2-en-1-ol | 0.2 | 0.3 | 0.2 | 0.3 | 0.3 |
| 1143 | camphor | 0.2 | - | - | 0.1 | - |
| 1148 | camphene hydrate | t | - | - | - | - |
| 1177 | terpinen-4-ol | 5.8 | 6.6 | 3.9 | 7.6 | 6.2 |
| 1179 | naphthalene | - | - | - | t | - |
| **1183** | **p-cymen-8-ol** | **t** | **0.1** | **-** | **-** | **-** |
| 1189 | α-terpineol | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 |
| **1191** | **myrtenol** | **-** | **0.1** | **0.1** | **-** | **-** |
| 1193 | cis-piperitol | 0.1 | t | 0.1 | 0.1 | 0.2 |
| 1196 | methyl chavicol | - | 0.1 | - | - | - |

| KI | Compound | SC | CM | BM | BH | BL |
|---|---|---|---|---|---|---|
| 1205 | trans-piperitol | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 |
| 1228 | citronellol | 0.5 | 0.1 | 0.1 | - | 0.1 |
| 1252 | piperitone | t | 0.2 | 0.2 | t | 0.1 |
| 1252 | trans-sabinene hydrate acetate | 0.1 | - | - | - | - |
| 1257 | 4Z-decen-1-ol | 0.1 | 0.2 | 0.2 | 0.1 | 0.1 |
| 1274 | pregeijerene B | 6.0 | 1.2 | 2.0 | 3.2 | 3.2 |
| 1285 | bornyl acetate | 0.7 | t | 0.2 | t | 0.2 |
| **1285** | **safrole** | **t** | **5.6** | **-** | **-** | **-** |
| 1287 | trans-linalyl oxide (pyranoid) | - | - | 0.1 | - | - |
| **1291** | **2-undecanone** | **t** | **0.3** | **0.1** | **-** | **-** |
| **1401** | **methyl eugenol** | **0.2** | **3.7** | **-** | **-** | **-** |
| 1418 | (E)-caryophyllene | 0.2 | 0.2 | 0.6 | 0.5 | t |
| 1442 | guaiadiene <6,9-> | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 |
| 1451 | (Z)-methyl iso-eugenol | 0.1 | - | - | 0.1 | 0.1 |
| 1455 | α-humulene | - | - | - | t | - |
| 1466 | 9-epi-(E)-caryophyllene | t | - | - | - | - |
| 1477 | γ-muurolene | 0.1 | - | - | t | - |
| **1480** | **germacrene D** | **0.1** | **0.2** | **0.2** | **0.3** | **0.1** |
| 1493 | epi-cubebol | 0.1 | t | 0.1 | 0.1 | - |
| 1499 | α-muurolene | 0.1 | 0.1 | 0.2 | 0.2 | t |
| **1513** | **γ-cadinene** | **0.2** | **0.2** | **0.5** | **0.3** | **-** |

| KI | Compound | SC | CM | BM | BH | BL |
|---|---|---|---|---|---|---|
| 1524 | δ-cadinene | 0.3 | 0.4 | 0.9 | 0.9 | t |
| 1535 | α-copaen-11-ol | 0.3 | t | 0.1 | 0.1 | 0.1 |
| 1538 | α-cadinene | t | t | 0.2 | 0.1 | t |
| 1549 | elemol | 4.3 | 2.1 | 2.2 | 4.0 | 2.5 |
| 1555 | elemicin | - | 0.5 | - | - | - |
| 1561 | germacrene B | 0.2 | t | - | 0.3 | 0.2 |
| 1564 | (E)-nerolidol | - | - | - | t | - |
| 1574 | germacrene D-4-ol | 0.8 | 1.0 | 2.6 | 0.9 | 0.1 |
| 1606 | β-oplopenone | 0.2 | 0.3 | 0.5 | 0.4 | - |
| 1630 | γ-eudesmol | 0.3 | 0.3 | 0.1 | 0.6 | 0.2 |
| 1640 | epi-α-cadinol | 0.5 | 0.3 | 0.5 | 0.5 | 0.2 |
| 1640 | epi-α-muurolol | 0.4 | 0.2 | 0.4 | 0.5 | 0.1 |
| 1645 | α-muurolol (=torreyol) | t | t | 0.1 | t | - |
| 1649 | β-eudesmol | 0.4 | 0.2 | 0.2 | 0.8 | 0.3 |
| 1652 | α-eudesmol | 0.6 | 0.4 | 0.7 | 1.1 | 0.3 |
| 1653 | α-cadinol | 0.5 | 0.4 | 0.7 | 0.8 | 0.2 |
| 1666 | bulnesol | 0.2 | 0.1 | 0.2 | 0.3 | 0.2 |
| 1689 | shyobunol | - | - | - | - | 0.2 |
| 1701 | cis-thujopsenol | - | t | - | - | - |
| 1739 | oplopanone | - | t | - | - | - |
| 1762 | 8-α-acetoxyelemo, isomer | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 |

| KI | Compound | SC | CM | BM | BH | BL |
|---|---|---|---|---|---|---|
| 1789 | 8-α-acetoxyelemol | 5.9 | 2.9 | 2.8 | 5.3 | 4.2 |
| **2055** | **manool** | **t** | **0.6** | **1.1** | **1.8** | **7.6** |
| 2087 | abietadiene | t | t | 0.1 | 0.2 | t |
| **2135** | **diterpene, 41,69,255,298** | **0.5** | **0.5** | **t** | **0.4** | **t** |
| **2283** | **sempervirol** | **t** | **t** | **0.4** | **0.2** | **0.5** |
| **2298** | **4-epi-abietal** | **0.1** | **0.1** | **0.5** | **0.5** | **0.4** |
| **2314** | **trans-totarol** | **t** | **t** | **0.2** | **0.1** | **0.2** |
| 2331 | trans-ferruginol | - | - | t | t | t |

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.