

## Volatile Terpenes in *Sequoia sempervirens* Foliage. Changes in Composition during Maturation

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Volatile components of foliage of coast redwood, *Sequoia sempervirens*, isolated by steam distillation and pentane extraction, were characterized by GC-MS, infrared spectra, and determination of Kovats' Indices. Thirty-five mono- and sesquiterpenes were identified, twenty of which had not been previously reported from coast redwood. Trees growing in an arboretum at Davis and in a natural forest setting were investigated. The compositions of volatile components in the essential oils from coast redwood new leaf growth and year-old growth from both areas were investigated over a growing season. A general trend observed in the analyses of both old and new growth for the forest redwood was a continual decrease in the level of volatile terpenes during the first 4 weeks after emergence of the new growth and then an increase during the next 14 weeks. No similar pattern of change in composition was evident in the terpenes from the arboretum redwood.

The coast redwood, *Sequoia sempervirens*, belongs to the class Coniferae, order Cupressales, and family Taxodiaceae. As part of an extensive investigation of the palatability of forest species to browsing ruminants (Longhurst et al., 1968), coast redwood was placed in the moderately palatable category with Douglas fir and white fir. It has been observed that browsing animals have a strong preference for young growing tips relative to mature foliage. The differences in composition of the volatile terpenes in the new growth relative to the mature growth and the changes in composition during maturation have been investigated for Douglas fir (Maarse and Kepner, 1970) and for a relatively unpalatable species, California Bay (Kepner et al., 1974). A similar investigation of composition differences in young and mature growth of coast redwood was also of interest.

Investigations of the chemical composition of both the heartwood (Erdtman, 1952) and pocket resin (Reifer et al., 1969) of coast redwood have been reported. In an investigation of the essential oil of coast redwood foliage from Oregon, Gregonis et al. (1968) reported the identification of  $\alpha$ -pinene, camphene,  $\beta$ -pinene, myrcene,  $\alpha$ -phellandrene,  $\alpha$ -terpinene, limonene,  $\beta$ -phellandrene,  $\gamma$ -terpinene, *p*-cymene, terpinolene, *cis*-hex-3-en-1-ol, camphor, terpinen-4-ol, bornyl acetate,  $\alpha$ -terpineol, terpinyl acetate, citronellol, geraniol, safrole, and  $\beta$ -eudesmol.

This paper presents the isolation and identification of additional components from the essential oil of *S. sempervirens* and a study of differences in composition between young tip growth and mature foliage over a growing season.

### EXPERIMENTAL SECTION

**Isolation and Identification.** Redwood foliage was collected from mature trees in the arboretum of the University of California at Davis. About 8 mL of essential oil was isolated by steam distillation of 2 kg of macerated needles, extraction of the steam distillate with *n*-pentane, drying of the *n*-pentane extract with anhydrous Na<sub>2</sub>SO<sub>4</sub>, and removal of solvent by rotary evaporation at 0 °C and 100 torr.

Individual components of the essential oil were isolated by preparative gas chromatography using 305 cm × 4 mm

i.d. glass columns of Carbowax 20M or SE-30 in a Hewlett-Packard 7620A gas chromatograph with FID. Fractions were collected by utilizing a 15:1 effluent splitter in 25 cm thin walled glass capillaries.

Infrared analyses were carried out as thin films on a Beckman IR-8 spectrophotometer using demountable sodium chloride microplates and a beam condenser.

Kovats' Indices were determined on 127 m × 0.75 mm i.d. open tubular stainless steel capillary columns in an F&M Model 810 gas chromatograph with FID, on Carbowax 20M [plus 5% (w/w) Igepal CO 880] isothermally at 70, 130 °C, or 190 °C and on SF-96(50) isothermally at 100 or 170 °C.

Mass spectra were run on a quadrupole Finnigan Model 3200 mass spectrometer coupled with a Finnigan Model 9500 gas chromatograph containing a Carbowax 20M glass capillary column (0.25-mm i.d.). The system was interfaced to a Finnigan Model 6000 data system having a Zeta Research, Inc., Series 100  $\zeta$  plotter. Spectra were run at 70 eV.

