Phytochemistry, 1968, Vol. 7, pp. 975 to 981. Pergamon Press. Printed in England

VOLATILE OILS FROM FOLIAGE OF COAST REDWOOD AND BIG TREE[1]

D. E. Gregonis,[2] R.D. Portwood, W. H. Davidson,
D. A. Durfee and A. S. Levinson[3]

Department of Chemistry, Portland State College, Portland, Oregon, U.S.A.

(*Received* 11 *October* 1967)

Abstract—The steam volatile oil from macerated foliage of coast redwood (*Sequoia sempervirens*) and big tree or giant sequoia (*Sequoiadendron giganteum*) have been examined by gas chromatography. Most major and many minor components of each of the oils have been isolated and identified by i.r. spectroscopy. A few components have been tentatively identified by retention times only.

The coast redwood (*Sequoia sempervirens*) and the big tree, or giant sequoia (*Sequoiadendron giganteum*), are members of the Taxodiaceae (order Cupressales). There is general agreement that each belongs to a monotypic genus.[4–6] The two genera were considered to be quite separate by Stebbins[6] with the *Sequoia* having greater similarity to *Metasequoia* than to *Sequoiadendron*. Agreement with this conclusion is not universal; Melchior and Werdermann[7] combine the genera *Sequoia* and *Sequoiadendron* in a tribe. The recent species of Taxodiaceae are only the scattered remnants of an old family and this necessarily complicates their classification.

Chemical investigation of the order Cupressales has long been an active area of research. The main concern has been with the large family Cupressaceae; the family Taxodiaceae has apparently not been studied extensively from the chemical point of view. Several excellent reviews covering the chemistry and chemotaxonomy of the Cupressales have been written by Erdtman.[8–10] With the objective of providing some chemical information which might be useful to botanical taxonomists we have initiated a study of the two species available to us, *Sequoia sempervirens* and *Sequoiadendron giganteum*.

Sequoia sempervirens grows within a narrow belt on the coast of California extending into southern Oregon. The most striking characteristics of the tree are its thick red bark, impressive height and longevity. The only previous work on the volatile oil of the foliage of

---

[1] Presented in part at the Twentieth Annual Northwest Regional Meeting of the American Chemical Society, Corvallis, Oregon, 14–15 June (1965).

[2] National Science Foundation Undergraduate Research Participant.

[3] To whom inquiries may be addressed.

[4] J. T. Buchholz, *Am. J. Botany* **26**, 535 (1939).

[5] G. N. Jones, *Science* **98**, 406 (1943).

[6] G. L. Stebbins, Jr., *Science* **108**, 95 (1948).

[7] H. Melchior and E. Werdermann, *A. Engler's Syllabus Der Pflanzenfamilien* (12th edition), p. 333. Gebruder Borntraeger, Berlin (1954).

[8] H. Erdtman and T. Norin, in *Fortschritte Der Chemie Organischer Naturstoffe* (edited by L. Zechmeister), Vol. XXIV, p. 206. Springer-Verlag, Vienna (1966).

[9] H. Erdtman, in *Chemical Plant Taxonomy* (edited by T. Swain), p. 89. Academic Press, London (1963).

[10] H. Erdtman, in *Progress in Organic Chemistry* (edited by J. W. Cook), Vol. 1, p. 22. Butterworths, London (1952).

this tree which we are aware of was reported by Severance[11] in a Master's Thesis in 1912 He isolated the volatile oil by steam distillation and obtained by fractional distillation one pure component, a $C_{10}H_{16}$ hydrocarbon, b.p. 157°.

Sequoiadendron giganteum grows on the western slope of the Sierra Nevada in California. It also is noted for its thick red bark, impressive height and longevity. The only previous work on the volatile oil of the foliage of this tree which we are aware of was reported by Lunge and Steinkauler in the last century.[12] By repeated fractional distillation of the steam volatile oil these authors obtained four components. The components were described as (1) a colorless oil, b.p. 155°; (2) a colorless oil, b.p. 227–230°; (3) a heavy yellow oil, b.p. 280–290°; and (4) a crystalline white solid, m.p. 105°, b.p. 290–300°, for which elementary analysis and molecular weight determination by the Victor Meyer method were consistent with a molecular formula of $C_{13}H_{10}$. They named the crystalline compound sequoien and showed that it behaved differently from the known fluorene on oxidation with chromic acid.

