AN ABSTRACT OF THE THESIS OF

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Title "The Nature of the Chemical Constituents of Incense Cedar, (Libocedrus Decurrens Torr.) Bark"

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The purpose of this investigation was to determine the nature of the chemical constituents of incense cedar bark with the hope that such an endeavor would spur future research, development and use of a now neglected natural raw material.

The bark from nine trees, which were separated into three age groups, was sampled from the bottom, middle and top sections. The age groups were 146-195 years, 261-285 years and 326-351 years. The nine samples were successively extracted with hexane, benzene, ethyl ether, hot water and 95 per cent ethanol to show the distribution and amounts of the extractives. The total extractive content varied between 13.70 per cent for the bottom sample of the oldest age group and 30.39 per cent for the top sample of the youngest age group. Similarly, the total extractive content of Port Orford cedar bark was found to range from 15.49 per cent to 25.69 per cent with the distribution corresponding closely to that of incense cedar. In both species, the greatest amount of extraneous material was removed with hot water and the variations between the total extractive content of the bottom and top samples were due primarily to the deviations in the amounts of this extract.

The hexane extract was found to comprise 3.53 per cent of the oven-dry unextracted bark. The components of the hexane extract and the yields based on the oven-dry weight of the hexane solubles were: volatile oil, 0.95 per cent; crystalline fatty ester, 5.83 per cent; free acids, 46.21 per cent; neutrals, 46.67 per cent; and undetermined, 0.34 per cent. Saponification of the crystalline fatty ester gave cerotic acid and an alcohol fraction equivalent to a mixture of behenyl and lignoceryl alcohols. The free acids were shown to contain 1.93 per cent resin acids, 1.11 per cent of a crystalline acid fraction equivalent to a mixture of behenic and lignoceric acids, and a fatty acid fraction, having a neutral equivalent of 435.8 and a Hanus iodine number of 41.2, which comprised 44.28 per cent of the hexane solubles. The neutral fraction contained 25.08 per cent combined acids, which had a neutral equivalent of 446.6 and a Hanus iodine number of 80.1, and 21.59 per cent unsaponifiables from which a crystalline phytosterol was isolated in a yield of 1.07 per cent of the hexane solubles.

The yield of benzene soluble material was 2.46 per cent of the oven-dry unextracted bark. The major components of this extract were resin acids, present in both the free and combined forms. The yield of free resin acids was 64.4 per cent of the benzene extract; the fraction melted

at 181-182° C., had a neutral equivalent of 347.7 and was optically active. The combined resin acids comprised 7.8 per cent of the benzene solubles. They melted at 151-152° C., had a neutral equivalent of 358.0 and were optically active. The remainder of the benzene extract was found to contain 8.2 per cent free fatty acids, 10.8 per cent combined fatty acids and 8.2 per cent unsaponifiables.

The tannin content of incense cedar bark ranged from 3.5 to 7.8 per cent, whereas that of Port Orford cedar varied between 4.2 and 7.0 per cent. These tannin yields are insufficient for economical commercial extraction.

A crude carbohydrate material was obtained with hot water in a yield of approximately nine per cent of the oven-dry weight of unextracted bark. It contained 65.9 per cent reducing sugars, 24.0 per cent tannin and 10.2 per cent insolubles. The composition of the reducing sugars based on the crude carbohydrate material was: pentosans, 13.46 per cent; mannans, 3.09 per cent; galactans, 21.50 per cent; uronic anhydride, 1.87 per cent; and glucosans, 25.98 per cent.

A composite sample of unextracted bark contained 14.93 per cent alcohol-benzene solubles; 3.04 per cent alcohol solubles, 7.47 per cent hot-water solubles, 37.06 per cent lignin, 36.40 per cent holocellulose, 8.51 per cent pentosans, 2.69 per cent methoxyl, 0.59 per cent acetyl, and 0.97 per cent ash.

THE NATURE OF THE CHEMICAL CONSTITUENTS OF INCENSE CEDAR, *LIBOCEDRUS DECURRENS* (TORR.) BARK

by

JAMES EDWARD SMITH

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Redacted for Privacy

Professor of Wood Chemistry

In Charge of Major

Redacted for Privacy

Head of Department of Chemistry

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

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THE NATURE OF THE CHEMICAL CONSTITUENTS OF INCENSE CEDAR, LIBOCEDRUS DECURRENS (TORR.) BARK

INTRODUCTION

Throughout regions of the far western states are found limited stands of incense cedar. These areas extend from Oregon southward through California into Mexico and Lower California. Stands are also found in western Nevada. The commercial range is confined largely to the Sierra-Nevada mountains in California and the mountain regions of northern California and southern Oregon. The stand of incense cedar in these three states has been estimated to be approximately eleven billion board feet by Betts (2). The tree is not a good lumber producer because of its short body, sharp taper, and susceptibility to a fungous disease known as "brown rot" after it attains an age of about 140 years. This fungus ceases its work when the tree is felled and from all observations has no further effect on the durability of the wood. Due to its high durability, ranking with cypress, redwood, black locust and other cedars, incense cedar has been used principally for commercial items requiring this property. Among these are included fence posts, railroad ties, cedarchests, sash and doors, and interior finishes.

The bark is deeply furrowed, two to three inches thick at the base and one inch thick midway of the trees height. On the mature trees the bark is shreddy and a cinnamon-brown color. The bark of the younger trees is thin, smooth, slightly scaly, a reddish-cinnamon or purplish-red color and has a silver sheen to the bark scales. Since the wood is not too valuable in conjunction with lumber production,

it is hoped that characterization of the chemical components of the bark may extend the demand for incense cedar.

Considerable importance has been attached to the extractive content of wood and barks. In some species, notably the Southern pines, the extractives supply an important source of chemicals, both from wood waste and from by-products of pulping operations. Recently, a pilot plant has been set up at Klamath Falls, Oregon with the object of recovering valuable chemicals from the extractives of Western pines. More complete utilization of Douglas fir through the extractives found in its bark appears probable (6, 10). Tannin, wax and dihydroquercetin were found and appear to have commercial potentialities. Douglas fir bark tannin has been used extensively to tan leather at a tannery in Dallas, Oregon. The wax obtained is comparable to high grade imported waxes. Dihydroquercetin is an antioxidant for the prevention of rancidity in fats and oils; in addition, it is experiencing an increased demand because of its pharmaceutical value. Products such as these are slowly helping to eliminate the problem of what to do with the vast amount of natural material not now utilized by the lumber industry.

A search of the literature revealed that little information has been published concerning the nature of the extractives from the bark of any of the cedars. Because of this, it was decided to make a comparison of the total extractives of incense cedar with another western cedar. Port Orford cedar (Chamaecyparis lawsoniana) was selected for this purpose. In this way, the possibility of utilizing cedars as a

source of extraneous material for chemical substances may be more readily ascertained.

The investigation of the chemical composition of incense cedar bark was therefore undertaken with the hope that such an endeavor would spur future research, development and use of a now neglected natural raw material.

EXPERIMENTAL PROCEDURE

I. COLLECTION AND PREPARATION OF SAMPLES.

The samples used in this work were collected May 25, 1950 from trees felled within one week along Weaver Creek east of Myrtle Creek in the Umpqua National Forest; T. 28S, Range 3W., Section 9. Table 1 shows a complete summary of the collection data. The shreddy, deeply-furrowed, cinnamon-colored bark samples from the various trees were compiled into three age groups, i.e., I, II, and III. For each age group there are three samples; bottom, top, and middle. Hence, for this species there were nine composite samples which were then ground in a Rietz Disintegrator to pass a No. 20 mesh screen. The ground bark was air dried at room temperature, placed in sealed glass jars for storage and labeled as to age group and section of the tree.

