Volatile Components of Douglas Fir Needles

T. Sakai,[1] H. Maarse,[2] R. E. Kepner, W. G. Jennings, and W. M. Longhurst

Volatile components of needles of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, isolated by steam distillation and ether extraction, were separated by repetitive gas chromatography on dissimilar substrates, utilizing both packed and wide-bore capillary columns. Individual components were characterized by relative retentions on several columns, Kovats' indices, and infrared spectroscopy. Compounds identified include α-pinene, camphene, β-pinene, 3-carene, myrcene, limonene, 2-hexenal, ethyl caproate, γ-terpinene, terpinolene, ethyl caprylate, citronellal, linalool, fenchyl alcohol, bornyl acetate, terpinen-4-ol, β-caryophyllene, citronellyl acetate, α-terpineol, citronellol, geranyl acetate, and farnesyl acetate; *p*-cymene and farnesol were identified on the basis of relative retentions only. Infrared spectra were obtained for two additional terpene alcohols and an aromatic alcohol which could not be identified.

The essential oils of coniferous plants represent fairly complex mixtures and have been the subject of many investigations. Cvrkal and Janák (1959), on the basis of gas chromatographic retention data, suggested identities for several constituents from the essential oils isolated from conifers of Czechoslovakia, as did Ognyanov and Tzankova (1966) for certain conifers of Bulgaria and Okay (1963) for various species of conifers. Juvonen (1966) made an exhaustive study of oil of the various parts of the branches of *Pinus silvestris* L. (Scotch pine) in Finland and determined the relative amounts of the various constituents as a function of the growing season. The essential oils isolated from the needles from several species of junipers have been investigated by Karlsen and Svendsen (1965), von Rudloff (1963), and von Rudloff and Couchman (1964). Von Rudloff (1961) utilized gas chromatographic data and infrared spectroscopy to identify a variety of terpenes from the volatile leaf oil from eastern white cedar; (1962a) from black, white, and Colorado spruce; and (1964) from Sitka and Engelmann spruce. Von Rudloff (1962a) observed that mature leaves contained more oil than young growth, and that the relative amounts of the various constituents varied as a function of the growing season. Later, Couchman and von Rudloff (1965) identified a variety of terpenes from the neutral oil of a creeping juniper, and von Rudloff (1966) examined the volatile oil components of two populations of red spruce. In 1964 and in each of the above two studies, von Rudloff suggested that the oil composition could form a basis for chemotaxonomy. Zavarin and Snajberk (1965) and Zavarin (1966) made a similar suggestion as a result of their studies of the composition of fir blister resins.

This study, a portion of a larger project concerned with palatability preferences of foraging ruminants, is directed to the investigation of the possible relationship of the volatile terpene content of Douglas fir needles to their acceptability by browsing animals. Early work on the essential oils of Douglas fir has been reviewed by Guenther (1952). Among the compounds reported were furfural, *l*-α-pinene, *l*-β-pinene, *l*- and *dl*-limonene, borneol, citral, geraniol, nerol, geranyl acetate, neryl acetate, camphene, and dipentene. Recently, Hancock and Swan (1965) investigated the composition of the oil isolated from Douglas fir wood and reported the identification of several terpenes.

This paper presents the results of the investigation of the volatile oil isolated from the mature needles of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco.

EXPERIMENTAL

Methods and Procedure. DOUGLAS FIR NEEDLE OIL. Mature needles from trees located in the Pacific Coast Range west of Ukiah, Calif., were picked, promptly slurried in a Waring Blendor with a minimum amount of distilled water, and steam distilled at atmospheric pressure for 4 hours. The distillates were saturated with sodium chloride and extracted with ether; the ether extract was dried over anhydrous $MgSO_4$, and the major portion of the ether was removed through an all-glass distillation column. Approximately 8 ml. of oil was obtained from 1.28 kg. of mature needles.

Apparatus and Materials. GAS CHROMATOGRAPHY. Initial separations utilized an Aerograph Autoprep, Model 700, fitted with an 18-foot × 1/4-inch O.D. stainless steel column packed with 10% Triton X-305 on 60- to 80-mesh Chromosorb W; injection temperature, 180° C.; He flow, 60 ml. per minute; column temperature programmed at 4° C. per minute from 58° to 235° C.; thermal conductivity detection; 100-μl. injections. Fractions were collected in thin-walled glass capillaries and subjected to repetitive separations (Sevenants and Jennings, 1966) prior to infrared spectroscopy. The repetitive separations were performed on a Beckman Thermotrac containing dual 13-foot × 1/8-inch O.D. stainless steel columns packed with 10% Apiezon L or 10% Carbowax 20M on 60- to 80-mesh Gas Pak F, and fitted with modified injection systems and Carle Microcell detection; injection temperature, 180° C.; He flow 30 ml. per minute; column temperatures were isothermal or nonlinear programmed runs selected to optimize separation of the peak in question. An F&M Model 810 gas chromatograph with dual flame ionization detection was used for determination of relative

Departments of Chemistry, Food Science and Technology, and Zoology, University of California, Davis, Calif. 95616.

[1] Present address, Institute for Food Research, Osaka, Japan.

