CHEMOSYTEMATICS OF *JUNIPERUS*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION

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ABSTRACT

The essential oils of leaves of *J. pinchotii* and *J. virginiana* tree were collected and analyzed as fresh vs. air dried then stored at ambient conditions (21º C) for various periods before extraction. The leaf oils of both species proved to be remarkably stable. For *J. virginiana*, ANOVA of the 58 components revealed only 9 significant and 4 highly significant differences among the 7 sample sets. PCO of the samples showed some clustering by length of storage, but considerable intermixing of samples. Drying *J. virginiana* leaves under extreme conditions (fresh vs. 30º C, 60º C, 100º C) revealed considerable changes in the oils at the highest drying temperature (100º C) with the loss of more volatile monoterpenes, and a relative increase (on a % total oil basis) of the diterpenes. Surprisingly, there was little change in the oils between the fresh, 30º C and 60º C samples. Comparison of *J. pinchotii* fresh and dried (2 weeks) oils revealed 5 significant and 3 highly significant differences, but the overall profile was little changed. It appears one can use the oils from dried leaves of *Juniperus* for chemosystematic studies. *Phytologia 92(2): 186-198 (August 2, 2010).*

KEY WORDS: *Juniperus pinchotii, J. virginiana,* oils from dried leaves, chemosystematics.

When collecting and transporting plant specimens across international borders, one encounters considerable difficulty with government customs agents. The transport of silica gel dried leaves (for DNA analyses) is generally easy. The importation of fresh plant

materials is subject to plant quarantine laws. However, herbarium vouchers are generally (in the author's experience) permitted without too much difficulty. Part of the ease of importing herbarium specimens is because specimens are often frozen to kill insects, then air dried.

A second problem of using fresh leaves is that refrigeration in the field is often impossible and international air travel using dry ice is getting to be extremely difficult. The use of air dried leaves for essential oils would make the problems much more manageable.

Recently, Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22º C, 16 days) leaves for *J. thurifera* L*., J. phoenicea* L. and *J. oxycedrus* L. The first two species are in section *Sabina* and have scale-leaves, whereas *J. oxycedrus* is in section *Juniperus* with awl-like leaves (Adams, 2008). They reported small to moderate changes in several components, however, no statistical data were published.

The purpose of this study was to determine if the changes in oil composition when using air dried leaves precludes their use in chemosystematics.

MATERIALS AND METHODS

Plant material - *J. pinchotii, Adams 11890,* 14.2 mi. s of Claude on TX 207, Armstrong Co., TX; *J. virginiana, Adams11768,* cultivated, nw corner of Gruver City Park, Hansford Co. TX, first sample for monthly drying test taken on 23 Apr 2009; 2nd sample for extreme drying tests taken on 10 May 2009 . Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (200 g.) and air dried (100 g) leaves 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 -20º C until analyzed. The extracted leaves were oven dried (48h, 100º C) for the determination of oil yields.

Analyses - 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, 2007 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. For the comparison of oils obtained from leaves stored for various periods, 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 the composition of the leaf oil of *J. virginiana* and a comparison of components over the 8 month storage period. The compounds are remarkably stable during the drying and storage tests. Only 9 compounds significantly differ: α-pinene, sabinene, β-phellandrene, terpinen-4-ol, γ-cadinene, δ-cadinene, elemol, germacrene D-4-ol and 4-epi-abietal. Four compounds differed highly significantly: pregeijerene B, safrole, methyl eugenol, and 8-α-acetoxyelemol. Interestingly, the per cent oil yield (on an oven dried, 100º C, 48h, wt. basis) did not vary significantly! One might expect that the more volatile monoterpenes (e.g., α-pinene, sabinene, limonene, β-phellandrene, etc.) would volatilize upon drying and storage, but in this instance the relative percentages were about the same after 8 mo. at 21º C as in the fresh leaves (Table 1). The leaf essential oils in *Juniperus* are stored in leaf glands. In *J. virginiana*, the leaf glands are generally not ruptured and often sunken beneath the waxy cuticle (Fig. 1). So volatilization in this instance seems to be minimized by the intact glands and waxy cuticle.

