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THE PETROLEUM ETHER SOLUBLE EXTRACTIVES OF BRITISH COLUMBIA COASTAL AND INTERIOR-TYPE DOUGLAS FIR

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Abstract—The light petroleum solubles of one Coastal- and two Interior-type Douglas firs (*Pseudotsuga menziesii* (Mirb.) Franco) have been examined by gas-liquid and thin-layer chromatography. This was an attempt to identify the underlying cause of gluing difficulties sometimes encountered in this species after high temperature drying of wood veneers. The total yield of light petroleum solubles and the qualitative, as well as quantitative yields of steam volatiles, fatty acids, resin acids, combined acids, and unsaponifiables varied greatly both between trees and within trees sampled. Interior-type fir wood differed mainly by having a significantly higher content of the even-numbered C_{20} to C_{24} fatty acids.

INTRODUCTION

IN A previous paper[1] the underlying cause for "inactivation", a heat-induced change in the surface of wood veneer that inhibits diffusion of moisture into the wood thereby causing subsequent gluing difficulties, had been investigated. It was demonstrated, by the use of Douglas fir† wood (*Pseudotsuga menziesii* (Mirb.) Franco), that the formation of an inactivated surface could be prevented by extraction with light petroleum, either before or after the wood was dried.

The objective of the work described here was to examine compositional differences of the light petroleum extracts from these veneers in order to find the agent(s) responsible for inactivation. Veneer was collected from British Columbia Coastal and Interior-type Douglas fir trees that exhibited no, moderate, or severe susceptibility to the development of inactivation (Table 1). The Coastal tree chosen for this investigation had shown no effect of high temperature;[1] it provided the nonsusceptible material. Veneer was collected from the sapwood, the outermost heartwood (Heart 1) and at an intermediate heartwood (Heart 3) position to provide replicates of this type of wood (see Ref. 1 for complete descriptions). Since the sapwood, outer heartwood (Heart 1) and intermediate heartwood (Heart 3) positions of the tree labelled Quesnel fir had shown varying susceptibility to inactivation,[1] veneer was collected from these positions. A sample was also taken from the very susceptible intermediate heartwood (Heart 4) position of the Interior-type specimen called Tree 3 and from the anomalous inner heartwood (Heart 5) position of the same tree which was not susceptible to inactivation.

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† Within the wide geographical distribution of this species two types are recognized by industry, Coastal Douglas fir which is generally easy to mill, glue and penetrate with preservatives and Interior Douglas fir which is generally denser and more difficult to mill and frequently is refractory in gluability and preservative penetration. Although there are many exceptions, most trees from the coastal region of British Columbia are of the Coastal type and those from east of the Coast Range of mountains are of the Interior type. This classification is used herein.

[1] W. V. HANCOCK, Ph.D. Thesis, University of British Columbia (1964).

TABLE 1. LIGHT PETROLEUM EXTRACT AND FRACTION YIELDS FROM THE VARIOUS COAST AND INTERIOR DOUGLAS FIR SAMPLES

Sample*	Yield of extract g/kg	Yield of components per cent total				
		Steam volatiles	Fatty acids	Combined acids	Resin acids	Unsaponifiables
Coast fir (not susceptible to inactivation)						
Sapwood	3·91	7·9	16·8	15·5	33·2	26·6
Heart 1	6·48	5·9	23·0	17·5	30·3	23·3
Heart 3	9·27	10·7	31·9	17·8	5·1	34·5
Interior fir Quesnel (susceptibility varies with position in tree)						
Sapwood	3·07	2·6	11·1	27·8	44·0	14·5
Heart 1	4·20	6·2	19·7	15·1	32·5	26·5
Heart 3	4·00	16·0	10·4	41·6	16·9	15·1
Interior fir Tree 3 (Heart 4—very susceptible. Heart 5—not susceptible)						
Heart 4	27·24	72·9	3·6	2·7	8·8	12·0
Heart 5	4·94	8·9	46·1	6·8	11·8	26·4
Heart 4 dried and extracted	2·81	7·8	11·3	32·5	27·1	21·3

* For description of tree sample see Ref. 1.

