Volatile terpenoids and tropolones in heartwood extracts of yellow-cedar, Monterey cypress, and their hybrid Leyland cypress

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ORIGINAL PAPER

Volatile terpenoids and tropolones in heartwood extracts of yellow-cedar, Monterey cypress, and their hybrid Leyland cypress

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Abstract

• ***Key message*** **Leyland cypress, an intergeneric hybrid, produces the same volatile heartwood compounds as its parental taxa, yellow-cedar and Monterrey cypress. However, the proportion of total sesquiterpenes and some of the individual components appear unique to their respective heartwoods.**

• ***Context*** Leyland cypress, x*Hesperotropsis leylandii* is an intergeneric hybrid between yellow-cedar, *Callitropsis nootkatensis,* and Monterey cypress, *Hesperocyparis macrocarpa.* Their heartwoods are protected by bioactive compounds and rated very durable to durable for products used above ground. Several compounds in yellow-cedar and Monterrey cypress heartwoods are also active against various fungi, bacteria, human insect pests, and plant pathogens, whereas Leyland cypress heartwood has never been thoroughly investigated.

• ***Aims*** The first aim for this study was to examine the volatile compounds in ethyl acetate extracts from the heartwood of all three tree species in Oregon. The second aim was to determine the extent Leyland cypress differs from its parental species, and further investigate any of its novel compounds for biological activity.

• ***Methods*** Ethyl acetate extracts of fresh heartwood were prepared for three trees of each species and analyzed by gas chromatography.

• ***Results*** Thirty-three compounds were detected at 0.5 % or greater abundance across all species, and 23 were identified. Carvacrol was the major monoterpene and nootkatin the most abundant tropolone in all three species. Valencene 11, 12-diol and nootkatone topped the list of sesquiterpenes in yellow-cedar and Leyland cypress, respectively, whereas no sesquiterpenes were detected in Monterrey cypress. This appears to be the first report of tropolones hinokitiol, procerin, and nootkatin in Leyland cypress, α-thujaplicinol, pygmaein, and procerin in Monterrey cypress, and hinokitiol in yellow-cedar.

• ***Conclusions*** Leyland cypress heartwood does not biosynthesize structurally unique compounds from those produced by its parental species, and is an unlikely source of novel biocides. However, the proportion of total sesquiterpenes and some of the individual components in Leyland cypress heartwood may distinguish it from the heartwood of its parental species.

Keywords Cupressaceae · *Callitropsis nootkatensis* · *Hesperocyparis macrocarpa* · x*Hesperotropsis leylandii* · Monoterpenes · Sesquiterpenes

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1 Introduction

Yellow-cedar (YC), *Callitropsis nootkatensis* (D. Don) Oerst. ex D.P. Little., also known as Alaska-cedar, Alaska yellow-cedar, or Nootka cypress, has experienced numerous nomenclature changes in recent years, as reviewed by Garland and Moore (2012). It is most abundant in the coastal forests of southeast Alaska and British Columbia with a southern extension of its distribution primarily in the Cascade Range through Washington and Oregon to northern California, usually at elevations above 600 m (Harris 1990; Murray 2010; Hennon et al. 2012). It is an ecologically and economically important tree that indigenous peoples have valued and used for centuries as a material resource (Stewart 1984; Hennon et al. 2012). Products made from its durable heartwood will last longer when used above ground, compared to those having constant contact with the soil (DeGroot et al. 2000; Hennon et al. 2007). Above ground performance is demonstrated by dead-standing YC trees that gradually lose their bark and sapwood, leaving predominately heartwood skeletons that can remain standing for up to a century after death with minimal loss in wood strength (McDonald et al. 1997). Heartwood durability is attributed to a mixture of various monoterpenes, sesquiterpenes, and tropolones (Carlsson et al. 1952; Duff et al. 1954; Erdtman and Topliss 1957; Erdtman and Hirose 1962; Norin 1964; Kelsey et al. 2005; Khasawneh and Karchesy 2011; Khasawneh et al. 2011) that have proven biological activity against various insects and microbes (Morales-Ramos et al. 2003; Arango et al. 2004; Panella et al. 1997, 2005; Taylor et al. 2006; Manter et al. 2006, 2007; Jordan et al. 2012).

