The Journal of Essential Oil Research
Vol. 24, No. 5, October 2012, 435–440

Taylor & Francis
Taylor & Francis Group

Alkanes and Terpenes in Wood and Leaves of *Pinus jeffreyi* and *P. sabiniana*

Robert P. Adams[a]* and Jessica W. Wright[b]

[a]*Biology Department, Baylor University, Waco, TX, USA;* [b]*Conservation Geneticist, Pacific Southwest Research Station, USDA Forest Service, Davis, CA, USA*

(*Received 7 December 2011; final form 16 March 2012*)

The wood oils of *Pinus jeffreyi* and *P. sabiniana* contain considerable amounts of heptane (76.6%, 92%), on a monoterpene basis. However, when entire wood extractables is considered, the amounts drop considerably (3.4%, 36.8%) with the major portion of the wood oils being diterpene acids. The leaf oil of *P. jeffreyi* is dominated by α-pinene (20.9%) and, a diterpene, thunbergol (9.2%) with moderate amounts of β-pinene, δ-3-carene, limonene, β-phellandrene, (*Z*)-β-ocimene, (*E*)-caryophyllene, δ-cadinene and cembrene. The leaf oil of *P. sabiniana* is dominated by α-pinene (39.1%) with moderate amounts of β-pinene, myrcene, limonene, β-phellandrene, (*Z*)-β-ocimene, methyl chavicol, decanal and thunbergol.

Keywords: *Pinus jeffreyi*; *Pinus sabiniana*; wood oils; leaf oils; heptane; alkanes; terpenes; diterpene acids

Introduction

The oleoresin oils (wood oils) of *Pinus* normally contain monoterpenes (turpentine) and non-volatile diterpene resin acids (rosin) (1). However, the turpentine of *P. jeffreyi* Grev. & Balif. (Jeffrey pine) and *P. sabiniana* Dougl. (gray or digger pine) is composed of 95 to 99% *n*-heptane (with small amounts of undecane and other alkanes) and only less than a few percent monoterpenes (2,3). Mirov (4) reported 5% undecane in *P. torreyana* Perry and less than 0.1% heptane and 5% heptane in *P. coulteri* D. Don. Smith (5) also reported varying amounts of heptane in the wood of *P. rudis* (0–32%) and *P. pseudostrobus* (0–47%). Smith (6) published a massive recompilation of data xylem monoterpenes in *Pinus*, but still reported that only the wood oils of *P. jeffreyi* and *P. sabiniana* contained high levels (95–99% *n*-heptane in the monoterpene fraction). It is important to note that the aforementioned analyses were computed on volatile alkanes/monoterpenes basis. The entire oil, including sesquiterpenes and diterpene acids, were not utilized in the computations.

Ekundayo (7) reviewed the volatile constituents of *Pinus* needle oils for thirty-three species. He reported the compositions (from the literature) for monoterpenes and sesquiterpenes but not for diterpenes. No data was presented for *P. jeffreyi* or *P. sabiniana*. Kurose et al. (8) analyzed leaf oils from nine *Pinus* species but not for *P. jeffreyi* or *P. sabiniana*.

Savage et al. (1) appear to be the first to compare the very volatile (monoterpenes and lower alkanes) of both wood and leaves of *Pinus jeffreyi*. They did not detect heptane in the needle oil, but did find a progressive increase from phloem (current 0.8%, basal, 33.1%), to xylem (current, 35.4%, sapwood, 80.6%, heartwood, 95.2%). No comparison appears to have been published of the leaf and wood oils of *P. sabiniana*.

The purpose of this study is to present the first complete analyses of both leaf and wood oils from *Pinus jeffreyi* and *P. sabiniana*.