TABLE 1

COLLECTION DATA ON INCENSE CEDAR BARK SAMPLES

Group No.	Age Years	Tree No.	Bottom Section Diameter outside bark In.		Middle Section Diameter outside bark In.		Top Section Diameter outside bark In.	
			(2.5 to 3.5 ft. height)		(29 to 30.5 ft. height)		(60.5 to 87.5 ft. height)	
I	195	20	23.0	17.5	14.0	12.5	5.8	5.0
	146	18	21.0	16.0	12.0	10.5	6.8	6.0
			(2.0 to 3.5 ft. height)		(38 to 65 ft. height)		(78 to 107 ft. height)	
	270	13	24.0	20.5	17.5	14.5	7.2	6.0
	263	15	30.5	23.0	15.5	13.5	9.0	7.5
II	261	16	37.0	30.0	21.5	19.0	7.0	6.0
	267	22	33.0	24.0	20.0	15.0	14.0	11.0
	285	23	44.5	35.5	20.5	17.5	12.5	11.0
			(2.0 to 2.5 ft. height)		(35 to 43.5 ft. height)		(125 ft. height)	
III	326	19	50.5	44.5	32.5	29.5	12.8	10.8
	351	21	38.5	32.5	23.5	20.5	----	----

II. DISTRIBUTION OF EXTRACTIVES IN SAMPLES.

An analysis was made on each sample (bottom, middle and top) from each age group for the following in the order given: hexane, benzene, diethyl ether, hot-water and 95 per cent ethanol soluble. The samples were initially extracted with hexane in order to remove oils, fats, waxes and resins. Benzene removes waxes and resins not previously removed by the hexane. These treatments were followed by extraction with diethyl ether which normally displaces any flavones or like coloring matter present. Hot water removes the tannin and carbohydrate materials which are contained in the bark. The final extraction is made with 95 per cent ethanol to indicate the amount of phlobaphenes.

With the exception of the hot water treatment, all extractions were carried out on 25-35 gram samples contained in cotton cloth bags in Pyrex glass Soxhlets. The samples were extracted continuously for eight hours at which time the fresh extract was colorless and in each case contained a negligible amount of soluble solids as determined by evaporation of the solvent and weighing the residue. The extract was filtered into a tared glass dish, evaporated on a steam bath in the hood and dried in an oven at 105° C for one hour. The amount of dry residue was obtained by cooling the dish in a desiccator and weighing.

Hot-water solubles were determined by a modification of the T.A.P.P.I. method T 1m-45 (19). The leaching time was extended to four hours and fresh water was added to the bark at the end of each hour following removal of the preceeding liquor by filtration. By this means, a more efficient extraction was achieved. During filtration,

the recommended sintered glass crucibles of (c) porosity became clogged to such an extent that it was almost impossible to filter and wash the bark with complete thoroughness. This difficulty was eliminated by filtering the bark liquor through No. 1 Whatman filter paper on a Buchner funnel with suction and retaining the filtrate. Upon completion of the leaching operation an aliquot 100 milliliter portion of the filtrate was placed in a tared weighing dish and evaporated to dryness on a steam bath. The weight of the residue was ascertained after drying in the oven at 105° C. followed by cooling in a desiccator and weighing. Knowing the total volume of the filtrate and the weight of the residue, calculation of the per cent hot-water solubles was completed. The bark remaining from the hot water extraction and the filter paper which retained it were placed in a cloth bag and the final Soxhlet extraction was made with 95 per cent ethanol.

The results of these extractions are shown in Table 2. The average moisture content of the air-dry bark was nine per cent. All percentages given are based on the oven-dry weight of unextracted bark. The total extractive content varied between 13.7 and 30.4 per cent.

Examination of Table 2 shows that the tops of the trees of all age groups contained the greatest amount of extraneous material. The total extractives increased progressively from the bottom to the top of trees in all age groups. With the exception of the hot-water solubles, extractive percentages obtained were comparatively low. Little variation was found in the amount of hexane solubles except for the tops of the younger trees. Here, the percentage increased

significantly. Distribution of the benzene solubles is similar to that of the hexane solubles except that the highest percentage was found at the tops of the oldest trees. It should be noted, however, that the large amount of benzene soluble material found in the tops of age group III may have resulted from defective sampling procedure. Reference to Table 1 will show that this sample was made up of bark from only one tree and therefore cannot be considered a representative sample.

The hexane extract was a soft resinous material varying in color from a light amber for the top sections to a slightly darker shade for the bottom sections. Its most noticeable physical property was the extreme tackiness exhibited.

Removal of the solvent from the benzene solubles resulted in a brown, amorphous, solid residue which melted between 105-115° C. The bright-red amorphous solid which was obtained as the ether residue is probably an essential part of the material which imparts the color to incense cedar. Its high melting point, approximately 235° C., is comparable to that of the flavone materials and related coloring matters found in other plant species (9, 15, p.161). The ethyl alcohol extract was an amorphous reddish-brown material and was considered to be essentially phlobaphene.

TABLE 2

EXTRACTIVE CONTENT OF INCENSE CEDAR BARK

(per cent of oven-dry weight of unextracted bark)

Age Group	Material	Bottom %	Middle %	Top %
I	Hexane Soluble	3.38	5.45	7.65
	Benzene Soluble	1.58	2.05	2.39
	Ether Soluble	1.22	1.14	0.91
	Hot-water Soluble	8.03	13.13	18.31
	Alcohol Soluble	1.94	1.55	1.13
	Total Solubles	16.15	23.32	30.39
II	Hexane Soluble	3.02	3.04	4.87
	Benzene Soluble	2.24	2.59	1.59
	Ether Soluble	0.50	0.84	0.62
	Hot-water Soluble	9.50	10.52	12.48
	Alcohol Soluble	1.38	1.62	1.67
	Total Solubles	16.64	18.61	21.23
III	Hexane Soluble	2.51	3.47	3.66
	Benzene Soluble	1.97	4.34	8.81
	Ether Soluble	1.05	1.34	1.83
	Hot-water Soluble	6.58	9.12	7.62
	Alcohol Soluble	1.59	1.94	2.87
	Total Solubles	13.70	20.21	24.79

III. COMPARISON OF EXTRACTIVES FROM BARKS OF INCENSE CEDAR AND PORT ORFORD CEDAR

Collection data for the Port Orford cedar bark is shown in Table 3. The samples were collected May 24, 1950 from timber felled within one week along Johnson Creek west of China Flat, Oregon; T. 32S, Range 12W, Sections 19 and 20. The bark was ground, screened and stored in the same manner as that previously described for incense cedar bark. The extraction procedure used was identical to that for incense cedar except that filtration difficulties were not encountered during the determination of the hot-water solubles. The hot-water extract was filtered through the sintered glass crucibles prescribed in the T.A.P.P.I. procedure T 1m-45 (19), thus eliminating the necessity of filtering the liquor through filter paper and using an aliquot portion of the filtrate for the analysis. Table 4 is a summary of the extractive percentages obtained. The total extractive content varied between 15.5 and 25.7 per cent.

One may easily note the close similarities between the amounts of extractives obtained from the two species. In each case the hot-water solubles were by far the largest singular extract acquired. The tops of the trees of both species yielded the greatest total extraneous material. The ethanol, ether and hexane solubles corresponded closely. Incense cedar bark contained slightly more benzene solubles than were found in Port Orford cedar. An over-all analysis of Tables 2 and 4 shows a close relationship between the extractive percentages of the two species.

The hexane extract of Port Orford cedar was a sticky, pitch-like amber material. The benzene solubles were composed of dark-brown resinous solids melting between 90 and 100° C. A red amorphous solid melting around 210° C. comprised the ethyl ether extract. Ethyl alcohol removed a reddish-brown phlobaphene material.

Physically and quantitatively there appears to be a close relationship between the extractives obtained from Port Orford cedar and incense cedar. However, chemical characterization of the extractives of the two species will be required to show the true correlation. Alloted time necessitated the exclusion of one of the species from further chemical investigation except for comparative tannin analyses. Hence, the chemical analysis of incense cedar bark was more fully pursued.

