THE WATER SOLUBLE POLYSACCHARIDE OF DOUGLAS FIR, PSEUDOTSUGA TAXIFOLIA

by

Albert Henry Roebuck

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THE WATER-SOLUBLE POLYSACCHARIDE OF DOUGLAS FIR, PSEUDOTSUGA TAXIFOLIA

INTRODUCTION

In 1916 A. W. Schorger and D. F. Smith[1] isolated a water-soluble polysaccharide from the wood of western larch, Larix occidentalis in amounts varying from 8 to 17 per cent of the weight of the wood. They reported that it consisted almost entirely of a galactan $(C_6H_{10}O_5)_n$ yielding only d-galactose on hydrolysis, and having a specific rotation of $+12.11^{\circ}$. They referred to it as e-galactan, and noted that when distilled with 12 per cent hydrochloric acid it gave furfural equivalent to 10.54 per cent pentosan. Since they were unable to detect a pentose among the sugars from the hydrolysis of the galactan they assumed that the furfural formed was due to the peculiar structure of the galactan molecule and not to the presence of a pentosan.

In 1930 L. E. Wise and F. C. Peterson[2] repeated this work. Their results were identical with those of the first two investigators except that l-arabinose was shown to be one of the products of hydrolysis. They therefore referred to the material as an arabogalactan. They also suggested that it was a definite chemical compound with a fixed structure because their analysis of material from an entirely different sample of western larch had been so nearly the same as that of Schorger and Smith. On

hydrolysis their material yielded l-arabinose and d-galactose in approximately the ratio of 1 mol to 6 mols. They assumed that the arabogalactan was a homogeneous polysaccharide with the empirical formula $\left[(C_5H_8O_4)(C_6H_{10}O_5)_6\right]_n$ and a minimum molecular weight of 1104.

In 1941 E. L. Hirst, J. K. N. Jones, and W. G. Campbell[3] studied the hydrolytic products and rates of hydrolysis of the methylated arabogalactan and concluded that it was not homogeneous, but probably a mixture of a galactan and an araban. They state:

> "The arabinose residues, as shown by their rate of hydrolysis to free arabinose, possess the furanose structure which has been found also in all naturally occuring arabans hitherto examined.[4] The galactan portion undergoes no appreciable change when subjected to the mild acid conditions which suffice to hydrolyze the araban. This is to be expected since all the galactose is present in the pyranose form. Futhermore, arabinose-free galactan thus obtained gives a fully methylated derivative having $[\alpha]_D$-18° in methyl alcohol which is identical with the substance obtained by separation of the methylated galactan from the mixture of methylated galactan and methylated araban produced by methylation of the original e-galactan."[5]

In 1940 F. C. Peterson, A. J. Barry, H. Unkauf, and L. E. Wise[6] prepared the propionate and the benzoate of the arabogalactan, and were able to obtain a partial separation of these substances by fractional precipitation. They were also led to believe that it was a mixture of an araban and a galactan rather than a homogeneous conpound.

L. E. Wise[7] points out that the acetyl derivatives are apparently non-homogeneous and that the arabogalactan is probably a mixture.

E. V. White[8,9,10] in his recent work on this material was unable to repeat the preferential hydrolysis of the furanopentose reported by Hirst, Jones, and Campbell. His study of the hydrolytic products of the methylated and acetylated arabogalactan has done much to establish its structure. After hydrolysis and oxidation he was able to separate a large portion of the crystalline amide of the corresponding acid of the arabofuranose unit. From this he concludes that there is a direct linkage of the arabinose fraction to the galactose. He states:

> "... it was shown that the methyl derivative of the polysaccharide, when subjected to complete hydrolysis and simultaneous glycoside formation, yielded the glycoside of 2,4-dimethyl-d-galactose, 2,3,4-trimethyl-d-galactose, and 2,3,5-trimethyl-l-arabinose in the approximate molecular ratio of 3 : 1 : 2 : 1, respectively. Futhermore, the isolation of a relatively large proportion of the terminal arabofuranose unit as the crystalline amide of the corresponding acid strongly suggested a direct linkage of the arabinose fraction to the galactose.
>
> "The validity of this assumption might be established by the preferential hydrolysis of the furanopentose under mild conditions leaving a galactan residue of unchanged structure. ... In the present experiments a successful partial hydrolysis of larch gum to arabinose and unchanged galactan has not been achieved as yet despite numerous attempts under a variety of conditions."[9]

White concludes that the molecule is a highly branched chain of galactose units, joined by oxygen linkages through the 1-3 and 1-6 positions and having as terminal residues arabofuranose and galactopyranose linked to the six position of the adjacent galactose anhydride units.

Apparently this arabogalactan represents a true polyose, but not a hemicellulose[11] because of its solubility in water. It is unique not only because of its role as a polysaccharide but also because of it galactose-arabinose association. This combination is relatively common in the annual plants and the structural similarity of the two monosaccharides has often been noted in connection with the explanation of the possible origin of l-arabinose in plants.

