THE NATURE OF THE CHEMICAL CONSTITUENTS OF GRAND FIR BARK (*Abies grandis* Lindl)

by

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THE NATURE OF THE CHEMICAL CONSTITUENTS OF GRAND FIR BARK (*Abies grandis* Lindl)

INTRODUCTION

The purpose of this investigation was to identify the principal constituents of grand fir bark (*Abies grandis* Lindl) with the hope that a more complete utilization can be made of the grand fir tree.

Grand fir is sometimes called white fir and is occasionally mistaken for the true white fir (*Abies concolor* Lindl and Gord). It is generally found on the lower slopes of the Cascades from Vancouver Island in Canada to northern California. It is usually in mixed stands, most commonly with Douglas fir, which is not a true fir. The annual cutting of grand fir would be difficult to determine because the true firs are not usually separated in logging operations.

An examination of the literature did not reveal any previous investigations of the chemical nature of grand fir bark. The wood of grand fir was investigated by Kurth (9) for extractive content and an analysis was given for the extractive-free wood. The bark of balsam fir, which is another true fir, was investigated by Hay and Lewis (6). They found a mucilage or gum-like material in their hot water extract, which appears to be similar to a material in

grand fir. It is possible that this material may be of commercial value, and it is also possible that it could be found in most of the true firs.

Grand fir and several other true firs are quite often cut along with Douglas fir to get them out of the way and are sold as low grade lumber. In the pulp industry grand fir is used but not particularly desired. In other words it is partially utilized but not sought after.

It is hoped that the information from this investigation will be used to instigate further study and eventually a more complete utilization of the grand fir tree.

EXPERIMENTAL PROCEDURE

I. SOURCE AND PREPARATION OF SAMPLES.

A complete list of the trees sampled is given in Table 1. The bark from tree No. 12 was collected May 25, 1950, at Weaver Creek, east of Myrtle Creek, from timber felled less than one week. The bark samples from the other nine trees were collected June 8, 1950, at Willow Creek, south of Oakridge, at 4000 feet elevation. The bark was room-dried and made into nine composite samples. The samples were made up by grouping the bark together with respect to age group and section of the tree. For example the bark, which was sampled from the bottom sections of trees No. 25, No. 26, and No. 31 at a height of 2.0 to 3.5 feet, was grouped together as one sample and designated age group III, bottom section.

The samples were then ground in a Rietz Disintegrator to pass a No. 20 mesh screen. There was a small amount of red cork-like material that would not pass through the Rietz Disintegrator because of its lightness. It was, however, fine enough to pass the No. 20 mesh screen so it was added to the samples. The bark from the bottom sections of the trees in all age groups contained more of this red cork-like material than the middle sections, and the middle sections more than the top sections. The presence of this

cork-like material was not obvious when a visual examination of the bark was made.

The samples were stored in glass jars and labelled as to age group and section of the tree.

TABLE 1

COLLECTION DATA ON GRAND FIR BARK SAMPLES

Group	Age	Tree	Bottom Section		Middle Section		Top Section	
			Diameter		Diameter		Diameter	
			Outside Bark	Inside Bark	Outside Bark	Inside Bark	Outside Bark	Inside Bark
No.	Years	No.	In.		In.		In.	
I			(2.5 to 4.0 ft. height)		(42 to 45 ft. height)		(95 to 115 ft. height)	
	170	34	17.2	15.2	15.8	14.8	9.0	8.6
	165	12	23.8	21.8	15.5	14.3	12.5	11.7
II			(1.0 to 3.0 ft. height)		(43 to 53 ft. height)		(85 to 112 ft. height)	
	196	27	22.5	20.5	17.5	15.8	11.2	10.0
	225	28	29.0	26.8	19.0	18.0	14.0	13.0
	220	29	18.7	17.5	16.8	16.0	8.1	7.5
	218	30	24.9	22.4	21.6	19.1	12.8	12.0
	202	32	31.1	28.6	24.0	22.8	17.2	16.0
III			(2.0 to 3.5 ft. height)		(44 to 68 ft. height)		(96 to 120 ft. height)	
	255	25	20.0	18.0	15.5	14.1	6.5	5.8
	257	26	30.5	27.2	18.5	17.0	16.0	15.0
	245	31	41.5	39.2	28.8	27.0	18.0	16.5