Monterey cypress (MC), *Hesperocyparis macrocarpa* (Hartw.) Bartel has also experienced numerous nomenclature changes in recent years (Garland and Moore 2012) and is endemic to small areas around Monterey Bay and the central coast of California. This tree has been cultivated in Hawaii and elsewhere around the world for wind breaks or an ornamental with potential as an important timber species in New Zealand (Little and Skolmen 1989; Nicholas 2014). MC heartwood, like YC is more durable for above ground products than those in contact with soil; it is rated very durable to durable against brown-rot decay (Jones et al. 2013). It is known to contain some of the same bioactive monoterpenes and tropolones as YC (Corbett and Wright 1953; Haluk and Roussel 2000; Igri et al. 1990; Zhang et al. 2012).

Leyland cypress (LC), x*Hesperotropsis leylandii* (A. B. Jacks. & Dallim.) Garland and Gerry Moore has also experienced nomenclature instability associated with phylogenetic research as reviewed by Garland and Moore (2012). Its origin as a putative spontaneous hybrid between cultivated YC and MC in the UK (Ovens et al. 1964) has been confirmed by genetic studies (Yamaguchi et al. 2000; Adams et al. 2006). Geographic separation has prevented the hybridization of these species in the wild. Until recently, LC was commonly considered sterile; it may be self-sterile, but can produce viable seeds when crossed with a compatible pollen donor (Armitage 2011). The 15 cultivars of LC (Armitage 2011) are propagated as clonal cuttings primarily for ornamental trees and shrubs, but it can be grown for timber (Nicholas 2014). Like the parental species, LC heartwood is best for products used above ground, and considered very durable to durable against brown-rot decay (Jones et al. 2013), but its chemical composition has not been thoroughly investigated.

Composition of the steam distilled essential oils, and ether extracts from one tree of each of the three taxa above have been examined (Liu 2009). However, oils collected after 6 h of distillation had a substantially different composition from oils collected from 6 to 12 h. This may be due to various factors, including nootkatin crystallization out of the essential oil (Liu 2009) and thermal instability of some compounds during steam distillation. The objectives for this study were to examine the volatile compounds in ethyl acetate extracts from all three species growing in Oregon to determine to what extent LC differs from its two parental species, and whether it contains any novel compounds worthy of investigating for biocidal activity.

2 Material and methods

2.1 Plant material

Heartwood samples were collected from three trees of each species at the locations listed in Table 1. The geographic

Table 1 Tree diameters and locations in Oregon

Species	Tree	Dbh (cm)	Location; latitude, longitude
YC	1	38.1	Corvallis, PA; 44.655793°, −123.234268°
	2	39.9	
	3	37.6	
MC	1	57.7	Newport, OCA; 44.616962°, −124.050060°
	2[a]	101.3	Newport, BHM; 44.631375°, −124.058188°
	3[a]	64.5	Newport, PMHC; 44.632637°, −124.048309°
LC	1	35.3	Dillard; 43.080601°, −123.409100°
	2	36.1	
	3	33.5	

Trees were sampled 18–24 October, 2013. All YC trees and MC tree 1 had multiple stems. The geographic origin of YC seeds was unavailable from the arboretum. LC was cultivar 'Leighton Green'according to their owner. This cultivar is believed to be an F1 hybrid (Ovens et al. 1964)

Dbh diameter at breast height (1.4 m), *YC* yellow-cedar, *MC* Monterrey cypress, *LC* Leyland cypress, *PA* Peavy Arboretum, *OCA* Oregon Coast Aquarium, *BHM* Burrows House Museum, *PMHC* Pacific Maritime and Heritage Center

[a] Heart rot in center of these stems, only the solid heartwood was used, tree 3 was sampled on the West side as the North side was not accessible

origins for the YC seed sources were unavailable from the arboretum allowing the possibility they are half-siblings from the same cone bearing tree, and the LC are clones of the same cultivar. Stem diameters were measured and cores taken at 1.4 m (breast height, DBH) with one core from the north and another from the south sides of each tree. The cores were 5 mm in diameter and penetrated to the center of each stem, except those with heartrot (Table 1). The heartwood was removed from each core and combined by tree into a sealed vial, then immediately frozen with dry ice for transport to the laboratory where they were stored frozen.