In the present work *Sequoia sempervirens* foliage was obtained from a population *ca.* 50 years old in Peavey Arboretum, Corvallis, Oregon, and from a population *ca.* 20 years old in the Hoyt Arboretum, Portland, Oregon. *Sequoiadendron giganteum* foliage was obtained from *ca.* 30-year-old trees along McLoughlin Boulevard, Milwaukee, Oregon.

The freshly collected foliage was macerated with water and the resulting mash was boiled under a continuous distillation-extraction head,[13] using cyclohexane as the extracting solvent and recycling the water. The material used in this study was obtained in the first 24 hr of distillation. Prolonged distillation, up to 30 days, still yielded some volatile material. In the case of *Sequoia sempervirens* most of the cyclohexane was removed by distillation. To prevent darkening of the oil distillation was discontinued when the mixture still contained 30 to 50 per cent cyclohexane. The per cent yield of oil (*ca.* 0·3 per cent) was obtained by subtraction of the weight of cyclohexane as determined by GLC. Analysis of the oil by GLC (Carbowax 20M) indicated a mixture with at least twenty-five components present in significant quantity (Fig. 1).

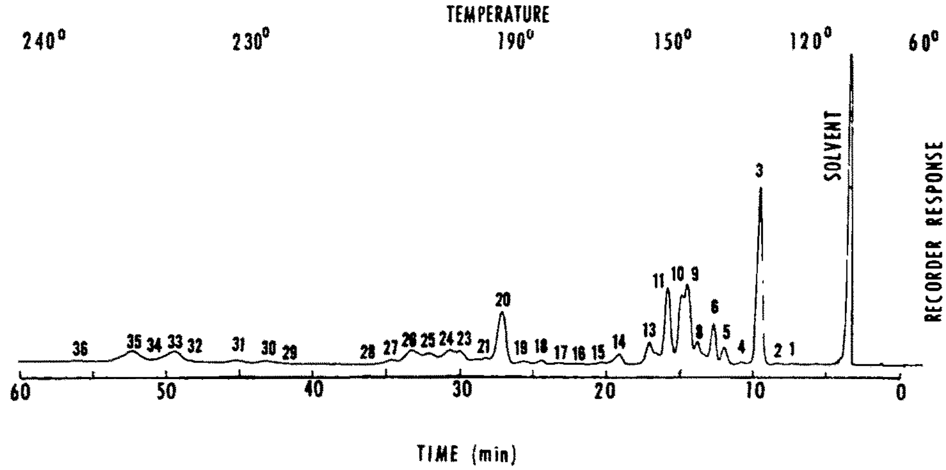

Fig. 1. Gas chromatogram of the volatile oil of *Sequoia sempervirens* on carbowax 20 M, helium 150 ml/min.

---

[11] H. M. Severance, Master's Thesis, University of California, Berkeley, California (1912).

[12] G. Lunge and T. Steinkauler, *Ber.* 1656 (1880); *Ber.* 2202 (1881).

[13] S. T. Likens and G. B. Nickerson, *Proc. Am. Soc. Brewing Chemists* 5 (1964).

The crude mixture was prefractionated by column chromatography. The difficulties encountered in other methods of prefractionation have been discussed by von Rudloff.[14] In the present study Woelm neutral alumina activity grade II was the adsorbent. Possible changes in some components of the mixture caused by the adsorbent cannot be precluded but there was no evidence that this had occurred. The desirability of a prefractionation step was demonstrated by preparative GLC on the whole mixture. The material obtained by this technique was less pure than the material collected after prefractionation of the mixture. Each fraction eluted from the column of alumina was examined by GLC, similar fractions were combined and the components of the combined fractions were separated by preparative GLC. Identification was based on identity of the i.r. spectra of the collected material with spectra of authentic samples or spectra from the literature. In most cases authentic samples were shown to have GLC behavior identical to the proper component of the mixture. Table 1 lists the components in *S. sempervirens* and their percentages calculated from the chromatogram.