Since it had been found possible to extract the causal agent of inactivation after drying the veneer, a further sample was collected from the Heart 4 position of Tree 3 and dried for 40 min beyond the oven-dry condition. If component changes taking place in the petroleum ether extractives are the causal agent, these should appear as differences in the extractives removed before and after drying from matched specimens.

The following extractives have been found previously in Douglas fir. Johnson and Cain[2] obtained a 3·29 per cent yield of volatile oil from the wood which contained 32·2 per cent α-terpineol, 30 per cent α-pinene, 14 per cent limonene, and 6 per cent camphene. Graham and Kurth[3] characterized the various extracts obtained with ether, acetone and cold water from three different samples of Douglas fir, but did not specify the area from which their samples were obtained. They were able to identify abietic, oleic, linoleic and lignoceric (tetracosanoic) acids, phytosterol, and a crystalline flavanone. The latter was shown by Pew[4] to be dihydroquercetin. Other phenolics found in the wood have been noted by Hergert.[5] Clark *et al.*[6] isolated eicosanoic, docosanoic, tetracosanoic, and oleic acids, eicosanol, docosanol, docosane, tetracosane and hexacosane in a study of the hydrolysis products of a lignin residue of Douglas fir; it was presumed that these compounds came from

[2] C. H. JOHNSON and R. A. CAIN, *J. Am. Pharm. Ass.* **26**, 623 (1937).
[3] H. M. GRAHAM and E. F. KURTH, *Ind. Engng. Chem.* **41**, 409 (1949).
[4] J. C. PEW, *J. Am. Chem. Soc.* **70**, 3031 (1948).
[5] H. L. HERGERT, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 558. Macmillan. New York (1962).
[6] I. T. CLARK, J. R. HICKS and E. E. HARRIS, *J. Am. Chem. Soc.* **70**, 3729 (1948).

the original wood. Marvell and Wiman[7] isolated 4-(*p*-tolyl)-1-pentanol from kraft liquor. Since this was not present in the wood, they suggested that it was formed from γ-curcumene. Dässler and Shiuă[8] found stearic acid to be the major constituent recovered from the benzene soluble acids of a European grown Douglas fir. In contrast to Graham and Kurth, [3]Dässler and Shiuă did not find linoleic or lignoceric acids, although sitosterol, stigmasterol, cetyl and lignoceryl alcohols were found.

The above literature survey gives only the compounds identified or presumed to be present and which would be expected to be present in light petroleum extracts.

RESULTS AND DISCUSSION

The extracts from the various veneer sets were fractionated according to the method outlined in Fig. 1. Yields of the various fractions are given in Table 1. The fractions obtained were examined using a variety of techniques as shown in Fig. 1.