Experimental

Plant specimens

Pinus sabiniana Dougl., Eddy Arboretum, United States Forest Service (USFS) Institute of Forest Genetics, Placerville, CA (Adams 12766-69), *Pinus jeffreyi* Grev. & Balf., Eddy Arboretum, USFS Institute of Forest Genetics, Placerville, CA (Adams 12770-75). Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Essential oil extraction

Fresh leaves were collected at 1.5 m high on the south facing side of each tree. A wood core (5 mm diameter × 20 cm) was taken at 1 m high from each tree and sealed in a 20 mm diameter 8 mL glass vial with a teflon coated compression cap. The wood and leaf samples were shipped by overnight express and kept at –20°C until extracted. The wood cores were extracted in diethyl ether on a shaker (48 hours). It should be noted that Smith (6) and his colleagues at USFS utilized a different protocol in collecting oleoresin. They

*Corresponding author. Email: Robert_Adams@baylor.edu

ISSN 1041-2905 print/ISSN 2163-8152 online

http://dx.doi.org/10.1080/10412905.2012.703512
http://www.tandfonline.com

Table 1. Wood and leaf volatile oil compositions (%) for *Pinus jeffreyi* and P. *sabiniana*.

Compound	KI Obs.	KI Litr.	Compound	*P. jeffreyi* wood	*P. jeffreyi* leaf	*P. sabiniana* wood	*P. sabiniana* leaf
1	700	700	*n*-heptane	3.4±0.08(76.6 ±1.5)	—	36.8±0.7(92 ±1.8)	t
2	900	900	*n*-nonane	0.1 (2.0±0.10)	—	0.4(1.0±0.03)	—
3	921	921	tricyclene	—	0.1	—	t
4	924	924	α-thujene	—	t	—	t
5	932	932	α-pinene	0.1 (2.7±0.0.5)	20.9 ±0.44	0.4(1.0±0.03)	39.1±0.09
6	946	946	camphene	—	1.6±0.05	—	0.5±0.01
7	953	953	thuja-2,4-diene	—	t	—	t
8	969	969	sabinene	—	0.1	—	t
9	974	974	β-pinene	0.1 (0.7±0.02)	6.7±0.16	—	3.3±0.06
10	988	988	myrcene	0.1 (1.3±0.04)	1.9±0.06	—	3.6±0.06
11	998	998	octanal	—	—	—	0.7±0.02
12	1000	1000	decane	—	—	0.1(0.3)	—
13	1002	1002	α-phellandrene	—	0.1	—	0.1
14	1008	1008	δ-3-carene	0.1 (2.0±0.12)	3.7±0.10	—	t
15	1014	1014	α-terpinene	—	t	—	—
16	1020	1020	*p*-cymene	—	t	—	t
17	1024	1024	limonene	0.1 (0.7±0.02)	5.0±0.13	t(0.2)	10.5±0.21
18	1025	1025	β-phellandrene	0.1 (4.0±0.11)	4.6±– 0.12	t(0.2	10.4±0.20
19	1032	1032	(*Z*)-β-ocimene	—	3.2±0.07	—	4.6±0.10
20	1044	1044	(*E*)-β-ocimene	—	0.6±0.01	—	0.2
21	1054	1054	γ-terpinene	—	0.1	—	—
22	1063	1063	octanol	—	—	—	0.2
23	1086	1086	terpinolene	—	0.6±0.01	—	0.3
24	1095	1095	linalool	—	1.7±0.05	—	0.2
25	1100	1100	undecane	0.1 (2.9±0.07)	—	0.2(0.6±0.02)	t
26	1100	1100	nonanal	—	t	—	t
27	1122	1122	α-campholenal	—	0.1	—	0.2
28	1136	1136	*trans*-pinocarveol	—	t	—	0.1
29	1141	1141	camphor	—	t	t(0.2)	0.1
30	1145	1145	camphene hydrate	—	t	—	t
31	1148	1148	citronellal	—	0.5±0.01	—	0.5±0.01
32	1158	1158	*trans*-pinocamphone	—	t	—	0.1
33	1165	1165	(3*E*,5*Z*)-1,3,5-undecatriene	—	0.7±0.02	—	0.6±0.01
34	1174	1174	terpinen-4-ol	—	t	t(0.2)	t
35	1186	1186	α-terpineol	—	0.3	—	0.3
36	1195	1195	methyl chavicol	0.1± (1.9±0.05)	1.4±0.03	0.1(0.4)	4.5±0.10
37	1200	1200	dodecane	—	t	t(0.2)	—
38	1201	1201	decanal	—	0.8±0.02	—	2.2±0.05
39	1215	1215	benzothiazole	0.1± (3.2±0.10)	—	0.2(0.6±0.02)	—
40	1223	1223	citronellol	—	0.3	—	0.2
41	1232	1232	thymol, methyl ether	—	—	—	0.1
42	1254	1254	2-phenyl ethyl acetate	—	0.1	—	—
43	1260	1260	2-decenal	—	—	—	0.1
44	1284	1284	bornyl acetate	—	1.2±0.03	—	—
45	1292	1292	(2*E*,4*Z*)-decadienal	—	—	—	0.1
46	1293	1293	2-undecanone	—	0.4	—	—
47	1315	1315	(2*E*,4*E*)-decadienal	—	—	—	0.1
48	1345	1345	α-cubebene	—	0.5±0.01	—	—
49	1350	1350	citronellyl acetate	—	0.2	—	—
50	1374	1374	α-copaene	—	0.5±0.01	—	—
51	1379	1379	geranyl acetate	—	0.1	—	—
52	1385	1385	octyl butanoate	—	—	—	0.1
53	1387	1387	β-cubebene	—	0.2	—	—
54	1389	1389	β-elemene	—	0.2	—	t
55	1396	1396	duvalene acetate	—	t	—	0.1