2.2 Extractions

In preparation for extraction, the cores from each tree were cut into thin disks (0.5–1.0 mm aprox.) and thoroughly mixed. Two-gram subsamples of MC and LC, and a 1 g subsamples of YC were sealed in smaller vials after adding 10 mL of ethyl acetate (EMD Chemicals, HPLC grade). Less YC tissue was used because of its higher extractive content (unpublished observations). The samples were extracted 16 h at room temperature and then transferred to a vial containing 1 g of anhydrous sodium sulfate powder (Acros, 99 %) to remove residual water.

2.3 GC-FID and GC-MS analysis

The percentage composition of volatiles in the extracts was determined on a Hewlett Packard (HP, currently Agilent, USA) 5890 Series II gas chromatograph (GC) with a DB-5 column (30 m×0.25 mm, 0.25-μm film thickness, J&W Scientific from Agilent, USA) connected to a flame ionization detector using helium as the carrier gas at 1.0 mL/min set at 60 °C. The injector and detector temperatures were 250 and 300 °C, respectively. The column oven program started at 60 °C and increased 3 °C/min up to 250 °C, with no final hold. Two microliter of extract was injected using the splitless mode. The mean (±standard error) percentage composition of each compound in a taxa are presented in Table 2 for those compounds averaging 0.5 % or higher in at least one species, but still reported in the other taxa if at lower amounts. One sample from each species was mixed with a solution containing multiple hydrocarbon standards and analyzed to calculate the arithmetic indices (AI) (Adams 2007).

Compound identity was achieved using the same GC with a DB-5MS column (30 m×0.25 mm, 0.25-μm film thickness, J&W Scientific from Agilent, USA) connected to an HP 5972 mass selective detector with injector and detector temperatures as above. The helium carrier flow was 0.5 mL/min set at 80 °C. The oven program was held 80 °C for 1 min then increased 5 °C/min up to 150 °C, then 3 °C/min up to 250 °C, with no final hold. One extract of each species was analyzed after concentration with a N_2 stream. Two microliter of each concentrated sample was injected using the splitless mode. Compounds were identified by comparison of their spectra with those in the Adams (2007) or NIST08 (U.S. Dept. Commerce 2008) libraries in conjunction with their AI values (Adams 2007).

3 Results

In total, there were 33 compounds representing three structural classes (monoterpenes, sesquiterpenes, or tropolones) at a mean 0.5 % composition or greater in at least one species (Table 2). Twenty-three compounds were identified, and 10 remain unknown. YC and LC heartwoods were chemically more diverse than MC, as they synthesized compounds in all three structural classes, while MC produced no sesquiterpenes. The heartwood chemistry of LC reflects its hybrid origin, yielding totals for each structural class intermediate between YC and MC. The proportions of most individual LC compounds were similar to one of the parental species, or intermediate between them, except α-terpineol, methyl carvacrol, procerin isomer, and unknown compounds number 6 and 18. LC produced only one compound not detected in either parental species (no. 12, unknown MW 204). The only compounds exclusive to YC and MC were β-bisabolenol and α-thujaplicinol, respectively.

Total monoterpenes were notably higher in MC heartwood extracts (53.2 %) compared to LC (34.5 %), or YC (8.9 %). Carvacrol was the most abundant monoterpene in all three species, followed by terpinen-4-ol. The individual compounds in this class were present more consistently in all species than compounds belonging to the other two classes.

Sesquiterpenes represented 56.8 % of the total volatile composition in YC heartwood and 18.2 % in LC. There were no sesquiterpenes produced in MC heartwood. Valencene 11, 12-diol (16.8 %), nootkatol (10.6 %), and nootkatone (8.2 %) were the three most abundant sesquiterpenes in YC extracts, compared with nootkatone (7.9 %), valencene 11, 12-diol (2.6 %), and valencene (2.3 %) in LC.

Total tropolones were most abundant (38.6 %) and structurally diverse in MC heartwood, including six identified compounds and one potential isomer of procerin, with nootkatin (23.3 %) and hinokitiol (7.2 %) in the highest amounts. LC had intermediate proportions of total tropolones (30.0 %) and produced the same compounds as in MC except for α-thujaplicinol, although α-thujaplicin and pygmaein were in low amounts. Nootkatin (14.5 %) and hinokitiol (8.4 %) were the two most abundant tropolones in LC. YC had the lowest total tropolone composition (13.8 %), and less structural diversity, producing only four of the compounds found in MC, including the procerin isomer. Nootkatin (6.4 %) and procerin (5.7 %) were the top two tropolones in