**Seasonal Variation Studies.** Foliage samples were collected from a 6-ft *S. sempervirens* growing on the Masonite property in the Pacific Coast Range west of Ukiah, CA. Sampling dates, beginning a few days after emergence of new foliage, were April 14, April 23, May 10, June 3, and Aug 8. Samples were wrapped tightly in aluminum foil and transported to Davis in an ice chest. Samples of redwood foliage were also gathered from a mature tree in the arboretum of the University of California at Davis on approximately the same dates. Small-scale steam distillation-extraction was carried out on the fresh foliage as described by Maarse and Kepner (1970) and Kepner et al. (1974) with the *n*-tetradecane internal standard added to the concentrated extract. Samples (1.0  $\mu$ L) were injected onto the 127 m × 0.75 mm i.d. Carbowax 20M column in the F&M Model 810 gas chromatograph with FID: injection temperature 180 °C; detector temperature 210 °C; He, He make-up gas, H<sub>2</sub>, and air flow rates were 8, 30, 23, and 300 mL/min, respectively. The oven temperature program was 5 min isothermal at 75 °C and then programmed at 2 °C/min to 145 °C and run isothermally thereafter. Peak heights of the components were calculated relative to the peak height of the internal standard, all at range 10, attenuation 32.

### RESULTS AND DISCUSSION

The components identified from the essential oil from the foliage of a coast redwood from the University of California, Davis, arboretum are listed in Table I. In an

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Table I. Components of Redwood<sup>a</sup> Needle Oil

component	identification			rel amount <sup>b</sup>
	Kovats' Indices		spectral	
	CW-20M	SF-96(50)		
$\alpha$ -thujene	1038	928	MS	T
$\alpha$ -pinene	1038	940	MS, IR	L
camphene	1082	952	MS	T
$\beta$ -pinene	1125	977	MS	S
sabinene	1134	972	MS	M
myrcene	1172	977	MS, IR	L
$\alpha$ -phellandrene	1176	985	MS	S
$\alpha$ -terpinene	1192	1051	MS	S
limonene	1211	1018	MS	L
$\beta$ -phellandrene	1219	1024	MS	M
<i>cis</i> - $\beta$ -ocimene	1240	1024	MS	S
$\gamma$ -terpinene	1254	972	MS, IR	L
<i>trans</i> - $\beta$ -ocimene	1255	1035	MS	T
<i>p</i> -cymene	1278	1015	MS, IR	S
terpinolene	1289	1049	MS	M
$\alpha$ -ylangene	1491		MS	T
$\delta$ -elemene	1501	1353	MS, IR	T
$\alpha$ -copaene	1533	1392	MS, IR	S
benzaldehyde	1553		MS	T
linalool	1553	1089	MS	T
bornyl acetate			MS	T
monoterpene alcohol			IR	T
$\beta$ -elemene	1617	1404	MS, IR	M
terpinen-4-ol	1627	1190	MS, IR	M
caryophyllene	1632	1440	MS, IR	S
<i>trans</i> -2-menthen-1-ol	1644		IR	S
$\gamma$ -elemene	1658		MS, IR	T
sesquiterpene hydrocarbon	1673		MS	T
$\alpha$ -humulene	1680	1469	MS	T
$\alpha$ -terpinyl acetate	1704	1345	IR	S
$\alpha$ -terpineol	1712	1200	IR	M
sesquiterpene hydrocarbon	1716	1481	MS, IR	S
germacrene D	1728	1497	MS, IR	M
$\alpha$ -muurolene	1740	1502	MS, IR	S
<i>cis</i> -piperitol	1749		IR	T
$\delta$ -cadinene	1767	1520	MS, IR	M
nerol	1786		IR	T
monoterpene alcohol	1798		IR	S
sesquiterpene hydrocarbon <sup>c</sup>	1846	1520	MS, IR	M
sesquiterpene alcohol	1987		MS, IR	M
elemol	2105	1760	MS, IR	S
sesquiterpene alcohol	2187	1836	MS, IR	S
$\beta$ -eudesmol			MS	T

<sup>a</sup> From the University of California, Davis, arboretum.

<sup>b</sup> Relative amounts: L = large; M = medium; S = small; T = trace. <sup>c</sup> Tentatively identified as germacrene B on the basis of the spectral data and thermal rearrangement to  $\gamma$ -elemene.

earlier investigation of the foliage of coast redwoods growing in an arboretum in Oregon, Gregonis et al. (1968) reported the identification of 21 volatile components, 5 by retention time only, with approximately an equal number listed as unidentified. The identification of some of these additional components was essential for other investigations which we had in progress. We have now identified 35 volatile components, 20 of which were not previously reported, and obtained spectral evidence for several additional sesquiterpene hydrocarbons and terpene alcohols which could not be positively identified.