II. EXTRACTIVE CONTENT OF SAMPLES.

The samples were successively extracted with hexane, benzene, diethyl ether, hot water and alcohol. This order of extraction was followed to show the distribution and amount of extractives present in the bark. Hexane was used first to remove the waxes, fats and oils; and benzene was used next to obtain any wax which was not removed by the hexane. Diethyl ether followed these extractions to remove any flavones or like coloring matter. The hot water extraction determined the amount of tannin and carbohydrates present, and lastly the alcohol indicated the amount of phlobaphenes. All of the extractions, with the exception of the hot water extraction, were done in a Pyrex Soxhlet. The weighed samples were placed in cloth bags and solvent extracted continuously for eight hours. The solution of solvent plus extractives was filtered into a tared glass dish and the solvent evaporated off in a steam bath. The dish was then placed in an oven at 105° C. for eight hours, cooled in a desiccator and weighed. For the hot water extractions, T.A.P.P.I. method T 1m-45 (15) was followed except that the leaching time was lengthened to four hours because it was impossible to filter the solution after three hours. The bark from the hot water extraction was placed in a cloth bag and final Soxhlet extraction was made with alcohol.

The results of these extractions are given in Table 2. The percentages are based on the oven-dry weight of the unextracted bark. The average moisture content of the air-dry bark samples was 8 per cent.

It may be noted in Table 2 that the extractive percentages do not vary a great deal and are rather small for all extractions except that of the hot water. The hot water soluble content was higher in the top sections of all age groups, and the middle sections were higher than the bottom sections. The content also increased for all sections going from the old trees of age group III to the young trees of age group I.

The hexane soluble material was a soft wax varying in color from a yellowish-green for the top section, to a light tan for the middle section, to a reddish tint for the bottom section.

The benzene solubles were a soft black resinous material and the ether soluble material was hard and dark red in color. Their appearance did not vary between sections or age groups. The alcohol soluble material was considered to be essentially phlobaphenes. An investigation of these materials was decided against due to their low yields.

TABLE 2

EXTRACTIVE CONTENT OF GRAND FIR BARK

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

Age Group	Section	Hexane Solubles	Benzene Solubles	Ether Solubles	Hot Water Solubles	Alcohol Solubles	Total Solubles
		%	%	%	%	%	%
I	Bottom	1.89	.86	.40	14.24	1.10	18.49
	Middle	2.23	1.10	.40	19.07	1.10	23.90
	Top	2.26	1.37	.15	24.62	.62	29.02
II	Bottom	2.45	1.95	.66	12.13	3.35	20.74
	Middle	2.33	.93	.76	15.44	2.32	21.78
	Top	2.41	1.17	.68	17.35	1.80	23.41
III	Bottom	2.53	1.13	1.02	10.97	2.67	18.32
	Middle	2.19	.97	.83	11.31	2.96	18.26
	Top	1.55	1.48	.30	13.46	2.28	19.07

III. CONSTITUENTS OF HEXANE SOLUBLES

A composite sample of bark was made up with equal parts of all nine samples. This was used for the subsequent analyses. Fifteen hundred grams of the composite sample were extracted with hexane (petroleum ether b.p. 60-70° C.) in a large Pyrex Soxhlet. The solvent was evaporated off in a steam bath and the extract recovered. The yield was 2.20 per cent, based on the oven-dry weight of bark. The extract was a light tan wax material with a melting point of 52-53° C. by the drop point method (2, p.214). To analyze the wax, a twenty gram sample was saponified with 150 ml of 2 per cent alcoholic potassium hydroxide. After saponification the alcohol was removed by repeatedly adding water and boiling off the alcohol. The unsaponifiables were removed from the water solution by shaking out with diethyl ether in a separatory funnel. The ether solution was washed with water and dried over anhydrous sodium sulfate. The ether was evaporated off in a steam bath in the hood and the unsaponifiables remained as a residue. The water solution, which contained the acid salts, was acidified by adding hydrochloric acid until the solution was definitely acid to litmus. The free acids, which were formed, were extracted with ether as described above for the unsaponifiables. The ether was removed and the acids

recovered. The composition of the wax was found to be: acids 92.5%; unsaponifiables 7.5%.

Acids.

A ten gram sample of the acids was dissolved in hot acetone and upon cooling a precipitate was obtained. Repeating this operation several times and finally purifying the precipitate with activated carbon produced a white powder. The material, which was soluble in cold acetone, was a black, sticky substance. It constituted 4 per cent of the acids and was not investigated.