[2] Present address, Central Institute for Nutrition and Food Research, TNO, Zeist, The Netherlands.

retention times, Kovats' indices, and analytical chromatograms. The instrument was fitted with two 500-foot × 0.03-inch I.D. stainless steel capillary columns, coated with Carbowax 20M + Igepal (20 to 1) and S.F. 96(50) + Igepal (20 to 1), respectively; injection temperature, 170° C.; detector temperature, 210° C.; column temperature, isothermal at 75°, 100°, 135°, or 200° C. for retention data (Table I) or programmed from 65° to 200° C. (Figure 1) for analytical runs; He, H_2, and air flows were 9, 23, and 300 ml. per minute, respectively.

INFRARED SPECTROSCOPY. Infrared spectra were obtained on thin films on ultramicro demountable cells of our manufacturing (Sevenants and Jennings, 1966) requiring 0.08 to 0.1 μl. samples, in a Beckman IR 8 spectrophotometer, fitted with a beam condenser.

RESULTS AND DISCUSSION

Figure 1 presents a chromatogram obtained by injecting 0.2 μl. of the essential oil from mature Douglas fir needles onto a 500-foot, wide-bore capillary column coated with Carbowax 20M. Table I lists the components which were isolated and purified by preparative gas chromatography using a variety of columns and identified by means of analytical gas chromatography and infrared spectroscopy. The relative retention times and Kovats' indices (Kovats, 1961) listed were determined under isothermal conditions on the 500-foot, wide-bore capillary columns and were comparable with the values for the known compounds. With the exception of *p*-cymene and farnesol, identification was substantiated by obtaining matching infrared spectra for the isolated compounds and the corresponding known compounds under identical experimental conditions.

Of the compounds characterized, the pinenes are the most abundant in the isolated oil, with β-pinene being present in approximately twice the amount of α-pinene. Other monoterpene hydrocarbons present in somewhat smaller amounts are limonene, 3-carene, *p*-cymene, myrcene, camphene, and γ-terpinene. Among the oxygenated monoterpenes, citronellyl acetate and α-terpineol are present in largest amounts. Peak 114 contains both geranyl acetate and citronellol which cannot be separated on Carbowax 20M. Separation of these components on the SF 96(50) column indicated that at least 75% of the material in this peak was the geranyl acetate. Several of the major components in this region of the chromatogram are still unidentified. The relative retention data and Kovats' indices for three of these components, corresponding to peaks 99, 121, and 130, are included in Table I. The infrared spectra indicate that these are two

Table I. Composition of Douglas Fir Oil from Mature Needles

Peak No.	Compound	Rel. Ret. Times[a] 20M	Rel. Ret. Times[a] SF96 (50)	Kovats' Indices 20M	Kovats' Indices SF96 (50)	References[b]
14	α-Pinene	0.56	0.68	1036	942	(1–14)
18	Camphene	0.63	0.72	1078	956	(1–14)
20	β-Pinene	0.72	0.81	1120	983	(1–9, 12, 13)
23	3-Carene	0.82	0.92	1156	1013	(4, 5, 7, 9, 12–14)
24	Myrcene	0.85	0.83	1166	988	(1, 4–6, 8, 9, 11–14)
30	Limonene	1.00	1.00	1204	1028	(1–14)
32	2-Hexenal	1.04	0.48	1212	840	
35	Ethyl caproate	1.13	0.82	1229	986	
38	γ-Terpinene	1.23	1.14	1247	1056	(1, 4, 5, 8–14)
39	*p*-Cymene[c]	1.36	0.97	1268	1018	(1, 4, 5, 8–14)
41	Terpinolene	1.43	1.27	1279	1074	(1, 4, 5, 8–12, 14)
61	Ethyl caprylate	0.81	1.32	1436	1183	
70	Citronellal	0.91	1.15	1491	1143	
79	Linalool	1.00	1.00	1533	1097	(1, 12–14)
84	Fenchyl alcohol	1.15	1.08	1580	1125	
85	Bornyl acetate	1.20	1.92	1599	1278	(4, 8, 9, 12)
89	Terpinen-4-ol	1.22	1.10	1601	1129	(1, 4, 8, 10–14)
93	β-Caryophyllene	1.32	0.68	1632	1455	(4, 6)
99	Terpene alcohol	1.40	1.24	1652	1165	
100	Citronellyl acetate	1.44	2.44	1662	1335	(13)
103	α-Terpineol	1.57	1.31	1685	1178	(4, 13)
114	Citronellol	1.96	1.37	1754	1192	(1, 11, 14)
114	Geranyl acetate	1.96	2.80	1754	1363	(3, 13)
121	Terpene alcohol	2.30	1.55	1796	1227	
130	Aromatic alcohol	2.61	1.35	1829	1188	
186	Farnesyl acetate	1.12	1.55	2225	1787	
196	Farnesol[c]	1.25	1.09	2277	1655	

[a] Peaks 14 through 41 relative to limonene (Carbowax 20M column at 75° C.; SF 96 (50) column at 100° C.); peak 61 through 130 relative to linalool (both columns at 135° C.); peak 186, 196, and β-caryophyllene on SF 96 (50) relative to cedrol (both columns at 200° C.)

