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Short Note

Composition of the heartwood essential oil of incense cedar (*Calocedrus decurrens* Torr.)

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Introduction

Incense cedar (*Calocedrus decurrens*) is a tree native to Oregon and California, perhaps best known for its aromatic wood and use in the manufacturing of pencils. The wood is also highly valued for its decorative appearance and durability in lumber, related sawmill products, and fence posts. Chemical investigations of heartwood extracts have shown the presence of carvacrol, thymoquinone, related *p*-cymene and *p*-menthane based phenols, and tropolones (Zavarin and Anderson 1955a,b; Zavarin 1958; Veluthoor et al. 2010). Heartwood durability was shown to be mainly due to the high concentration of the *p*-cymene phenols, such as carvacrol, but as the wood ages these are converted to non-fungicidal compounds, resulting in decreasing durability (Anderson et al. 1963). Recent investigations have found the steam distilled essential oil from heartwood to have significant biological activity against arthropods of public health importance such as fleas, ticks and mosquitoes (Dolan et al. 2007), as well as *Phytophthora ramorum*, the organism responsible for Sudden Oak Death (Manter et al. 2006). In this paper we report on the GC-MS analysis of this essential oil. Nakatsuka and Hirose (1954) were first to describe the essential oil of incense cedar wood, but the oil had not yet been analyzed by modern methods. In the present paper the GC-MS analysis of this essential oil will be reported.

Materials and methods

Trees of *Calocedrus decurrens* Torr. were collected from a logging and saw mill operation at Warm Springs, Oregon in June 2004. A voucher specimen is deposited in the Oregon State University Herbarium. Heartwood shavings (1206 g) were steam distilled for 6 h in a glass steam distillation unit yielding 7.5 g of deep red oil.

GC-MS analysis: Hewlett Packard 5890 Series II GC (Angilent Technologies, Inc., Santa Clara, CA, USA) equipped with an Agilent (J&W Scientific) DB-5MS column (30 m×0.25 mm, 0.25 μm film thickness) connected to an HP 5970 mass selective detector (He flow 1.0 ml min^{-1}; split 1:10). Quantification of the oil components was achieved on the same instrument equipped with an Agilent DB-5 column connected to a flame ionization detector with a 1.0 ml min^{-1} He flow and a 1:50 split. Injector and detector temperatures for both analyses were: 250°C; the oven program as described by Adams (2007). Five μl of neat essential oil was diluted in 1.0 ml ethyl acetate and 1.0 μl of this solution injected. For a more rapid routine GC analysis of the oil, the following oven program was optimal: starting at 100°C with a 1.0 min hold, then programmed 5°/min to 150° followed by 3°/min to 300° with a 10 min hold. Compounds were identified by comparison of their spectra with those in the Wiley and Adams libraries and comparison of their Arithmetic Indices (Adams 2007). Some compounds were further confirmed by standard addition co-chromatography with authentic samples and by NMR spectra (^1H and ^{13}C). Samples for NMR analyses were isolated as described in the literature (Zavarin and Anderson 1955a,b; Veluthoor et al. 2010).

Results and discussion

Steam distillation of the heartwood gave an oil in 0.62% yield composed almost entirely of *p*-cymene and *p*-menthane derivatives (88.7%) as shown in Table 1 and Figure 1. Thymoquinone and carvacrol account for 65.1% of the oil, *p*-methoxythymol 11% and *p*-methoxycarvacrol 3.2%. These four compounds alone dominate the GC scan as shown in Figure 2. The tropolone β-thujaplicin (hinokitiol) was only present in trace amounts. The high concentrations of thymoquinone and carvacrol alone can account for the previously observed high bioactivity of this oil. Carvacrol is well known for its antimicrobial, antioxidant, insecticidal, and repellent activities (Panella et al. 2005; Dietrich et al. 2006; Veldhuizen et al. 2006) and is a component in several other important agricultural based essential oils such as those from

Table 1 Composition of the heartwood essential oil from *Calocedrus decurrens*.

Compound	AI[a]	Concentration (%)[b]	Identification[c]
1,4-Cineole	1009	Traces	MS, ST
p-Cymene	1018	0.8	MS, ST
Limonene	1023	Traces	MS
m-Cymenene	1083	0.3	MS
Terpinen-4-ol	1172	2.4	MS, ST
α-Terpineol	1185	1.6	MS, ST
Thymoquinone	1250	35.9	MS, NMR
Piperitone[d]	1253	0.8	MS
Carvenone	1256	1.1	MS
Carvacrol	1300	29.2	MS, NMR
Unknown	1367	1.0	*m/z* 178
2,5-*p*-Dimethoxycymene	1416	0.4	MS, NMR
p-Methoxythymol	1484	11.0	MS, ST, NMR
p-Methoxycarvacrol	1491	3.2	MS, ST, NMR
β-Thujaplicin	1485	Traces	MS
Thymohydroquinone	1553	0.3	MS
Libocedroquinone	2337	Traces	MS, ST, NMR
Heyderiol	2346	0.5	MS, NMR
Libocedrol	2386	1.2	MS, NMR
Total		89.7	

[a]AI=Arithmetic Index on DB-5 Column (Adams 2007).
[b]Determined by GC-FID analysis, unidentified compounds less than 0.5% are not reported.
[c]ST=standard addition co-chromatography with authentic compound. NMR, isolation and identification by ^{1}H and ^{13}C NMR.
[d]Tentatively identified.