TABLE 1. COMPOSITION OF THE VOLATILE OIL OF THE FOLIAGE OF *Sequoia sempervirens*

Peak No.	Compound*	%†	Peak No.	Compound*	%†
1	Unidentified	Trace	19	Camphor	0·4
2	Unidentified	0·1	20	1-Terpinen-4-ol Bornyl acetate	8·8
3	α-Pinene	19·7			
4	Camphene	0·3	21	Unidentified	0·3
5	β-Pinene	2·2	22	Unidentified	0·1
6	Myrcene	4·8	23	α-Terpineol	2·9
7	α-Phellandrene (r.t.)	0·8	24	Terpinyl acetate	3·9
8	α-Terpinene (r.t.)	3·2	25	Citronellol	2·4
9	Limonene	10·1	26	Unidentified	3·9
10	β-Phellandrene (r.t.)	7·7	27	Geraniol (r.t.)	1·0
11	γ-Terpinene	9·8	28	Safrole	Trace
12	*p*-Cymene	1·0	29	Unidentified	Trace
13	Terpinoline (r.t.)	3·1	30	Unidentified	0·7
14	*cis*-Hex-3-ene-1-ol	1·5	31	Unidentified	1·2
15	Unidentified	0·1	32	Unidentified	Trace
16	Unidentified	Trace	33, 34	Unidentified	4·1
17	Unidentified	0·1	35	β-Eudesmol	4·8
18	Unidentified	0·5	36	Unidentified	0·4

* The compounds tentatively identified by retention times only are designated by (r.t.).
† Peak areas were determined by the height × half-width method.

In the case of *Sequoiadendron giganteum* removal of the cyclohexane by careful fractional distillation yielded *ca.* 0·6 per cent of golden yellow oil. Further distillation yielded (+)-α-pinene in 57 per cent yield. Analysis of the distillation residue by GLC indicated a mixture with about fifteen components present in significant quantity (Fig. 2). Further analysis of the mixture was performed similarly to the procedure described above. In this case the prefractionation yielded directly three pure components, safrole, elemicin and α-terpineol.

---

[14] E. VON RUDLOFF and F. M. COUCHMAN, *Can. J. Chem.* **42**, 1890 (1964).

Direct collection on the GLC without prefractionation was more successful here than in the previous case. However, prefractionation followed by collection on the GLC was the preferred method for isolation of β-caryophyllene, humulene, O-methyl eugenol and benzyl

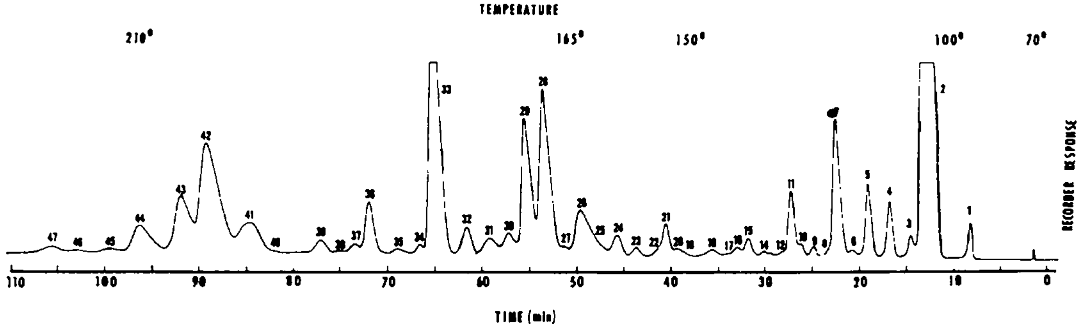

FIG. 2. GAS CHROMATOGRAM OF THE VOLATILE OIL OF *Sequoiadendron giganteum* ON CARBOWAX 20 M, HELIUM 150 ml/min.

TABLE 2. COMPOSITION OF THE VOLATILE OIL OF THE FOLIAGE OF *Sequoiadendron giganteum*

Peak No.	Compound*	Dist. residue (%)†	Total (%)‡	Peak No.	Compound*	Dist. residue (%)†	Total (%)‡
1	Unidentified	0·7	0·3	26	1-Terpinen-4-ol	3·1	1·3
2	α-Pinene	27·6	69·2	27	Unidentified	Trace	
3	Unidentified	0·5	0·2	28	α-Terpineol Caryophyllene	8·0	3·4
4	Unidentified	1·6	0·7				
5	Myrcene	2·2	0·9	29	Humulene	6·3	2·7
6	Unidentified	0·2	0·1	30	Citronellol (r.t.)	1·1	0·5
7	Limonene	4·8	2·0	31	Unidentified	0·8	0·3
8	Unidentified	0·2	0·1	32	Anethole (r.t.)	1·1	0·5
9	Unidentified	0·3	0·1	33	Safrole	12·0	5·1
10	*p*-Cymene	0·3	0·1	34	Unidentified	0·3	0·1
11	Terpinoline (r.t.)	2·1	0·9	35	Unidentified	0·2	0·1
12	Unidentified	Trace		36	O-Methyl eugenol	2·1	0·9
13	Unidentified	Trace		37	Unidentified	0·2	0·1
14	Unidentified	Trace		38	Unidentified	Trace	
15	Unidentified	0·5	0·2	39	Unidentified	0·6	0·3
16	Unidentified	Trace		40	Unidentified	Trace	
17	Unidentified	Trace		41	Eugenol (r.t.)	3·5	1·5
18	Unidentified	0·3	0·1	42	Elemicin	10·4	4·4
19	Unidentified	Trace		43	β-Eudesmol	3·3	1·4
20	Unidentified	Trace		44	Unidentified	2·2	0·9
21	Unidentified	1·1	0·5	45	Unidentified	0·3	0·1
22	Unidentified	Trace		46	Unidentified	Trace	
23	Unidentified	0·3	0·1	47	Unidentified	0·5	0·2
24	Unidentified	1·0	0·4	48	Benzyl benzoate	Not obsed. in GLC under these cond.	
25	Unidentified	Trace					

