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A Re-examination of the Volatile Leaf Oils of *Pinus Ponderosa* Dougl. Ex. P. Lawson using Ion Trap Mass Spectroscopy

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The composition of the volatile leaf oil of *Pinus ponderosa* Dougl. Ex. P. Lawson was re-examined by fused silica capillary gas chromatography–ion trap mass spectroscopy. The leaf oil from Washington was high in α- and β-pinenes (9·1% and 37·9%), car-3-ene (19·3%), α-terpineol (6·2%), myrcene (4·1%) and terpinolene (3·1%). A total of 52 components were identified. This analysis is compared and contrasted with the previous analyses which reported from 8 to 17 monoterpenoids plus estragole in Ponderosa pine leaf oil. The utility of ion trap mass spectroscopy is shown to be of general use for the identification of flavours and fragrances.

KEY WORDS Essential oil Monoterpenes Sesquiterpenes Ponderosa pine *Pinus ponderosa* Dougl. ex. P. Lawson Pinaceae Ion trap mass spectroscopy

INTRODUCTION

Ponderosa pine (*Pinus ponderosa* Dougl. ex. P. Lawson) is one of the most common pines in North America, occurring from British Columbia southward in the Rocky Mountains into central Mexico.[1] The volatile oil of the leaves has been little investigated, although Schorger[2] made a very early report (1919) that the oil was composed of α-pinene (2%), β-pinene (75%), limonene (6%), borneol (7%), bornyl acetate (2%) and 'green oil' (3%). Analyses using modern gas chromatography have reported similar results,[4–6] except for the identification of estragole (= methylchavicol) as a sometimes major constituent of the oil.[3,5,6]

The previous analyses have been from three native stands: the San Bernardino mountains of southern California;[3] Sierra Nevada mountains of central California;[6] and British Columbia,[5] as well as a sample from cultivated Ponderosa pine grown in the USSR.[4]

Ion trap mass spectroscopy (ITMS) has not been widely utilized in essential oil analyses as it has only recently become widely available. Just as in quadrupole mass spectroscopy, the molecules are introduced (via helium carrier gas and a capillary column) into an electron beam and ionized by interaction with electrons. Ion trap mass spectroscopy depends on the ability of helium to focus ions in an RF (radio frequency) trap. As the RF voltage is increased, each class of mass ions becomes unstable in the trap and is released on to an electron multiplier which produces a current proportional to the number of ions detected. The total ion chromatogram and mass spectrum are comparable to quadrupole EI mass spectroscopy although occasionally the spectra are rather different. Thus, it is important to build a library of ITD mass spectra. The principal advantages of ITMS are: it is much cheaper to purchase and maintain; it is much easier to operate (one does not need a full time, dedicated operator); and the data system is based on an IBM PC XT computer (or compatible). Since the library and search system are PC-based, libraries can be copied to floppy discs and easily shipped anywhere. Raw data can be collected and then analysed in another laboratory or country on any PC-compatible machine. It is the transportability of the data and the user-friendliness of the software that sets the ITMS apart from quadrupole mass spectroscopy systems.

The purpose of this paper is to report on both the major and minor components of the volatile

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leaf oil of Ponderosa pine from Washington, to compare the composition with previous analyses of Ponderosa pine leaf oils from different locations and to demonstrate the utility of ITMS for routine volatile oil analysis.

EXPERIMENTAL

The samples of plant material consisted of approximately 100 g of fresh foliage (needles), with two samples collected from each of 20 trees (40 samples total) from a population of *Pinus ponderosa* 1 km north of Dryden, Washington (Chelan Co.), July 1987, and kept cool on ice until frozen. A voucher specimen is on deposit at the herbarium at the University of Utah. The leaves were then kept at −20° C until steam distilled. The leaves were cut into 1-cm segments just prior to steam distillation using a modified Clevenger apparatus with a floating trap of diethyl ether.[7] Steam distillations were performed for 2 h and 24 h to determine yields. The oils were concentrated with nitrogen, tightly sealed in glass vials with foil lined caps and stored at −20° C until analysed.

