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THE VOLATILE OIL OF THE LEAVES OF *CHAMAECYPARIS NOOTKATENSIS**

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Abstract—Analysis of the volatile leaf oil of the Alaska (yellow) cedar showed (−)-α-pinene, (+)-3-carene, and (+)-limonene to be the main constituents. In addition, nine monoterpene hydrocarbons, seven oxygenated monoterpenes, six sesquiterpene hydrocarbons, nerolidol, cedrol, bisabolol, α-cadinol, nine diterpene hydrocarbons (including one new one), manoyl-, 13- and 8-epimanoyl- and 8,13-diepimanoyl oxides, phyllocladol, six *n*-alkanes, three alkanals and nine C_5-branched chain or aromatic esters of isovaleric, 3-methyl-2-, and 3-methyl-3-butenoic acids were identified (Table 1A–C). The structures of the esters were confirmed by synthesis. Many of the sesquiterpene and diterpene hydrocarbons, cedrol and phyllocladol are typical constitutuents of the order Cupressales, but the new oxides and esters, the *n*-alkanes, alkanals, and possibly 1-methyl-4-isopropenyl- and 1-methyl-(α-hydroxyisopropyl)-benzene appear to be characteristic of this cedar species. The quantitative composition of the leaf oil from different trees varied little when the leaves were collected in winter, although some variation from one population to another may exist. Large losses of the low boiling components may ensue if the foliage is transported in an unsealed container.

INTRODUCTION

THE ALASKA, or yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach (Cupressaceae) occurs mainly along the west coast of North America ranging from Washington and British Columbia to Alaska.[1] Botanically it is classified in the genus *Chamaecyparis*, but Erdtman and Norin[2] expressed the opinion that this assignment should be reconsidered, the heartwood extractives of the Alaska cedar differing considerably from other *Chamaecyparis* species.

Previously, we found that the composition of the leaf oils of conifers[3–8] can be used effectively in chemosystematic studies at the species level. A survey of the literature[9] indicates that the leaf oil of the Alaska cedar was analyzed in 1926,[10] but no more recent analysis appears to have been reported.‡ A detailed analysis employing GLC was, therefore, undertaken to establish a more accurate knowledge of the composition of this cedar leaf oil. From a chemotaxonomic point of view it is important to know the degree of variability which may

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‡ See however Ref. 16.

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[2] H. ERDTMAN and T. NORIN, *Prog. Chem. Org. Nat. Prod.* **24**, 257 (1966).
[3] E. VON RUDLOFF, *Can. J. Chem.* **41**, 1737, 2876 (1963).
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[8] E. VON RUDLOFF, *Can. J. Botany* **45**, 891, 1703 (1967).
[9] E. GILDEMEISTER and FR. HOFFMANN, in *Die aetherischen Oele* (edited by W. TREIBS and K. BOURNOT), Vol. 4, p. 271, Akademie Verlag, Berlin (1956).
[10] R. H. CLARK and C. C. LUCAS, *Trans. R. Soc. Can. Sect. III* **20**, 423 (1926).

be encountered from tree to tree and from population to population. Freshly cut foliage of the Alaska cedar loses its aroma fairly rapidly[11] and this can make quantitative analysis difficult since serious losses of the more volatile constituents may be encountered in the transportation of the cedar foliage. For this reason, the present study was carried out in two parts. First, a large batch of oil from the foliage of very old trees growing on Vancouver Island was analyzed quantitatively and qualitatively. Thereafter, the effect of transportation and storage on the leaf oil composition was determined on a number of leaf samples obtained from several collection sites. In this manner it was hoped to obtain both qualitative and quantitative data which could form the basis of a more extensive chemotaxonomic investigation of the Alaska cedar and related cedar species.

The analytical techniques employed in this study were described in detail before.[6,8,12,13] However, the leaf oil of the Alaska cedar was found to be unusually complex and several modifications and improvements had to be made to arrive at satisfactory results.

RESULTS AND DISCUSSION

A typical gas chromatogram of the volatile leaf oil of the Alaska cedar as obtained on our standard polyethylene glycol (5% PEG 20 M) column is shown in Fig. 1. The percentage composition of the oil from five different sources is shown in Table 1 A–C. This leaf oil is characterized by the large amounts of (−)-α-pinene, (+)-3-carene, and (+)-limonene and the

TABLE 1. % COMPOSITION OF THE LEAF OIL OF THE ALASKA CEDAR (*Chamaecyparis nootkatensis*)

Peak No.	Compound	Leaf oil sample No. *				
		1	2	3	5b	6b
A: Monoterpenes						
1	(−)-α-Pinene	38·8	33·8	32·0	32·8	33·0
2a	(Fenchene)†	1·1	0·8	1·1	0·8	0·7
2b	Camphene	0·5	0·3	0·2	0·3	0·2
3a	β-Pinene	2·0	2·4	1·7	2·6	2·6
3b	(Sabinene)	0·2	0·5	0·2	0·5	0·3
4a	(+)-3-Carene	30·8	36·5	32·4	24·8	25·3
4b	Myrcene	2·3	2·5	2·8	3·8	4·2
5	(α-Phellandrene)	0·5	0·3	0·1	0·4	0·1
6a	(+)-Limonene	9·2	12·1	18·2	23·4	23·5
6b	β-Phellandrene	2·9	1·3	1·5	1·9	2·8
7	(α-Terpinene)	0·2	0·1	0·1	0·3	0·3
8	*p*-Cymene	0·3	trace	trace	0·1	0·1
9	Terpinolene	2·0	3·8	2·8	2·4	2·5
14	1-Methyl-4-isopropenyl benzene	0·3	0·6	0·1	0·3	0·3
21	Bornyl acetate	0·2	0·2	0·2	0·1	0·2
22	Terpinen-4-ol	0·2	0·1	0·2	0·7	0·7
26	α-Terpineol	0·5	0·5	0·3	0·3	0·1
27	α-Terpinyl acetate	1·2	0·9	0·9	1·1	0·3
28	Piperitone	0·1	0·1	0·1	0·1	0·2
30b	Citronellol	0·1	trace	0·1	0·1	0·1
34b	1-Methyl-4-(α-hydroxy isopropyl)benzene	trace	trace	trace	trace	trace