Table 2 The volatile composition (mean %±SE) of ethyl acetate extracts from heartwood of yellow-cedar (YC), Leyland cypress (LC), and Monterey cypress (MC) growing in Oregon (n=3 trees per species)

No.	Compound	YC	LC	MC	AI		Class
					Measured	Adams[e]	
1	Terpinen-4-ol	1.05±0.04	2.45±0.02	2.99±0.16	1179	1174	M
2	α-Terpineol	0.29±0.04	0.51±0.01	0.38±0.08	1193	1186	M
3	Methyl carvacrol	0.38±0.19	1.25±0.22	0.73±0.28	1245	1241	M
4	Carvacrol	7.13±2.25	30.26±1.19	49.06±5.67	1302	1298	M
5	Unknown MW 168	0.09±0.06	0.85±0.04	1.27±0.10	1309		
6	Unknown MW 170	0.27±0.02	0.74±0.03	0.23±0.09	1323		
7	Unknown MW 170	0.51±0.05	0.54±0.03	0.27±0.10	1374		
8	α-Thujaplicin	0±0	0.10±0.00	1.50±1.15	1417	1410	T
9	Unknown MW 166	0.54±0.08	0±0	0.52±0.12	1464		
10	Unknown MW 166	0.89±0.07	0.80±0.01	0±0	1466		
11	Hinokitiol (β-thujaplicin)	0.93±0.13	8.38±0.66	7.21±1.86	1484	1475	T
12	Unknown MW 204	0±0	0.58±0.01	0±0	1492		
13	Valencene	2.69±0.20	2.25±0.11	0±0	1500	1496	S
14	β-Bisabolene	1.11±0.17	0.48±0.01	0±0	1513	1505	S
15	α-Thujaplicinol	0±0	0±0	2.35±1.21	1515	1509	T
16	Nootkatene	2.71±0.31	0.66±0.03	0±0	1522	1517	S
17	7-epi-α-Selinene[a]	0.65±0.07	0.15±0.01	0±0	1531	1520	S
18	Unknown MW 198	0.63±0.11	1.46±0.02	0±0	1563		
19	Pygmaein	0±0	0.03±0.01	0.82±0.35	1588	1581	T
20	α-Cadinol[a]	0.84±0.03	0.27±0.02	0±0	1664	1652	S
21	Intermedeol[a]	0.59±0.03	0.25±0.01	0±0	1667	1665	S
22	epi-Nootkatol	2.83±0.36	0.58±0.04	0±0	1707	1699	S
23	Nootkatol	10.59±2.17	2.16±0.15	0±0	1723	1714	S
24	Valencene 13-ol	7.33±2.16	0.52±0.03	0±0	1778	1767	S
25	β-Bisabolenol	0.79±0.18	0±0	0±0	1794	1789	S
26	Nootkatone	8.19±0.26	7.94±0.13	0±0	1817	1806	S
27	Kudtdiol	1.75±0.07	0.38±0.00	0±0	1919	1912	S
28	Valencene 11,12-diol[b]	16.77±0.49	2.56±0.24	0±0	1928	1914	S
29	Procerin	5.73±1.30	2.65±0.17	0.02±0.02	1942	1931	T
30	Unknown MW?	0.83±0.05	0.16±0.00	0±0	1954		
31	Nootkatin	6.44±0.87	14.53±0.37	23.29±4.04	1970	1959	T
32	Procerin isomer[c]	0.71±0.13	4.31±0.25	3.37±1.05	2008		T
33	Unknown MW 234	0.90±0.26	0.20±0.00	0.05±0.02	2123		
	Total monoterpenes[d]	8.85±2.45	34.47±1.21	53.16±5.69			
	Total sequiterpene	56.83±1.76	18.20±0.42	0±0			
	Total tropolones[d]	13.82±1.67	29.99±1.30	38.57±4.68			
	Total for extract[d]	84.17±1.07	88.01±0.19	94.07±0.74			

Compounds in this study were identified by GC-MS whereas compounds 1–4, 13, 16, 22–24, 26–28, and 31 in YC were previously identified by co-chromatography with authentic standard, or isolation and identification by ^{1}H and ^{13}C NMR (Khawawneh et al. 2011; Khasawneh and Karchesy 2011)

MW molecular weight, *M* monoterpene, *S* sesquiterpene, *T* tropolone

[a] Tentative identity

[b] Khasawneh and Karchesy (2011) note this could be tedonodiol isolated by Guerreiro et al. (1979), but the later authors did not determine the stereochemistry

[c] MW=230 with ion fragmentation very similar to procerin

[d] Structural class totals do not include the unknowns, except for the procerin isomer that was included in the tropolones. The total for extract includes all compounds, including the unknowns

[e] Adams (2007)

YC, making nootkatin the most abundant tropolone in all three species.