To estimate the impact of the utilization of oils from fresh versus dried and stored leaves, principal coordinates analysis (PCO) was performed. The PCO (figure 1) shows that the samples do cluster somewhat by sample date, but there is considerable interspersion of samples. The first 9 eigenroots of the similarity matrix accounted for 14.7, 11.6, 11.0, 9.7, 7.38, 6.93, 5.62, 4.67 and 3.86% of the variance. It is obvious from the eigenroots (and the PCO, figure 2) that the oils from fresh leaves did

J. virginiana |'''¦'''¦'''| 1 mm
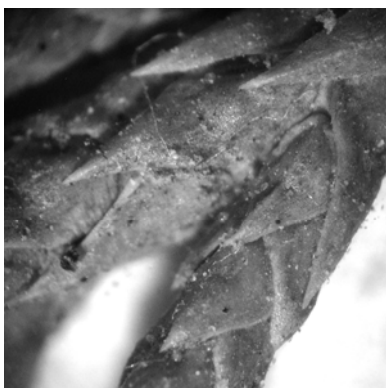
Figure 1. Leaves of *J. virginiana*.

not account for a large portion of the variance as one might have expected if large changes in composition occurred between the fresh leaves and the first and second weeks of drying.

Because the changes in composition from fresh to air dried leaves were so small, it seemed of interest to investigate the effects of extreme drying on oils. A second sample of *J. virginiana* leaves was collected (10 May 2009) and subjected to 4 treatments: fresh and dried at: 30º C (24h), 60º C (24h), and 100º C (24h). A comparison of these oils is shown in table 2. As one would expect, there was a significant decrease in the percent oil yields with increased drying temperatures (Table 2). Five (5) compounds varied significantly and twenty five (25) compounds varied highly significantly (Table 2). Several of the major compounds were stable even upon drying at 60º C, then decreased at 100º C: sabinene, limonene, β-phellandrene, pregeijerene B. The opposite trend (increased at 100º C) was found for: safrole, elemol and 8-α-acetoxyelemol (Table 2). Only one new compound was formed, cyclohexadecanolide (trace at 60º C, 0.5% at 100º C). The stability of compounds *in situ* even at extreme drying was surprising.
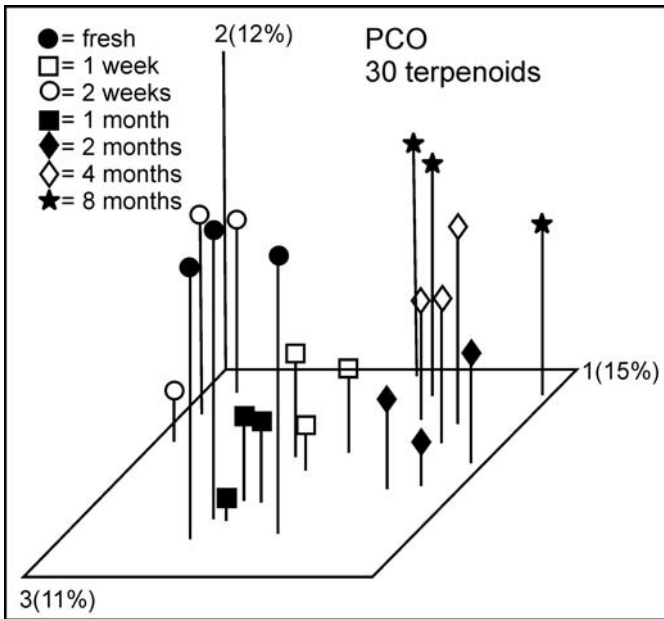
Figure 2. PCO based on 30 terpenoids of the oils from J. virginiana samples dried for periods of 1 week to 8 months at 21º C.