The white powder melted at 69-70° C. A Liebermann-Storch test (8, p.358) for resin acids was negative and a test for unsaturation with cold permanganate solution was negative. These tests, combined with the fact that the acids were isolated from a wax-type material implied they were a mixture of saturated, aliphalic or hydroxy, fatty acids. The low melting point of the acids indicated they were aliphatic rather than hydroxy acids. The neutral equivalent was 466, which was determined by dissolving the white powder in a warm mixture of 50 per cent alcohol and benzene and titrating against a standard alcoholic KOH solution. The neutral equivalent indicated the acids were equivalent to a mixture of C_{30} and C_{32} aliphatic fatty acids. No attempt was made to separate this natural mixture of acids.

Unsaponifiables.

One gram of the unsaponifiable material was dissolved in hot acetone and a white precipitate resulted upon cooling the solution. This precipitate was dried to a white powder. The amount of material soluble in the cold acetone solution was negligible.

The white powder melted at 70-71° C. An acetate was formed when it was refluxed with acetic anhydride in anhydrous pyridine. The acetate melted at 53-54° C. This indicated it was a saturated fatty alcohol.

An oxidative fusion of the alcohol was carried out by fusing with potassium hydroxide (7, p.1737). The fusion mixture was dissolved in water and the solution extracted with ether. The ether solution was allowed to evaporate in the hood and the remaining residue was negligible in amount. This denoted the absence of hydrocarbons. The water solution was acidified and the resulting free acid removed by ether extraction. The acid product was precipitated by dissolving in hot acetone and cooling the solution. It was dried in a vacuum oven at 60° C. to a white powder. It melted at 73-74° C. and the neutral equivalent was 397. Cerotic acid has a neutral equivalent of 396.68, which indicated the original alcohol to be a C_{26} (Ceryl) alcohol.

IV. HOT WATER SOLUBLES.

Tannin.

The original objective of this part of the investigation was to determine the amount of tannin in the bark, but it was found that the method of tannin extraction presented a problem.

The first attempt at analysis was made using a 30 gram composite sample of unextracted bark. A percolation type extractor, recommended by the American Leather Chemists Association (1, p.14), was used. This did not prove entirely successful because after one-half hour of operation a gelatinization of the sample occurred and the liquor would not drain through the sample. Graham and Rose (5) experienced the same difficulty with balsam fir bark. They claimed that the gelatinization was due to a pectinous material. Hay and Lewis (6), working with balsam fir bark, found that the pectinous gel could be removed by heating a water solution of bark in a steam bath for three or four days. The liquor, which was obtained before the gelatinization took place, was analyzed for tannin in accordance with the standard methods of the American Leather Chemists Association (1, p.7). This analysis appears in Table 3.

Another percolation extraction was made on a 30 gram composite sample of unextracted bark, using 95 per cent ethanol instead of water. The extraction time was 8 hours.

The liquor obtained from this extraction was poured into an evaporating dish and placed in the hood. It was allowed to remain at room temperature for several days until the alcohol was almost completely evaporated off. The very thick syrup remaining was added to cold water, heated to 60° C. with constant stirring and cooled to room temperature. This solution was analyzed for tannin by the standard method and the results appear in Table 3. Since this extraction was made with alcohol, the values given for this analysis cannot be compared with the other analyses, except for the tannin value.

A 30 gram composite sample of unextracted bark, which had been ground to pass a No. 4 mesh screen, was used for the final percolation extraction. Water was the solvent as in the first extraction. The previously reported gelatinization did not occur in this coarse ground bark, and the liquor drained through the sample in a normal manner. The liquor received from this extraction was a dark red color and was analyzed for tannin. This analysis also appears in Table 3. The optimum time for tannin extraction was found to be 10 hours. This was determined by extractions of coarse ground bark samples for various periods of time, from 4 hours to 14 hours. The liquors from these extractions were analyzed for tannin and the 10 hour period gave the maximum amount.

It should be noted in Table 3 that 89 per cent of the tannin was removed in the first one-half hour.

TABLE 3

TANNIN ANALYSES

(Percentages based on oven-dry weight of unextracted bark.)

	1. Gelatinized Sample (½ hour)	2. Alcohol Extraction (8 hours)	3. Sample Passing #4 Mesh Screen
	%	%	%
Total Solids	12.14	15.35	15.55
Soluble Solids	11.75	11.22	14.55
Insoluble Solids	.39	4.13	1.00
Non Tannin	5.39	4.66	7.42
Tannin	6.36	6.56	7.13

Carbohydrate Material.

