THE CONSTITUENTS OF DOUGLAS FIR BARK EXTRACTIVES (*PSEUDOTSUGA TAXIFOLIA* BRITT.)

by

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(PSEUDOTSUGA TAXIFOLIA BRITT.)

INTRODUCTION

In such industries as Pulp and Paper, Plywood, and Lumbering, much of the waste produced is bark. Most of this material is used as fuel or is burned as a means of disposal. The need for more efficient utilization of lumber has fostered the development of mechanical debarking methods and has stimulated research to find economically sound means for utilizing the bark produced.

Some recent uses of bark are based upon the mechanical separation of the material into cork, fiber and powder. These fractions are employed mainly as fillers in the Linoleum and Plastics industries. Another recent industrial use of waste wood and bark is in the production of ethanol by the fermentation of sugars obtained by saccharification of the wood wastes. Other possible means of utilizing bark may be in the extractives obtained by the action of neutral solvents on the bark. In the case of the Southern pines, the extractives serve as an important source of chemicals both from waste wood and from by-products of pulping operations. In general, the extractives are present as minor constituents, however, they have much influence upon the commercial utilization of the wood. The resistance to decay, the color, odor and the medicinal value of various woods and barks are directly due to the extractives present.

Douglas fir is the major lumber species in the Pacific Northwest, and is one of the most important lumber species in the total production of lumber and plywood in the United States. Because of the vast supply of Douglas fir bark available as a source of raw material, this investigation was undertaken to develop further information on the distribution and chemical nature of the materials soluble in organic solvents and water. Particular emphasis was placed upon the waxy components of the bark because of the possible utility of these substances.

HISTORICAL BACKGROUND

Not a great deal of information concerning the extractives from Douglas fir bark has been published. In previous work upon the extractives from Douglas fir bark, Warth (18, p. 327) reported the presence of a wax which can be removed by means of hexane. An ester of melissic acid and an unsaturated alcohol, as well as free melissic acid were indicated as possible constituents of this wax. Weiser (19, p. 61) reported the presence of docosanol-1 and lignoceryl alcohol in a high boiling, petroleum ether extract of Douglas fir bark. Other components which have been examined include the volatile oils obtained from the bark. These were characterized as terpenes by Johnson and Cain (9, p. 495). Since the nature of the essential oil has been established, the present work has not dealt with them other than to report the yield obtained.

EXPERIMENTAL PROCEDURE

The Samples

Samples of the bark used in this investigation were collected from trees in the area of Molalla, Oregon in August 1947. These trees had been felled one month prior to the time of sampling. The specimens analyzed were taken from the bases and tops of trees in the three age groups shown in Table 1.

Table 1

Age Group	Average Age of Trees Sampled	Average Height of Top Sample
57-70 years	62 years	30 ft.
80-95 years	88 years	54 ft.
150-260 years	200 years	116 ft.

Five pounds of bark were taken from the base of each tree and a like quantity was taken from the top at the average height indicated in the table. This same procedure was followed for each of five trees in the particular age group; composite samples were thereby obtained totaling 25 pounds each. These samples were ground to pass a No. 12 Standard U. S. sieve and then air dried to a moisture content of about 8 per cent.

Distribution of Extractives

The distribution of various extractives with respect to age groups and position of the bark on the tree was determined in the

following manner. Duplicate 40 gram samples of the air dried bark containing a predetermined amount of moisture were extracted successively with hexane (Skelly Solvent B), and then with acetone. Soxhlet type extractors were used in these determinations. The solvents were removed from the extractives by evaporating the extract to dryness followed by one hour in an oven at 100°C.

Water soluble contents of the solvent extracted bark residues were determined by the Technical Association of the Pulp and Paper Industry Method, T 1 m, (16).

Hexane was selected because of its solvent action upon fatty acids, oils and waxes; acetone was used as a means of removing the more polar substances such as tannins, phlobaphenes and resins. Water also removes some tannins along with the salts and carbohydrate materials. The experimental findings are shown in Table 2.

Table 2

Distribution of Extractives in Douglas Fir Bark
Per Cent of Oven Dry Weight of Bark

Age Group	Sample	Hexane	Acetone	Water	Sum of 3 Extractives
57-70 years	Top	3.54	13.70	14.75	31.99
	Bottom	4.76	17.14	7.14	29.04
80-95 years	Top	3.98	16.10	9.61	29.69
	Bottom	5.47	16.48	9.14	31.09
150-260 years	Top	3.84	12.60	11.50	27.94
	Bottom	5.96	16.48	7.20	29.64

No large difference in the sum of the three extractive contents was observed between the old and young trees. The sum of the

extractive contents was found to be approximately 30 per cent in the top and bottom samples. The hexane extractive was a light-colored, waxy material, melting point 59 to 64°C. as determined by means of a Fisher Scientific Co. melting point block. Yields of this extractive varied from 3.54 per cent in the top bark of the youngest trees to 5.96 per cent in the bottom bark from the oldest trees.