Most, but not all, of the compounds identified by Gregonis et al. (1968) were again identified in our investigations. Camphor, *cis*-hex-3-en-1-ol, citronellol, geraniol, and safrole were identified from the Oregon redwood oil but could not be detected in the essential oil which we isolated from the coast redwood in the arboretum at Davis. These

Table II. Changes in Composition of Redwood<sup>a</sup> Foliage

component	needle age <sup>b</sup>	rel peak heights <sup>c</sup> at collection dates				
		Apr 14	Apr 23	May 10	May 31	Aug 8
		$\alpha$ -pinene	N	12.2	9.7	5.8
	O	29.5	21.0	6.6	7.6	23.2
camphene	N	0.2	0.2		0.1	0.1
	O	0.2	0.1			0.1
$\beta$ -pinene	N	0.5	0.4	0.2	0.5	0.7
	O	1.7	1.3	0.4	0.5	1.6
sabinene	N	2.6	2.1	1.1	2.2	2.9
	O	4.9	3.4	0.9	1.4	3.6
myrcene	N	1.9	1.8	1.1	2.6	3.6
	O	8.1	6.4	2.0	2.7	6.8
$\alpha$ -phellandrene	N	0.2	0.3	0.2	0.4	0.7
	O	1.0	0.9	0.3	0.3	0.9
$\alpha$ -terpinene	N	0.7	0.7	0.5	0.9	0.8
	O	1.1	0.9	0.3	0.4	0.9
limonene	N	6.6	4.4	2.1	2.9	3.6
	O	3.9	3.1	1.0	2.2	3.3
$\beta$ -phellandrene	N	1.7	2.0	1.0	2.7	5.5
	O	11.4	9.1	2.9	3.7	10.0
<i>cis</i> - $\beta$ -ocimene	N	0.4	0.6	0.6	2.1	3.1
	O	1.2	0.8	0.4	0.6	1.7
$\gamma$ -terpinene	N	10.7	6.7	4.4	8.7	10.0
	O	16.4	10.5	3.6	5.0	10.1
<i>p</i> -cymene	N	0.2	0.5	0.2	0.5	1.0
	O	0.8	0.6	0.3	0.4	1.3
terpinolene	N	0.9	0.9	0.6	1.1	1.7
	O	2.6	2.3	0.8	1.1	2.3
$\delta$ -elemene	N					
	O	0.2	0.4	0.3	0.3	0.2
$\alpha$ -copaene	N	0.2	0.3	0.2	0.2	0.6
	O	0.9	0.9	0.5	0.7	0.9
$\beta$ -elemene	N				0.1	0.7
	O	0.5	0.5	0.3	0.5	0.9
terpinen-4-ol	N	3.1	3.1	2.2	2.2	3.0
	O	3.1	2.8	1.3	1.8	2.5
<i>trans</i> -2-menthen-1-ol	N	0.1	0.1		0.2	0.5
	O	1.0	0.9	0.4	0.6	0.8
$\alpha$ -terpinyl acetate	N	1.1	1.1	1.2	1.3	2.3
	O	5.9	5.3	3.1	4.9	3.1
germacrene D	N	1.2	1.0	0.8	0.8	4.0
	O	3.0	2.8	1.7	2.9	4.9
$\alpha$ -muurolene	N	0.1	0.1	0.1	0.1	1.2
	O	0.5	0.4	0.2	0.2	0.4
<i>cis</i> -piperitol	N			0.1	0.1	2.3
	O	0.7	0.6	0.3	0.3	0.5
$\delta$ -cadinene	N	0.8	0.7	0.7	0.7	1.6
	O	2.0	2.0	0.8	0.8	1.7
nerol	N					0.1
	O	0.2	0.2	0.1	0.1	0.3
sesquiterpene hydrocarbon <sup>d</sup>	N					1.9
	O	2.4	2.4	1.3	1.3	3.0

<sup>a</sup> From the Masonite property west of Ukiah. <sup>b</sup> N =

new tip growth; O = year-old growth. <sup>c</sup> Listed peak heights were calculated relative to the peak heights of the internal standard, all at range 10, attenuation 32.