A large sample of carbohydrate material was desired, so a thirty pound composite sample of unextracted bark was made up. The sample was divided into three, ten pound samples and placed in wooden kegs with approximately eight gallons of water to each keg. The water and bark mixtures were heated by steam coils to a 90-100° C. range which was maintained for four hours. The liquor was drained off at

the end of this time. Fresh water was added and the leaching operation repeated two more times. The liquor collected from these extractions was concentrated to a thick syrup by vacuum evaporation. The evaporator employed for this operation was a vertical, long tube, natural circulating type, which was constructed by the Precision Scientific Company. It had a three liter batch capacity and could be set up for a continuous operation. Vacuum was applied by means of a water type aspirator and the boiling point of the evaporating solution was never allowed to exceed 70° C. The thick syrup was then added to four volumes of 95 per cent ethanol. The carbohydrate material precipitated and the tannins and coloring matter remained in solution. After the precipitate had settled, the alcohol-water solution was decanted and more alcohol was added to the precipitate. This was repeated until the alcohol was practically colorless. The mixture was then filtered by suction on a Buchner funnel. The precipitate was dissolved in water, once more precipitated with alcohol and filtered. The precipitate was not allowed to remain on the filter funnel for more than a few minutes either time, because it turned black and hardened upon standing. It was placed in a vacuum desiccator in a semi-dry state. A vacuum was applied by means of a laboratory type, water aspirator and maintained until the moisture

content was reduced to 8 per cent. The yield of this carbohydrate powder, based on the oven-dry weight of unextracted bark, was 2.36 per cent.

The powder was a light tan color. It was only slightly soluble in cold water and almost completely soluble in hot water. When added to 72 per cent sulfuric acid a few black particles were formed which were insoluble. An analysis of the carbohydrate material was made in the following manner:

A five gram sample of carbohydrate powder was dissolved in three hundred mls. of water heated to 60° C. When the solution had cooled to room temperature, it was analyzed for tannin by a method of the American Leather Chemists Association (1, p.7). The tannin content was 17.7 per cent based on the oven-dry weight of carbohydrate material. This proved the material was not pure carbohydrate and indicated the tannin was not entirely soluble in alcohol.

The detanninized solution from the tannin analysis was refluxed with 2 per cent sulfuric acid for 14 hours and filtered. The insoluble material amounted to 8.2 per cent of the carbohydrate material. It was a black amorphous substance, insoluble in 72 per cent sulfuric acid, and appeared to be a lignin or phlobaphene type material.

The hydrolyzed solution, from which the insolubles were removed, was neutralized with sodium hydroxide and

utilized for a total reducing sugar determination by the Somogyi method (14). The carbohydrate material was found to contain 47.7 per cent reducing sugars after hydrolysis. The fourteen hour hydrolysis time was found to give the maximum total reducing sugar value as shown in Table 4.

TABLE 4

TOTAL REDUCING SUGAR DETERMINATIONS

(Percentages are based on the oven-dry weight of carbohydrate material.)

Time	Total Reducing Sugars %
4 hours	44.8
8 hours	46.0
14 hours	47.7
18 hours	47.3

A total of the tannin, insolubles and total reducing sugars did not account for all of the material in the carbohydrate powder. An explanation for this was found in the following work:

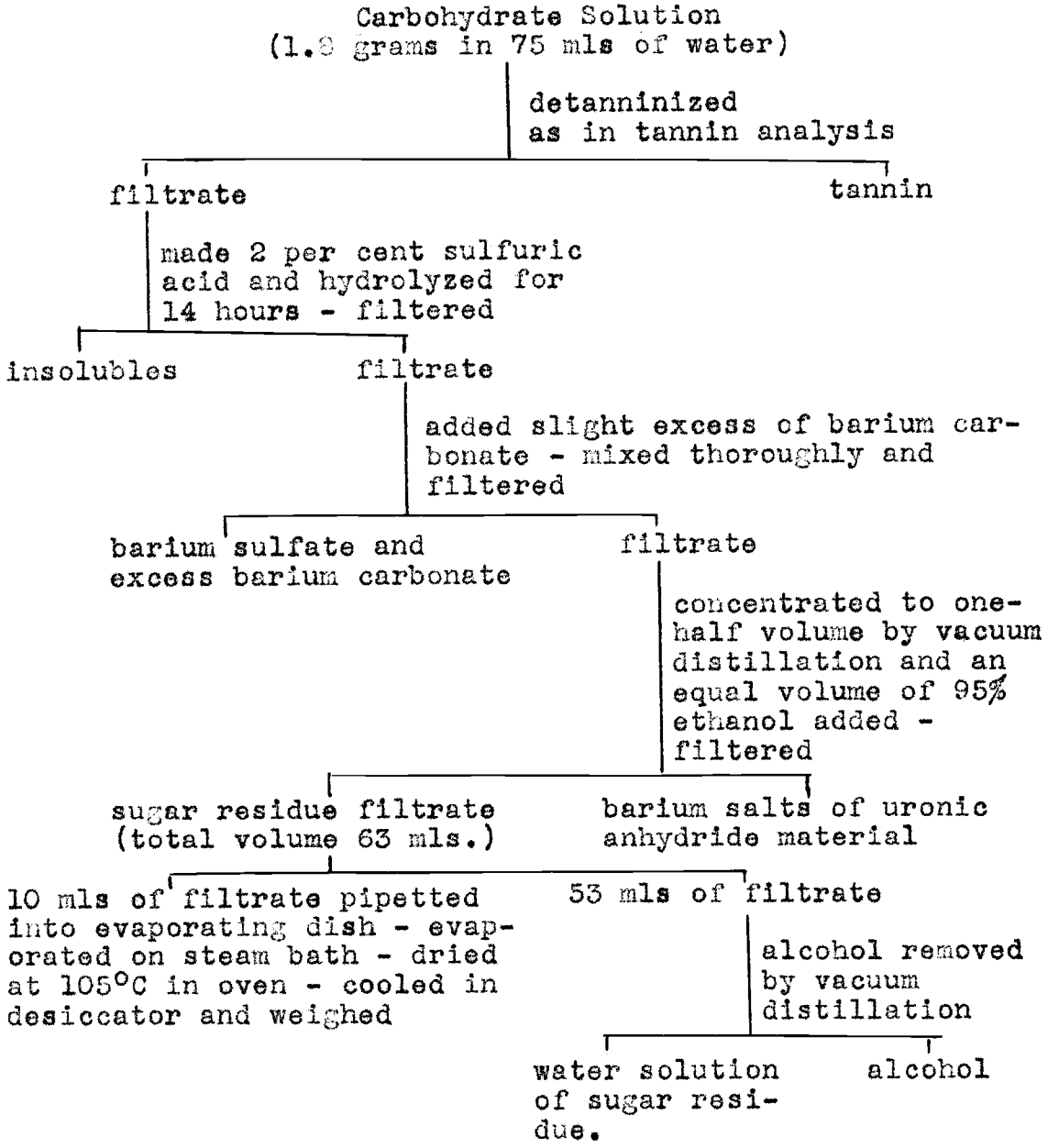
Pigman and Goepp, Jr. in their book on carbohydrate chemistry (13, p.304) and (13, p.608) revealed the resistance to acid hydrolysis of polyuronide material in general

and particularly polygalacturonide residues. A method devised by Mather (11) was used to determine the uronic anhydride content of the carbohydrate material. In this method carbon dioxide was evolved by heating the carbohydrate powder with 12 per cent hydrochloric acid. The carbon dioxide was bubbled through a dilute solution of barium hydroxide and the unreacted base was titrated against a standard acid. From the data obtained the uronic anhydride content was calculated to be 15.6 per cent.

It was necessary, then, to determine whether or not the uronic anhydride portion of the carbohydrate material contributed to the total reducing sugar value. This was accomplished by treatment of the carbohydrate material as described in Figure 1. The total sugar residue was calculated from the 10 ml aliquot. In order to determine if the uronic anhydride material was removed completely, the sum of the tannin, insolubles, and sugar residue was subtracted from the weight of carbohydrate material. This indicated 16.6 per cent uronic anhydride was removed which was a reasonable check with the previous value of 15.6 per cent. The final water solution of the sugar residue was utilized for the total reducing sugar determination by the Somogyi method (14). The total reducing sugar, calculated on the basis of the total carbohydrate material, was 42.4 per cent, which was only 5.3 per cent lower than the value obtained

FIGURE 1

PROCESS FOR DETERMINING TOTAL REDUCING SUGARS AFTER REMOVAL OF URONIC ANHYDRIDE



without removing the uronic anhydride. It was obvious, therefore, that the uronic anhydride did not appreciably contribute to the total reducing sugar. The loss of sugars could have been due to removal of the barium sulfate as shown in Figure 1.

The composition of the carbohydrate material is given in Table 5.