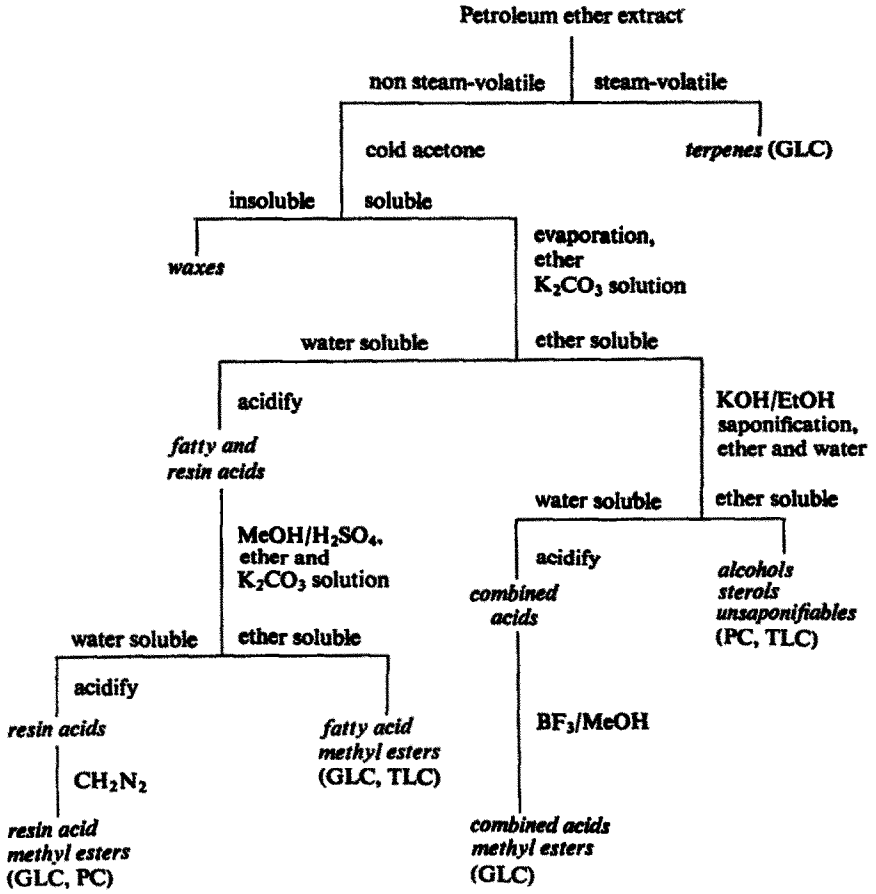

FIG. 1. FRACTIONATION METHOD USED FOR PETROLEUM ETHER EXTRACTS.

Abbreviations: GLC—gas-liquid chromatography; PC—paper chromatography; TLC—thin-layer chromatography; these indicate the methods subsequently used to identify subfraction components. Pure reference samples were used for characterization of unknowns by the various chromatographic techniques.

[7] E. N. MARVELL and R. WIMAN, *J. Org. Chem.* **28**, 1542 (1963).
[8] H. G. DÄSSLER and W. D. SHIUĂ, *Flora* **153**, 326 (1963).

A great variation in extractive yields was found within the species and in different growth zones at the same level of a single stem (Table 1). The variation in percentage compositions of the major fractions between trees and position with a tree was also remarkable. For example, the sample from Tree 3—Heart 4, which showed the highest degree of susceptibility to inactivation, also had an inordinately large steam-volatile content (19·87 g of volatiles compared with 7·37 g of non-volatiles per kg of wood). Although there was no apparent correlation between the type of volatiles found and inactivation, a second sample from this material was examined and found to contain 9·19 g of total extract per kilogram of wood, most of which was non-volatiles. It would appear, then, that the first sample contained a "pocket" of volatile material.

(a) *Steam volatiles.* The major component was α-pinene although many other terpenes were detected. Twelve unknown oxygenated terpenes or sesquiterpenes were also detected (unknowns A–N, Table 2). There was no pattern within the volatile fraction that was

TABLE 2. STEAM VOLATILE COMPONENTS IN LIGHT PETROLEUM EXTRACTS FROM VARIOUS POSITIONS* IN COASTAL AND INTERIOR DOUGLAS FIR

	Coast fir			Interior fir Quesnel			Interior fir Tree 3		
Component (b)	Sapwood	Heart 1	Heart 3	Sapwood	Heart 1	Heart 3	Heart 4	Heart 5	Heart 4 dried and extracted
α-Pinene	+	+	+	+		+	+		
Camphene	+	+	+		+	+	+	+	+
β-Pinene	+	+	+		t	+	+	+	+
4-Carene						+			
3-Carene	+	+	+			+	+		
α-Phellandrene			+		+		+	+	+
α-Terpinene	+			t		+	+	+	
β-Phellandrene	+	+	+	+		+	+		
3,8-Menthediene			+			+	+		
p-Cymene		+	+		+	+			
Terpinolene						+	+		
Unknown A			+	+	+				
Unknown B	+	+	+	+		+			
Unknown C	+	+	+	+	+	+			
Unknown D			+						
Bornyl alcohol	+	+	+	+	+	+		+	+
Unknown F	+	+	+	+	+			+	
Unknown G			+					+	+
Unknown H	+	+	+	+	+	+		+	
Bornyl acetate	+	+	+	+					
Unknown J	+				+				
Unknown K	+								
Unknown L	+								
Unknown M	+								
Unknown N	+	+	+				+		