4 Discussion

We believe this is to be the first report of hinokitiol in heartwood of YC, although its presence was suspected based on biosynthetic considerations (Little et al. 2004; Little 2006). This also appears to be the first report of α-thujaplicinol, pygmaein, and procerin in heartwood of MC, or hinokitiol, procerin, and nootkatin in LC, based on the review of tropolones in Cupressales (Haluk and Roussel 2000), and our additional literature search. Furthermore, the compound we suspect to be a procerin isomer is potentially a new tropolone for all three species.

Our results show valencene 11, 12-diol, and nootkatone as the major sesquiterpenes in YC and LC, respectively. Live YC trees in southeast Alaska forests contained much lower amounts of valencene 11, 12-diol, ranging from 0 to 2.4 % with a mean of 1.2 % (authors unpublished data). The YC trees used in the present study may denote a different chemotype since their geographic origin is unknown. Carvacrol was the most abundant monoterpene in all three taxa and the major volatile component in MC heartwood as observed in other studies that include trees growing in Morroco (Igri et al. 1990; Zhang et al. 2012).

In general, the proportions of most individual compounds in LC heartwood extracts were similar to the amounts in one or the other parental species, or intermediate between them. When the compounds were totaled into monoterpene, sesquiterpene, or tropolone structural classes, the proportions for LC were intermediate between the two parental species, as might be anticipated from an F1 hybrid. The absence of sesquiterpenes in MC makes it easily distinguishable from the other two species that are chemically more similar one to one another. But, the proportions of total sesquiterpenes might also be a diagnostic characteristic for separating YC and LC heartwoods. In all samples of YC, the total sesquiterpenes have exceeded 35 %. From our results here, they averaged 56.8 % (Table 2). In 12 live trees from natural populations across a 260-km distance in southeast Alaska, they ranged from 37.8 to 53.6 % (41.6 % mean, authors unpublished data), and in 0–6 and 6–12-h steam distilled essential oils, they were 47.7 and 43.32 %, respectively (Liu 2009). In contrast, the LC here contained only 18.2 % total sesquiterpenes and 11.98 and 12.39 % in the 0–6 and 6–12-h oils (Liu 2009). If LC produces no more than half of the total sesquiterpenes as their YC parent, they would always contain less than 30 % based on the YC values above. This would make LC readily distinguishable from YC with total sesquiterpenes greater than 35 %. Proportions of carvacrol, hinokitiol, and nootkatin might also help separate LC and YC heartwoods.

Unlike heartwood, the major sesquiterpene in LC foliage, (+)-dauca-5,8-diene is not present in either parental species (Cool 2001) making it a unique chemical characteristic. Also, the monoterpene hydrocarbon mixtures in essential oils from LC foliage are sufficiently unique to allow identification of some clones, and the grouping of others (Scheffer et al. 1980). Whether the volatile extractives in LC heartwood can provide similar clonal discrimination remains to be evaluated.

The accumulation of bioactive compounds that contribute to heartwood durability of YC and MC most likely evolved as a chemical defense against various natural enemies (Morales-Ramos et al. 2003; Arango et al. 2004; DeGroot et al. 2000; Kelsey et al. 2005; Taylor et al. 2006; Hennon et al. 2007; Zhang et al. 2012). Many of these compounds may also have value as natural repellents or biocides with potential use for protecting humans, crops, and forest products from insect and microbial pests (Panella et al. 1997, 2005; Zhu et al. 2001; Haluk and Roussel 2000; Dietrich et al. 2006; Manter et al. 2006, 2007; Saniewski et al. 2007; Baser 2008; Dolan et al. 2009; Jordan et al. 2012).

5 Conclusion

The intergeneric hybrid gene recombination in LC does not lead to the biosynthesis of structurally unique compounds from those produced by the parental species, and therefore is an unlikely source of novel biocides. However, the proportions of total sesquiterpenes, or some of the individual compounds may provide useful chemical characteristics for separating heartwood of LC from its parental taxa.

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