Mass spectra were recorded with a Finnigan Ion Trap (ITD) mass spectrometer, model 700, directly coupled to a Varian 6500 gas chromatograph, using a J & W DB5, 30 m × 0·26 mm i.d., 0·25 μm coating thickness, fused silica capillary column. The GC–ITD was operated under the following conditions: injector temperature: 220° C; transfer line: 240° C; oven temperature programmed: 60° C to 240° C at 3° C/min; carrier gas: He at 31·9 cm/s or 1·017 ml/min (at 210° C); injection size: 0·1 μl (10% solution), split 1:20, 500 ng on column. Tuning values for the ITD were 100, 100, 100, 100 using cedrol as a tuning standard. We initially used cedrol as a tuning standard because its mass spectrum was very sensitive to tuning and other parameters. However, we have recently found that using the new automatic gain control (AGC) software (version 3·00), cedrol is now little affected by tuning values. Internal standards (*n*-octane and *n*-eicosane) were added to each sample to aid in the standardization of retention times. Identifications were made by library searches of our volatile oil library, LIBR(TP) using the Finnigan library search routines based on fit. Additional searches were made of the EPA/NIH mass spectral data base.[8,9]

Quantification was made by FID using a DB5 column (see above for conditions) in a Varian 6500 gas chromotograph with He as the carrier gas (as above). The FID was operated at 240° C; otherwise all temperatures and conditions were the same as the GC–ITD (see above). Peak areas were quantitated using a Columbia Scientific Industries Supergrator-2, electronic digital integrator, with output (per cent area) to an Edge Technology floppy disk. These data were then read into an IBM PC XT computer for processing.

RESULTS AND DISCUSSION

The total ion count (TIC) mass chromatogram of the voltale leaf oil of Ponderosa pine reveals a dominance by the monoterpenes (Figure 1).

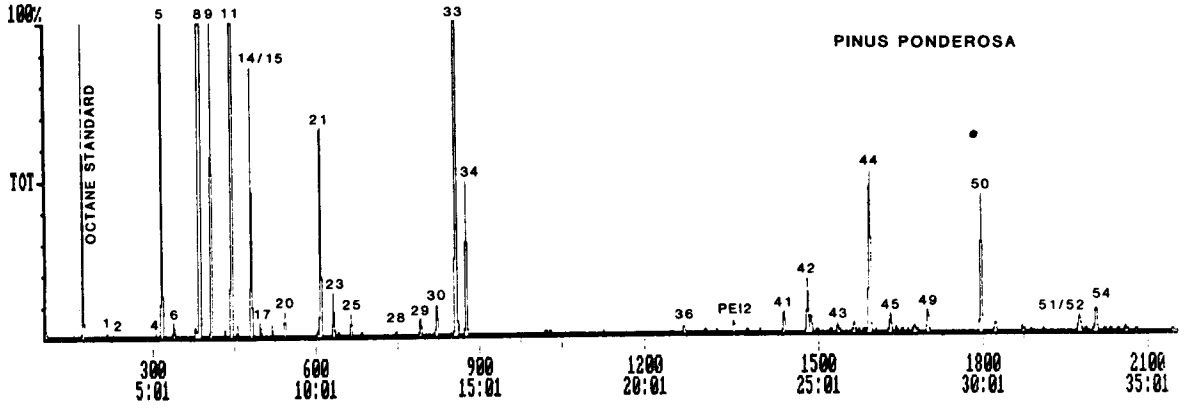

Fig. 1. TIC chromatogram of the steam volatile leaf oil of *Pinus ponderosa* run on a capillary DB5, 30 m column direct coupled to a Finnigan Ion Trap Detector. Peak numbers refer to components in Table 1