Juniperus pinchotii is in the serrate-leaf margined *Juniperus* group and has oil glands that rupture with white exudate on the leaves (Fig. 3). The white crystalline exudate is composed mostly of camphor and bornyl acetate (Adams, 2008). A comparison of the leaf oils of *J. pinchotii* from fresh vs. air dried (21º C, 2 weeks) leaves is shown in Table 3.
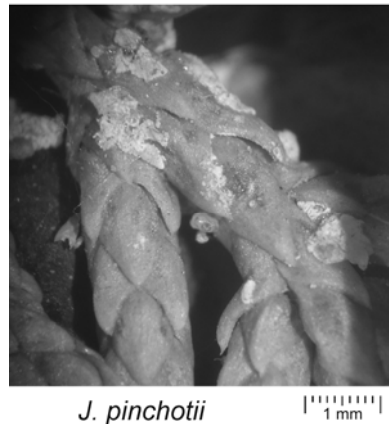
J. pinchotii

Figure 3. Exudate on *J. pinchotii*.

Surprisingly, the oil from fresh and dried leaves are very similar (Table 3). The largest volatile terpene, sabinene had a non-significant change upon leaf drying. However, the percent oil yield (oven dry wt. basis) showed a highly significant decline (Table 3) in contrast to *J. virginiana* that displayed no significant changes in percent oil yields during drying and storage (Table 1). For the *J. pinchotii* oils, only five compounds varied significantly: tricyclene, limonene, camphor, bornyl acetate and β-eudesmol and two components varied highly significantly: borneol and terpinen-4-ol (Table 3). In spite of the large decrease in oil yield with drying, the overall composition of *J. pinchotii* leaf oil remained relatively unchanged.

CONCLUSIONS

For *J. virginiana* and *J. pinchotii* (and other *Juniperus* species) the leaf oils appear to be sufficiently stable in dried leaves (21º C and 30º C) to justify their use in chemosystematic studies. For studies of infraspecific geographical variation, it appears that if all the samples were subjected to the same drying regime, the oils would be comparable. Chemosystematics among species with large differences in the essential oil compositions appear to be valid, even if oils from both fresh and air dried leaves were utilized.

ACKNOWLEDGEMENTS

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Table 1. Comparison of the composition of leaf oils from fresh leaves of *J. virginiana* vs. leaves dried and stored at 21º C. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	F sig
	percent yield	0.55	0.52	0.48	0.51	0.48	0.56	0.53	ns
924	α-thujene	0.4	0.4	0.5	0.5	0.4	0.4	0.5	ns
932	α-pinene	0.7	0.7	0.9	0.7	0.5	0.6	0.8	*
945	α-fenchene	t	t	t	t	t	t	t	nt
969	sabinene	18.0	17.7	19.8	17.1	15.5	17.9	17.6	*
974	β-pinene	0.2	0.2	0.3	0.2	0.2	0.2	0.3	ns
988	myrcene	1.2	0.9	1.1	0.8	0.7	0.7	0.5	ns
990	<u>74</u>,87,43,115	0.5	0.3	0.4	0.3	0.4	0.3	0.4	ns
1008	3-carene	0.6	0.6	0.6	0.5	0.5	0.7	0.9	ns
1014	α-terpinene	0.4	0.3	0.3	0.4	0.3	0.4	0.4	ns
1024	limonene	14.4	14.2	15.6	13.8	14.0	14.4	14.6	ns
1025	β-phellandrene	9.6	9.3	10.4	9.2	7.9	9.5	9.7	*
1054	γ-terpinene	0.6	0.5	0.5	0.6	0.5	0.6	0.5	ns
1065	cis-sabinene hydrate	0.5	0.5	0.5	0.5	0.6	0.6	0.5	ns
1086	terpinolene	0.8	0.7	0.8	0.7	0.7	0.8	0.7	ns
1096	trans-sabinene hydrate	0.3	0.2	0.2	0.2	0.3	0.3	0.3	ns
1097	linalool	0.4	0.3	0.6	0.5	0.5	0.7	0.5	ns
1100	n-nonanal	t	t	0.2	t	0.2	t	t	ns
1118	cis-p-menth-2-en-1-ol	t	t	t	t	t	0.2	t	nt
1136	trans- p-menth-2-en-1-ol	t	t	t	t	t	t	t	nt
1148	citronellal	0.2	t	t	t	t	t	t	nt
1174	terpinen-4-ol	1.3	0.8	0.8	0.9	1.1	1.2	0.9	*
1186	α-terpineol	t	t	t	t	t	t	t	nt
1195	methyl chavicol	0.1	0.2	t	0.2	0.2	0.2	t	ns
1223	citronellol	0.2	t	t	t	0.2	0.2	t	ns
1261	<u>152</u>,123,81,77, aromatic	0.4	0.4	0.3	0.4	0.3	0.4	0.3	ns
1274	pregeijerene B	10.2	11.7	10.7	10.6	9.4	8.7	8.3	**
1285	safrole	11.6	9.1	9.6	10.9	10.0	8.5	9.9	**
1322	methyl geranate	0.1	t	t	t	0.1	0.1	t	nt
1350	citronellyl acetate	t	t	t	t	t	t	t	nt
1379	geranyl acetate	t	t	t	t	t	t	t	nt
1403	methyl eugenol	2.4	2.0	1.6	2.7	2.3	2.0	2.2	**