* For description of samples see Ref. 1 and Table 1.
(b) Identified by GLC using standards.
\+ Definite peak on recorder.
t Trace only present.

consistent with the susceptibility of the various wood samples to inactivation. All of the components found in Tree 3—Heart 4 and Quesnel fir—Heart 3 were present in one or more of the samples from the Coast except terpinolene and this was absent from Tree 3—Heart 4, after heating.

The volatile fraction did not give the same results with GLC and TLC. The much wider range present in the fraction heated in the chromatograph probably contained products of rearrangement or polymerization due to the high temperatures employed. Evidence for this was that the material from Tree 3—Heart 4 that was heated for a total of 120 min at 185° contained only these higher-boiling materials, most of them unknowns.

(b) *Resin acids.* It was shown that all resin acid methyl ester fractions contained the same major components in the same relative amounts. The esters of abietic, dehydroabietic, pimaric, and isopimaric acids were present in all fractions, together with traces of dehydroabietic acids and of seven unknown components. On the paper chromatogram three major components appeared in all samples. By comparison with published data[9] and commercial standards, these were shown to be the dehydroabietate, the abietate and the pimarate-isopimarate methyl esters. There was complete agreement between these data and those obtained from GLC.

(c) *Combined fatty acids.* These acids, analysed by GLC as the methyl esters, were a mixture of the saturated monocarboxylic acids containing from 14 to 19 carbon atoms. In addition, the esters of oleic, linoleic, and linolenic acids were also found. All materials had exactly the same composition except the anomalous Tree 3—Heart 5, and Tree 3—Heart 4 which had possible traces of a C_{20} saturated acid in the dried portion, although this was probably an artifact. The presence of fatty acids with an odd number of carbon atoms was not surprising.

(d) *Combined alcohols and unsaponifiables.* Again, all fractions were found to contain the same major components in similar amounts. Compounds identified as present were glycerol and sitosterol. The presence of another sterol (probably stigmasterol) and other fatty alcohols were indicated together with several unidentified compounds present in minor amounts. There was no minor component restricted to the susceptible veneer.

Paper chromatography showed the presence of glycerol in all fractions. Therefore, the fats present in the wood were presumably all glycerides. TLC indicated the presence of sitosterol and another plant sterol, as well as unidentified alcohols.

(e) *Free fatty acids.* In general, the major component of the free fatty acids was linoleate although reasonable amounts of stearic and palmitic acid esters were also found. Table 3 contains the complete list of all fatty acids found.

Only in the fatty acids was there a pattern that correlated with the degree of susceptibility to inactivation. The Coastal Douglas fir, which was apparently insusceptible to inactivation, contained mainly shorter-chain, saturated acids, some C_{18} unsaturated or saturated acids and only traces of saturated acids above C_{18}. The Quesnel fir, which showed some effect of over-drying (inactivation), contained amounts of the C_{20}, or higher, saturated acids which were of greater concentration in the more susceptible material. Tree 3—Heart 4, which was very susceptible to the development of inactivation, contained a relatively large amount of eicosanoic (C_{20}) and docosanoic (C_{22}) acids, and the latter homologue was present in relatively large amounts in the hearted material, together with some tetracosanoic (C_{24}) acid. The anomalous Tree 3—Heart 5 sample contained only traces of fatty acids with no measurable amount of any one acid.