TABLE 3

COLLECTION DATA ON PORT ORFORD CEDAR BARK SAMPLES

Group	Age	Tree	Bottom Section		Middle Section		Top Section	
			Diameter outside bark	inside bark	Diameter outside bark	inside bark	Diameter outside bark	inside bark
No.	Years	No.	In.		In.		In.	
			(1.0 foot height)		(42 ft. height)		(83 ft. height)	
	161	7	27.2	19.2	13.3	11.8	6.6	6.0
I	97	7N	----	----	----	----	----	----
	93	6N	----	----	----	----	----	----
	98	1N	----	----	----	----	----	----
			(2.0 foot height)		(32 to 43 ft. height)		(65 to 91 ft. height)	
	242	8	32.5	25.0	17.0	14.0	10.5	9.5
II	257	2	26.5	22.0	15.7	14.5	7.6	7.0
	257	9	17.3	15.8	12.5	11.5	6.1	5.5
	253	10	29.5	22.0	19.2	16.2	8.9	8.3
			(2.5 to 3.5 ft. height)		(39.5 to 45.5 ft. height)		(85.5 to 133.5 ft. height)	
	328	1	57.0	42.5	29.5	25.0	17.5	15.7
	297	3	30.0	25.0	13.1	12.6	6.0	5.5
III	291	4	43.0	35.5	19.5	18.0	7.0	6.5
	336	5	52.0	40.0	30.0	26.3	11.7	10.5
	327	6	48.0	38.0	31.0	26.7	23.0	19.6
	285	11	29.5	24.0	18.1	16.3	7.6	7.0

TABLE 4

EXTRACTIVE CONTENT OF PORT ORFORD CEDAR BARK

(per cent of oven-dry weight of unextracted bark)

Age Group	Material	Bottom %	Middle %	Top %
	Hexane Soluble	5.05	6.13	4.63
	Benzene Soluble	1.78	0.98	0.79
I	Ether Soluble	1.78	2.12	1.24
	Hot-water Soluble	8.82	13.76	12.39
	Alcohol Soluble	3.31	2.70	1.42
	Total Solubles	20.74	25.69	20.47
	Hexane Soluble	3.40	3.00	4.56
	Benzene Soluble	0.83	1.23	1.55
II	Ether Soluble	1.51	1.11	1.29
	Hot-water Soluble	8.22	8.62	13.06
	Alcohol Soluble	3.26	2.52	1.62
	Total Solubles	17.22	16.48	22.08
	Hexane Soluble	3.65	2.95	3.14
	Benzene Soluble	1.11	0.94	0.90
III	Ether Soluble	1.64	1.35	0.94
	Hot-water Soluble	7.85	8.47	11.82
	Alcohol Soluble	1.86	1.78	1.51
	Total Solubles	16.11	15.49	22.08

IV. CONSTITUENTS OF THE EXTRACTIVES FROM INCENSE CEDAR BARK

Hexane Solubles.

A composite sample of the nine individual bark fractions previously ground to pass a No. 20 mesh screen was prepared. Four-hundred gram batches of this bark were placed in a cotton cloth bag in a Soxhlet-type extractor and extracted with hexane for 15 hours. The combined extract from two kilograms of bark was filtered and the solvent removed on a steam bath. The residue, after drying in the vacuum oven at 30° C. for one hour, comprised 3.53 per cent of the oven-dry weight of the unextracted bark.

Volatile oil. The volatile oil from the hexane extract was removed by steam distillation. The distillate was shaken with three portions of ethyl ether in a separatory funnel, the ether solution dried over anhydrous sodium sulfate overnight, filtered, evaporated to dryness under reduced pressure in a vacuum oven at 35° C. and the residue of volatile oils weighed. The pale greenish-yellow liquid isolated as the volatile oil comprised 0.95 per cent of the hexane soluble material. This bark oil has been previously characterized by Schorger (17), the major components being furfural, alpha pinene, dipentene, bornyl acetate and free borneol. For this reason, no further work was carried out on this component.

Crystalline ester. The non-volatile hexane soluble material which remained in the distillation flask following the removal of the

volatile oil was dissolved in ether. The ether solution was dried over anhydrous sodium sulfate, filtered and the solvent removed under reduced pressure. Upon dissolving the non-volatiles in warm acetone and placing the solution in the refrigerator at 4° C. for 24 hours, light yellow crystals separated. These crystals were removed by filtration and recrystallized from hot ethyl acetate and then from hot ethanol. The resulting white crystals were present in a yield of 5.83 per cent of the hexane extract. The melting point of the purified material was 70-71° C. Approximately four grams of the material were saponified for seven hours with six per cent alcoholic potassium hydroxide. The saponification mixture was evaporated to 35 milliliters on a steam bath, diluted with 125 milliliters distilled water and again evaporated to 35 milliliters. After four additional dilutions and evaporations, the solution was considered to be alcohol-free and it was extracted with ether to remove the alcohols. The potassium salt of the acid was soluble in water.

Alcohols from ester. The ether extract, which contained the alcohols resulting from saponification of the crystalline ester, was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was a white powder. This material was recrystallized from warm solutions of acetone and ethyl acetate respectively. It melted at 61-62° C. An acetate derivative formed by refluxing the alcohol with acetic anhydride and anhydrous pyridine melted at 49-50° C.

An oxidative fusion of the alcohol crystals was carried out by fusion with potassium hydroxide (5, p1737). The fusion mixture was dissolved in water and the solution extracted with ether. No significant residue was obtained on evaporation of this extract which indicated that the fusion was complete. The aqueous solution was acidified with dilute mineral acid and the resulting precipitate removed by ether extraction. After evaporation of the ether, the product was recrystallized from warm acetone. It melted at 68-69° C. and had a neutral equivalent of 343.7. This is equivalent to a mixture of behenic and lignoceric acids and indicated that the alcohol fraction of the ester was a mixture of behenyl and lignoceryl alcohols.

Acids from ester. The aqueous solution containing the potassium salts of the acids from the saponification of the ester was acidified to pH 4 with dilute mineral acid. The white precipitate formed was removed by filtration and washed thoroughly with distilled water. It was dissolved in hot acetone, filtered and the filtrate cooled in the ice box. The acid crystals which separated from the cold acetone melted at 74° C., gave a neutral equivalent of 396.0, and the molecular weight as determined by the Rast camphor method (16) was 388 and 418. These data suggest that the product may be cerotic acid, which has a neutral equivalent of 396.68, or a mixture of near homologues.

Free acids. Removal of the crystalline ester from the hexane solubles left an acetone soluble residue which was then dissolved in ether and shaken with five portions of a five per cent potassium carbonate solution. The neutral material remained in the ether layer

while the free acids were found in the aqueous solution as potassium salts. The aqueous solution was acidified with dilute hydrochloric acid, transferred to a separatory funnel and shaken with several portions of ethyl ether to remove the free organic acids. The ether solution was dried over anhydrous sodium sulfate overnight, filtered into a tared glass dish, evaporated to dryness under reduced pressure and the weight of free acids determined. The yield was 46.21 per cent of the hexane solubles.

Two grams of the free acids were treated with methanol and sulfuric acid according to the Wolff-Scholze method (23) for the isolation of resin acids. In this procedure one can achieve a quantitative separation of resin and fatty acids through a preferential esterification treatment. Fatty acids can readily be converted to methyl esters using absolute methanol containing a small amount of concentrated sulfuric acid. Resin acids are not esterified under these conditions. The resin acids obtained comprised 1.93 per cent of the hexane extract. The brown amorphous material melted at 182-184 ° C. This melting point is quite similar to that of the resin acids isolated from the benzene soluble free acids which are more fully described in later sections of this paper. This suggests the possibility that the resin acids of incense cedar bark are only slightly soluble in hexane and the major portion is extracted with benzene. For this reason, the hexane soluble resin acids were not characterized further.

The methyl esters of the fatty acids were saponified for two hours with 150 milliliters of 0.5 normal alcoholic potassium hydroxide. The

saponification mixture was acidified to pH 5 with dilute hydrochloric acid and the solution made essentially alcohol-free as previously described. The aqueous solution was transferred to a separatory funnel and shaken with three portions of ether. Upon removing the solvent from the ether extract, the free fatty acids were obtained as a tacky amber solid in a yield of 44.28 per cent of the hexane solubles.

The free fatty acids were dissolved in warm acetone and this solution placed in the refrigerator for 48 hours. A small amount of crystalline precipitate, comprising 1.11 per cent of the hexane solubles, was removed by filtration. Recrystallization of this material from dilute ethanol and ethyl acetate gave a product melting at 83.5-84.5° C. The neutral equivalent of this acid was 358.3 which is intermediate between the values for behenic and lignoceric acids.

The acetone-soluble, free, fatty acids were obtained as an amber-colored resinous solid. No significant crystalline material resulted from attempted precipitations from a variety of solvents. This fatty acid fraction had a melting point of 50-53° C., a neutral equivalent of 435.8 and an iodine number of 41.2 as determined by the Hanus method (7, p344).

Neutral fraction. Following isolation of the free acids, the ether was removed from the neutral fraction of the hexane solubles under reduced pressure. The neutrals were obtained in a yield of 46.67 per cent of the hexane solubles. This material was saponified for four hours with 130 milliliters of five per cent alcoholic potassium hydroxide. The saponification mixture was made

essentially alcohol-free as previously described. The aqueous solution was transferred to a separatory funnel and shaken with five portions of ether. The potassium salts of the acids remained in the water layer whereas the unsaponifiables dissolved in the ether.