* The compounds tentatively identified by retention times only are designated by (r.t.).

† Determined by the height × half-width method.

‡ Total per cent composition calculated from distillation residue composition and distillation data.

benzoate. Table 2 lists the identified components in *S. giganteum* and the per cent composition of the distillation residue and the original mixture.

Approximately 90 per cent of the material in each of the oils has been identified. The major component of each, α-pinene, constitutes 69 per cent of *S. giganteum* oil and 20 per cent of *Sequoia sempervirens* oil. Other monoterpene hydrocarbons constitute an additional 43 per cent of *S. sempervirens* oil but only an additional 9 per cent of *Sequoiadendron giganteum* oil. *Sequoia sempervirens* elaborates a number of monoterpene hydrocarbons in significant amounts while *Sequoiadendron giganteum* does not.

Another difference between the oils is the presence of allyl phenylethers in significant quantity in *S. giganteum* and their almost total absence in *Sequoia sempervirens*.

The presence of caryophyllene and humulene in *Sequoiadendron giganteum* is the second report of the presence of these compounds in Taxodiaceae.[15]

It is interesting to note that the properties of the component of *Sequoia sempervirens* isolated by Severance[11] in 1912 correspond well with those of the major component, α-pinene. Further, α-pinene, safrole and elemicin, the major components of *Sequoiadendron giganteum*, may be three of the components described by Lunge and Steinkauler[12] in the last century as occurring in *S. giganteum*. Their fourth component, the $C_{13}H_{10}$ hydrocarbon, has not been encountered yet.

EXPERIMENTAL

Gas–liquid chromatography (GLC) was performed with an Aerograph Autoprep Model 700 equipped with a thermal conductivity detector and a non-linear temperature programmer. A 3 m × 9 mm o.d. column having 20 per cent polyethylene glycol (Carbowax 20M) as liquid phase and Chromosorb W (60–80 mesh) as solid support was used for both analytical and preparative work. I.r. spectra were obtained as films between sodium chloride plates with a Perkin–Elmer Infracord Model 137B. Optical rotations were measured on undiluted samples with a Kern Full Circle Polarimeter. Densities were determined with a Fisher–Davidson Gravitometer.

Sequoiadendron giganteum

Isolation of the Oil

Freshly collected *S. giganteum* foliage (18·5 kg) was macerated in a Waring blendor in 2·3 to 4·5 kg batches with 8 to 10 l. of water. The resulting mash was boiled 24 hr under a continuous distillation-extraction head[13] using cyclohexane as the extracting solvent. The cyclohexane solutions from each batch were combined, extracted with 100 ml of 5 per cent $NaHCO_3$, dried ($MgSO_4$), filtered and distilled through a 90 cm column packed with glass helices to remove solvent. The resultant golden yellow oil (111 g, 0·6 per cent yield) had $n_D^{25°}$ 1·4779, $d^{27°}$ 0·883 and $[\alpha]_D^{26°}$ +43·0°. Further distillation yielded 62·2 g (57·4 per cent of the oil), b.p. mainly 151–156°, with $n_D^{23°}$ 1·4655, $d^{25°}$ 0·856 and $[\alpha]_D^{26°}$ +53·7°. The i.r. spectrum was identical to that of α-pinene. Reported physical constants for (+)-α-pinene: b.p. 156°, $d^{15°}$ 0·8620, $n_D^{12°}$ 1·4650 and $[\alpha]_D$ 48·85°, $[\alpha]_D^{25°}$ +51·1° and $[\alpha]_D^{25°}$ +53·0°.[16] A chromatogram of the distillation residue is shown in Fig. 2.