[11] J. WALTERS, personal communication.
[12] A. R. VINUTHA and E. VON RUDLOFF, *Can. J. Chem.* **23**, 3743 (1968).
[13] E. VON RUDLOFF, in *Recent Advances in Phytochemistry* (edited by M. K. SEIKEL and V. C. RUNECKLES), Vol. II, p. 127, Appleton-Century-Crofts, New York (1969).

TABLE 1—*cont.*

Peak No.	Compound	Leaf oil sample No.* 1	2	3	5b	6b
B: Sesqui- and diterpenes						
16	α-Cubebene	trace	trace	trace	trace	?
18	α-Copaene	trace	trace	trace	trace	trace
24	Thujopsene	0·1	trace	trace	trace	trace
30a	γ + δ-Cadinene	0·1	0·1	0·1	0·1	0·1
31	*ar*-Curcumene	trace	?	0·1	trace	0·1
34a	Calamenene	0·1	trace	trace	trace	0·1
38	Nerolidol	0·4	0·3	0·6	0·4	0·1
41	Cedrol	trace	trace	trace	trace	trace
45	(+)-Bisabolol	0·7	0·3	0·6	0·2	0·3
46b	α-Cadinol	0·3	0·1	0·2	0·1	trace
44a	Diterpene I	0·5	0·2	0·2	trace	0·2
44b	Isopimara-8(9),15-diene	0·3	0·1	0·1	trace	0·1
46a	Isohibaene	0·3	0·1	0·1	trace	0·1
47a 47b	Sandaracopimaradiene Isophyllocladene	0·1	trace	trace	trace	0·1
48a	Isopimaradiene	0·1	trace	trace	trace	0·1
49b 49c	Manoyl oxide 13-Epimanoyl oxide	0·3	0·6	0·5	0·2	0·5
50	Phyllocladene	0·1	0·1	0·2	trace	0·1
51	Abieta-7,13-diene	trace	trace	trace	trace	trace
52a	Dehydroabietadiene	trace	trace	trace	?	trace
53	8-13-Diepimanoyl (IV)	trace	trace	trace	trace	trace
54	8-Epimanoyl (III)	1·1	0·5	1·7	0·6	0·5
55	Phyllocladan-16-ol	0·3	0·1	0·1	trace	0·1
C: Branched-chain esters and non-terpenoid compounds						
11	3-Methyl-3-butenyl isovalerate	0·4	0·2	0·1	0·2	0·3
12	Pelargonaldehyde	0·2	0·1	trace	0·2	0·2
13	3-Methyl-2-butenyl isovalerate	0·2	0·1	0·1	0·1	0·1
15	3-Methyl-2-butenyl-(3-methyl)-2-butenoate	trace	trace	trace	trace	trace
19	*n*-Pentadecane	trace	trace	trace	?	?
20	3-Methyl-3-butenyl-(3-methyl)-2-butenoate	0·2	trace	0·1	0·1	0·3
23	3-Methyl-2-butenyl-(3-methyl)-2-butenoate	trace	trace	trace	trace	trace
29	*n*-Heptadecane	trace	trace	trace	0·1	0·1
35	Benzyl isovalerate	0·1	0·1	trace	0·1	trace
36a 36b	*n*-Nonadecane *n*-Tetradecanal	trace	trace	trace	trace	?
37	β-Phenylethyl isovalerate	0·1	trace	trace	0·1	?
40	Benzyl-(3-methyl)-2-butenoate	0·1	0·1	trace	0·1	trace
42	*n*-Heneicosane	trace	?	trace	trace	?
43	Pelargonic acid	0·1	—	trace	—	—
44c	β-Phenylethyl-(3-methyl)-2-butenoate	0·2	0·1	trace	trace	trace
48b	*n*-Tricosane	trace	trace	?	trace	0·1
52b	*n*-Pentacosane	trace	trace	trace	trace	trace
56	*n*-Docosanal	trace	?	?	?	?

* (1) Large batch (commercial distillation); Vancouver Island location. (2) Young tree growing in greenhouse, November 1968; Vancouver nursery stock. (3) Combined oil from five young trees growing in greenhouse, March 1969, Vancouver nursery stock. (5) Sealed sample from medium-sized tree at Forbidden Plateau, August 1968. (6) Sealed sample from 50–55-year-old tree, Research Forest of the University of British Columbia at Haney, B.C., Canada, March 1969.

† Names in parentheses represent tentatively identified compounds. Peak No. 10, 25, 32, 33, 39, 41b and 50 (cf. Fig. 1) could not be identified.