[9] P. DANIELS and C. ENZELL, *Acta Chem. Scand.* 16, 1530 (1962).

TABLE 3. FREE FATTY ACIDS FOUND IN PETROLEUM ETHER EXTRACTS FROM VARIOUS POSITIONS IN COASTAL AND INTERIOR DOUGLAS FIR

Sample*	Acid** Lauric C_{12}	Myristic C_{14}	Palmitoleic C_{16}	Palmitic C_{16}	Oleic C_{18}	Linoleic C_{18}	Linolenic C_{18}	Stearic C_{18}	Eicosanoic C_{20}	Docosanoic C_{22}	Tetracosanoic C_{24}
Coast fir											
Sapwood	+	t	+	++		++	t	++	t		
Heart 1	++			++			+				
Heart 3	++		+	++	++	++	t	t	t	t	
Interior fir Quesnel											
Sapwood	++		++	++		++		++	t		
Heart 1		++		++		++		++	+	+	
Heart 3	+	+	++	++		++	++	++	t	+	
Interior fir Tree 3											
Heart 4	+	+	+	+		++		++	++	++	
Heart 5	Traces only—no clear evidence of any one acid										
Heart 4 dried and extracted		t	++	++	++	++		t	t	++	++

* For description of samples see Ref. 1 and see Table 1. ** Identified by GLC using known standards.
++ Large peak.
+ Small peak.
t Present only in trace amount.

The thin-layer chromatogram gave results that agreed with those of the gas-liquid chromatograph. For example, the Coast-Sapwood sample showed definite spots for C_{12}, C_{14}, C_{16}, C_{18} and C_{20} acids with the last a very faint spot. Tree 3—Heart 4 showed spots for C_{12}, C_{14}, C_{16}, C_{18} and clearly visible spots for C_{20} and C_{22}.

These results show that the discrepancy between the results of Graham and Kurth[3] and Dässler and Shiuă,[8] noted in the introduction, could have been another manifestation of the different fatty acid balance between types of Douglas fir. Graham and Kurth, who found oleic, linoleic, and lignoceric acids, could have studied the Interior-type tree. Conversely, Dässler and Shiuă who found stearic acid could have studied a Coastal-type tree.

CONCLUSIONS

The total petroleum ether extract yield and the yields of steam volatiles, fatty acids, resin acids, combined acids, and unsaponifiables varied greatly between trees and within tree samples.

Stearic, oleic, and smaller amounts of linoleic and linolenic acids were common to all samples. The Coastal Douglas fir contained these acids and the lower, even-numbered homologues with from ten to sixteen carbon atoms. The Interior tree samples, while they

too contained these homologues, also contained the higher even-numbered homologues, C_{20} to C_{24}. Although several components of the remaining fractions (steam volatiles, resin acids, combined acids, and unsaponifiables) were identified, there were no differences between fractions from each of the two types of Douglas fir. The steam volatiles contained the terpenes α- and β-pinene, camphene, α-phellandrene, and *p*-cymene as major components, together with small or trace amounts of most of the other common cyclic terpenes (perhaps from isomerization) and approximately twelve unknown oxygenated terpenes or sesquiterpenes. Resin acids present were the common abietic, dehydroabietic, pimaric, and isopimaric. Insignificant amounts of waves were present so the combined acids were present as glycerides. These acids were odd and even carbon number fatty acids from C_{14} to C_{19} inclusive. Glycerol was identified from the saponification of these fats. The unsaponifiables included sitosterol, another unidentified sterol (probably stigmasterol) and other unidentified alcohols.

EXPERIMENTAL

Sample Preparation

The acquisition and description of veneer samples have been described.[1] Twelve veneer sheets from each wood sample were extracted for 12 hr in two lots of light petroleum, maintained just below the boiling point. The solvent was combined and recovered on a flash evaporator under vacuum. The veneer was washed with fresh solvent, the washings were added to the main body, and a small amount of water was removed using a separatory funnel. The viscous, brown extract was lyophilized and kept at about $-36°$.