(*Continued*)

Table 1. (*Continued*).

Compound	KI Obs.	KI Litr.	Compound	*P. jeffreyi* wood	*P. jeffreyi* leaf	*P. sabiniana* wood	*P. sabiniana* leaf
56	1400	1400	tetradecane	t	—	0.2	—
57	1403	1403	methyl eugenol	—	—	—	0.3
58	1407	1407	longifolene	0.3	0.4	—	—
59	1408	1408	dodecanal	—	1.1±0.03	0.4	1.0±0.03
60	1417	1417	(*E*)-caryophyllene	—	4.2±0.10	—	t
61	1439	1439	2-phenyl ethyl butanoate	—	2.8±0.08	—	1.5±0.04
62	1448	1448	*cis*-muurola-3,5-diene	—	0.1	—	—
63	1451	1451	*trans*-muurola-3,5-diene	—	0.7±0.02	—	—
64	1475	1475	*trans*-cadina-1(6),4-diene	—	0.1	—	—
65	1478	1478	γ-muurolene	—	0.3	—	—
66	1484	1484	germacrene D	—	1.0±0.03	—	t
67	1486	1486	phenyl 2-me-butanoate	—	0.4	—	—
68	1493	1493	*trans*-muurola-4(14),5-diene	—	0.2	—	—
69	1493	1493	epi-cubebol	—	1.0±	—	—
70	1500	1500	bicyclogermacrene	—	0.9±0.03	—	—
71	1500	1500	pentadecane	—	—	0.1	—
72	1500	1500	α-muurolene	—	0.5±0.01	—	—
73	1508	1508	germacrene A	—	0.2	—	t
74	1513	1513	γ-cadinene	—	0.9±0.02	—	t
75	1514	1514	cubebol	—	1.0±0.03	—	—
76	1522	1522	δ-cadinene	—	2.4±0.07	—	0.1
77	1533	1533	*trans*-cadina-1,4-diene	—	0.1	—	—
78	1537	1537	α-cadinene	—	0.1	—	—
79	1561	1561	*E*-nerolidol	—	0.6±0.01	—	—
80	1562	1562	geranyl butanoate	—	t	—	—
81	1574	1574	germacrene-d-4-ol	—	1.0±0.03	—	0.1
82	1582	1582	caryophyllene oxide	—	0.2	—	—
83	1594	1594	ethyl dodecanoate	—	—	—	0.1
84	1600	1600	hexadecane	—	—	0.1	—
85	1611	1611	tetradecanal	—	0.1	—	t
86	1627	1627	1-epi-cubenol	—	0.1	—	—
87	1638	1638	epi-α-muurolol	—	0.4	—	—
88	1640	1640	phenyl ethyl hexanoate	—	0.4	—	0.3
89	1644	1644	α-muurolol	—	0.1	—	—
90	1652	1652	α-cadinol	—	0.7±0.02	—	0.1
91	1715	1715	(2*Z*,6*E*)-farnesol	—	0.4	—	—
92	1795	1795	ethyl tetradecanoate	—		—	t
93	1800	1800	octadecane	—	—	t	—
94	1814	1814	hexadecanal	—	0.1	—	t
95	1846	1846	phenyl ethyl octanoate	—	0.3	—	—
96	1900	1900	nonadecane	—	—	t	—
97	1937	1937	cembrene	—	1.9±0.05	—	0.6±0.02
98	1943	194312	iso-cembrene	—	1.2±0.03	—	0.3
99	1959	1959	hexadecanoic acid	1.5±0.04	—	0.1	—
100	1965	1965	(3*Z*)-cembrene A	—	0.2	—	—
101	1987	1987	manoyl oxide	—	0.2	—	0.5±0.01
102	2000	2000	eicosane	—	—	0.1	—
103	2048	2048	thunbergol	—	9.2±0.21	—	4.7±0.11
104	2105	2105	isoabienol	—	—	9.2±0.24	0.2
105	2132	2132	linoleic acid	33.2±0.66	—	1.1±0.03	—
106	2141	2141	oleic acid	18.0±0.16	—	2.0±0.06	—
107	2149	2149	abienol	—	1.3±0.04	2.6±0.09	1.3±0.04
108	2200	2200	docosane	—	—	0.5±0.01	—
109	2218		41,55,187,286,302,diterpene	2.0±0.06	—	—	—
110	2232	2232	3,5-dimethoxy stilbene	1.8±0.05	—	—	—
111	2293		4-methoxy-2-hydroxy-Stilbene[a]	5.1±0.16	—	—	—
112	2297	2297	methyl isopimerate	—	—	—	t
113	2298	2298	4-epi-abietal	—	—	—	t
114	2300	2300	tricosane	—	—	0.7±0.02	—