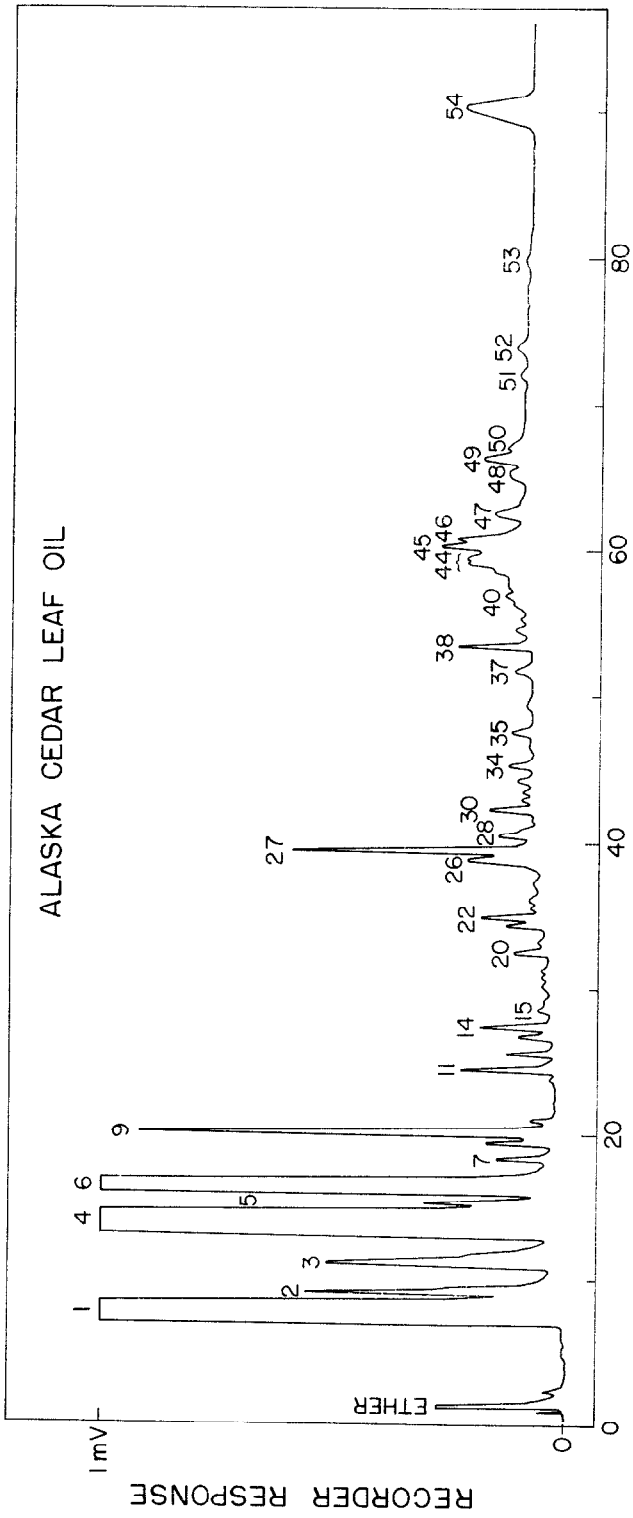

FIG. 1. GAS CHROMATOGRAM OF THE LEAF OIL OF *Chamaecyparis nootkatensis* L. (SAMPLE 1, LARGE BATCH FROM VANCOUVER ISLAND). 350 × 0·4 cm o.d., 5% PEG 20 M on Aeropak 30 (70–80 mesh); PTGC 50 to 215° at 2·9%/min.

unusually large number of minor constituents as well as the variety in their chemical structure. In addition to the mono- and sesquiterpenes (Tables 1A, 1B) which are usually found in most conifer leaf oils,[3-9,12,14] nine diterpene hydrocarbons, phyllocladan-16-ol, (+)-manoyl oxide, (+)-13-epimanoyl oxide, two new diterpenoids (–)-8-epimanoyl (III) and (+)-8,13-diepimanoyl (IV) oxide[15] (Table 1B), and a new series of branched-chain and aromatic esters of isovaleric, 3-methyl-2-butenoic (senecic), and 3-methyl-3-butenoic acids (Table 1C) were isolated.

N. H. Andersen and D. D. Syrdal,[16] report in considerable detail on the sesquiterpenes of this leaf oil and found, in addition to the compounds listed in Table 2, α-ylangene, longifolene, α-bisabolene, α-, β-, and γ-curcumenes, and β-farnesene. They also found α-pinene, 3-carene, and limonene to be the major components, but did not investigate the diterpenoid and non-terpenoid components.

The mixture of diterpenes could be resolved satisfactorily on the OV-17 (a phenyl methyl silicone polymer[17] and PEG-20 M columns, the former resolving also manoyl- and 13-epimanoyl oxide. In addition to the eight known hydrocarbons a new hydrocarbon (peak 44a) was isolated. This hydrocarbon had the typical spectral properties (see Experimental) of an isopimaradiene, but these (as well as the GLC retention) were not identical with those of isopimaradiene, sandaracopimaradiene, and isopimara-8(9),15-diene. Catalytic hydrogenation gave mainly isopimar-8(9)-ene and isomerization with anhydrous hydrogen chloride gave the same four hydrocarbons in approximately the same ratio as produced by isomerization of isopimaradiene. Hence, this hydrocarbon can only have structure I (two possible epimers) or II. Since the latter is subject to considerable strain, I is the more likely.