Fractionation and Identification Methods

Pure reference samples were used for characterization of unknowns by various chromatographic techniques (see Fig. 1). These were obtained from the following sources; terpenes from K. & K. Laboratories, Inc.; abietic acid fatty acids and their esters from Nutritional Biochemicals Co.

(a) *Steam volatiles.* The extract was steam distilled until no further material came over. The distillate was washed with ether into a separatory funnel at 1° and separated. The water phase was chilled and washed with ether twice more. The ether washings were combined, dried over Na_2SO_4 and the ether removed on a flash evaporator at 21° in a tared flask. This fraction was examined by gas-liquid chromatography (GLC) using the method of Haslam and Jeffs.[10] Also, the higher boiling components of this fraction were examined by GLC using a 12 ft × ¼ in. (O.D.) S.S. column packed with 14 per cent silicone gum QF-1 on Gas Chrom CLA at 160° and a flow rate of 60 ml per min of helium.

(b) *Waxes.* The dried non-volatile extract from the previous separation was dissolved in acetone and kept at about $-12°$ for four hr. The precipitate (waxes) was recovered by centrifuging. The yields were too small for further investigation.

(c) *Free acids.* The residual extract was recovered from the acetone of the wax precipitation, dissolved in ether (25 ml) and washed with 5 per cent potassium carbonate solution (3 × 20 ml). The free acids were recovered from the aqueous layer by acidification, ether extraction, drying with sodium sulphate, and evaporation. The fatty acids were separated from the resin acids by the method of Wolff and Schölze[11] in which the fatty acid methyl

[10] J. Haslam and A. R. Jeffs, *Analyst* **87**, 658 (1962).
[11] H. Wolff and E. Schölze, *Chemiker-Zt.* **38**, 369 (1914).

esters are formed. After the separation, the resin acids were methylated with diazomethane in ether.

GLC of the fatty acid methyl esters was accomplished using two techniques: (a) a 10 ft × ¼ in. (O.D.) S.S. Column packed with polyester LAC–2R–446 (30 per cent) on Gas Chrom CLA at 230° and 144 ml/min of helium, and (b) a 6 ft × ¼ in. (O.D.) aluminum column packed with silicone oil SE–30 on firebrick with the temperature increasing linearly from 150° to 300° in 15 min at a helium flow rate of 75 ml/min. Thin-layer chromatography (TLC) was carried out on silica gel SG-DF5 (Camag) with hexane:ether:acetic acid (85:15:1) as developing solvent and concentrated sulphuric acid:nitric acid (1:1), followed by heat, as detecting reagent.

The resin acid methyl esters were examined by the paper chromatographic (PC) technique of Daniels and Enzell.[9] GLC was also employed, using a 6 ft × ¼ in. (O.D.) S.S. column packed with neopentyl glycol adipate (15·9 per cent) on Gas Chrom CLA at 220° and a helium flow rate of 140 ml/min.

(d) *Saponification of fats; unsaponifiables.* The ether layer from the free acid separation was evaporated and the residue saponified by refluxing with 5 per cent ethanolic potassium hydroxide (100 ml). The ethanol was evaporated and the residue partitioned between ether and water. The water layer was acidified to yield the combined acids. They were taken up in ether and methylated with boron trifluoride-methanol reagent.[12] The combined alcohols from the fat saponification and the unsaponifiables were determined by PC with Whatman No. 1 paper, butanol:acetic acid:water (4:1:5 upper phase) developer, and periodate-permanganate spray detecting reagent. The temperature programmed GLC method described above and TLC on silica gel SG-DF5 using ether:hexane:acetic acid (50:50:1) as developing solvent, together with the previously described detecting reagent, were also used. The combined acids from the saponification of the fats were examined by the GLC methods described above.

[12] L. D. METCALFE and A. A. SCHMITZ, *Anal. Chem.* 33, 363 (1961).