Table 1. Volatile oil composition of leaves of *Pinus ponderosa* Dougl. ex. P. Lawson based on the average for 20 trees from Washington state. Compounds are listed in order of their elution from a DB5 column. Compounds in parenthesis are tentatively identified. Previous analyses are from plants from: British Columbia;[5] Southern[3] and Central[6] California; and cultivated in Russia.[4] The data for the per centage concentrations of each component for central and southern California have been renormalized to include estragol (= methylchavicol) in the total

	Per cent total oil				
Compound	Washington	British Columbia[5]	California South[3]	California Central[3]	Russia (cult.)[4]
1. Hexan-3-ol	0·2	—	—	—	—
2. Hexanol	0·2	—	—	—	—
3. Tricyclene	T	—	—	—	0·4
4. α-Thujene	T	—	—	—	—
5. α-Pinene	9·1	11	12·5	11·2	37·2
6. Camphene	0·3	T	T	—	10·1
7. Sabinene	0·2	—	—	—	—
8. β-Pinene	37·9	55	62·7	66·0	30·0
9. Myrcene	4·1	2	7·4	4·7	4·2
10. α-Phellandrene	0·1	—	—	—	—
11. Car-3-ene	19·3	10	0·2	7·5	5·4
12. α-Terpinene	0·2	—	—	—	5·1
13. *p*-Cymene	T	—	—	—	—
14. Limonene	1·9	2	0·6	1·7	T
15. β-Phellandrene	1·8	2	1·3	2·1	3·0
16. 1,8-Cineole	—	—	T	—	T
17. *cis*-Ocimene	0·3	T	T	—	—
18. *trans*-Ocimene	T	T	—	—	—
19. Benzaldehyde	0·3	—	—	—	—
20. γ-terpinene	0·4	T	—	—	T
21. Terpinolene	3·1	2	—	—	
22. Fenchone	—	T	—	—	—
23. Linalol	0·5	T	—	—	—
24. Nonanal	T	—	—	—	—
25. α-Fenchol	0·3	—	—	—	—
26. *cis*-Pinene hydrate	T	—	—	—	—
27. *trans*-Pinene hydrate	T	—	—	—	—
28. Camphene hydrate	0·1	—	—	—	—
29. Borneol	0·2	—	—	—	—
30. Terpin-4-ol	0·4	T	—	—	—
31. Naphthalene	T	—	—	—	—
32. *p*-Cymen-8-ol	T	—	—	—	—
33. α-Terpineol	6·2	10	—	—	T
34. Estragol	1·8	8	20·4	6·0	—
35. Bornyl acetate	T	T	—	—	0·1
36. α-Terpinyl acetate	0·2	—	—	—	—
37. Neryl acetate	T	—	—	—	—
38. Dodecanal	T	—	—	—	—
39. Geranyl acetate	0·1	—	—	—	—
PE12. Phenyl ethyl acetate isomer #2	0·2	—	—	—	—
40. Longifolene	0·2	—	—	—	T
41. Caryophyllene	0·4	—	—	—	T
42. *trans*-α-Bergamotene	0·8	—	—	—	—
43. *cis*-β-Farnesene	0·2	—	—	—	—
44. Germacrene-D	1·7	—	—	—	—
45. γ-Elemene	0·2	—	—	—	—
46. α-Muurolene	0·2	—	—	—	T?
47. α-Farnesene	T	—	—	—	—
48. γ-Cardinene	0·2	—	—	—	0·3
49. δ-Cadinene	0·4	—	—	—	T
50. Nerolidol	1·7	—	—	—	—
51. τ-Cadinol	0·4	—	—	—	—
52. τ-Muurolol	T	—	—	—	—
53. Torreyol	T	—	—	—	—
54. α-Cadinol	0·4	—	—	—	—

T = trace, < 0.1% of total oil.