TABLE 5

COMPOSITION OF THE CARBOHYDRATE MATERIAL

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

	%
Tannin	17.7
Insolubles	8.2
Total Reducing Sugar	47.7 (after hydrolysis)
Uronic anhydride	15.6 (unhydrolyzed)
Undetermined	10.8

The carbohydrate material was further analyzed to determine the identity of the sugars present. The pentosan content was determined by a method of the Association of Official Agricultural Chemists (10, p.412) and corrected for the uronic anhydride present. Norris and Resch (12)

found that the ratio of uronic anhydride present to furfural phloroglucide formed was 2.39. The calculations for the corrected pentosan value are as follows:

sample weight = 1.355 grams (oven-dry weight)

1.355 x .156 = .2115 grams of uronic anhydride

$\frac{.2115}{2.39}$ = .0885 furfural phloroglucide formed from uronic anhydride

Total weight furfural phloroglucide = .3247

corrected weight = .3247 − .0885

" " = .2362

pentosans = (wt. of furfural phloroglucide + .0052) .8824

" = (.2362 + .0052) .8824

" = .2130

% pentosans = $\frac{.2130}{1.355}$ x 100

% pentosans = 15.72

The mannan content was determined by hydrolyzing a one gram sample of the carbohydrate powder with 3 per cent sulfuric acid for six hours, and neutralizing the solution with barium carbonate to approximately a pH of 6. The mannose formed was determined by precipitation as mannose phenylhydrazone by the method of Wise, Ratliff and Browning (16). The mannan content was 1.11 per cent.

The galactan content was determined by the action of 25 per cent nitric acid on the carbohydrate powder. The purpose of this was to oxidize the galactan material to mucic acid, which was precipitated from solution at 15° C. This method was outlined in the National Bureau of Standards circular "Polarimetry, Saccharimetry and the Sugars" (3, p.218). The determination indicated the galactan content to be 4.96 per cent. The fact that the carbohydrate powder contained uronic anhydride material complicated this determination because the mucic acid could have been formed from galacturonic anhydride. The absence of galacturonic anhydride was shown by a qualitative test on the barium salts of the uronic acids. This test is as follows:

One gram of carbohydrate material was hydrolyzed with 50 mls. of 5 per cent sulfuric acid for six hours, and the solution was filtered to remove the insolubles. Barium carbonate was added to the filtrate and the solution was heated until it tested neutral. The barium sulfate and excess barium carbonate were removed by filtration. One and one-half volumes of alcohol were added and the barium salts of the uronic acids were precipitated and recovered by filtration. The salts were then dissolved in twenty-five mls. of water, and five drops of bromine and one-half ml. of hydrobromic acid were added to the solution. The solution was allowed to remain over night at room temperature and

the last few hours at 15° C. No mucic acid was formed, which indicated the absence of galacturonic anhydride in the carbohydrate material. A bromine solution of this type oxidizes galacturonic acid to mucic acid but not galactose to mucic acid.

The results of these analyses appear in Table 6.

TABLE 6

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

(Percentages are based on the oven-dry weight of carbohydrate material.)

	%	
Pentosans	15.72	(corrected for uronic anhydride)
Mannans	1.11	
Galactans	4.96	
Glucosans	25.91	(by difference)
Total Reducing Sugars	47.70	(after hydrolysis)

A purified carbohydrate material was prepared in the following manner:

The carbohydrate material was obtained by the method outlined at the first of this section, except that the final precipitate was dissolved in water instead of being placed in a desiccator for drying. The solution was made slightly

acid with hydrochloric acid and heated on a steam cone for one hour. A black precipitate was formed and, when an equal volume of alcohol was added to the solution, more black material precipitated. This material constituted 34 per cent of the total carbohydrate material. The solution was filtered and the filtrate added to two volumes of alcohol. This produced an orange colored precipitate which was obtained by suction filtration on a Buchner funnel. The orange colored precipitate was dissolved in water and a few drops of liquid bromine were added until the solution was yellow in color. The solution was heated in a steam bath for one-half hour and then added to alcohol. A light orange colored precipitate was formed and recovered by filtration. The final precipitate was dried in a desiccator without discoloration.

This purified carbohydrate powder was similar in appearance to the gum material described in the next section and the powdered particles were observed to be semi-transparent. It was found to be almost completely free of tannin. The uronic anhydride content (11) of the purified carbohydrate was 18.4 per cent; whereas, the unpurified material contained 15.6 per cent.

Gum Material.

The bark, which had been extracted with hot water as described in the carbohydrate section, was left in the wooden kegs; and an additional extraction was made with 0.5 per cent ammonium oxalate solution. The method used for this extraction was the same as that used for the hot water extraction, except that there were two four-hour leaching periods instead of three. The liquor obtained from these extractions was concentrated to a thick syrup by vacuum evaporation as described in the carbohydrate section. The thick syrup was added to three volumes of 95 per cent ethanol and acidified with enough hydrochloric acid to react with the ammonium oxalate. The ammonium chloride and oxalic acid formed were soluble in the alcohol-water solution. The solution was stirred thoroughly and the precipitate of crude gum was allowed to settle and then filtered. The precipitate was dissolved in water, reprecipitated with alcohol, filtered and washed with alcohol to complete the removal of the ammonium chloride and oxalic acid.