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	F sig
1417	(E)-caryophyllene	t	t	t	t	t	t	t	nt
1447	43,105,149,178, aromatic	0.3	0.3	0.3	0.2	0.3	0.3	0.3	ns
1465	cis-muurola-4(14),5-diene	t	t	t	t	t	0.2	t	nt
1491	epi-cubebol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	ns
1500	α-muurolene	0.2	0.2	0.2	0.3	0.2	0.2	0.3	ns
1513	γ-cadinene	0.3	0.4	0.5	0.6	0.5	0.5	0.4	*
1522	δ-cadinene	0.8	0.7	0.8	1.0	0.8	0.9	0.9	*
1539	α-copaen-11-ol	t	0.3	t	t	t	t	t	nt
1548	elemol	5.1	5.3	5.1	7.2	5.4	5.5	5.8	*
1555	elemicin	0.8	0.8	0.5	0.8	0.9	0.7	1.1	ns
1565	(3Z)-hexenyl benzoate	0.2	t	0.2	0.2	0.3	0.2	t	ns
1574	germacrene-D-4-ol	2.8	3.4	3.4	2.6	3.5	3.0	3.8	*
1630	γ-eudesmol	0.3	0.3	0.2	0.3	0.3	0.3	0.2	ns
1638	epi-α-cadinol	0.6	0.6	0.5	0.6	0.6	0.6	0.6	ns
1638	epi-α-muurolol	0.6	0.6	0.5	0.7	0.6	0.6	0.7	ns
1649	β-eudesmol	0.4	0.5	0.4	0.5	0.2	0.6	0.6	ns
1652	α-eudesmol	0.6	0.7	0.6	0.6	0.7	0.7	0.8	ns
1652	α-cadinol	1.0	1.0	0.8	1.0	1.0	1.1	1.2	ns
1670	bulnesol	0.5	0.4	0.4	0.3	0.5	0.5	0.6	ns
1688	shyobunol	t	t	t	t	0.2	0.2	t	ns
1746	8-α-11-elemodiol	t	t	0.2	t	0.3	0.4	0.3	ns
1761	iso to 8-α-acetoxyelemol	0.2	0.3	0.2	0.2	0.3	0.3	0.3	ns
1792	8-α-acetoxy-elemol	8.1	9.3	6.3	7.5	12.3	10.5	10.7	**
2054	41,81,137,270,	0.2	0.2	t	0.3	0.3	0.3	0.3	ns
2087	abietadiene	t	t	t	t	t	t	t	nt
2298	4-epi-abietal	0.4	0.3	0.3	0.2	0.4	0.4	0.3	*
2312	abieta-7,13-dien-3-one	t	t	t	t	t	t	t	nt

AI = Arithmetic Index on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Comparison of the leaf oils of *J. virginiana* under extreme drying conditions: 24hrs @ 30º C, 60º C,100º C. * = significant, ** = highly significant, ns = not significant, nt =not tested. Any values that have the same letter (a, b, c, d) following it are not significantly different by the SNK multiple range test (P=0.05).