thyme and oregano (Baser 2008). Manter et al. (2007) have demonstrated the biocidal activity of thymoquinone against *P. ramorum*. Thymoquinone is also the bioactive constituent of the oil of black cumin (*Nigella sativa*), which has been used since antiquity in the Middle East for the treatment of many human diseases (Padhye et al. 2008). This compound has been shown to have antioxidant, anti-inflammatory, and promising anticancer activities in both *in vitro* and *in vivo* studies (Gali-Mahtasib et al. 2006). According to Anderson et al. (1963) *p*-methoxythymol and *p*-methoxycarvacrol are anti-fungal against wood decay organisms. The quoted authors also indicated that β-thujaplicin is the strongest anti-fungal compound of the group. However, due to its low concentration this compound probably plays only a minimal role in incense cedar wood durability, or essential oil bioactivity.

Calocedrus decurrens is one of only three species in the genus *Calocedrus* (Chen et al. 2009). The other two, *C. formosana* and *C. macrolepis*, are divergent species native to Asia. No analyses have been reported for the steam distilled essential oils from the heartwoods of these trees. However, Wang et al. (2006) have reported on the GC-MS of the solid phase micro extraction (SPME) of *C. macrolepis* wood. The major compounds were the volatiles *p*-cymene (24.4%), terpinen-4-ol (16.6%) and α-terpineol (12.5%). These are all present in *C. decurrens* oil, but in much lower concentrations (0.8, 2.4, and 1.6%, respectively). Carvacrol, thymoquinone, *p*-methoxythymol, *p*-methoxycarvacrol and their associated derivatives were not found in *C. macrolepis* by SPME and there seems to be only minimal overlap of other compounds.

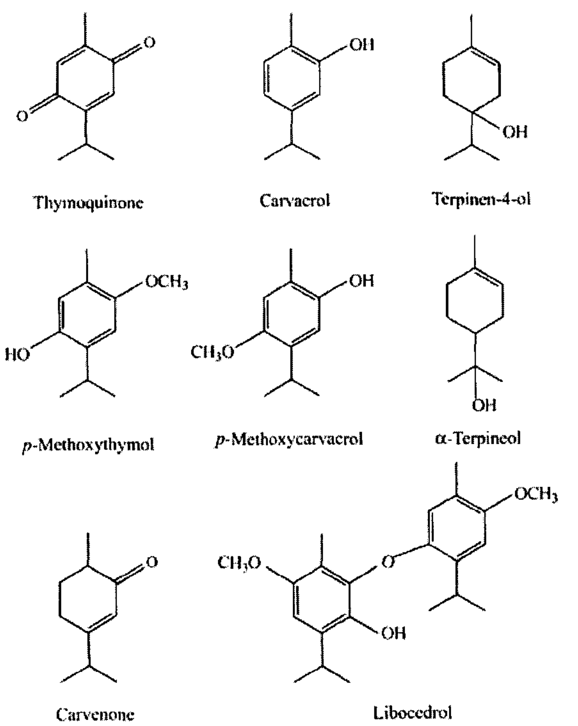

Figure 1 Main constituents in the heartwood essential oil of *C. decurrens*.

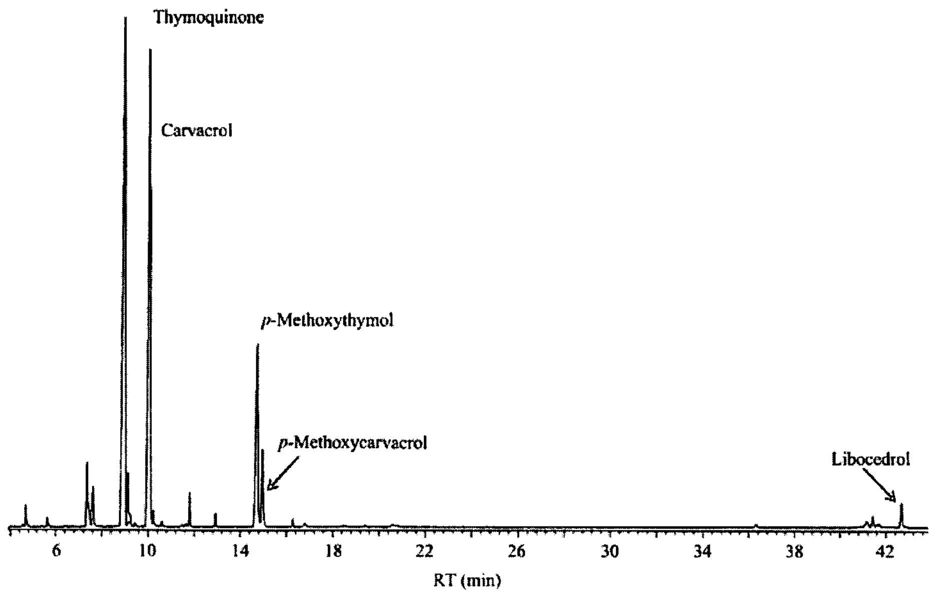

Figure 2 GC scan of *C. decurrens* heartwood essential oil.

Conclusions

There is presently an interest in more efficient utilization of forest resources and the development of new biocides and repellents that are safe for both the environment and people. The composition of *C. decurrens* wood essential oil combined with biological studies give insight into the development of such biocides. There is also potential for utilization of this oil from mill residues in limited, small specialty operations.

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