Fifty-two components were identified from the oil (Table 1). In general, the analysis agrees with the previous reports (Table 1) as far as the monoterpenes are concerned. There are, however, large quantitative differences in several components. The cultivated Ponderosa pine from the USSR had very high amounts of α-pinene (37·2%), camphene (10·1%) and α-terpinene (5·1%), compared to any of the analyses from United States stands (Table 1). Our samples were much higher in car-3-ene than any previous reports and lower in β-pinene (37·9%) and estragole (1·8%) than samples from other native stands (Table 1). Zavarin, *et al.*[6] reported large variations in estragole (methylchavicol) among 30 trees, with a range of from about 3% to over 40% of the total volatile oil. The population we sampled exhibited variability with estragole ranging from 0·4% to 5·3% total oil. It is possible that the analyses of Ponderosa pines from central and southern California[3,6] (see compounds 33/34 in Table 1) may have failed to resolve α-terpineol from estragole since neither study reported α-terpineol.

In order to examine the relative variability among populations, we re-computed coefficients of variation from three studies[3,6] that reported on variation. Comparisons are made in Table 2. In general, the Washington population had much less variation than the California populations, particularly for estragole, car-3-ene, limonene and myrcene. Both α- and β-pinenes had about the same variation in all populations. The Washington population was more variable in its sesquiterpenoids (*trans*-α-bergamotene, germacrene-D, nerolidol and α-cardinol) than in its monoterpenoids (Table 2). However, it is possible that part of these larger coefficients of variation may be due to numerical rounding errors in handling the smaller values for the sesquiterpenes. The studies cited did not report on the variation in the sesquiterpenoids, so we cannot determine if this pattern holds for the California plants.

In addition to the identified components, we also found four minor components that appear to be a series of isomers related to phenylethyl acetate. The second isomer (PEI2), which eluted between geranyl acetate and longifolene, had the largest concentration (0·2%). Mass spectrum for the unknown, PEI2: MW?; *m/z* (%) 104 (100), 91(16), 78(12), 76(10), 65(8), 51(7), 43(54), 41(22).

A study[3] of the effects of air pollution on the volatile oil of Ponderosa pines suggested that injured trees had a lower concentration in estragole (derived from the phenyl-propanoid pathway) in the volatile oil than that found in healthy trees. This may be important for our future study, because we will be examining the incidence of scale insects on the Ponderosa pine in the stand as well as cross-compatibility of scale insect colonies with pine chemotypes.

Table 2. Comparisons of variation in the major components of the leaf oil of Ponderosa pine from Washington (20 trees, 2 samples/tree), South[3] (20 healthy and 20 injured trees), Central[6] (25 trees) California. Coefficients of variation were computed as: (standard deviation/population average) × 100.

	Washington Average Range	South California Healthy CV	South California Injured CV	Central California CV	CV
α-Pinene	9·1 (14·9–5·7)	10·1	27	38	12
Camphene	0·3 (1·3–0·1)	26·3	—	—	—
β-Pinene	37·9 (50·6–23·3)	7·1	6	12	9
Myrcene	4·1 (13·8–1·5)	14·2	66	121	74
Car-3-ene	19·3 (39·9–8·5)	6·4	150	170	35
Limonene	3·6 (6·7–2·6)	6·9	62	44	61
β-Phellandrene	(with Limonene)		144	54	68
Terpinolene	3·1 (4·6–1·9)	5·8	—	—	—
Linalol	0·5 (2·3–0·1)	23·6	—	—	—
Terpin-4-ol	0·4 (0·7–0·2)	11·0	—	—	—
α-Terpineol	6·2 (11·2–2·8)	12·3	—	—	—
Estragol	1·8 (5·3–0·4)	18·5	75	130	90
trans-α-Bergamotene	0·8 (3·1–0·7)	47·8	—	—	—
Germacrene-D	1·7 (9·9–0·1)	57·9	—	—	—
Nerolidol	1·7 (5·2–0·1)	40·5	—	—	—
α-Cadinol	0·4 (1·7–0·1)	43·1	—	—	—

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