AI	compound	fresh	30º C	60º C	100º C	F sig.
	percent yield	0.54a	0.50b	0.42c	0.36a	**
924	α-thujene	0.5a	0.5a	0.6a	1.1b	**
932	α-pinene	0.7	0.8	1.0	0.7	**
945	α-fenchene	t	t	0.1	t	nt
969	sabinene	19.6a	21.5a	20.2a	13.9b	**
974	β-pinene	0.3	0.1	0.1	t	nt
988	myrcene	1.3a	1.2a	1.1a	0.3b	**
990	74,87,43,115	0.5a	0.4a	0.3a	0.3a	**
1008	3-carene	0.5a	0.7b	2.0c	0.6a	**
1014	α-terpinene	0.5a	0.4ab	0.3b	0.5ab	ns
1024	limonene	15.0a	15.0a	15.0a	13.3b	*
1025	β-phellandrene	10.8a	10.5a	10.8a	8.8b	*
1054	γ-terpinene	0.7a	0.6a	0.5a	0.9b	**
1065	cis-sabinene hydrate	0.6a	0.5b	0.4b	0.4b	**
1086	terpinolene	0.8a	0.8a	1.0b	0.8a	*
1096	trans-sabinene hydrate	0.3	0.3	0.2	0.2	nt
1097	linalool	0.6	0.4	0.2	0.2	nt
1100	n-nonanal	t	t	t	t	nt
1118	cis-p-menth-2-en-1-ol	0.2	0.1	t	t	nt
1136	trans- p-menth-2-en-1-ol	0.1	t	t	t	nt
1148	citronellal	0.1	t	t	t	nt
1174	terpinen-4-ol	1.7a	1.1b	0.7b	1.0b	**
1186	α-terpineol	t	t	t	t	nt
1195	methyl chavicol	0.1	t	t	0.1	nt
1223	citronellol	t	t	t	0.1	nt
1261	152,123,81,77, aromatic	0.3	0.3	0.3	0.4	nt
1274	pregeijerene B	8.3a	9.3b	8.4a	4.0c	**
1285	safrole	9.0a	8.2a	9.0a	10.7b	**
1322	methyl geranate	0.1	t	0.1	0.1	nt
1350	citronellyl acetate	t	t	0.1	t	nt
1379	geranyl acetate	t	t	t	t	nt
1403	methyl eugenol	1.7a	1.8a	2.1b	2.5c	**
1417	(E)-caryophyllene	t	t	0.7	0.2	nt
1447	43,105,149,178, aromatic	0.2	0.3	0.3	0.2	nt