Combined acids. The potassium salts of the combined acids were acidified to pH 3 with dilute hydrochloric acid and the solution made alcohol-free as described earlier. The liberated acids were extracted with ether. The ether solution was dried over anhydrous sodium sulfate, filtered into a tared glass dish, the ether removed under reduced pressure and the combined acids found to comprise 25.08 per cent of the hexane extract. The soft dark-brown material could not be crystallized from a variety of solvents. The acids had a neutral equivalent of 446.6 and a Hanus iodine number of 80.1.

Unsaponifiables. The ether extract, containing the unsaponifiables, was dried over anhydrous sodium sulfate overnight, filtered and the solvent removed under reduced pressure. A white crystalline phytosterol was obtained by dissolving the material in warm acetone and placing the solution in the refrigerator at 0° C. for 96 hours. The colorless needle-like crystals were removed by filtration, air dried and weighed. The yield was 1.07 per cent of the hexane solubles. The sterol crystals melted at 137.5-138.5° C. following recrystallization from 95 per cent ethanol. They gave a positive Liebermann-Burchard test (4) which is characteristic of sterols. An acetate derivative, melting at 125-126° C., was formed with acetic anhydride and anhydrous pyridine. The specific rotation of the sterol in chloroform was found

to be $[\alpha]_D^{20°C.} = -19.2 \pm 1°$. A similar phytosterol, suggested to be a mixture of alpha and beta sitosterol, was isolated from Sierra juniper wood (11).

The filtrate from the precipitation of the phytosterol yielded a resinous gum and was not further investigated.

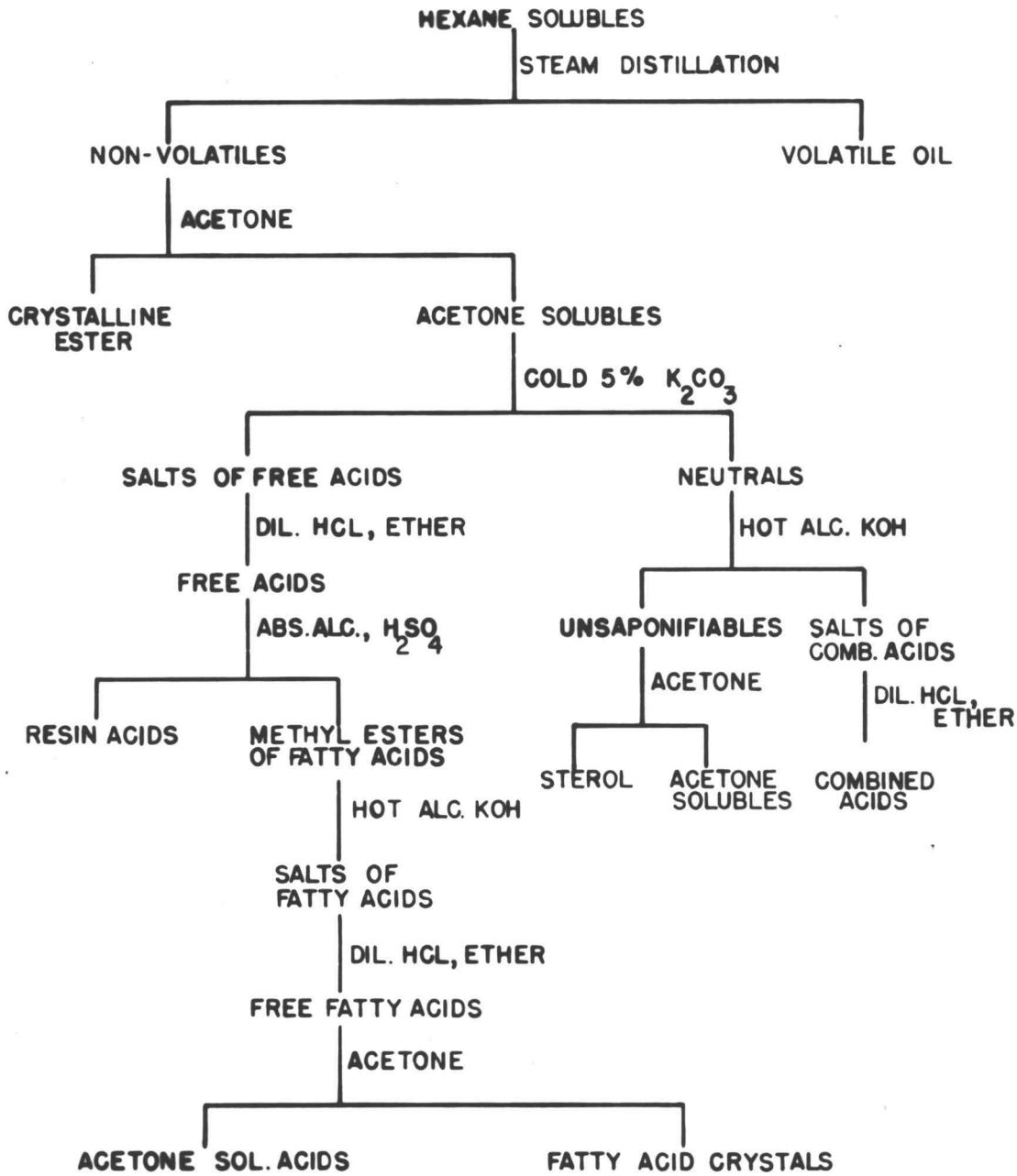
The scheme of separation of the hexane extract is shown in Figure 1. The percentages of the various components are listed in Table 5.

TABLE 5

COMPONENTS OF THE HEXANE SOLUBLES

Component		Per cent
Volatile Oil		0.95
Crystalline Ester		5.83
Free Acids		46.21
Resin Acids	1.93	
Fatty Acids	44.28	
Neutrals		46.67
Combined Acids	25.08	
Unsaponifiables (by difference)	21.59	
Undetermined		0.34
		100.00

FIGURE 1



SEPARATION OF INCENSE CEDAR BARK
HEXANE SOLUBLE COMPONENTS

Benzene Solubles.

The bark used in this portion of the work was previously extracted with hexane. It was further extracted with benzene in the Pyrex glass Soxhlet extractor for 15 hours. The yield of benzene soluble material, following removal of the solvent on a steam bath and drying the residue to constant weight in a vacuum oven at 40° C., was 2.46 per cent.

Free acids. Thirty grams of the benzene soluble extract were dissolved in ethyl ether and shaken with six portions of a five per cent potassium carbonate solution. The free acids were found in the carbonate layer as potassium salts and the neutrals remained in the ether layer. The aqueous extract was boiled gently to remove any ether present. After cooling, acidification was carried out by pouring dilute hydrochloric acid slowly into the mixture with constant stirring. The acid solution was then shaken with ether in a separatory funnel to remove the free organic acids. The ether solution was dried over anhydrous sodium sulfate overnight, filtered into a tared glass dish and the solvent removed under reduced pressure. The benzene solubles contained 72.8 per cent free acids. The amber-colored amorphous acids melted at approximately 160° C.

The resin acids in the free acid fraction were found to comprise 64.4 per cent of the benzene extract by the method of Wolff and Scholze (23). These resin acids melted at 181-182° C., and had a neutral equivalent of 347.7. They gave a positive Liebermann-Storch test (7, p358) and the specific rotation in absolute ethanol was

found to be $[\alpha]_D^{20°C.} = +19.5 \pm 1°$. Attempts to recrystallize the resin acids from hexane, ethyl acetate, acetone, methanol and dilute ethanol failed to yield a precipitate either at room temperature or in the cold.

The neutral equivalent of the free resin acid fraction is higher than that of abietic acid, a common resin acid which has the empirical formula $C_{20}H_{30}O_2$. However, this fraction was optically active, gave a positive Liebermann-Storch test and was not esterified under the conditions set forth by Wolff and Scholze. Resin acids of similar properties and molecular weight have been isolated from Ponderosa pine bark (9).

The methyl esters of the free fatty acids were saponified with 0.5 normal alcoholic potassium hydroxide for two hours. The saponification mixture was acidified to pH 5 with dilute hydrochloric acid. This acid solution was evaporated to 35 milliliters on a steam bath and then diluted with 125 milliliters distilled water. The resulting solution was again evaporated to 35 milliliters and an additional 125 milliliter portion of water added. This treatment was repeated five times and the final solution considered to be alcohol-free. The aqueous solution was shaken with three portions of ether in a separatory funnel to extract the free fatty acids. Upon removal of the solvent from the ether extract, the free fatty acids were obtained as a dark-brown resinous material melting at 66-68° C. The yield was 8.2 per cent of the benzene solubles.