Acetone solubles were found to range from 12.60 per cent in the bark from the tops of old trees to 17.14 per cent in the bark from the bottom of the young trees.

Water soluble extractives were found to range from 7.14 per cent in the bottom bark of the youngest trees to 14.75 per cent in the top bark of the youngest trees.

The distribution data indicate that the highest content of the hexane extractives is found in the bottom bark samples and increases in amount with the age of the tree. The bottom bark also exhibits the highest acetone soluble portion with the highest percentages being found in the specimens from the youngest trees. The highest water extractive contents were exhibited by the top bark samples with the greatest quantity being present in the youngest sample. It is interesting to note that the 80 to 95 year bark did not show much difference in the extractive distribution between the top and bottom bark samples.

Extraction of the Sample for Analysis

One kilogram of the bottom sample of bark from trees in the 80 to 95 year age group was extracted successively with hexane, chloroform, ethyl ether, acetone and water as shown in Table 3. Prior to extraction, the sample was air-dried, ground in a Gruendler hammer mill and then further ground in a Wiley mill to pass a No. 12 Standard U. S. sieve. The moisture content at the time of extraction was 6.93 per cent. The solvents were selected and used in the order given because fairly clear cut fractions were found to result when the bark was extracted in this manner. The solvents were removed from the bark between each successive extraction by spreading out the bark in a ventilating hood until the odor of the solvent had disappeared. All extractions except the water extraction were carried out in a Pyrex glass Soxhlet extractor. The extractor used had a capacity of approximately 500 grams of bark and employed three liters of solvent. Heating was done by means of an electric hot plate with rheostat control. The yields of extractives are given in Table 3.

Table 3

Douglas Fir Bark Extractives 80 to 95 Year Bark
Per Cent of Oven Dry Weight of Bark

Solvent	Yield
1. Hexane	4.18
2. Chloroform	2.52
3. Ethyl Ether	5.95
4. Acetone	7.70
5. Water	6.68
Total	27.03

Hexane Soluble Components

The solution of hexane soluble extractives was cooled to room temperature and a white crystalline solid was found to separate out. This precipitate was filtered, washed with hexane and dried to constant weight in a vacuum oven at 50°C. The yield, 14.2 grams, amounted to 36.4 per cent of the extractive and was analyzed as a separate fraction, B, of the hexane soluble components.

The filtrate and washings were reduced in volume to 300 ml. by means of a hot water bath and reduced pressure. This concentrate was evaporated to dryness and then placed in a vacuum oven at 50°C. for one hour. The yield was 24.7 grams, 63.6 per cent of the extractive, and was designated fraction A of the hexane soluble components.

Fraction A, Soluble in Hexane at Room Temperature

The weighed extract was placed in a flask with 200 ml. of distilled water and steam distilled to remove the volatile oils. The volatile oils were extracted from the distillate with ethyl ether, dried over anhydrous sodium sulfate, and weighed; the yield was 1.75 per cent, based on the total hexane soluble components of the bark. These oils were not examined further since they have already been characterized as terpenes by Johnson and Cain (9, p. 495-497).

After removal of the volatile oils, the light-colored, waxy residue was dissolved in ethyl ether and dried over anhydrous sodium sulfate. The ether was then removed by means of reduced pressure

and a hot water bath, and an analysis was made on the residue for the usual constants determined on fats and waxes. All titrations were performed by means of a Beckmann, type H, pH meter in the manner described by Dole (3, p. 139). Saponification number and Hanus iodine number determinations were made according to the methods of the Association of Official Agricultural Chemists (2). Melting points were determined on a Fisher Scientific Company melting point block. Results of these determinations were as follows:

Acid number	48.0
Iodine number	21.9
Saponification number	138.5
Melting point	50-52°C.
Color	light brown

Separation of A into Component Fractions

Ten grams of the wax were dissolved in 400 ml. of ethyl ether and free acidic compounds were extracted with four 400-ml. portions of aqueous 5 per cent potassium carbonate solution in a separatory funnel. A three layer system developed in which the center layer consisted of the ether and water insoluble potassium salts of fatty acids. The water layer and the insoluble salts were drawn off from the ether layer and acidified with dilute hydrochloric acid. The released acids were then separated from the water by several extractions with ether. The ether solution of acids was dried over anhydrous sodium sulfate, evaporated to dryness, and the residue

dried to constant weight in a vacuum oven at 50°C. The yield was 29.9 per cent free acidic material.

The ether solution containing the neutral substances was evaporated to dryness, and the residue was saponified in a solution of 100 ml. of benzene and 250 ml. of 0.7 normal alcoholic potassium hydroxide. After refluxing the saponification mixture four hours in a boiling water bath, the solution was taken to dryness by means of reduced pressure. The residue was then extracted with four 100 ml. portions of boiling ethyl ether to remove the unsaponifiable compounds. The ether solution was washed with water, dried over anhydrous sodium sulfate and then evaporated to dryness. The residue of unsaponifiables was dried to constant weight in a vacuum oven at 50°C.; a yield of 35.0 per cent was obtained.