[b] Reference code:

(1) Couchman and von Rudloff (1965).
(2) Cvrkal and Janák (1959).
(3) Guenther (1952).
(4) Juvonen (1966).
(5) Karlsen and Svendsen (1965).
(6) Ognyanov and Tzankova (1966).
(7) Okay (1963–1964).
(8) von Rudloff (1961).
(9) von Rudloff (1962a).
(10) von Rudloff (1962b).
(11) von Rudloff (1963).
(12) von Rudloff (1964).
(13) von Rudloff (1966).
(14) von Rudloff and Couchman (1964).

[c] On basis of retention times only.

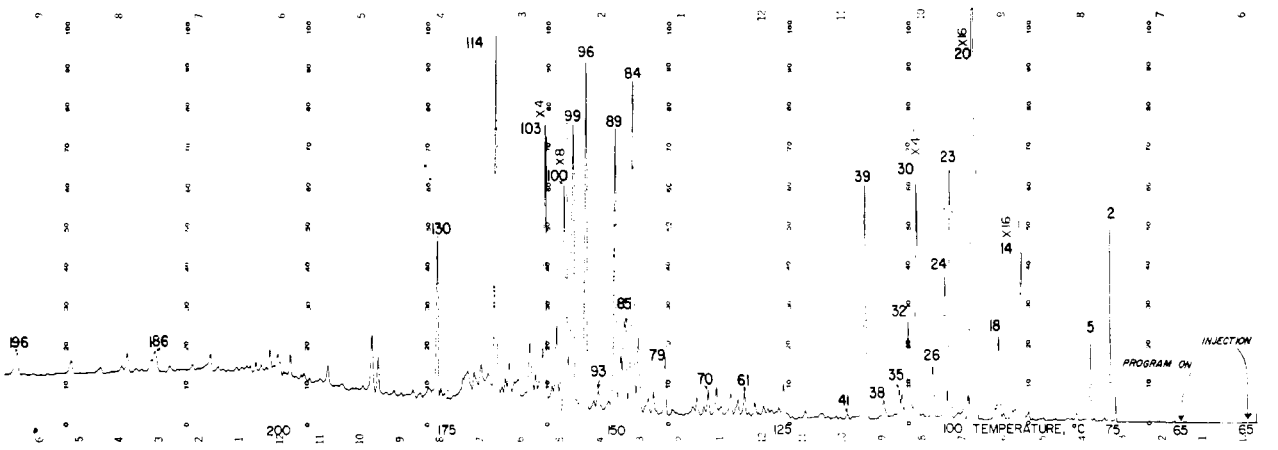

Figure 1. Gas chromatogram of 0.2 μl. of Douglas fir needle oil

500-foot × 0.03-inch I.D. Carbowax 20M column; temperature isothermal at 65° C. for 5 minutes then programmed at 2° C. per minute to 200° C. and run isothermal thereafter; chart speed, 20 inches per hour; He flow rate, 9 ml. per minute; range, 10^3; attenuation, 1 except as indicated

terpene alcohols and an aromatic alcohol, respectively. The only sesquiterpenes identified were β-caryophyllene, farnesyl acetate, and farnesol, although evidence of additional sesquiterpenes was obtained. Farnesol was identified on the basis of relative retention times and Kovats' indices only, but its presence is reasonable in view of the identification of farnesyl acetate. The only compounds identified which were not terpenes were ethyl caproate, ethyl caprylate, and 2-hexenal. The possibility that the 2-hexenal is an artifact formed during the isolation of the oil is suggested by the investigations of Major *et al.* (1963), who observed that 2-hexenal was formed when the leaves of *Ginkgo biloba* L. were ground in an air atmosphere. Many of the trace peaks shown in Figure 1 may be artifacts owing to isomerizations, etc., which occurred during isolation and subsequent processing of the Douglas fir oil. The problems of artifact formation and ratio changes are always factors which must be considered when volatile compounds are isolated and concentrated by classical techniques. Because of these factors the chromatographic trace can give a rough estimate of the relative amounts of the components in the isolated oil, but to relate these back to concentrations in the original fir needles with any degree of certainty is not possible. The question of artifact formation in the Douglas fir oil is under investigation using gas chromatographic head space techniques.

Most of the components identified in the Douglas fir oil have been previously reported as present in other conifers, as indicated in the references in Table I. Not all of the compounds reported in Guenther's summary of the early work on Douglas fir species have been identified in the present investigation of Douglas fir volatiles. Possibly, some of these additional components may be present in some of the peaks which are still not identified in this study, or some of them may be artifacts resulting from the classical isolation and fractional distillation processes utilized in the earlier investigations.

Thus far, most of the major components and several of the minor components of the Douglas fir oil have been characterized. The effect of these components on sheep and deer rumen microbial activity, as part of an investigation of animal preferences and palatability of various browse species, has been studied and is being reported by Oh *et al.* (1967). These investigations, however, are concerned mainly with the major constituents. The animal's sensory response and threshold values for these volatile components may be such that the minor components could be the most important ones as regards palatability of various browse species.

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