No crystalline product of the free fatty acids could be isolated from a variety of solvents including acetone, ethyl acetate, methanol,

dilute ethanol and chloroform. These acids decolorized aqueous potassium permanganate, absorbed bromine and had a neutral equivalent of 329.6. The presence of unsaturated acids was clearly demonstrated when an iodine number of 83.6 was obtained by the Hanus method (7, p344). The acids were oxidized by cold alkaline potassium permanganate according to the method of Lapworth and Mottram (13) but no crystallizable material resulted. Because of the small amount of these acids present in the bark, no further attempts were made to characterize them.

Neutral fraction. The neutral fraction which remained following the isolation of the free acids was found to comprise 27.2 per cent of the benzene solubles. The soft resinous material was saponified with 100 milliliters of five per cent alcoholic potassium hydroxide by refluxing for four hours. The saponification mixture was made essentially alcohol-free by five successive evaporations and dilutions with distilled water. The aqueous solution was transferred to a separatory funnel and shaken with successive portions of ether until the ether layer was colorless. The ether extracted the unsaponifiable material while the potassium salts of the combined acids remained in the aqueous layer.

Combined acids. The potassium salts of the combined acids were acidified with dilute hydrochloric acid and the liberated acids extracted with ether. The reddish-brown acids obtained following removal of the solvent melted at approximately 136° C. The yield was 18.6 per cent of the benzene solubles.

A combined resin acid fraction was obtained by treating two grams of the combined acids with methanol and concentrated sulfuric acid according to the method of Wolff and Scholze. The resin acids obtained were found to comprise 7.8 per cent of the benzene solubles. The dark-brown amorphous material melted at 151-152° C., gave a positive Liebermann-Storch test and had a neutral equivalent of 358.0. The specific rotation in absolute ethanol was $[\alpha]_D^{20°C.} = +20.2 \pm 1°$.

The presence of a combined resin acid fraction in the incense cedar bark benzene solubles, in addition to the free resin acid fraction previously described, shows the benzene extract to contain 72.2 per cent total resin acids. This was further verified by direct saponification of the benzene solubles and preferential esterification of the resulting acid fraction. In this work, 20 grams of the dry benzene solubles were saponified by dissolving the material in 150 milliliters of seven per cent alcoholic potassium hydroxide and refluxing for six hours. The saponification mixture was made alcohol-free as previously described. The resulting aqueous solution was shaken in a separatory funnel with five portions of ethyl ether, the unsaponifiables dissolving in the ether and the potassium salts of the acids remaining in the water layer. The ether solution was dried, the solvent removed and the percentage unsaponifiables determined. Acidification of the aqueous solution with dilute hydrochloric acid produced a cream-colored amorphous precipitate of acids. This acid mixture was removed from the water by shaking with portions of ethyl ether until the aqueous layer was colorless. The ether solution

was dried over anhydrous sodium sulfate overnight, evaporated to dryness in a tared glass dish under reduced pressure and the weight of acids determined.

Two grams of the acids resulting from saponification of the benzene solubles were treated according to the preferential esterification procedure of Wolff and Scholze. The methyl esters of the fatty acids which resulted were saponified with 0.5 normal alcoholic potassium hydroxide and the percentage fatty acids determined as previously described. The resin acids were found to comprise 72.1 per cent of the benzene solubles. This mixture of both the free and combined resin acids melted at 145° C., gave a positive Liebermann-Storch test and had a neutral equivalent of 342.1. The specific rotation in absolute ethanol was $[\alpha]_D^{20°C.} = +23.3 \pm 1°$.

The amber colors and the specific rotations of the combined and free resin acids correspond quite closely. However, the lower melting point and higher neutral equivalent of the combined form indicates definite dissimilarities between the two fractions. The higher neutral equivalent suggests the possibility of additional functional groups in the combined resin acids and it may be through this means that combination occurs. Combination is also possible through the carboxyl group of the acid although it is known that esterification of this group is quite difficult as evidenced by the Wolff-Scholze method of isolating resin acids. A slight lowering of the neutral equivalent and a decisive decrease in the melting point of the resin acids obtained by direct saponification of the benzene solubles indicates that the strong

alkali treatment may have altered the structure of the resin acids obtained by the more mild method of isolation.

None of the resin acid fractions could be crystallized from a variety of solvents. Each fraction gave a neutral equivalent which was somewhat higher than that for abietic acid ($C_{20}H_{30}O_2$). However, each fraction was optically active, gave a positive Liebermann-Storch test and was not esterified under the condition prescribed by Wolff and Scholze. It is evident that further work is necessary to more fully characterize the resin acids of incense cedar bark.

Table 6 summarizes the properties of the three resin acid fractions obtained. Components of the benzene solubles as determined by direct saponification of the benzene extract are shown in Table 7.

TABLE 6

PROPERTIES OF RESIN ACID FRACTIONS

	Neutral Equivalent	M.P. (°C.)	Optical Rotation
Free Resin Acids	347.7	182	$+19.5 \pm 1^{\circ}$
Combined Resin Acids	358.0	151	$+20.2 \pm 1^{\circ}$
Resin Acids (from direct saponification)	342.1	145	$+23.3 \pm 1^{\circ}$

The methyl esters of the combined fatty acids which resulted from the isolation of the combined resin acids were saponified with 100 milliliters of 0.5 normal alcoholic potassium hydroxide for two hours, acidified with dilute mineral acid and the liberated acids extracted

with ether. Removal of the solvent resulted in a fatty acid mixture comprising 10.8 per cent of the benzene soluble extract. These acids had a melting point of 63-64° C. and gave a neutral equivalent of 366.5. They absorbed bromine and decolorized an aqueous solution of potassium permanganate. Absorption of iodine from a Hanus solution showed the combined fatty acids to have an iodine number of 101.1. Oxidation with cold alkaline permanganate failed to yield any significant crystallizable product. Separation of this material into saturated and unsaturated fatty acids would enable one to characterize these acids more thoroughly but the insignificant amount present in incense cedar bark does not warrant such an undertaking at this time.

Unsaponifiables. Separation of the combined acids from the neutral fraction of the benzene solubles left an ether soluble fraction composed of unsaponifiable material. The ether solution was dried over anhydrous sodium sulfate overnight, filtered, evaporated under reduced pressure and the percentage unsaponifiables determined. This dark-red amorphous material was found to comprise 8.2 per cent of the benzene extract. It melted at 68-70° C., decolorized an aqueous solution of potassium permanganate and absorbed bromine. A Hanus iodine number of 88.5 indicated the presence of unsaturated materials which may be unsaturated alcohols or hydrocarbons. No crystalline products were obtained from this fraction by attempted precipitation from acetone, ethyl alcohol, methanol, ethyl acetate, hexane and chloroform.

Figure 2 summarizes the scheme of separation of the incense cedar bark benzene soluble extract. The percentages of the various

components are listed in Table 8.

TABLE 7

COMPONENTS OF THE BENZENE EXTRACT
(direct saponification)