Salts of the acids remaining in the saponification flask were treated with dilute hydrochloric acid, and the released acids were extracted with three 100 ml. portions of ethyl ether. The ether extract was dried over anhydrous sodium sulfate, evaporated to dryness and taken to constant weight in a vacuum oven at 50°C. The yield of combined acids was 21.7 per cent. Remaining in the saponification flask was a dark-colored residue which was no longer soluble in ether or hexane. This material was dissolved in acetone, dried over anhydrous sodium sulfate and evaporated to constant weight; the yield obtained was 11.2 per cent.

Chemical Nature of the Component Fractions of A

Each of the component fractions separated in the manner described in the foregoing section was examined in an effort to identify its chemical constituents.

Free acidic material: An attempt was made to separate fatty acids from resin acids by means of the preferential esterification method of Wolff and Scholze (20), whereby the fatty acids are converted to methyl esters. The resin acids and phenols are not esterified. The resin acids if present may then be isolated by the formation of their cyclohexylamine salts which are insoluble in acetone (7, p. 335). No precipitate of resin acid salts was obtained, and a 17.4 per cent yield of amorphous phenolic materials resulted. Since no crystalline products could be separated by crystallization methods, nothing further was done with this fraction.

The fatty acid esters obtained in this separation were then separated into saturated and unsaturated acids by means of the lead salt-ether method (8, p. 405). This separation is based upon the difference in solubilities of the lead salts in ether; the lead salts of the higher saturated acids are insoluble in cold ether, whereas those of the unsaturated acids are quite soluble. A yield of 1.1 per cent unsaturated acids was obtained. These were brown amorphous materials, neutral equivalent 340.1, iodine number 34.0. The neutral equivalent obtained on the mixture indicated that acids of higher molecular weight than oleic acid (Neutral equivalent 282) were present.

Because of the relatively small amount of these acids available, no attempt was made to separate the mixture by distillation. Instead the mixture was oxidized with dilute cold alkaline permanganate (11, p. 1629) to obtain crystalline hydroxy derivatives which may be separated by means of their differences in solubility in water. The presence of oleic acid was indicated in the unsaturated acids by the isolation of dihydroxystearic acid (m. p. 129-131°C., literature values 131-132°C.).

The yield of saturated fatty acids obtained from the lead salt-ether separation amounted to 11.4 per cent based upon the yield of fraction A. These acids were recrystallized from acetone and then hexane; a white crystalline product was obtained, melting point 73 to 74°C., neutral equivalent 360.1. This neutral equivalent is intermediate between behenic acid ($C_{22}H_{44}O_2$, neutral equivalent 340.6) and lignoceric acid ($C_{24}H_{48}O_2$, neutral equivalent 368.6). These acids when prepared synthetically melt at 79.9°C. and 84°C. respectively. It has been shown however, that mixtures of these acids cannot be satisfactorily separated by crystallization methods or fractional distillation of esters, and that when obtained from natural sources, the melting point is always low (4, p. 215). An equimolecular mixture of lignoceric acid and behenic acid melts at 74.5°C. (12, p. 121). A p-bromo phenacyl ester of the acids was prepared, melting point 86 to 88°C. The p-bromo ester of pure lignoceric acid melts at 90 to 91°C. (12, p. 121).

Neutral fraction of A: The combined acids obtained by

saponification of the neutral fraction were recrystallized from acetone and hexane. A white crystalline solid was obtained, melting point 73 to 74°C., iodine number 0.2, neutral equivalent 358.7. These data indicated that the combined acids were essentially the same mixture of near homologues of lignoceric acid found in the free acids.

The unsaponifiables from the neutral fraction were dissolved in acetone and cooled in the refrigerator. A white crystalline solid precipitated from the solution and was filtered off, melting point 68 to 69°C. An acetyl number determined by the method of Lewkowitsch (8, p. 403) indicated that the material was an alcohol, molecular weight 360.6. Oxidation of the alcohol to an acid by fusion with alkali (14, p. 1738) yielded a crystalline acid, melting point 71°C., neutral equivalent 371. These values indicate that the alcohol is possibly a mixture of near homologues of lignoceryl alcohol, $C_{24}H_{50}O$ (4, p. 215). An ether extraction of the alkaline fusion mixture prior to acidification yielded only traces of residue in the ether extract. This indicated a practically complete conversion of the alcohols to acids and also indicated the absence of hydrocarbons in the unsaponifiables.