(*Continued*)

Table 1. (*Continued*).

Compound	KI Obs.	KI Litr.	Compound	*P. jeffreyi* wood	*P. jeffreyi* leaf	*P. sabiniana* wood	*P. sabiniana* leaf
115	2330		41,55,121,287.302,diterpene	2.7±0.08	—	—	—
116	2342		41,55,268,286,314,diterpene	—	0.1	—	3.2±0.11
117	2391		41,55,241,287,338, acid	6.9±0.15	—	3.7±0.07	—
118	2395		β-pimaric acid[b]	5.4±0.13	—	12.0±0.31	—
119	2438		dehydro-abietic acid[b]	1.7±0.04	—	6.6±0.17	—
120	2473		256, 179,152,302, acid	2.4±0.07	—	—	—
121	2476		abietic acid[b]	6.2±0.13	—	14.4±0.31	—
			volatile alkanes/monoterpenes	4.4	56.8	39.2	82.9
			less volatile alkanes/ sesquiterpenes	0.3	24.1	—	3.8
			diterpene/acids	86.9	14.4	52.8	10.8
			% total oil	91.6	95.3	92.0	97.5

Notes: Wood components in parentheses are percent concentrations based on monoterpenes only. Percentage by FID peak area normalization without the use of response factors.% peak ± standard deviation, $n = 3$. KI, Kovats Index (linear) on DB-5 column [Obs., observed; Litr., from the literature (10)]. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. [a]Tentatively identified. [b]NIST 05 library.

used a brace and bit to drill a 0.56 in. hole through the bark and phloem thence about 0.25 in. into the xylem. A 5-cc shell vial was inserted into the hole and the resin was collected for 8–48 hours. After 8–48 hours, the vial was removed, tightly capped, taken to the laboratory and diluted 1:1 with pentane before analysis.

Fresh leaves (200 g) were steam distilled for 2 hours using a circulatory Clevenger-type apparatus with a layer of diethyl ether as an oil trap (9). The oil samples were concentrated (diethyl ether removed) with nitrogen and the samples stored at –20°C until analyzed.

Gas chromatography (GC) and GC–mass spectrometry (GC–MS) analysis

The oils were analyzed on a Hewlett–Packard (HP) 5971 MSD mass spectrometer, scan time 1/second,