Although the diterpenes with the isopimaradiene skeleton predominate, those of the pimaradiene and phyllocladene type are also elaborated in the leaves of the Alaska cedar. The isolation of phyllocladene (peak 49a) and isophyllocladene (peak 47b) from the polar (methanol) fraction in the prefractionation procedure indicates that dehydration of phyllocladan-16-ol has taken place, and it is possible that these two hydrocarbons, as isolated from the hydrocarbon fraction, are also artifacts. It is also noteworthy that isohibaene (peak 46a) does not appear to have been isolated from a natural source before; however, it was synthesized by Wenkert *et al.*[18] Identification of mg amounts of isohibaene and the related diterpenes presented no serious obstacles because of their characteristic NMR spectra.[18-23]

Of considerable interest is the isolation of a number of non-terpenoid components (see Table 1C). An unusual series of esters was isolated in addition to the homologous series of odd-carbon number hydrocarbons (C_{23}–C_{25}; peaks 19, 29, 36b, 42, 48b and 52b) and the three *n*-alkanals pelargonaldehyde (peak 12), *n*-tetradecanal (peak 36a), and *n*-docosanal (peak 56a). The 3-methyl-3- and 3-methyl-2-butenyl esters of isovaleric acid (peaks 11 and

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[16] N. H. Andersen and D. D. Syrdal, *Phytochem.* **9**, 1325 (1970).
[17] Pierce Chemical Co., Rockford, Illinois, U.S.A.
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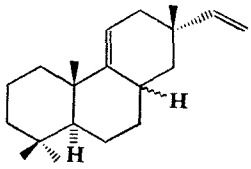
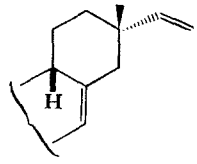
[22] Y. Kitahara and A. Yoshikoshi, *Tetrahedron Letters* 1771 (1964).
[23] J. W. Ap-Simon, W. G. Craig, P. V. Demarco, D. W. Mathieson and W. B. Whalley, *Tetrahedron* **23**, 2375 (1967).

13), 3-methyl-3-butenoic acid (peak 15), and 3-methyl-2-butenoic acid (senecic acid) (peaks 20 and 23), as well as benzyl- and β-phenyl ethyl isovalerate (peaks 35 and 37) and β-phenyl ethyl-(3-methyl)-2-butenoate (peak 44c) do not appear to have been isolated from conifers or other natural sources before. Benzyl-(3-methyl)-2-butenoate (peak 40) was prepared by Yamada *et al.*[24] The spectra of the natural and synthetic esters (see Experimental) were completely superimposable and the peak assignment in Table 1 was confirmed by co-injection. The characteristic NMR signals are summarized in Table 3. There appears to be an asymmetry effect in the NMR spectra of the branched-chain esters; the chemical shifts of the two methyl groups of the 2-butenoates differ by 25–30 ppm, and that of the 2-butyl esters by 3–5 ppm. Some fine-splitting of the 3-proton signals was also detected.



(I)



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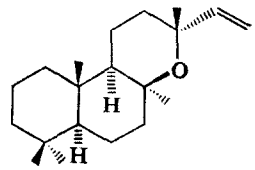
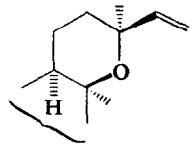


(III)



(IV)

This series of esters represents a new group of components of the leaf oils of conifers, and the finding of isopentenyl esters may be significant in that they may be looked upon as hemiterpenoids. Hence, it is possible that in the Alaska cedar an early branch in the mevalonic acid pathway of terpene synthesis is operative. Under the conditions of isolation of the individual components of the Alaska cedar leaf oil no evidence of the free isopentenyl alcohols was obtained (the gas chromatograms were devoid of peaks in the isoamyl alcohol range). The fact that free mono-, sesqui-, and diterpenoid alcohols as well as some acetates were isolated, but not the esters of the isopentenyl acids and these terpene alcohols indicates that two different sites of biosynthesis, compartmentalization, or unusual enzyme specificity are involved in the formation of these esters.

The *n*-alkanes and *n*-alkanals are not typical components of conifer leaf oils or other essential oils, and from the work of Eglinton and co-workers[25] one may assume these to be derived from the cuticle wax. Such components of the cuticle wax were not encountered in our previous analyses of conifer oils. The presence of pelargonic acid (peak 43) may be explained by autoxidation of pelargonaldehyde. On standing in the cold and dark for many weeks the oil showed an increase of the pelargonic acid with concomitant reduction of pelargonaldehyde; several other autoxidation products (e.g. *p*-cymene) became noticeable too.

[24] S. YAMADA, M. TANIGUCHI and K. KOGA, *Tetrahedron Letters* 25 (1969).

[25] G. EGLINTON and R. J. HAMILTON, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 187, Academic Press, London (1963).

From the chemotaxonomic and phylogenetic points of view the presence of the branched-chain esters is significant in that they may form a unique biochemical character. To these one may add the related series of diterpenes, the four epimeric manoyl oxides, the *n*-alkanes, and the *n*-alkanals, since these too have not been detected in conifer leaf oils before. A detailed analysis of the leaf oils of other *Chamaecyparis* species should reveal whether these are unique to the Alaska cedar alone, and hence justify the botanical reclassification as suggested by Erdtman and Norin,[2] or whether these are typical constituents of the genus. The earlier analyses of the leaf oils of the Asiatic cedar species[9] do not permit a conclusion on this point. Work on the leaf oil of one other North American *Chamaecyparis* species, *C. lawsonia* (Port Orford Cedar) has been commenced in this laboratory and a preliminary GLC analysis indicates that the new diterpenoid oxides and at least some of the branched-chain esters are absent.