AI	compound	fresh	30º C	60º C	100º C	F sig.
1465	cis-muurola-4(14),5-diene	t	t	0.1	t	nt
1491	epi-cubebol	t	0.3	0.2	0.2	nt
1500	α-muurolene	t	0.3	0.2	0.3	nt
1513	γ-cadinene	0.3a	0.5b	0.6bc	0.6bc	**
1522	δ-cadinene	0.6a	1.1c	1.0b	1.1c	**
1539	α-copaen-11-ol	t	t	0.2	0.1	nt
1548	elemol	5.1a	4.7a	4.7a	16.0c	**
1555	elemicin	0.6a	0.6a	0.5ab	0.5b	*
1565	(3Z)-hexenyl benzoate	0.3	0.1	t	t	nt
1574	germacrene-D-4-ol	2.6a	2.8a	3.4b	1.5c	**
1630	γ-eudesmol	0.4	0.4	0.3	t	nt
1638	epi-α-cadinol	0.6	0.7	0.5	0.7	ns
1638	epi-α-muurolol	0.6	0.7	0.5	0.7	ns
1649	β-eudesmol	0.5	0.6	0.4	0.4	ns
1652	α-eudesmol	0.6	0.9	0.6	0.6	ns
1652	α-cadinol	1.2	1.0	0.7	0.7	ns
1670	bulnesol	0.5a	0.4b	0.3c	-d	**
1688	shyobunol	0.2	t	t	t	ns
1746	8-α-11-elemodiol	t	0.1	0.1	0.3	ns
1761	iso to 8-α-acetoxyelemol	0.2	0.1	0.1	0.3	ns
1792	8-α-acetoxyelemol	8.2ac	7.0bc	4.9b	10.5a	*
1933	cyclohexadecanolide	- a	- a	t a	0.5b	**
2054	41,81,137,270, diterpene	0.1a	0.1a	0.1a	- b	**
2087	abietadiene	t a	t a	t a	0.1b	**
2108	71,43,57,123, unknown	t a	t a	0.3b	0.2b	**
2142	41,69,255,298,unknown	t a	t a	0.3b	0.3b	**
2188	E-phytol acetate	t	t	0.1	0.1	ns
2298	4-epi-abietal	0.4	0.4	0.5	0.6	ns
2312	abieta-7,13-dien-3-one	0.1a	0.1a	t b	t b	**
2363	abienol isomer	t a	t a	0.1b	0.1b	**

AI = Arithmetic Index on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 3. Comparison of the composition of leaf oils obtained from fresh leaves of *J. pinchotii* vs. leaves dried and stored at 21º C for 2 weeks. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

AI	Compound	Fresh	Dried	F sig
	percent yield	1.49	1.10	**
921	tricyclene	0.3	0.2	*
924	α-thujene	1.0	0.8	ns
932	α-pinene	1.5	1.0	ns
946	camphene	0.4	0.3	ns
969	sabinene	27.5	27.1	ns
974	β-pinene	t	t	nt
988	myrcene	2.8	2.9	ns
1002	α-phellandrene	t	t	nt
1008	δ-3-carene	t	t	nt
1014	α-terpinene	2.6	2.5	ns
1020	p-cymene	0.2	0.2	ns
1024	limonene	3.1	3.3	*
1054	γ-terpinene	4.1	3.9	ns
1065	cis-sabinene hydrate	1.6	1.5	ns
1086	terpinolene	1.7	1.4	ns
1098	trans-sabinene hydrate	1.8	1.5	ns
1118	cis-p-menth-2-en-1-ol	0.7	0.7	ns
1141	camphor	22.7	26.0	*
1145	camphene hydrate	0.9	0.9	ns
1148	citronellal	1.1	1.2	ns
1165	borneol	1.4	1.9	**
1174	terpinen-4-ol	10.1	7.5	**
1186	α-terpineol	0.5	0.4	ns
1195	cis-piperitol	0.2	0.8	ns
1207	trans-piperitol	0.3	0.3	ns
1219	coahuilensol, me-ether	t	t	nt
1223	citronellol	3.7	4.0	ns
1284	bornyl acetate	3.6	2.5	*
1298	carvacrol	t	t	nt
1374	α-copaene	t	t	nt
1548	elemol	2.9	3.0	ns
1559	germacrene B	0.2	0.1	ns
1627	1-epi-cubenol	0.1	0.1	ns
1630	γ-eudesmol	0.5	0.5	ns

AI	Compound	Fresh	Dried	F / sig
1649	β-eudesmol	0.4	0.6	*
1652	α-eudesmol	0.7	0.6	ns
1670	bulnesol	0.3	0.2	ns
1987	manoyl oxide	0.2	0.1	nt
2055	abietatriene	0.1	0.1	nt
2087	abietadiene	0.2	0.1	nt
2???	abietal	0.1	0.1	nt
2312	abieta-7,13-dien-3-one	0.2	0.1	nt

AI = Arithmetic Index on DB-5 column. *Tentatively identified.
Compositional values less than 0.1% are denoted as traces (t).
Unidentified components less than 0.5% are not reported.