The crude material was again dissolved in water and the solution made slightly acid. It was warmed on a steam cone for one hour and a black precipitate resulted which was removed by filtration. This material constituted 12 per cent of the crude gum. The filtrate was added to three volumes of alcohol and a dark orange precipitate was formed.

It was recovered by suction filtration and redissolved in water. A few drops of liquid bromine were added until the solution was yellow in color. The solution was heated in a steam bath for one-half hour and added to alcohol. The resulting precipitate was recovered by filtration and dried in a vacuum desiccator to a light orange powder of negligible moisture content. The yield of this purified gum was 1.37 per cent.

The purified gum was semitransparent. It decomposed upon heating above 90° C. The specific rotation of a .4 per cent water solution of the gum at 25° C. was +175° using a sodium lamp. A 5 per cent solution of the gum in cold water was quite viscous after 24 hours. A thick paste of the gum was effective as a glue for paper.

The procedure for analyzing the gum material was the same as that used for the carbohydrate material. The composition of the gum material is shown in Table 7.

TABLE 7

COMPOSITION OF THE GUM MATERIAL

(Percentages are based on the oven-dry weight of gum material)

	%	
Uronic Anhydride	48.3	(unhydrolyzed)
Total Reducing Sugar	44.3	(after hydrolysis)
Insolubles	5.8	
Undetermined	1.6	

The insoluble material appeared to be the same as that found in the carbohydrate material.

Further analysis of the purified gum gave a pentosan content of 7.8 per cent which was corrected for uronic anhydride, and the mannan content was 14.0 per cent. Treatment with 25 per cent nitric acid indicated the presence of 44.8 per cent galactans.

V. ANALYSIS OF EXTRACTIVE-FREE BARK.

The extractive-free bark was prepared from a composite sample of unextracted bark by successive extractions with alcohol-benzene mixture, alcohol and hot water. These extractions were done in accordance with T.A.P.P.I. method T 12m-45 (15). The extractive contents appear in Table 8.

The extractive-free bark was used for the following analyses and the values, given in Table 8, were obtained by exact duplication of the methods referred to: lignin, T.A.P.P.I. method T 13m-45 (15); methoxyl, T.A.P.P.I. method T 2m-43 (15); acetyl, (4); pentosans, (10, p.412). The holocellulose determination was made by a modification of the sodium chlorite method (17) for wood.

In this determination the extractive-free bark samples were prepared from bark, which passed a No. 40 mesh screen and was retained on a No. 60 mesh screen. Six treatments of sodium chlorite and glacial acetic acid were necessary to isolate the holocellulose. The first two treatments were of one-half hour duration and the remaining four were one hour in length. The isolated holocellulose samples were analyzed for lignin and found to contain an average of 4.05 per cent lignin based on the oven-dry weight of extractive-free bark. This value was subtracted

from the average amount of isolated holocellulose and the difference reported as pure holocellulose in Table 8.

The ash content of the unextracted bark was determined by T.A.P.P.I. method T 15m-45 (15) and appears in Table 8.

The analyses in this section were conducted as part of course Ch. 471, "Chemical Analysis of Wood and Related Products".

TABLE 8

ANALYSES OF EXTRACTIVE-FREE BARK

(Percentages are based on oven-dry weight of unextracted bark)

	%
Pentosans	8.02
Methoxyl	3.40
Acetyl	.71
Ash Content	1.80
Alcohol-Benzene Solubles	10.02
Alcohol Solubles	6.07
Hot Water Solubles	7.20
Lignin	31.01
Holocellulose (corrected for lignin)	45.66
	99.96

DISCUSSION

The grand fir bark samples were collected from trees of three age groups and were sampled from the bottom, middle and top sections of a total of ten trees. The total extractive content varied from 18.26 per cent to 29.02 per cent of the oven-dry weight of bark. This wide variation in extractive content was essentially due to the distribution of hot water soluble material. The top sections of all age groups were higher in content of this material than the middle sections, and the middle sections higher than the bottom sections. Also, the content increased for all sections going from the old trees of age group III to the young trees of age group I.

The hexane soluble material was the next major constituent and did not vary a great deal in content between samples. The older trees of age group III contained slightly more of this material at the bottoms of the trees, whereas the young trees of age group I contained slightly more at the tops of the trees. The trees of age group II did not show an appreciable variation between sections.