The filtrate from the precipitate of alcohols was evaporated to dryness and the residue was dissolved in hot 95 per cent ethanol, diluted with water and set aside to crystallize. A yield of 0.3 per cent white needle shaped crystals was obtained. These were recrystallized from dilute ethanol and a melting point of 134 to 135°C.

was obtained. A violet color appeared when the Liebermann-Burchard Test (8, p. 363) was applied, and crystals were obtained with digitonin solution. These data indicated that the material was a phytosterol. By boiling the phytosterol with acetic anhydride, the acetate was formed and was found to melt at 124 to 125°C. A purified sample of the phytosterol was intimately mixed with an equal portion of the phytosterol isolated from Douglas fir wood (6, p. 409), and the three melting points were determined together. All three melted simultaneously. From these results it can be concluded that this phytosterol or mixture of phytosterols is similar to the one found in Douglas fir wood.

Phenolic material in unsaponifiables of fraction A: As was previously mentioned in the foregoing section dealing with the separation of A into component fractions, a yield of 11.2 per cent of an amorphous substance was obtained in the saponification flask. This material was found to be insoluble in hot methanol; soluble in aqueous 3 per cent sodium hydroxide and acetone. Attempts to crystallize it from various solvents were unsuccessful; apparently it consisted of oxidized or polymerized substances of a phenolic nature as indicated by the solubility in dilute alkali and the formation of a dark color with dilute ferric chloride in an acetone-water solution.

Since the presence of phenolic, amorphous material of this type is generally undesirable in waxes used commercially, an effort was made to remove it from the whole wax fraction A. Five grams of A were placed in 100 ml. of boiling methanol, and the phenolic

substances went to the bottom of the beaker. By decanting off the hot methanol solution, evaporating it to dryness and then to constant weight in a vacuum oven, 62.7 per cent of A could be obtained in the form of a light-colored wax which possessed properties of possible commercial significance. The following constants were determined upon the wax purified in this manner.

Acid number	34.4
Iodine number	6.1
Saponification number	191.0
Melting point	59-63°C.
Color	light yellow

A summary of the separation scheme used in the analysis of A, the fraction of hexane soluble extractives soluble at room temperature, is shown in Figure 1. The yields of the various constituents found are presented in Table 4.

Figure 1

Separation of Hexane Soluble Extract, Fraction A
Per Cent Based on Total Hexane Extract