The presence of sesquiterpenes derived from both *cis*- and *trans*-farnesol according to the scheme of Hendrickson[26] deserves some comment. In the leaf oils of the genus *Picea* only the cadinane types (*cis*-farnesol derived) have been found[6,8] but in the genus *Juniperus* both cadinane- and selinane-type (elemol, eudesmol, etc.; *trans*-farnesol derived) were encountered.[4,5,12,14] In a very detailed study of the composition of the blister resins of the genus *Abies*, Smedman *et al.*[27,28] have found both *cis*- and *trans*-farnesol derived sesquiterpenes, and this holds also for the heartwood extractives of the order Cupressales.[2] Hence, the type of sesquiterpene found in conifers does not appear to offer an aid to chemotaxonomic analysis. However, the presence of thujopsene (peak 24) and cedrol (peak 41) as well as the diterpene alcohol phyllocladan-16-ol (peak 55) is typical for a member of this natural order. Again it remains to be determined whether the presence in the Alaska cedar leaf oil of cadinane-type sesquiterpenes, as well as the typical monoterpenes of the order *Pinales* (myrcene, 3-carene, β-phelladrene, α-terpinene, terpinolene, bornyl acetate, α-terpineol and piperitone[6] is exceptional or typical for the genus *Chamaecyparis*. The chemical composition of the leaf oil of the Alaska cedar shows more similarity with those of the genus *Juniperus* than with the genus *Thuja*. In the latter genus, compounds of the thujene-thujone type appear to be characteristic.[29,30] Sabinene and thujene have been found in the leaf oils of *C. taiwanensis* and *C. formosensis*,[9] whereas in that of *C. nootkatensis* we found only a small amount of sabinene; thujene could be present as a trace component.

The results obtained with the leaf samples collected from different trees (Tables 1 and 2) show that the variation from tree to tree is small provided the foliage is picked during the dormant season. The samples collected from two trees on Forbidden Plateau late in August show a relatively large quantitative difference (samples 4 and 5). This difference can be a result of the time of collection,[8] August being a time of the growing season at which the stable quantitative composition of the dormant season may not have been reached.

For reproducible results it is essential that precaution against evaporation losses of the lower boiling terpenes are taken. When the leaf samples were transported in a sealed container virtually identical quantitative results with those of the samples distilled near the site of collection were obtained (Table 2, samples a and b). When the leaves were enclosed in polythene bags (sample c) or packed unsealed into the cooler box (sample 6d) considerable losses of α-pinene etc. were evident.

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[27] L. A. SMEDMAN, E. ZAVARIN and R. TERANISHI, *Phytochem.* **8**, 1457 (1968).
[28] L. A. SMEDMAN, K. SNAJBERK, E. ZAVARIN and T. R. MON, *Phytochem.* **8**, 1471 (1968).
[29] E. VON RUDLOFF, *Can. J. Chem.* **39**, 1200 (1961).
[30] E. VON RUDLOFF, *Phytochem.* **1**, 195 (1962).

TABLE 2. EFFECT OF TRANSPORTATION AND STORAGE ON THE % COMPOSITION OF THE LEAF OIL OF THE ALASKA CEDAR

Peak No.	Compound	Sample No.* 4 *a*	4 *b*	4 *c*	5 *a*	5 *b*	5 *c*	6 *a*	6 *b*	6 *c*	6 *d*
1	α-Pinene	14·7	15·5	8·0	32·3	32·8	17·7	31·5	33·0	18·6	16·3
3	β-Pinene (+ sabinene)	1·1	1·2	0·5	3·0	3·1	1·1	2·8	2·9	1·0	0·8
4a	3-Carene	23·1	24·8	21·8	25·1	24·8	26·9	25·1	25·3	28·5	22·5
4b	Myrcene				3·7	3·8	3·7	4·0	4·2	2·2	3·3
6	Limonene (+β-phellandrene)	48·0	48·3	58·5	25·5	25·3	46·6	23·8	26·3	38·2	46·0
9	Terpinolene	2·8	2·5	2·3	2·3	2·4	2·8	2·5	2·5	2·6	2·2
22	Terpinene-4-ol	1·2	0·9	1·2	0·7	0·7	1·1	0·7	0·7	0·8	0·9
27	α-Terpinyl acetate	1·1	0·8	1·2	1·0	1·1	1·6	1·0	1·0	1·1	1·1
38	Nerolidol	0·1	0·1	0·2	0·4	0·4	0·6	0·1	0·1	0·1	0·2
45	Bisabolol	0·1	0·1	0·2	0·3	0·3	0·4	0·2	0·3	0·4	0·5
53	8-Epimanoyl oxide	0·9	0·8	1·2	0·6	0·7	1·1	0·4	0·5	0·6	0·7

* Nos. 4 and 5: collected on Forbidden Plateau, Vancouver Island, August 1968. No. 6: collected near Placid Lake, Research Forest of the University of British Columbia at Haney, March 1969.

(*a*) Steam-distilled near site of collection within 1 hr of cutting foliage.

(*b*) Transported in sealed glass insert of steam-distillation apparatus in an insulated cooler.

(*c*) Transported in polyethylene bags in an insulated cooler.

(*d*) Transported unsealed in an insulated cooler.

In Table 1 the percentage composition of the leaf oil from old and young trees (samples 1–3) is compared and no significant differences can be detected. Similarly, the variation from one young tree to another was found to be very small (samples 2, 3 and Experimental), and collection in November (sample 2) or March (sample 3) does not appear to affect the composition to any noticeable degree except that there was a change in the content of limonene. A somewhat larger difference was found in the oil originating from the trees growing at Forbidden Plateau (sample 5) and at Haney (sample 6) as compared with the large sample, and such differences may represent variations from one population to another. The data obtained suggest that the leaf oil of the Alaska cedar is very well suited for detailed chemotaxonomic studies, both as far as variation within populations is concerned, as well as a possible correlation with related, or presumably related cedar species.