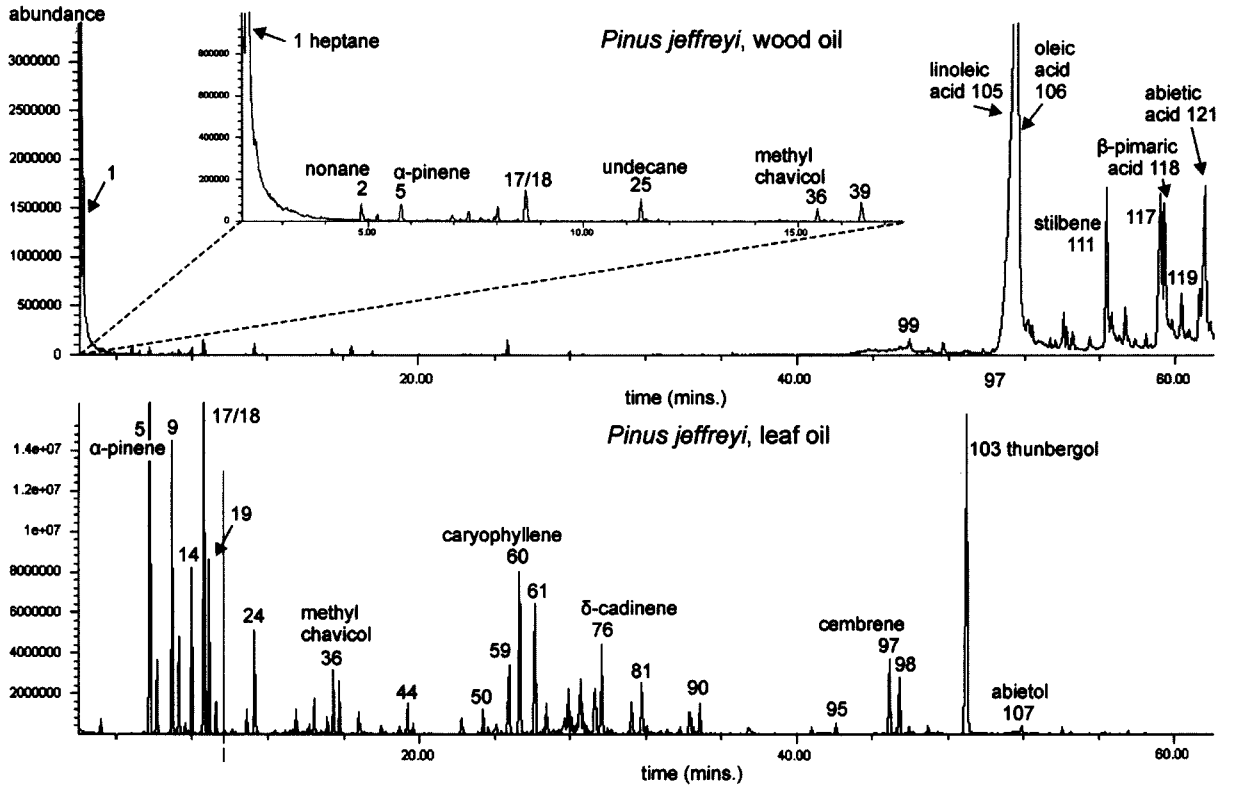

Figure 1. Mass spectroscopy total ion chromatograms for *Pinus jeffreyi* wood and leaf oils. Peak numbers correspond to Table 1.

directly coupled to a HP 5890 gas chromatograph, using a J&W DB-5, 0.26 mm × 30 m, 0.25 micron coating thickness, fused silica capillary column [see Adams (10) for operating details]. The oils were run at both 60–246°C/3°C/minute and at 40°C, isothermal, 4 minutes, then 3°C/minute to 246°C in order to resolve heptane and diethyl ether. Identifications were made by library searches of our volatile oil library (10), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds and the NIST database. Quantitation was by flame ionization detection (FID) on an HP 5890 gas chromatograph using a J&W DB-5, 0.26 mm × 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software without FID response factors.