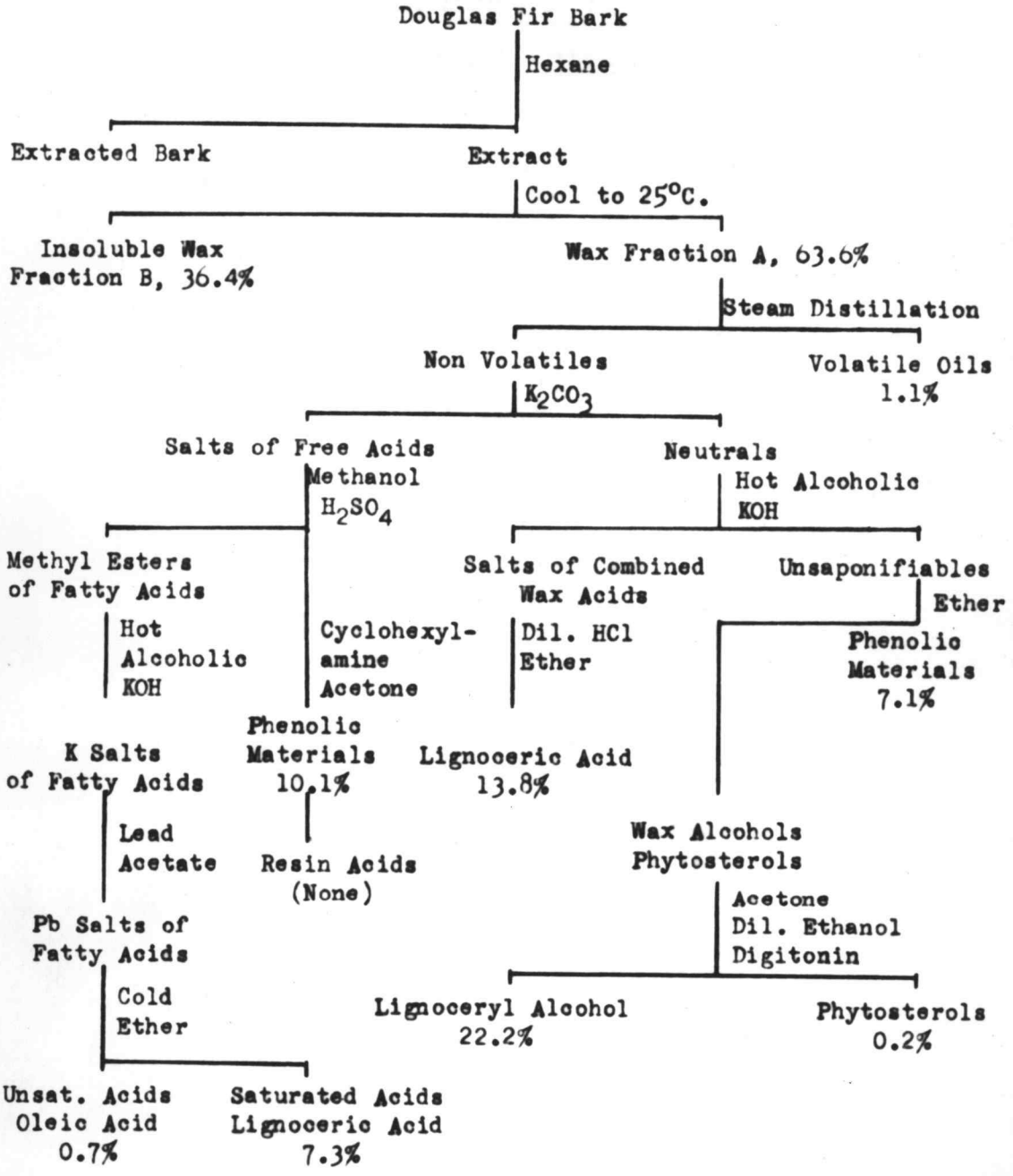

Table 4

Composition of Hexane Soluble Extractive,
Fraction A, Soluble at 25°C.
Per Cent of Fraction A

Free Acids	
Saturated fatty acids (Lignoceric acid)	11.4
Unsaturated acids (Oleic acid present)	1.1
Resin acids	0.0
Total	12.5
Combined Acids	
Saturated fatty acids (Lignoceric acid)	21.7
Unsaponifiables	
Fatty alcohols (Lignoceryl alcohol)	34.7
Phytosterols	0.3
Total	35.0
Phenolic Material	28.6
Loss (difference)	2.2

Fraction B, Insoluble in Hexane at Room Temperature

This fraction of hexane soluble extractives had separated from the hexane upon cooling the extract to room temperature as shown previously in Figure 1. The yield obtained after drying to constant weight in a vacuum oven at 50°C. was 36.4 per cent of the hexane extractives. In appearance it was a light yellow, waxy material; constants determined on the crude wax were as follows:

Acid number	65.5
Iodine number	17.7
Saponification number	219.0
Melting point	61-67°C.

This material was found to be soluble in ether, chloroform, boiling acetone, and partially soluble in boiling methanol. Extraction of an ether solution of the wax with 5 per cent aqueous sodium bicarbonate in a separatory funnel dissolved 20.1 per cent. Potassium carbonate in the same concentration dissolved 41.7 per cent. Boiling methanol was found to dissolve most of the waxy constituents, but an amorphous phenolic fraction was insoluble. A sample of the wax was purified by dissolving it in boiling methanol and filtering on a steam heated funnel. The residue remaining in the beaker was extracted several times with boiling methanol in this manner. The filtrate was evaporated to dryness and taken to constant weight in a vacuum oven at 50°C. A yield of 68.4 per cent methanol soluble wax was obtained. Its properties as a wax were improved over the crude material in that it was now found to have a higher melting point and a lighter color. Constants determined on the purified wax were as follows:

Acid number	20.7
Iodine number	2.4
Saponification number	203.1
Melting point	68-70°C.
Color	light yellow

The analysis for constituents of fraction B was carried out on the crude wax rather than that purified by means of boiling methanol because it was found that a portion of the combined acids and alcohols remained in the material insoluble in boiling methanol.

Separation of B into Component Fractions

This fraction of hexane soluble extractives was separated into component fractions and analyzed in the same manner which was employed in examining fraction A, discussed in the foregoing section. An outline of the analytical scheme is shown in Figure 2. The yields of the various constituents obtained are presented in Table 5.

The analytical data obtained indicate that fraction B of the hexane soluble extractives differs in the following respects from fraction A, the portion soluble in hexane at room temperature. Fraction B contains much more of the free fatty acids as would be expected from the increase in solubility of fatty acids in hexane with increases in temperature (12, p. 204). Phytosterols and oleic acid were not indicated in fraction B, possibly because of their solubility in hexane and the relatively small amounts of these materials found in the extractives.

Figure 2

Separation of Hexane Soluble Extract, Fraction B
Per Cent Based on Total Hexane Extract