The hexane soluble material was found to be a light tan wax melting at 52-53° C. The yield of this wax was 2.20 per cent based on the oven-dry weight of a composite sample of unextracted bark. It was found to contain 92.5

per cent acids which were chiefly saturated fatty acids. This mixture of fatty acids melted at 69-70° C. and gave a neutral equivalent of 466. The remaining 7.5 per cent of the wax was principally a saturated fatty alcohol which melted at 70-71° C. The alcohol was oxidized to the corresponding acid. The acid gave a neutral equivalent of 397 and melted at 73-74° C. This wax appeared to be suitable for commercial usage.

The benzene soluble material was a soft black resinous substance and was not investigated because of its low content. The ether soluble material was found to be low in content and contained 50 per cent tannin, so it was not investigated. The alcohol soluble material was a hard black amorphous substance and varied in content from .62 per cent to 2.67 per cent of the oven-dry bark. It was not investigated.

The tannin content of a composite sample of the unextracted bark was 7.13 per cent of the oven-dry weight. The optimum time of extraction was 10 hours, and it was determined that 89 per cent of the tannin was removed in the first one-half hour of extraction. It was discovered that the usual sample, ground to pass a No. 20 mesh screen, could not effectively be used in the standard percolation type extractor. A gelatinization occurred within the sample and prevented the proper drainage of the liquor. The

gelatinization did not take place when a sample was used, which had been ground to pass a No. 4 mesh screen, and the tannin liquor drained through the sample in a normal manner.

The carbohydrate component of the hot water soluble material was obtained by adding a hot water extract to 95 per cent ethanol and filtering off the crude precipitate. The yield of this material was 2.36 per cent based on the oven-dry weight of unextracted bark. Although referred to as a carbohydrate material, it was found to contain 17.7 per cent tannin and 8.2 per cent of an insoluble material. The total reducing sugar content was 47.7 per cent after 14 hours of refluxing with 2 per cent sulfuric acid. It was ascertained that the uronic anhydride, which was present in the amount of 15.6 per cent, was not hydrolyzed to any extent and did not contribute to the total reducing sugar value. The remaining 10.8 per cent of the carbohydrate material was undetermined. The composition of the material represented as total reducing sugars was as follows: pentosans 15.72 per cent; mannans 1.11 per cent; galactans 4.96 per cent; and glucosans 25.91 per cent.

Purification treatments of the carbohydrate material with dilute acid and liquid bromine produced a material which was free of tannin and resembled in appearance the

later described gum material. It had a uronic anhydride content of 18.4 per cent as compared to 15.6 per cent for the unpurified material.

A gum material was obtained from the hot water extracted bark by an additional extraction with 0.5 per cent ammonium oxalate solution. This material was believed to be the cause of the gelatinization, which occurred during tannin extraction. The gum was semitransparent and decomposed upon heating above 90° C. The specific rotation of a 0.4 per cent water solution of the gum at 25° C. was +175° as determined with a sodium lamp. A 5 per cent solution of the gum in cold water was quite viscous after 24 hours. A thick paste was effective as a glue for paper. The composition of the gum was as follows: uronic anhydride 48.3 per cent; total reducing sugar 44.2 per cent; insolubles 5.8 per cent; undetermined 1.7 per cent. The composition of the material represented as total reducing sugars was not accurately determined. It was found, however, that 7.76 per cent pentosans and 14.03 per cent mannans were present. Another determination indicated the presence of 44.85 per cent galactans which implied that the uronic anhydride was at least in part galacturonic anhydride.

SUMMARY

The grand fir bark was sampled by removing bark from the top, middle and bottom sections of ten trees divided into three age groups. These age groups were: 165-170 years; 196-225 years; and 245-257 years. The nine samples were successively extracted with hexane, benzene, diethyl ether, hot water and alcohol to show the distribution and amount of extractives. Further analysis showed the hexane soluble material to be principally saturated fatty acids with a small amount of saturated fatty alcohol. The benzene, ether and alcohol soluble materials were of low content and were not investigated. The tannin content was 7.13 per cent of the oven-dry weight of unextracted bark. The carbohydrate material, obtained from the hot water extract, contained uronic anhydride, pentosans, glucosans, mannans, galactans and tannin. A method was developed to purify the carbohydrate material, which in effect removed the tannin. A gum material was extracted with 0.5 per cent ammonium oxalate solution from bark which had been previously extracted with hot water. This material was discovered to be almost one-half uronic anhydride with some pentosan and mannan present.

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