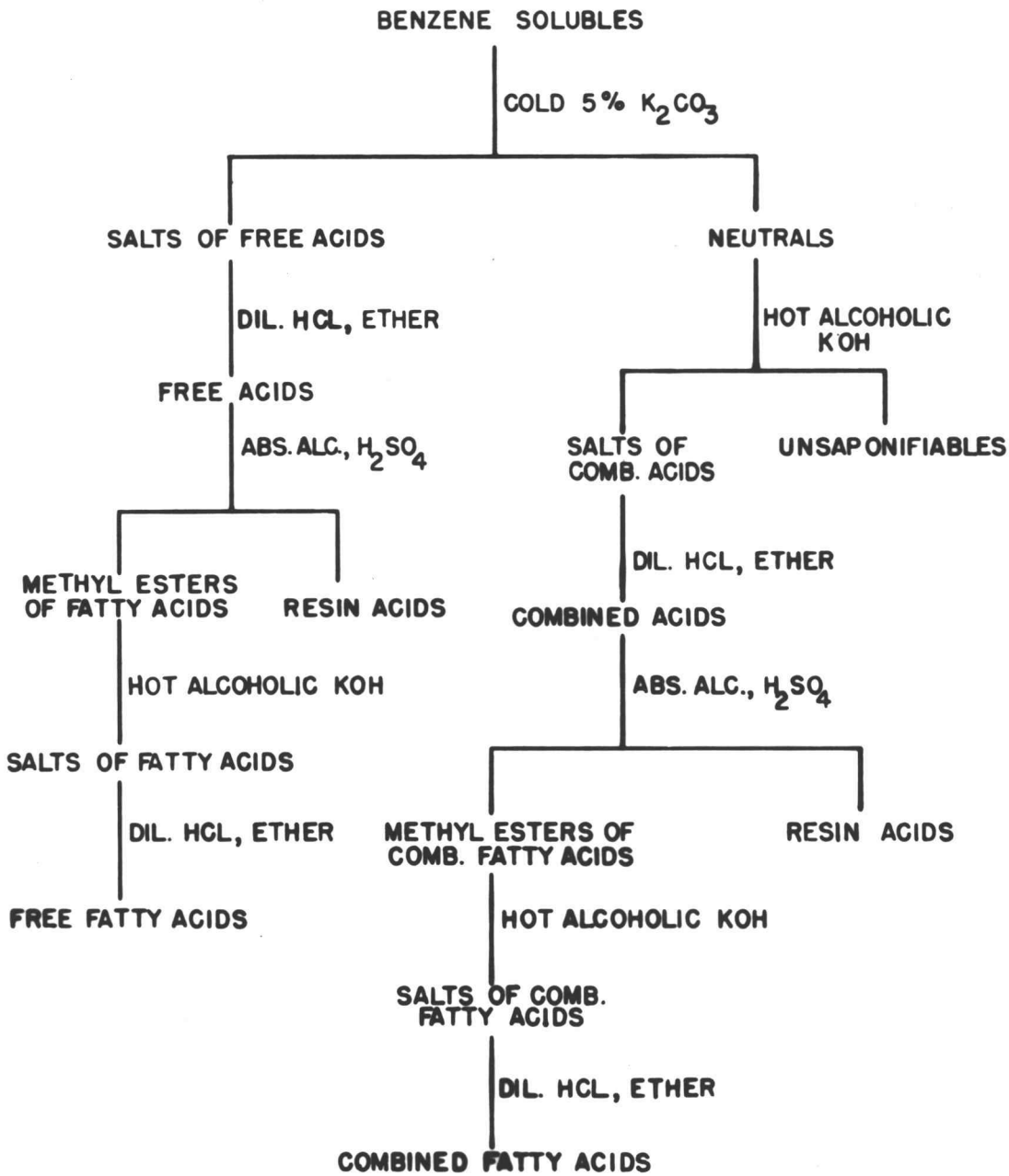
	per cent	
Acids		91.1
Resin Acids	72.1	
Fatty Acids	18.5	
Undetermined	0.5	
Unsaponifiables		8.9
		100.0

TABLE 8

COMPONENTS OF THE BENZENE EXTRACT
(K_2CO_3 treatment)

	per cent	
Free Acids		72.8
Resin Acids	64.4	
Fatty Acids	8.2	
Undetermined	0.2	
Neutrals		27.2
Resin Acids (combined)	7.8	
Fatty Acids (combined)	10.8	
Unsaponifiables	8.2	
Undetermined	0.4	
		100.0

FIGURE 2



SEPARATION OF INCENSE CEDAR BARK
BENZENE SOLUBLE COMPONENTS

Hot Water Solubles.

Tannin. Analysis of the bark for its tannin content was made by the hide powder method of the American Leather Chemists' Association (20, p10). A bark sample of approximately 50 grams was placed in the recommended percolation-type extractor. To the reflux flask, 750 milliliters of water were added. This water was enough to allow for the moisture absorbed by the bark and gave a final volume, which contained the soluble material, of not more than 600 milliliters. This gave a tannin concentration of about four grams per liter as required in the recommended procedure.

As shown in Table 10, the extent of the extraction time had a significant effect on the percentage tannin obtained. Extraction of the bark for a period of ten hours gave the maximum tannin yield. Further increases in this time either failed to increase the yield materially or a decrease in the tannin content was noted.

Table 9 summarizes the tannin analyses made on the nine composite samples. The tannin concentration is greatest from bark samples selected near the tops of incense cedar trees. The tops of the youngest trees provided the best yield of tannin while the bottom sample of the oldest trees was the least productive. The tannin content ranged from 3.5 to 7.8 per cent, which is less than that required for economical commercial extraction.

In order to facilitate a further comparison between the extractives of incense cedar and Port Orford cedar barks, analysis of Port Orford cedar bark for its tannin content was carried out similarly by

TABLE 9

ANALYSES OF TANNIN EXTRACTS FROM INCENSE CEDAR BARK

(percentages based on oven-dry weight of unextracted bark)

Age Group	Sample	Total Solids (%)	Soluble Solids (%)	Insol-ubles (%)	Tannin (%)	Non-Tannin (%)	Tannin / Soluble Solids
I	Bottom	10.35	9.54	0.81	3.93	5.61	0.41
	Middle	17.89	16.50	1.39	5.74	10.76	0.35
	Top	21.79	20.39	1.40	7.78	12.61	0.38
II	Bottom	14.27	12.38	1.89	4.30	8.08	0.35
	Middle	13.07	11.41	1.66	4.10	7.31	0.36
	Top	19.08	17.35	1.73	6.07	11.28	0.35
III	Bottom	8.68	8.09	0.59	3.54	4.55	0.44
	Middle	14.10	11.75	2.35	4.32	7.43	0.37
	Top	13.66	11.05	2.61	4.48	6.57	0.41

TABLE 10

EFFECT OF EXTRACTION TIME ON TANNIN YIELDS OF INCENSE CEDAR BARK

(Age Group II Middle)

(per cent of oven-dry weight of unextracted bark)

Component	Time				
	4 hrs. %	6 hrs. %	8 hrs. %	10 hrs. %	14 hrs. %
Tannin	3.58	4.02	4.12	4.30	4.18
Non-Tannin	6.64	6.13	7.35	8.08	7.57
Soluble Solids	10.22	10.15	11.47	12.38	11.75
Insoluble Solids	1.48	0.48	1.76	1.89	1.53
Total Solids	11.70	10.63	13.23	14.27	13.28
Tannin/Soluble Solids	0.35	0.40	0.36	0.35	0.36

TABLE 11

EFFECT OF EXTRACTION TIME ON TANNIN YIELDS OF PORT ORFORD CEDAR BARK

(Age Group II Tops)

(per cent of oven-dry weight of unextracted bark)

Component	Time			
	4 hrs. %	5 hrs. %	8 hrs. %	10 hrs. %
Tannin	4.48	5.18	6.28	5.68
Non-Tannin	7.38	6.01	7.89	7.91
Soluble Solids	11.86	11.19	14.17	13.59
Insoluble Solids	0.64	0.30	1.31	1.62
Total Solids	12.50	11.49	15.48	15.21
Tannin/Soluble Solids	0.38	0.46	0.44	0.42

TABLE 12

ANALYSES OF TANNIN EXTRACTS FROM PORT ORFORD CEDAR BARK

(percentages based on oven-dry weight of unextracted bark)

Age Group	Sample	Total Solids (%)	Soluble Solids (%)	Insol-ubles (%)	Tannin (%)	Non-Tannin (%)	Tannin / Soluble Solids
I	Bottom	11.47	10.98	0.49	5.21	5.77	0.48
	Middle	17.90	16.42	1.48	7.01	9.41	0.43
	Top	14.21	12.79	1.42	5.47	7.32	0.43
II	Bottom	9.57	8.67	0.90	4.60	4.07	0.53
	Middle	10.80	9.55	1.25	4.23	5.32	0.44
	Top	15.48	14.17	1.31	6.28	7.89	0.44
III	Bottom	9.98	9.17	0.81	4.87	4.30	0.53
	Middle	10.09	8.57	1.52	4.22	4.35	0.51
	Top	14.85	13.63	1.22	5.61	8.02	0.41

the hide powder method. The maximum tannin yield was obtained with an extraction time of eight hours, as shown in Table 11.

Table 12 shows the complete tannin analyses of the nine composite samples. The largest tannin yield was obtained from the tops of Port Orford cedar trees. The yield ranged from 4.2 to 7.0 per cent.

Comparison of Table 9 with Table 12 shows a similar distribution of tannin in the barks of the two cedar species. These results are in close agreement with the tannin distribution found in Douglas fir bark (6). The tannin extract from both cedars gave a precipitate with gelatin, a green color with ferric chloride and a precipitate when boiled with dilute sulfuric acid. These tests indicated the tannin to be of the phlobatannin class.