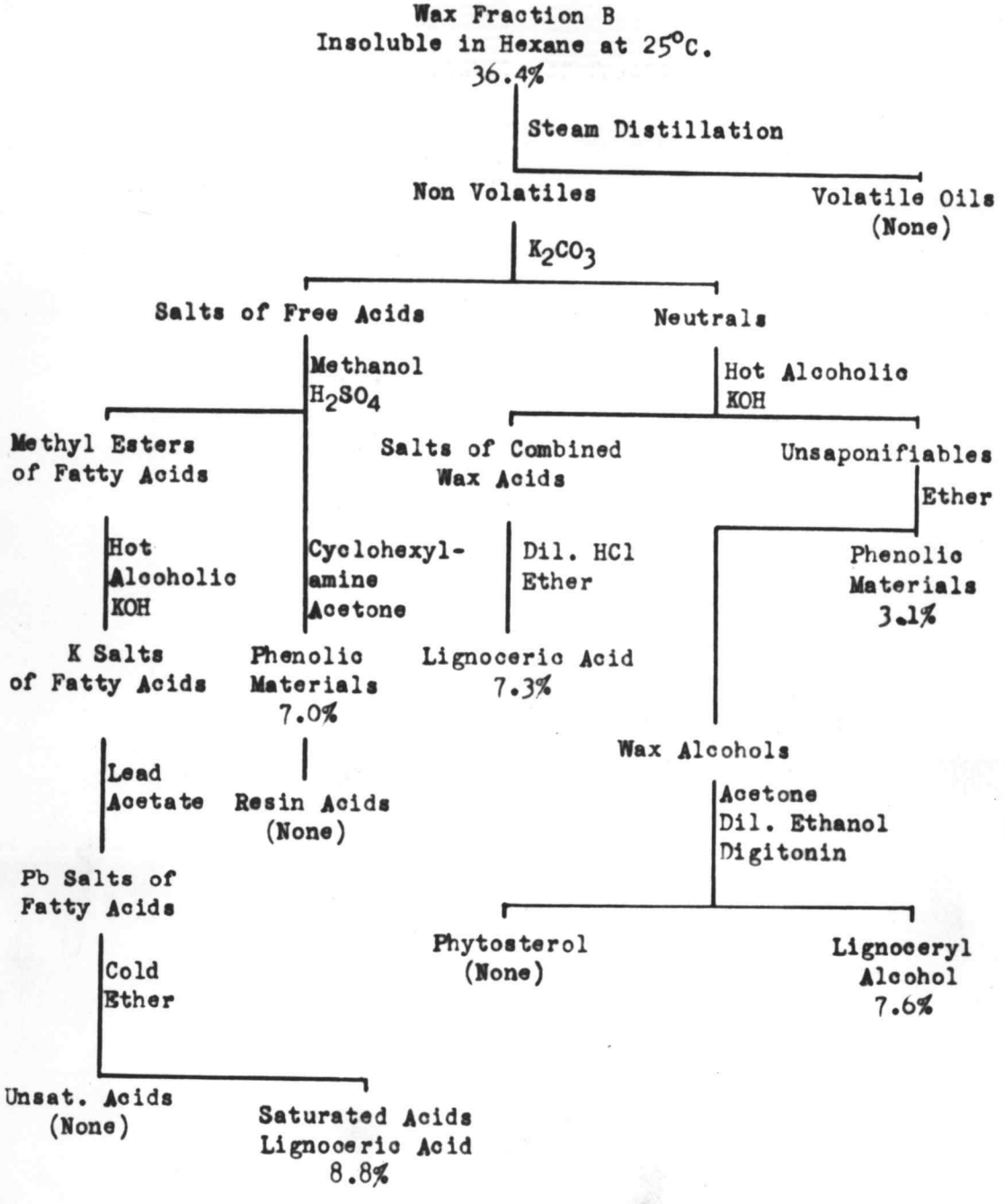

Table 5

Hexane Soluble Extractive
Composition of Fraction B, Insoluble at 25°C.
(Per Cent of Fraction B)

Free Acids	
Saturated fatty acids (Lignoceric acid)	24.0
Unsaturated acids	0.0
Resin acids	0.0
Total	24.0
Combined Acids	
Saturated fatty acids (Lignoceric acid)	20.0
Unsaponifiables	
Fatty alcohols (Lignoceryl alcohol)	21.0
Phenolic Material	27.5
Loss (difference)	7.5

Chloroform Soluble Components

Some of the phenolic materials in the bark were extracted by hexane as was indicated in Tables 4 and 5, but these substances were found to be much more soluble in chloroform and were investigated further as the chloroform extractives.

The bark which had previously been extracted with hexane was spread out in a ventilating hood until the odor of hexane had disappeared. It was then replaced in the extractor and extracted with chloroform for six hours. Most of the chloroform was removed from the extract by distillation over a hot water bath, and the residue was taken to constant weight. A yield of 30.4 grams or 3.27 per cent

was obtained.

It was found that 23.1 per cent of the chloroform extractives were soluble in hexane and proved to be of the same nature as the hexane solubles previously extracted. Evidently, the hexane extraction had been incomplete, and the chloroform, being an excellent solvent for waxy materials, had removed the remaining portion of the hexane soluble material. This hexane soluble portion of the chloroform extractives was included in the yield of hexane soluble extractives presented in Table 3.

The 76.9 per cent of the chloroform soluble extractives which remained was a dark brown amorphous substance. This material was extracted in a beaker with several portions of hot water to remove small amounts of water soluble materials, especially dihydroquercetin, which was found to be present in the subsequent ether extraction of the bark. The water washings were decanted; the residue was dissolved in ether, and then dried over anhydrous sodium sulfate. Constants determined on the material were as follows:

Acid number	65.0
Iodine number	20.2
Saponification number	216.0
Melting point	57°C.