<sup>d</sup> Tentatively identified as germacrene B on the basis of rearrangement to  $\gamma$ -elemene.

differences may be the result of genetic variability or environmental conditions, or both, in the foliage samples investigated. The absence of camphor, reported as 0.4% of the essential oil from the Oregon redwoods, in our studies may be due to differences in isolation techniques in the two investigations.

Shown in Table II are the changes in composition of essential oil over the growing season for the foliage from the redwood tree growing under natural conditions in the Pacific Coast Range on the Masonite property. The amount of essential oil from the new foliage was considerably less than the amount in mature growth. This was

previously observed for *Mentha arvensis* (von Rudloff and Hefendehl, 1966), for Douglas fir (Maarse and Kepner, 1970), and for white, black, and blue spruce (von Rudloff, 1972, 1975a,b). An exception to this generality in the present study was limonene which was present in higher concentrations in new than in mature growth throughout the period of the investigations. The terpenes present in highest amounts in the essential oil from the mature growth were  $\alpha$ -pinene,  $\alpha$ -terpinene,  $\beta$ -phellandrene, myrcene, and  $\alpha$ -terpinyl acetate in that order. As a general trend, the total amount of essential oil from both new growth and old growth decreased during the first 4 weeks after emergence of the new growth and then increased during the next 14 weeks of the study.

In an earlier study (Maarse and Kepner, 1970) of the changes in composition of the volatile terpenes in Douglas fir new tip growth, it was observed that acyclic monoterpenes were almost completely absent in the new growth as it first appeared, while cyclic monoterpenes were immediately present in amounts comparable to those in year-old growth. Of the acyclic monoterpenoids in redwood, *cis*- $\beta$ -ocimene and nerol are present in very low concentration in newly emerged growth and increase during the growing season. Myrcene, the only acyclic monoterpene present in either old or new growth in appreciable amounts in redwood, shows the same general seasonal variation as the cyclic monoterpenes. *trans*- $\beta$ -Ocimene in trace amounts was the only other acyclic monoterpene detected in the essential oils from redwoods in this study. These results suggest that neryl pyrophosphate is the primary precursor of the predominantly cyclic monoterpenes (Cramer and Rittersdorf, 1967; Loomis, 1967; Miller and Wood, 1964; Rittersdorf and Cramer, 1967, 1968; Beytia et al., 1969) identified in this study as redwood volatiles and that geranyl pyrophosphate is not important as a precursor in this system (Maarse and Kepner, 1970).

The redwood tree in the University of California, Davis, arboretum, which was used for the compound identification studies, was also monitored for changes in terpene composition during the growing season. The arboretum tree was given regular irrigation and fertilization treatment during the growing season, cultural conditions markedly different from the natural conditions of the tree growing on the Masonite property. The arboretum tree produced twice the length of new growth and matured later than the foliage on the Masonite tree. No definitive pattern of changes in terpene composition could be detected in the essential oil samples from the arboretum tree, such as was observed for the tree growing under the natural conditions on the Masonite property. The terpenes present in largest amounts in the essential oil from the mature growth of the arboretum tree were  $\gamma$ -terpinene,  $\alpha$ -pinene, limonene, myrcene, the sesquiterpene hydrocarbon tentatively identified as germacrene B, and  $\beta$ -elemene in that order (Ellison, 1973).

Qualitative and quantitative differences in composition between the essential oils from the Davis arboretum tree

mature foliage and the Masonite tree mature foliage were observed as well as differences between the present study and the study of the Oregon redwood terpenes (Gregonis et al., 1968). At the species level in conifers, von Rudloff (1975c) has observed that essential oil compositions are qualitatively the same and thus under strict genetic control. Quantitative differences in composition are undoubtedly influenced by environmental conditions such as soil quality, sunlight, water, fertilization, climate, etc. (Longhurst et al., 1968). Essential oil composition is also dependent on the plant age, foliage age, and the location of the foliage on the tree. Thus, both qualitative and quantitative composition differences can be attributed to both genetic variability and environmental conditions. Two distinct species of coast redwood may exist that are taxonomically indistinguishable but chemically different, as in the case of some western junipers (Vasek and Scora, 1967).

The amounts of monoterpene alcohols and carbonyls, compounds found by Oh et al. (1967) to be highly inhibitory to the functioning of the rumen microorganisms of deer and sheep, are very small in the coast redwoods investigated in this study. This result is consistent with the concept that the level of inhibitory compounds present is related to the palatability of the range forest species to browsing ruminants (Longhurst et al., 1968).

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