EXPERIMENTAL

The GLC apparatus and methods of column preparation and analyses, as well as most of the columns, have been described before.[6,8,12–14] In addition, columns with OV-17 phenyl methyl silicone polymer[17] (1 or 5% on Aeropak 30, 15% on Gas Chrom P) were employed. Preparative separations were carried out in two stages; a preliminary fractionation with 50–200-μl aliquots on the preparative gas chromatograph (90–180 × 0·9 cm o.d. columns with 15–25% liquid phase) using 1 ml glass traps cooled in ice water, and smaller scale fractionation of 5–25-μl aliquots on 180–200 × 0·6 cm o.d. columns using teflon tubing as traps[31] (hand-collection).

I.r. spectra were either obtained as films between NaCl plates (Perkin–Elmer model 21 spectrophotometer) or in AgCl microcells (0·01 mm path length extro cells, Beckmann Instruments Co.; Perkin-Elmer model 257 spectrophotometer with beam condenser). NMR spectra were measured in CCl_4 with tetramethylsilane as internal standard (Varian A-100 100 MHz instrument). Optical rotations were determined with the undiluted oil samples and in $CHCl_3$ (1–5%) on components which were isolated in sufficient amounts.

[31] R. TERANISHI, R. A. FLATH, T. R. MON and K. L. STEVENS, *J. Gas Chromatog.* 3, 206 (1965).

Plant Material and Recovery of the Volatile Leaf Oil

The main batch of cedar foliage was collected on 5 April 1967 near Robertson and Flat creeks, Mesatchie Lake district, Vancouver Island, from trees which were over 100 years old. The foliage was stored in the cold until commercial steam-distillation (Kent Chemicals Ltd., Vancouver; special contract) was carried out. This oil had n_D^{25} 1·4727, d_4^{25} 0·874, $[\alpha]_D^{25}$ − 0·2° (sample 1). All other leaf oil samples were obtained by laboratory-scale steam-distillation (0·1–1 kg leaves) in the apparatus described previously.[8,13] Samples 2 and 3 were obtained from young trees (6–10 yr old) growing in the greenhouse of this laboratory and originating from a Vancouver nursery (H. M. Eddie & Sons, Ltd.). Sample 2 was derived from the foliage of a single tree steam-distilled in November 1968. In March 1969 the foliage from five individual trees was steam-distilled. The quantitative composition was virtually the same for all five samples and hence these were combined to give sample 3. To test the effect of transportation on the leaf oil composition small-scale steam-distillation (0·1 kg) was carried out at the site of collection on Forbidden Plateau, Vancouver Island, August 1968. The foliage from a young and a medium-sized tree (samples 4 and 5) was divided into three equal portions, the first (a) was steam-distilled near the site of collection, the second (b) was placed into the glass insert of the steam-distillation apparatus,[13] which was sealed temporarily at each end, and the third (c) was placed in polythene bags. The latter two samples were stored in an insulated box under ice and were transported to this laboratory, where they were stored at −10° until steam-distillation 1–2 weeks after collection. An additional batch of yellow cedar foliage was collected near Flatiron Lake of the Research Forest of the University of British Columbia at Haney (sample 6) in March 1969. It was divided into four parts, three smaller portions (1 kg each) being treated or packed as above (a)–(c) and the fourth (5 kg) was transported unsealed in the cooler box (d). The duration of the distillation was 4–6 hr in small-scale experiments and 8–16 hr in large-scale work. Further distillation did not increase the yield of oil markedly but some increase in the higher boiling components was obtained. The yield of oil from sealed samples varied from 1·8 to 2·4% whereas that from the unsealed leaf samples was 1·5–1·8%. The percentage composition of each oil sample was determined from duplicate runs on the 5% PEG- 20 M analytical column (5-μl aliquots, internal normalization method, peak area integration with a Varian-Aerograph model 471 digital integrator) and the results obtained are shown in Tables 1 and 2. The physical constants of the oil samples were: n_D^{25} 1·472–1·473; d_4^{25} 0·873–0·875; $[\alpha_D^{25}]$ − 0·2–3·5°.

TABLE 3. NMR SIGNALS OF THE ESTERS OF BRANCHED-CHAIN (C_5) ACIDS (100 MHz, IN CCl_4)

Moiety	Signal (ppm)	Coupling constant (Hz)
CH_3 CH_3>CH—	near 1·3 (1 H, m) 0·91–0·95 (6 H, d*)	 7
CH_3 CH_3>C=CH—CH_2O	5·25–5·26 (1 H, s*) 4·43–4·48 (2 H, d†) 1·70–1·87 (6 H, d)	7, 2 7 2–4
CH_3 CH_3>C=CH—CO—O—	5·54 (1 H, s*) 2·13 (3 H, d) 1·86 (3 H, d)	1 1 1
CH_2=C— CH_3	4·69–4·70 (2 H, m) 1·76 (3 H, s†)	
—CH_2—CH_2O—	4·09 (2 H, t*) 2·27–2·28 (2 H, t*)	7 7
—CH_2—CO—O—	2·06–2·13 (2 H, m)	2
Phenyl—CH_2O—	7·24 (5 H, s) 5·00 (2 H, s)	
Phenyl—CH_2CH_2O—	7·14 (5 H, s) 4·18 (2 H,) 2·84 (2 H,)	 7 7

* With fine splitting.
† Relatively broad signal.