It was found to be soluble in benzene, acetone, and ethyl ether. When dissolved in ether and extracted with aqueous 5 per cent potassium carbonate in a separatory funnel, 66.4 per cent was dissolved; sodium bicarbonate in the same concentration removed 6.2 per

cent. In alcoholic solution with ferric chloride, a greenish black coloration was produced, indicating phenolic constituents. This was also indicated by the appreciable solubility in potassium carbonate.

No crystalline products could be obtained from a variety of solvents. A methoxyl determination by a revised method of Zeisel (16, 2 m) indicated a methoxyl content of 5.43 per cent. Methylation of the material in 25 per cent potassium hydroxide with dimethyl sulfate indicated an increase of 4.77 per cent methoxyl more than the unmethylated material. This was equivalent to 2.62 per cent free hydroxyl groups.

Five grams of the extractive was then refluxed in a two per cent alcoholic solution of hydrochloric acid. Evaporation of the alcohol filtrate yielded a dark residue with a distinct odor of vamillin. Hydrochloric acid and phloroglucinol reagent produced a bright red coloration which is indicative of the presence of vanillin. This was suggestive that a glucoside, coniferin, or its polymerization products were present in the chloroform extractive, since its presence has been shown in some conifers (10, p. 465), (17, p. 613). However, confirmatory evidence for the presence of coniferin could not be obtained either in the preliminary water washings of the chloroform extractive or in the hydrolyzed residue. The exact nature of this extractive remains to be determined.

Ethyl Ether Soluble Components

After the bark had been successively extracted with hexane and

chloroform, it was removed from the extractor and placed in a fume hood, where it remained until the odor of chloroform had disappeared from the bark. An extraction with ethyl ether was then made. Upon completion of a seven hour refluxing period, the ether extract was concentrated to 800 ml. and dried over anhydrous sodium sulfate. This solution was then taken to dryness by means of a hot water bath, and the residue was dried to a constant weight in a vacuum oven at 50°C. The yield of crude ether extractives was 5.95 per cent based on the oven dry weight of unextracted bark.

In appearance the ether extractive was a light tan colored powder which melted with decomposition at 222-230°C. It was soluble in acetone, alcohol, and hot water. From a dilute alcohol or hot water solution it crystallized in the form of needle shaped crystals. Further purification was accomplished by the use of activated charcoal in a hot water solution of the compound. This treatment yielded a white crystalline product with a melting point 240 to 242°C.

The compound gave a greenish black coloration with ferric chloride in aqueous solution. On reduction in alcoholic solution with magnesium and hydrochloric acid, an intense purple-red color developed. This color reaction is suggestive of a flavanone structure. Pew (13, p. 3031) and Graham (6, p. 409) have characterized a flavanone, dihydroquercetin, which occurs in Douglas fir wood. A mixed melting point with the dihydroquercetin from the wood indicated that the compound in the bark was identical. Oxidation of the flavanone by Pew's method (13, p. 3031) yielded quercetin (melting

point 315 to 317°C. dec.) which verified the identity of the flavanone as dihydroquercetin.

The ether extractive was found to be predominately dihydroquercetin. An exact percentage was difficult to obtain because it was not known how much dihydroquercetin was included in the material absorbed by the charcoal during purification. A 95 per cent recovery was obtained from the charcoal treatment.

Acetone Soluble Components

The fourth successive extraction of the bark was made with acetone, which is a solvent for tannins, phlobaphenes, and coloring matters. After concentrating the extract to 800 ml. by means of reduced pressure and a hot water bath, an aliquot portion was dried to constant weight. The yield of acetone extractives was found to be 7.7 per cent based upon the oven dry weight of the bark.

Separation of the Extract into Component Fractions

The concentrated acetone extract was poured into a liter of distilled water and the solution was subjected to distillation under reduced pressure to remove the acetone. After the precipitated material had settled, the clear liquid was decanted off, and the precipitated phlobaphene material was dried to constant weight in a vacuum oven at 80°C. The water soluble portion was then analyzed for tannins according to the method of the American Leather Chemists Association (1). In this method the tannins are determined by ab-

sorption on hide powder, and the non-tannins are obtained by difference. Results of this determination are shown in Table 6.

Table 6

Acetone Extractives, Per Cent of Extractive by Weight

Tannins	23.8
Non-Tannins (water soluble)	12.1
Non-Tannins (phlobaphenes)	64.1

The water solution of the material gave a precipitate with gelatine, a green color with ferric chloride, and a precipitate in boiling dilute sulfuric acid. These tests indicated the tannin to be of the phlobatannin classification.

Water Soluble Components

The final extraction on the bark was made with distilled water. This extraction was carried out in a covered, enamel-ware kettle equipped with a copper steam coil, which maintained the mixture at 70 to 80°C. Seven extractions were made using a four liter portion of water for each extraction. Each portion of water was in contact with the bark for six hours. After each extraction the mixture was filtered, and the combined filtrates from the seven extractions were reduced in volume to 2250 ml. by means of reduced pressure and a hot water bath. An aliquot portion of the concentrated extract was evaporated to constant weight, and the total water extractives based on the oven dry weight of bark was found to be 6.68 per cent.