Carbohydrate material. Isolation of the carbohydrate material was accomplished by leaching the bark with hot water and pouring the concentrated extract into a large volume of 95 per cent ethanol. One hundred gram batches of unextracted bark ground to pass a No. 20 mesh screen were placed in a five liter round-bottom flask together with two liters of distilled water and heated for 24 hours on a steam cone. The liquor was removed by filtration and the bark leached with an additional two liter portion of water for 24 hours. The extract from 500 grams of bark was then evaporated at room temperature to a thick syrup by means of a forced air draft from an ordinary fan. The water soluble material was separated into two fractions by pouring this syrup into five volumes of 95 per cent ethanol. Under these conditions, the tannin and other colored substances were soluble in the ethanol while

the carbohydrate fraction precipitated. The carbohydrate was removed by filtration, redissolved in hot water and precipitated from a fresh portion of alcohol. After five precipitations from ethanol, a light pink-colored carbohydrate product was obtained in a yield of approximately 9 per cent of the oven-dry unextracted bark.

It was found that special precautions must be observed in drying the carbohydrate precipitate. Attempts to dry the material on a Büchner funnel with suction from a laboratory type, water aspirator resulted in a brown amorphous gum, a large part of which would not redissolve in water. Similar unsatisfactory results were noted when attempts were made to dry the material in a vacuum oven at 55° C. Best results were produced by filtration on a Büchner funnel with suction and then removing the suction as soon as the standing liquid had passed through. Following three washings with 100 milliliter portions of ethanol, the thoroughly moist precipitate was placed in a desiccator until dry.

The dried carbohydrate material began to decompose at 260° C. Optical rotation measurements were attempted but no significant results could be obtained due to the turbid water solution which resulted from the presence of small insoluble particles.

After hydrolysis, the Somogyi-Shaffer procedure (18) demonstrated the presence of 65.9 per cent reducing sugar in the carbohydrate material, expressed as glucose. Maximum reducing sugar was obtained after eight hours hydrolysis as shown in Table 13.

TABLE 13

TOTAL REDUCING SUGAR DETERMINATIONS
(percentages based on oven-dry weight of carbohydrate material)

Length Of Hydrolysis Treatment (hours)	Total Reducing Sugars (per cent)
0	0.00
4	61.63
8	65.90
15	62.76
21	60.71

In attempts to account for the non-reducing fraction of the carbohydrate material, a tannin analysis was made on the alcohol insoluble fraction according to the hide powder method of the American Leather Chemists' Association (20, p.10). Results showed 24.0 per cent tannin and 10.2 per cent insolubles. Thus, an alcohol insoluble tannin material is present in the crude carbohydrate fraction. This tannin comprises almost 40 per cent of the total tannin present in the hot-water extract. The insolubles are apparently due to drying methods used in procuring the original carbohydrate material since it was previously demonstrated that extreme care must be taken to prevent formation of a water insoluble substance. The composition of the crude carbohydrate material is given in Table 14.

The non-tannin solution from the tannin analysis of the crude carbohydrate material was evaporated to a syrup under the fan and poured into five volumes of 95 per cent ethanol. The white precipitate obtained was removed by filtration on a Büchner funnel and dried in

the desiccator. This purified carbohydrate fraction dissolved readily in water. It showed 95.7 per cent reducing sugar after eight hours hydrolysis. Optical rotation determinations gave duplicate values showing $[\alpha]_D^{20°C.} = +133.2 \pm 1°$.

Table 15 gives the approximate composition of the total reducing sugars present in the crude carbohydrate material. Pentosans, as determined by the method of the Association of Official Agricultural Chemists (12), comprised 13.46 per cent of the crude carbohydrate. Uronic anhydride was found, by Maher's method (14), in a yield of 1.87 per cent. One gram of carbohydrate material was oxidized with nitric acid according to the Bureau of Standards procedure (1, p218). The yield of mucic acid showed 21.50 per cent galactans to be present. Mannans were shown to comprise 3.09 per cent of the carbohydrate material by precipitation as the usual mannose phenylhydrazone (22). Glucosans were calculated by difference.

TABLE 14

COMPOSITION OF THE CRUDE CARBOHYDRATE MATERIAL

(percentages based on the oven-dry weight of carbohydrate material)

Component	Per cent
Tannin	24.0
Insolubles	10.2
Total Reducing Sugar (after hydrolysis)	65.9
Total	100.1

TABLE 15

THE APPROXIMATE COMPOSITION OF THE TOTAL REDUCING SUGARS OF THE CARBOHYDRATE MATERIAL

(percentages based on the oven-dry weight of carbohydrate material)

Component	Per cent
Pentosans	13.46
Mannans	3.09
Galactans	21.50
Uronic Anhydride	1.87
Glucosans (by difference)	25.98
Total Reducing Sugars	65.90

V. ANALYSIS OF EXTRACTIVE-FREE BARK

The conventional wood analysis method for the determination of extractive content consists of successive extractions with ethyl ether, 95 per cent ethanol, and hot water. Extractive-free wood is prepared according to the T.A.P.P.I. method T 12m-45 (19) which consists of making successive extractions with alcohol-benzene, alcohol, and hot water. This wood is then analyzed for various constituents. These analyses were carried out on incense cedar bark in conjunction with work done in course Ch. 471, "Chemical Analysis Of Wood And Related Products."

Analysis of the extractive-free bark for lignin and methoxyl were made in accordance with the T.A.P.P.I. methods T 13m-45 and T 2m-43 (19) respectively. The acetyl determination was made according to the method outlined by Freudenberg and Harder (3). Pentosans were ascertained by Tollens' procedure (19). Values obtained by these determinations are given in Table 17.

Because of filtration difficulties, holocellulose could not be prepared by means of the monoethanolamine and chlorination procedure T 9m-45 (19). Similar results were found when analyzing Douglas fir bark bast fibers for holocellulose (8). Efforts to prepare holocellulose by a modification of Jayme's sodium chlorite method (21) proved more successful. In order to establish the most satisfactory conditions for the analysis, a series of determinations was made. Duplicate 2.5 gram samples of extractive-free bark ground to pass a

No. 40 mesh screen were subjected to a varying number of treatments in the following manner. The sample was placed in a 250 milliliter Erlenmeyer flask with 160 milliliters of water, 10 drops of glacial acetic acid and 1.5 grams of sodium chlorite. The flask was then placed in the hood in a hot water bath which was maintained at 70 to 80° C. for the duration of the treatment. At the end of one-half hour another addition of the same amounts of acetic acid and sodium chlorite was made, taking care to add the acetic acid first since the solution must be acid at all times. Following an additional one-half hour period, the same amounts of acetic acid and sodium chlorite were introduced. Thereafter, these additions were made at the end of every one hour period. The total length of treatment of the various samples is shown in Table 16.

Upon completion of the three and four hour treatments, the flask was removed from the water bath, cooled to room temperature and the holocellulose filtered on a sintered glass crucible. The precipitate was washed with ice water, acetone and ether. Drying of the holocellulose was carried out in a vacuum oven at 50° C. which resulted in the soft fluffy holocellulose desirable for subsequent lignin determinations. The samples treated for five and six hours were allowed to stand overnight at room temperature before being filtered. This evidently led to continued degradation of the holocellulose which accounts for the large difference in the total lignin and holocellulose obtained in the four and five hour determinations.

TABLE 16

CHLORITE HOLOCELLULOSE DETERMINATIONS

(per cent of oven-dry weight of extractive-free bark)

Time Of Treatment (hrs.)	Holocellulose (%)	Lignin On Holocellulose (%)	Holocellulose Corrected For Lignin (%)	Total Lignin Plus Holocellulose (%)
3	57.83	5.57	52.26	98.75
4	54.54	2.97	51.57	98.06
5	47.10	0.82	46.28	92.77
6	45.80	0.85	44.95	91.44

The lignin remaining in the holocellulose following the three hour treatment was almost nine per cent of the uncorrected holocellulose. Table 16 shows the four hour treatment to be the most efficient since holocellulose containing less than six per cent lignin resulted without appreciable degradation of the holocellulose. Treatments of longer duration resulted in considerable destruction which substantiated the experiences of other holocellulose investigators.

Total lignin, as determined by the 72 per cent sulfuric acid method previously cited, accounted for 46.49 per cent of the extractive-free bark. This value is used in making the calculations in Table 16. The ash content of the unextracted bark was found to be 0.97 per cent and is included in Table 17.