Prefractionation and Isolation of Individual Components

The leaf oil (10·0 g) was chromatographed on deactivated silicic acid (120 g).[5,32] Elution with petrol (Skelly solve F, b.p. 40–50°, 500 ml) gave a hydrocarbon fraction A (8·6 g), elution with CH_2Cl_2 (450 ml) gave a mid-fraction B (0·75 g), and elution with methanol (500 ml) a polar fraction C (0·7 g). It was found that the amounts of the higher boiling trace components obtained in these fractions was rather small. Hence, a major portion of the low-boiling hydrocarbons was removed by distilling the oil fairly rapidly *in vacuo* (30 mm Hg) until about two-thirds had distilled over. The residue (b.p. > 80°/30 mm) was then chromatographed as described above. GLC analysis showed that no significant rearrangements had occurred during the distillation.

A. Hydrocarbons

Fraction A was prefractionated on a 90 × 0·9 cm o.d. 25% SE-30 column (non-linear temperature programmed from 80 to 210°). Three monoterpene-(A-I–A-III), a sesquiterpene-(A-IV) and a diterpene fraction (A-V) were collected. The monoterpenes were separated on a 200 × 0·6 cm o.d. 15% PPE (MCS-562) column (isothermal at 85, 90 and 108°) and each isolated fraction was purified further by GLC on a 15% PEG 20 M column. α-Pinene, $[\alpha]_D^{23} - 57{\cdot}0°$ (peak 1), camphene (peak 2b), β-pinene (peak 3a), 3-carene, $[\alpha]_D^{24} + 9{\cdot}5°$ (peak 4a), myrcene (peak 4b) limonene, $[\alpha]_D^{24} + 11{\cdot}8°$ (peak 6a), β-phellandrene (peak 6b), *p*-cymene (peak 8), terpinolene (peak 9) and 1-methyl-4-isopropenyl benzene (peak 14) were identified (i.r., NMR, u.v. spectra, retention times), whereas peaks 2a, 3b, 5 and 7 could only be identified tentatively by their relative retention times as fenchene, sabinene, α-phellandrene and γ-terpinene respectively.

Fraction A-IV was prefractionated on a 180 × 0·6 o.d. 15% PEG 20 M column (150° isothermal) and each subfraction collected was purified by GLC on 400 × 0·45 cm o.d. 5% PEG 20 M. α-Cubebene (peak 16), α-copaene (peak 18) *n*-pentadecane (peak 19), thujopsene (peak 24), *ar*-curcumene (peak 31), and calamenene (peak 34a) were identified. Peak 30a was found to be a mixture of γ- and δ-cadinene (i.r., NMR); this pair of isomers was encountered previously in juniper leaf oils[4,11,15] and could not be resolved on these GLC columns on a preparative (3–10 mg) scale.

Fraction A-V (2·1 g) was chromatographed on a column of freshly prepared silicic acid (30 g), which was eluted with petrol (Skelly solve F, b.p. 40–50°). The first and second subfractions, AV-1 and AV-2, were eluted with 30 ml solvent each, and a third subfraction, AV-3, was obtained by eluting with a further 300 ml. GLC on the 180 × 0·6 o.d. 15% PEG 20 M column (192° isothermal) and repurification on the OV-17 column resulted in the isolation of *n*-heptadecane (peak 29), *n*-nonadecane (peak 36a), *n*-heneicosane (peak 42), isohibaene (peak 46a), isophyllocladene (peak 47b), *n*-tricosane (peak 48b) and *n*-pentacosane (peak 52b) from fraction AV-1; diterpene I (peak 44a), isopimara-8(9),15-diene (peak 44b), isohibaene (peak 46a), sandaracopimaradiene (impure, peak 47a) isophyllocladene (impure, peak 47b), isopimaradiene (peak 48a) phyllocladene (peak 50), abieta-7,13-diene (peak 51) and dehydroabietadiene (peak 52a) from fractions AV-2 and AV-3.

B. Mid-fraction B

The mid-fraction B was prefractionated on the 25% SE-30 column (80–210°) and five crude subfractions (B-I–B-V) were collected. Subfractions B-I and B-II were chromatographed on the 15% PEG 20 M column 125° and 158° respectively) and the following compounds were isolated sufficiently pure to permit positive identification: 3-methyl-3-butenyl isovalerate (peak 11), pelargonaldehyde (peak 12), 3 methyl-2-butenyl isovalerate (peak 13), 3-methyl-3-butenyl-(3-methyl)-3-butenoate (peak 15), 3-methyl-3-butenyl-(3-methyl)-2-butenoate (peak 20), bornyl acetate, $[\alpha]_D + 27°$ (peak 21), 3-methyl-2-butenyl-(3-methyl)-2-butenoate (peak 23), α-terpinyl acetate, $[\alpha]_D - 0{\cdot}8°$ (peak 27), benzyl isovalerate (peak 35), *n*-tetradecanal (peak 36b), β-phenyl ethyl isovalerate (peak 37), nerolidol (peak 38), benzyl-(3-methyl)-2-butenoate (peak 40), β-phenyl-ethyl-(3-methyl)-2-butenoate (peak 44c) and bisabolol (peak 45). The reference spectra for the new esters were obtained from purified synthetic reference compounds.

Subfraction B-III was further fractionated on a 15% QF-1 column (180 × 0·6 o.d., 80–200° non-linear programming) and the resulting fractions were purified on the 15% PEG 20 M column, when diterpene I (peak 44a), isopimara-8(9,15-diene (peak 44b), sandaracopimaradiene (peak 47a), isopimaradiene (peak 48a), and a mixture of manoyl and 13-epimanoyl oxides (peaks 49b and c) were isolated. The latter oxide mixture could be resolved on the 15% OV-17 column (198°) to give manoyl oxide, $[\alpha]_D + 21{\cdot}8°$ and 13-epimanoyl oxide, $[\alpha]_D + 34°$.