The concentrated extract was then analyzed for tannins by the method of the American Leather Chemists Association (1). By means of this method the tannins are absorbed on specially prepared hide powder; the non-tannins remain in solution and are determined by difference. Results of the tannin analysis are shown in Table 7. The tannin solution gave a green color with ferric chloride, a precipitate with gelatine and a precipitate when boiled with dilute sulfuric acid. These tests indicated that the tannin was of the phlobatannin class, similar to the tannin removed by acetone.

The water soluble extractives were also analyzed for reducing and non-reducing sugars. A 400 ml. aliquot of the water soluble extractives was treated with 50 ml. of saturated lead acetate solution to precipitate the tannins. The precipitate was filtered off, and the excess lead in the filtrate was precipitated by adding 10 grams of dipotassium phosphate and again filtering to remove the precipitated lead phosphate. This clarified, deleaded solution was then analyzed for reducing sugars by means of the Somogyi method (15, p. 599), employing copper reagent which was reduced by the reducing sugar. The difference between a blank and the titration of a determination was equivalent to the copper reduced and thus to the reducing sugars. A 0.005 N sodium thiosulfate solution was used for the titration.

To determine the total sugars an aliquot portion of the clarified, deleaded solution was hydrolyzed with dilute hydrochloric acid. A reducing sugar determination was then made upon the

hydrolyzed solution. The per cent of non-reducing sugars is the difference between reducing sugars and the total sugars. Results of the sugar analyses are shown in Table 7.

Table 7

Composition of Water Soluble Extractives
(Per Cent of Extractives)

Tannins	35.6
Non-Tannins (soluble)	57.0
Non-Tannins (insoluble)	7.4
Reducing Sugars	18.2
Non-Reducing Sugars	10.8
Total Sugars (as glucose)	29.0

Summary and Discussion

When determined by the methods indicated, the sum of the extractive contents of the selected Douglas fir bark samples was found to be approximately 30 per cent. These samples of bark were selected with respect to age groups and position of the bark on the tree trunks. Although the sums of the extractives in each case were found to be similar, the distribution of the extractives varied with respect to solubility in different solvents.

Waxy constituents soluble in hexane were found to be present in the highest percentage in the bark samples taken from the base of the oldest trees. Water soluble extractives were found in greatest amount in the youngest bark samples taken from the top portion of the tree trunks. Acetone solubles were found in the highest yield in the bottom bark of the youngest trees.

Douglas fir bark was extracted with solvents in the following order: hexane, chloroform, ethyl ether, acetone, and distilled water. Materials found in the hexane extractives were lignoceric acid ($C_{24}H_{48}O_2$ and near homologues), lignoceryl alcohol ($C_{24}H_{50}O$ and near homologues), lignoceryl cerate, oleic acid, and a mixture of phenolic materials non-crystallizable from a variety of solvents. The removal of the phenolic materials from the hexane extractive yielded a wax consisting mainly of free and combined lignoceric acid and lignoceryl alcohol. This wax had a light color and high melting point. Chloroform was found to be a good solvent for the amorphous phenolic substances which were removed in part by hexane. A subsequent extraction

with ethyl ether removed dihydroquercetin in almost pure form. Acetone was found to remove tannins and phlobaphenes. A water extraction of the bark removed sugars and tannins; the tannins were of the phlobatannin class.

Warth (18, p. 327) reported the presence of free and combined melissic acid and an unidentified, unsaturated alcohol in a hexane extract of Douglas fir bark. These substances were not found in the samples examined in this investigation. Melissic acid has a neutral equivalent of 466.8 as compared with 368.6 for lignoceric acid. This difference in neutral equivalents made the possibility for the presence of melissic acid rather remote because the neutral equivalent of the saturated acids isolated in this investigation ranged from 360.1 to 365.8. No unsaturated fatty alcohols were found in the hexane extractives; it is possible that the unsaturation observed by Warth was due to the phytosterol constituents in the alcohol fraction, which he did not describe other than to report that an unsaturated alcohol was present.

Weiser (19, p. 61) reported the presence of docosanol-1 in a hexane extract of Douglas fir bark. It is highly probably that some docosanol-1 was present in the alcohol fraction. However, it seems improbable that a pure fraction of an individual, long chain alcohol such as docosanol-1 could be isolated as such by crystallization from the lignoceryl alcohol near homologues. It has been shown that almost without exception, long chain fatty acids and alcohols obtained in nature are mixtures of near homologues which cannot be

effectively separated by crystallization or distillation procedures.

From the data obtained Douglas fir bark appears to be a rich source of extractives; some of these extractives have commercial value and could therefore aid in attaining more complete utilization of the trees taken for lumber or pulp.

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