Results and discussion

As anticipated, the wood oils of *Pinus jeffreyi* and *P. sabiniana* contain considerable amounts of heptane (76.6%, 92%, on a monoterpene basis, Table 1). However, when entire wood extractables is considered, the amounts drop considerably (3.4%, 36.8%, Table 1). The reports in the literature are on a 'monoterpene' basis, so our data seem consistent with Smith (6) and others. The method of wood extraction (shaking wood cores in diethyl ether) removes only the 'constitutive' oil. Drilling, then collecting oleoresin directly into tubes for 8 to 48 hours would remove both 'constitutive' and possibly some 'wound induced' components. Keeling and Bohlmann (11) note that both constitutive and induced terpenoids in conifers are important in insect defense. Whether the 8–48 hours oleoresin collection time is sufficient to induce significant amounts of terpenes/alkanes in *P. jeffreyi* and *P. sabiniana* is not known. The leaf oil of *P. jeffreyi* is dominated by α-pinene (20.9%) and a diterpene, thunbergol (9.2%) with moderate amounts of β-pinene, δ-3-carene, limonene, β-phellandrene, (*Z*)-β-ocimene, (*E*)-caryophyllene, δ-cadinene and cembrene. The leaf oil of *P. sabiniana* is dominated by α-pinene (39.1%) with moderate amounts of β-pinene, myrcene, limonene, β-phellandrene, (*Z*)-β-ocimene, methyl chavicol, decanal and thunbergol.

The whole oil of *Pinus jeffreyi* wood is dominated by diterpene acids (90.3%, Table 1), whereas the leaf oil is somewhat balanced between monoterpenes

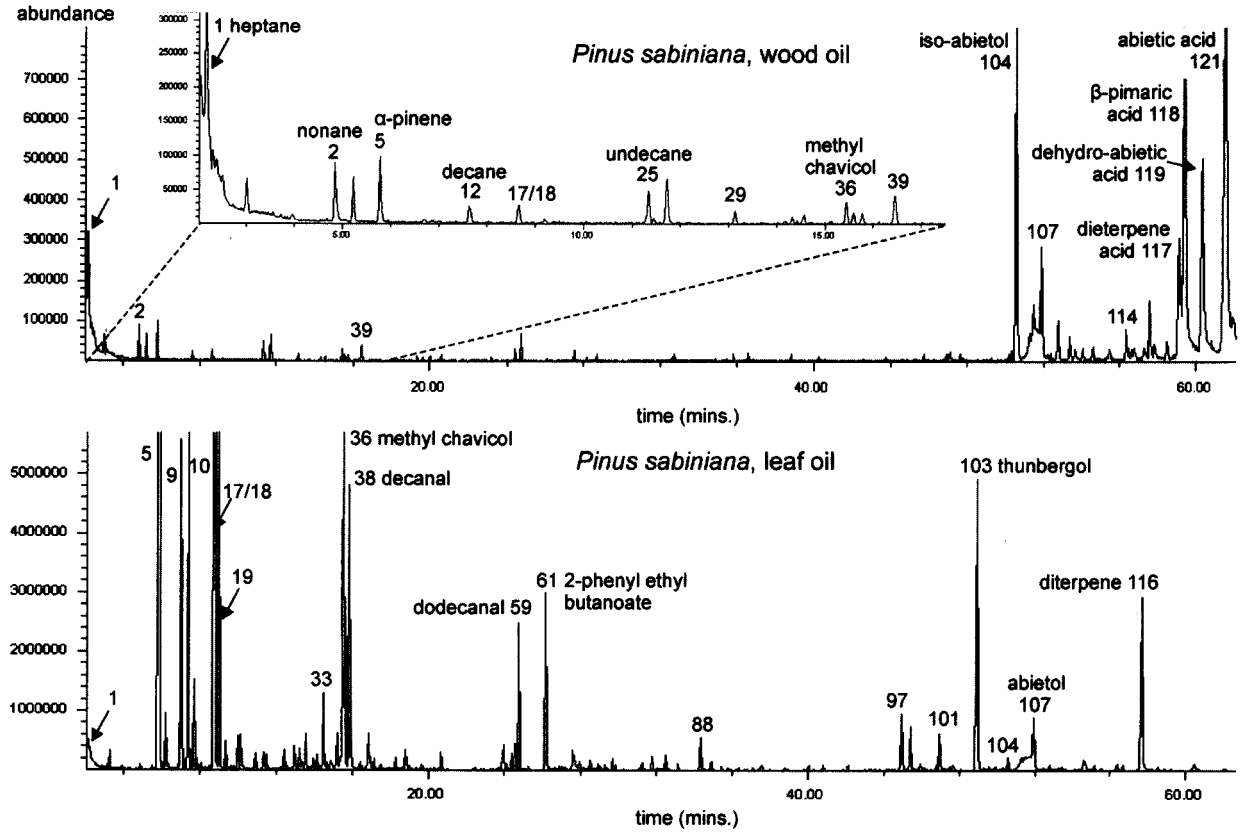

Figure 2. Mass spectroscopy total ion chromatograms for *Pinus sabiniana* wood and leaf oils. Peak numbers correspond to Table 1.