Fraction B-IV and B-V were purified on the 15% PEG 20 M column and 8-epimanoyl oxide (III)[15] m.p. 44–45° (peak 54), *n*-docosanal (peak 56), and impure 8,13-diepimanoyl oxide (IV) (15) (peak 53) were isolated.

Diterpene I (peak 44a) was obtained in trace amounts only and could be separated from isopimara-8(9),15-diene only with difficulty, using either the PEG or OV-17 columns. Its i.r. and NMR spectra are

[32] E. KUGLER and E. SZ. KOVATS, *Helv. Chim. Acta* **46**, 1480 (1963).

typical of the isopimaradiene-type of diterpene: ν_{max}^{liq} 3080, 1818, 1630, 1408, 985, 900 cm^{-1} (—CH=CH_2); 3020, 810 cm^{-1} (—CH=C<); 1385, 1365 cm^{-1} (>$C(CH_3)_2$); δ 5·68 ppm, quartet, 1H, $J = 11$, 19 Hz, δ 4·81 ppm, 2 overlapping doublets, 2H, $J = 11$, 19, 2 Hz (—CH=CH_2), δ 5·29 ppm, broad singlet with fine splitting, 1H (—CH=C<), 1·04, 0·97, 0·89, 0·85 ppm, singlets, 3H each. Catalytic hydrogenation (Brown microhydrogenator) gave mainly isopimar-8(9)-ene. Isomerization with anhydrous HCl in $CHCl_3$[33] gave a mixture of the 8(9),15-; 7,15; and 8(14),15-isomers and an unknown hydrocarbon in the ratio 78:8:9:5. Under the same conditions isopimaradiene gave the same mixture of isomers in a ratio of 7:1:1:1.

C. Polar Fraction

Fraction C solidified on standing and repeated recrystallization from methanol gave phyllocladan-16-ol (peak 55); white needles, m.p. 190–1°, $[\alpha]_D + 18·6°$ (lit.[34] m.p. 184–518°, $[\alpha]_D + 16°$;[35] m.p. 181–182°, $[\alpha]_D + 14·5°$). The mother liquors were combined and fractionated on the 25% SE-30 column (80–310°). The crude fractions were fractionated further on the 15% PEG 20 M column and the following components were isolated sufficiently pure to permit positive identification: Terpinen-4-ol, $[\alpha]_D - 15·8°$ (peak 22); α-terpineol, $[\alpha]_D - 31·1°$ (peak 26), piperitone, $[\alpha]_D + 8·0°$ (peak 28), citronellol (peak 30b) nerolidol, $[\alpha]_D + 14·3°$ (peak 38), cedrol (peak 41), pelargonic acid (peak 43), bisabolol, $[\alpha]_D + 50·7$ (peak 45), α-cadinol (peak 46b), isophyllocladene (peak 47b) and phyllocladene (peak 49a). Peak 34b was also obtained almost pure and its identity with 1-methyl-4-(α-hydroxy isopropenyl)-benzene was confirmed by dehydration over active alumina[36] (Woelm, almost neutral, Grade I) to 1-methyl-4-isopropenyl benzene.

Peaks 10, 17, 25, 32, 33 and 39 could not be obtained in sufficient amounts or purity to permit identification.

Synthesis of Branched-Chain and Aromatic Esters

3-Methyl-3-butenyl-, 3-methyl-2-butenyl-, and benzyl isovalerate, 3-methyl-3-butenyl-, 3-methyl-2-butenyl-, and benzyl-(3-methyl)-2-butenoate were prepared by Fischer's method.[37] The latter ester has been prepared by Yamada *et al.*[22] Each ester was purified by GLC and the i.r. and NMR spectra were recorded.

3-Methyl-3-butenyl-(3-methyl)-3-butenoate was prepared as follows: a mixture of 3-methyl-3-butenyl alcohol (0·8 g), acetic acid (0·5 ml) and chromic acid in dil. H_2SO_4 (5 ml; 1·0 g CrO_3, 1·8 ml H_2SO_4 in 7·5) was allowed to react for 20 min at 10°. Aq. NaOH (1·4 ml, 50%) was added and the mixture was extracted with ether. The ethereal solution was dried and evaporated to give the crude ester, which was purified by GLC.

β-Phenylethyl isovalerate was prepared as follows: isovaleric acid (1·0 g) was dissolved in 3·5 N NH_4OH and to this was added aq. $AgNO_3$ (1·7 g in 2 ml H_2O). The precipitated salt was filtered off, washed with water, and dried *in vacuo* to constant wt. The silver salt (0·7 g) was mixed with β-phenylethyl bromide (0·7 g) and benzene (1 ml) and the mixture was heated on a steam bath for 5 hr. Excess water was added and the mixture was extracted with ether. The ethereal solution was washed with aq. $NaHCO_3$ dried and evaporated to give the crude ester, which was purified by GLC. β-Phenylethyl-(3-methyl)-2-butenoate was prepared from senecic acid (3-methyl-2-butenoic acid) and β-phenylethyl bromide as above.

The i.r. spectra of these esters showed the typical ester carbonyl (1735 or 1715 cm^{-1}), vinylidene (3080, 1650, 890 cm^{-1}), trisubstituted ethylenic (1675–1650 and 850–825 cm^{-1}), and monosubstituted phenyl (1605, 1585, 1500, 740 and 690 cm^{-1}) absorption bands. The characteristic NMR signals are summarized in Table 3.

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