Prefractionation

Crude big tree oil (3·0 g) was chromatographed on Woelm neutral alumina grade II (75 g). Elution was carried out with pure cyclohexane, benzene–cyclohexane mixtures, pure benzene, benzene–methanol mixtures and pure methanol. Twenty-seven fractions of 50 ml volume each were collected, concentrated and examined by GLC. There was recovered 2·6 g of material (87 per cent recovery). Material distribution is summarized below.

---

[15] Ref. 8, p. 266.

[16] J. L. Simonsen and L. N. Owen, *The Terpenes*, Vol. II, 2nd edition, revised, p. 132. Cambridge University Press, Cambridge (1957).

TABLE 3

Fraction No.	Solvent system	Type of material
2	Cyclohexane	Hydrocarbons, safrole
3	Cyclohexane	Safrole
4–7	Cyclohexane	Negligible quantity
8, 9	Cyclohexane–benzene mixtures	O-methyl eugenol An alcohol Benzyl benzoate
10	Cyclohexane–benzene mixtures	Elemicin (impure)
11	Benzene	Elemicin
12–14	Benzene	Elemicin+alcohols
15	Benzene	α-Terpineol
16–24	Benzene	Alcohols
25–27	Benzene–methanol thru methanol	Alcohols

Direct identification of pure column fractions. Pure samples of safrole, α-terpineol and elemicin were obtained directly as column fractions and the compounds were identified by comparison of i.r. spectra with those of authentic samples in the former two cases and with a literature spectrum in the case of elemicin.[17]

Collection by GLC of Prefractionated Material

Fraction No. 2 containing safrole and hydrocarbons was injected into the GLC in aliquots at 190°. Peaks No. 28 and 29 (Fig. 2) were collected. The i.r. spectrum of peak No. 28 was identical to that of an authentic sample of β-caryophyllene. The i.r. spectrum of peak No. 29 was identical to that of humulene published by Sorm and co-workers;[18] this differs from the spectrum of Sutherland and Waters[19] by the presence of a peak at 890 cm^{-1}, and is a mixture of α and β humulenes.[20]

Several minor components, probably peaks 31, 32, 37 and 39 (Fig. 2), are concentrated in fraction No. 2 and will be examined in the future.

Fractions 8 and 9 were combined and injected into the GLC at 230°. O-methyl eugenol (peak 36) and benzyl benzoate (r.t. 29 min, He flow rate *ca.* 300 ml/min) were collected and identified by their i.r. spectra. A third component (r.t. 14 min) was collected but has not been identified.

Direct collection. Repeated injections of 150-μl aliquots of the original oil into the GLC with temperature programming allowed manual collection of peaks 5, 7, 21, 26, 36, 37, 39, 43, 44, 45, 46 and 47. Good i.r. spectra were obtained for peaks 5, 7, 26, 36, 39, 43 and 47. The spectra for peaks 39 and 47 were not identified; the other spectra matched those of authentic samples (see Table 2) or published spectra.[18]

Sequoia sempervirens

Isolation of the Oil

Freshly collected *S. sempervirens* foliage was treated by the method described above. There was obtained 6·8 g of yellow oil from 1·6 kg of plant material. The oil so obtained was 30 per cent cyclohexane by GLC analysis (0·3 per cent yield). (Further heating to remove the remaining solvent in earlier experiments led to considerable discoloration of the sample.) A chromatogram of the crude oil is shown in Fig. 1.

Prefractionation

Crude redwood oil (8·6 g, 42 per cent cyclohexane by GLC) was chromatographed on Woelm neutral alumina grade II (100 g). Elution was carried out with pure cyclohexane, benzene–cyclohexane mixtures, pure benzene, benzene–ether mixtures, pure ether, ether–methanol mixtures and finally with pure methanol. Seventeen fractions of 50 ml volume each were collected, concentrated and examined by GLC. Similar fractions were combined. There was recovered 4·9 g of material (98 per cent recovery). Material distribution is summarized below.

---

[17] A. T. SHULGIN, *Can. J. Chem.* 43, 3439 (1965). The Depository of Unpublished Data, National Science Library, National Research Council, Ottawa, Canada.

[18] J. PLIVA, M. HORAK, V. HEROUT and F. SORM, *Die Terpene. Der Spektrum und phys. Konstanten. Teil I. Sesquiterpenes.* Akademie-Verlag, Berlin (1960).