(56.8%), sesquiterpenes (24.1%) and diterpenes (14.4%) (Figure 1). The whole oil of *P. sabiniana* wood is somewhat similar to *P. jeffreyi* in having mostly diterpene acids (55.8%), however it also contains two major neutral diterpenes (isoabienol, 9.2%; abienol, 2.6%). The balance of the wood oil is heptane/alkanes/monoterpenes (39.1%) with no sesquiterpenes (Table 1). The leaf oil of *P. sabiniana* is in contrast to its wood oil, having 82.9% monoterpenes, 3.8% sesquiterpenes and 10.8% diterpenes (Table 1) (Figure 2).

Clearly, the wood and leaf oils of *Pinus jeffreyi* and *P. sabiniana* differ completely in their compositions. Keeling and Bohlmann (11) review defense chemicals (largely terpenoids) and offer insightful theories which the reader is referred to. The role of chemicals in a tree trunk in resisting insects and diseases for many (often hundreds) years, must be quite different from the role of chemicals in the leaves (needles) that have a short (and expendable) life span of only a few years. It seems unusual that only very few *Pinus* species have evolved the production of heptane in the wood (and not in the leaves), whereas most of the other species appear to have wood oil that is similar to the leaf oil. Diterpene acids are very common in *Pinus* (12) and even in the two species in this report with large amounts of heptane. As the flow of oleoresin is important in expelling bark beetles (and the associated fungi), it may be that heptane acts as a solvent for the diterpene acids to increase flow much in the manner that monoterpenes (cf. α-pinene, etc.) may act as a solvent for the diterpene acids (rosin) in other pines.

Acknowledgements

This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for laboratory assistance.

References

1. T.J. Savage, B.S. Hamilton and R. Croteau, *Biochemistry of short-chain alkanes.* Plant Physiol., **110**, 179–186 (1996).
2. N.T. Mirov, *The terpenes (in relation to the biology of the genus Pinus).* Ann. Rev. Biochem., **17**, 521–540 (1948).
3. R.H. Smith, *Variations in the monoterpene composition of the wood resin of Jeffrey, Washoe, Coulter and Lodgepole pines.* For. Sci., **13**, 246–252 (1967).
4. N.T. Mirov, *Composition of Gum Turpentine of Pines*, USDA FS Technical Bulletin 1239, 895 pp., USDA, Albany, CA (1961).
5. R.H. Smith, *Xylem Monoterpenes of Some Hard Pines of Western North America: Three Studies*, Research Paper PSW-160, 7 pp., Pacific Southwest Forest and Range Experimental Station, USFS, USDA, Albany, CA (1982).
6. R.H. Smith, *Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, and Function.* General Technical Report PSW-GTR-177, 454 pp., Pacific Southwest Research Station, USFS, USDA, Albany, CA (2000).
7. O. Ekundayo, *Volatile constituents of* Pinus *needle oils.* Flav. Frag. J., **3**, 1–11 (1988).
8. K. Kurose, D. Okamura and M. Yatagai, *Composition of the essential oils from the leaves of nine* Pinus *species and the cones of three* Pinus *species.* Flav. Frag. J., **22**, 10–20 (2007).
9. R.P. Adams, *Cedarwood oil – Analysis and properties.* In: *Modern Methods of Plant Analysis, New Series: Oil and Waxes.* Edits., H.-F. Linskens and J.F. Jackson, pp. 159–173, Springler-Verlag, Berlin (1991).
10. R.P. Adams, *Identification Of Essential Oil Components by Gas Chromatography/Mass Spectrometry.* 4th edn. Allured Publ. Corp, Carol Stream, IL (2007).
11. C.I. Keeling and J. Bohlmann, *Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. Tansley Review.* New Phytologist, **170**, 657–675 (2006).
12. E. Duquesnoy, V. Castola and J. Casanova, *Composition and chemical variability of the twig oil of Abies alba Miler from Corsica.* Flav. Frag. J., **22**, 293–299 (2007).