During the course of the analysis of extractive-free bark, the total extractive content of incense cedar bark was ascertained according to the conventional wood analysis method which consists of making successive extractions with diethyl ether, 95 per cent ethanol, and hot water. If the bark resulting from these determinations could be considered extractive-free, the preparation of extractive free bark by the T.A.P.P.I. method could be eliminated and the initial bark sample used for the complete analysis for components given in Table 17. Thus, the procedure for the analysis of extractive-free incense cedar bark would be simplified considerably.

A comparison of the amount of material removed in each extraction procedure is shown in Table 18. This table indicates that the T.A.P.P.I. procedure provides a more efficient method for the removal

TABLE 17

ANALYSES OF EXTRACTIVE-FREE BARK

(per cent of oven-dry weight of unextracted bark)

Component	Per cent
Pentosans	8.51
Methoxyl	2.69
Acetyl	0.59
Ash Content	0.97
Alcohol-Benzene Solubles	14.93
Alcohol Solubles	3.04
Hot-Water Solubles	7.47
Lignin	37.06
Holocellulose (corrected for lignin)	36.40
	98.90

of the total extractives. However, closer examination of this data reveals that the organic solvents used in the T.A.P.P.I. procedure did not remove as much extraneous material from the bark as did the organic solvents used in the conventional method. The major difference between the two methods appears to be in the amounts of material removed in the hot water extraction.

The determination of the hot-water solubles by the T.A.P.P.I. procedure for the preparation of extractive-free bark is as follows. Bark previously extracted with alcohol-benzene and alcohol is treated in a flask with three portions of distilled water, heating each change of water for one hour in a hot water bath at 100° C. The hot-water solubles are then determined by evaporating an aliquot portion of the total extract to dryness on a steam bath and drying the residue in the oven at 105° C. for one hour. In the determination of the hot-water solubles by the conventional T.A.P.P.I. method T 1m-45 (19), a similar procedure is followed. However, only one portion of water is used to extract the bark; that is, no change of water took place every hour. It must be assumed that in using fresh portions of water during the extraction, the hot-water solubles are removed more efficiently than when the bark merely soaks in one portion of water for three hours. This might account for the difference in the amount of hot-water solubles obtained by the two methods. In order to obtain a more accurate comparison between the two procedures, the conventional method of analysis was modified so as to provide three water portions during the determination of the hot-water solubles. This eliminated the

discrepancies originally present.

The T.A.P.P.I. method for the preparation of extractive-free bark removed 0.77 per cent more material from the bark than did the modified conventional procedure. Thus, this approved method should be used for the preparation of extractive-free incense cedar bark in preference to the modified T 1m-45 procedure described. If this is not done, material not removed will later appear as lignin and an error of over 1.5 per cent in the lignin determination will result.

As was previously noted, the amount of ether-alcohol soluble material extracted was greater than that removed with alcohol-benzene and alcohol. This difference evidently arose from the fact that ether removed some water soluble material which was insoluble in alcohol-benzene.

TABLE 18

COMPARISON OF METHODS TO REMOVE TOTAL EXTRACTIVES

(per cent of oven-dry weight of unextracted bark)

	one portion of water	three portions of water
	per cent	per cent
Ether Solubles	9.24	9.24
Alcohol Solubles	9.42	9.42
Hot-Water Solubles	5.05	6.01
Total Extractives	23.71	24.67
Alcohol-Benzene Solubles	-----	14.93
Alcohol Solubles	-----	3.04
Hot-Water Solubles	-----	7.47
Total Extractives		25.44

DISCUSSION

As has generally been the case with other barks, the total extractives obtained from incense and Port Orford cedar barks varied according to the sample on which the analysis was made. In both species the greatest extractive content was found in the bark from the tops of the younger trees; bark from the bottoms of the oldest trees contained the least extraneous material. The hot water extract was by far the largest singular component obtained from both species. Insignificant amounts of alcohol and ether solubles were found. The total extractive content of incense cedar bark varied between 13.70 and 30.39 per cent, that of Port Orford cedar between 15.49 and 25.69 per cent. It is interesting to note that the physical properties of the various extractives of the two species were quite similar.

The hexane extract from incense cedar bark contained small amounts of volatile oil, resin acids, phytosterol, crystalline fatty acid and a crystalline ester. Larger quantities of free and combined acids showing unsaturated properties were isolated along with a tacky, amber, unsaponifiable fraction. Attempts to obtain crystalline derivatives of these components by precipitation from a variety of solvents were unsuccessful. The extreme tacky nature of the hexane extract must result, in part, from the presence of these unsaturated materials.

The benzene solubles contained over 72 per cent resin acids. It should be noted in Table 7 that these acids were present in both the free and combined forms. Both fractions were optically active, were not esterified under the conditions prescribed by Wolff and Scholze (23)

and gave positive Liebermann-Storch tests (7, p358). This information is significant in that previous investigations of resin acids do not show them to be present in the combined form. Both resin acid fractions had higher neutral equivalents than abietic acid, a common resin acid, but these values correspond quite closely to a material isolated from Ponderosa pine bark (6). Considerable work is still necessary to more fully characterize these acids. As in the case of the hexane solubles, the benzene extract contained significant amounts of unsaturated materials. For this reason, crystalline precipitates were not obtained from a variety of solvents. Oxidation of the free and combined fatty acids with cold alkaline potassium permanganate failed to yield any crystalline derivative indicating the absence of oleic and linoleic acids.

Tannin analyses of the hot water extract of both incense and Port Orford cedar barks showed the tannin content to be less than that required for feasible commercial use at this time. The yield from incense cedar varied between 3.5 and 7.8 per cent; that from Port Orford cedar ranged from 4.2 to 7.0 per cent, the optimum extraction times being ten and eight hours respectively. Bark from the tops of the younger trees of both species gave the largest yields while the bottom samples of the oldest trees gave the smallest. Since incense cedar trees are distinguished by their large butts and sharp taper, a large majority of the bark comes from the lower portions of the tree, further decreasing the commercial possibilities of using the bark as a source of tannin.

Attempts to obtain a pure carbohydrate fraction from the hot water extract by precipitation from large volumes of 95 per cent ethanol were hindered by the presence of an alcohol insoluble tannin material. Removal of this tannin with hide powder made it possible to obtain a carbohydrate which showed 95.7 per cent reducing sugar. Analysis of the crude carbohydrate material for individual sugars gave the following results: pentosans, 13.46 per cent; mannans, 3.09 per cent; galactans, 21.50 per cent; glucosans, 25.98 per cent; and uronic anhydride, 1.87 per cent.

An attempt to prepare extractive-free incense cedar bark by successive extractions with ethyl ether, 95 per cent ethanol and hot water was unsuccessful. It was found that this method removed 0.77 per cent less extraneous material than did the conventional T.A.P.P.I. procedure which utilizes alcohol-benzene, 95 per cent ethanol and hot water as the solvents. Hence, in the preparation of extractive-free incense cedar bark, the standard T.A.P.P.I. procedure was used.

Because of filtration difficulties, holocellulose determinations by the T.A.P.P.I. method T 9 m-45 employing monoethanolamine were not applicable to incense cedar bark. The chlorite method (21) was found to be more successful but the lignin content of the holocellulose could not be lowered below 5.6 per cent without appreciable degradation of the holocellulose. Lignin-free holocellulose comprised 36.4 per cent of the oven-dry unextracted bark.

SUMMARY

Incense cedar bark was collected from the bottom, middle and tops of nine trees which were divided into three age groups: 146-195 years, 261-285 years and 326-351 years. The nine samples were successively extracted with hexane, benzene, ether, hot water and alcohol in order to show the distribution of the extractives. A comparison of the extractives of this bark was made with the extraneous material from the bark of Port Orford cedar, another western species. The physical properties, distribution and the amounts of the extracts were quite similar. Further chemical investigation of incense cedar bark extractives showed the hexane extract to contain small amounts of volatile oil, phytosterol, resin acids, crystalline fatty acid and a crystalline ester. Other fatty acids of varying degrees of unsaturation were shown to be present.

The benzene extract contained a large amount of resin acids, present in both the free and combined forms. Fatty acids and unsaponifiables showing strong unsaturated properties were also isolated. The presence of oleic or linoleic acid could not be shown. The ether and alcohol extracts were not characterized because of relatively low yields. Analysis of both incense and Port Orford cedar barks showed the tannin content of each species to be less than that necessary for economical commercial extraction at this time. A crude carbohydrate fraction containing a small amount of an alcohol insoluble tannin was isolated from the hot water extract, other components being pentosans,

mannans, galactans, glucosans and uronic anyhdride, Extractive-free bark was prepared and analyzed for various constituents by the conventional methods of wood analysis.

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