[19] M. D. SUTHERLAND and O. J. WATERS, *Australian J. Chem.* 14, 596 (1961).

[20] V. BENESOVA, V. HEROUT and F. SORM, *Coll. Czechoslov. Chem. Commun.* 26, 1826 (1961).

Fraction No.	Solvent system	Type of material
2	Cyclohexane	Hydrocarbons
3, 4	Cyclohexane	Esters, safrole
5, 6	Cyclohexane–benzene mixtures	Esters
7	Cyclohexane–benzene mixtures	Esters, camphor
8–15	Benzene thru methanol–ether	Alcohols

Collection and Identification

Hydrocarbons. Preparative GLC of the hydrocarbon fractions from several different column chromatography separations (repeated injections 80 μl or 100 μl isothermally at 115° and 105° respectively) resulted in the isolation in traps at Dry Ice–acetone temperature of pure samples of α-pinene, camphene, β-pinene, myrcene, limonene and *p*-cymene whose i.r. spectra were identical to published spectra.[21] A sample of γ-terpinene was collected which was impure, as indicated by the presence of a weak broad peak at 9·0 μ not present in the spectrum of the pure compound. Injection of a mixture of the crude oil and pure γ-terpinene resulted in the establishment of identical retention times for γ-terpinene and the No. 11 peak (Fig. 1). Similarly, retention time correspondence was obtained for α-phellandrene, β-phellandrene, α-terpinene and terpinoline. After the monoterpenes were collected from each injection the column temperature was raised to 230°. The higher molecular weight hydrocarbons were not collected. (Samples of the hydrocarbon-containing column fraction after 3 months' storage contained much less β-phellandrene, γ-terpinene and terpinoline and more limonene and *p*-cymene than the original material.)

Esters, safrole, camphor. Column fractions 3 and 4 were injected in aliquots at 185°. Three components were collected in sufficient quantity for i.r. determination. These components gave i.r. spectra identical to those of bornyl acetate, terpinyl acetate and safrole. Column fractions 5 and 6 combined injected in aliquots at 220° gave more bornyl acetate and terpinyl acetate. No other pure material could be collected. Column fraction 7 was injected in aliquots at 158°. Four components were collected all of which had carbonyl absorption in the i.r. Slightly impure bornyl acetate and camphor were identified by their i.r. spectra.

Alcohols. Column fractions 8, 9, 10, 11 and 12 were injected separately at 205° to 232°. Each fraction yielded 1-terpinen-4-ol. In addition, a total of approximately fifteen different alcohols were collected which were not identified. Fraction 13 injected at 220° yielded α-terpineol and β-eudesmol which were identified by their i.r. spectra. Five other alcohols which were not identified were also collected. Fraction 14 injected at 160° yielded more α-terpineol, citronellol, identified by its i.r. spectrum, and four additional alcohols which were not identified. Fraction 15 treated similarly yielded *cis*-3-hexene-1-ol (identified by i.r. spectrum) and three other alcohols not identified.

Direct collection. Repeated injections of *ca.* 150-μl aliquots of the original oil into the GLC with temperature programming allowed manual collection of peaks 27, 28, 31, 32, 33, 35 and 36. Good i.r. spectra were obtained for all except 27 and 28. All the spectra had adsorption in the —OH region and weak adsorption in the carbonyl region probably due to contamination. Only β-eudesmol, peak 35, was identified by this technique.

Retention time identifications. The tentative identifications made on the basis of retention times were performed by injecting into the GLC mixtures of the original oil with a sample of the suspected compound and noting peak enhancement.

Acknowledgements—Acknowledgement is made to the donors of The Petroleum Research Fund, administered by the American Chemical Society, to the Undergraduate Research Participation Program of the National Science Foundation and to the Portland State College Research Committee for support of this research.

The gift of terpene samples by Mr. Gordon E. Fisher, Weyerhauser Company, Longview, Washington, and by Waldorf Hoerner Paper Products Company, Missoula, Montana, is gratefully acknowledged.

For permission to collect biological materials we are grateful to Mr. E. Fischer, Curator, Hoyt Arboretum, Portland, Oregon, Mr. A. E. Johnson, Division Engineer, State of Oregon Highway Dept., Portland, Oregon, and Dr. R. Dilworth, Head, Dept. of Forest Management, School of Forestry, Oregon State University, Corvallis, Oregon.

---

[21] B. M. MITZNER, E. T. THEIMER and S. K. FREEMAN, *Appl. Spectry* **19**, 169 (1965).