Recently Anderson, Cullen, and Rosenblatt[12] isolated a mixture of carbohydrate materials from the water extract of Douglas fir. Calcium pectate was isolated from this mixture by treatment with a solution of calcium chloride. Hydrolysis of the residual impure material yeilded d-galactose, l-arabinose and a small amount of d-mannose. In their investigation they did not determine whether the polysaccharide material remaining after the removal of the calcium pectate was a mixture of an arabogalactan and a mannan, or a complex polysaccharide containing the three different sugars.

The purpose of the present investigation is to isolate and purify this polysaccharide, and to determine its composition

and relation to the arabo galactan of western larch.

EXPERIMENTAL

Description of Wood

The Douglas fir heart wood used in this investigation came originally from the Pacific Coast of Oregon. It was supplied in the form of a coarse sawdust by The Institute of Paper Chemistry, Appleton, Wisconsin.

Extraction of Wood

Extraction with acetone and alcohol.

The powdered wood, in 500 gram amounts, was placed in six liter flasks, covered with acetone and heated under reflux in water baths for ten to fifteen hours. The solvent was filtered off at the pump, and the extraction repeated twice. After extraction with acetone the material was allowed to dry in the air, returned to the flasks, covered with 95 per cent ethanol, and heated under reflux in a water bath for ten to fifteen hours. The solution was filtered from the solid and the extraction with ethanol was repeated twice. The acetone and alcohol extracts were not studied futher.

Extraction with water.

After extraction with acetone and alcohol the wood was dried and returned to the flask. It was covered with water and heated in a bath of boiling water for ten to fifteen hours with frequent shaking. The solution was filtered from the solid and the extraction with hot water was repeated twice. The filtrates from successive

extractions with water were kept separate and concentrated in vacuo from a volume of 6 liters to approximately 100 ml. or until they became thick sirups.

Isolation of the Water Extract

The arabogalactan is insoluble in 95 per cent ethanol. To isolate it, ten volumes of ethanol were added to the sirup from the concentrated water extract, and the mixture was allowed to stand until the solid had settled. It was then filtered from the solution and washed with ethanol and ether, and dried in vacuo. The yields of crude extract are given in table 1.

TABLE 1

AMOUNTS OF CRUDE MATERIAL OBTAINED FROM THE WATER EXTRACT

				Total
Wood extracted gs.	1500	1500	1000	4000
Dried extract gs.	30	23	20.2	73.2
Dried extract per cent	2	1.53	2.02	1.83

The acetone and alcohol extractions remove gums, tars, resins and other materials soluble in these solvents. The polysaccharide is insoluble in both solvents. The purpose of removing these materials is to prepare the wood for the water

extraction by removing any substance which might interfere with the extraction of the polysaccharide, and the removal of materials which might be extracted with it. In previous work[13] these materials were observed to hinder the purification of the polysaccharide.

From the first 1500 grams of wood, 15 grams of material were obtained from the first water extraction, 11 grams from the second, and 4 grams from the third and last extraction. The second 1500 grams of wood yielded 13 grams of material from the first extraction, 5 grams from the second, and 5 from the third. Some of the extract was lost in concentration the solution from the second 1500 gram lot, and the yield was correspondingly low. In a later extraction of 1000 grams of wood, four water extractions were made, and yielded 20.2 grams of material. It appears that very close to two per cent of the wood is water soluble, and that the amount of material obtained depends to some extent on the number of extractions made.

Purification of the Water Extracts

Preliminary treatment.

The dry extracts were kept separate, and purified in the following manner. The material was dissolved in 30 to 40 parts of cold one per cent hydrochloric acid by rubbing in a large mortar. This required approximately one hour, and gave a viscous brownish solution which contained some suspended material. This was separated by centrifuging and filtering. The filtrate

was treated with bromine in the cold for one hour with occasional stirring. This partially decolorized the solution. The supernatant liquid was decanted from the excess bromine and added to 8 to 10 volumes of ethanol to precipitate the polysaccharide. The mannan and pectic materials which are present are also precipitated. The supernatant solution was siphoned off. The precipitate was centrifuged and filtered off at the pump. This was washed with 95 per cent alcohol and ether, dried in vacuo, and weighed. This treatment is to decolorize the material. The 72.76 grams of crude material treated in this manner yielded 61.4 grams of partially purified material. The results are summarized in table 2.

TABLE 2

YIELDS OF PARTIALLY PURIFIED EXTRACTS FROM DOUGLAS FIR

Extraction	1	2	3	4	5	Total
Material treated, gs	13	5.26	14.3	20	20.2	72.76
Material obtained, gs	11.9	4.5	11.0	16.5	17.5	61.4
Material remaining, per cent	91.5	85.5	77.0	82.5	86.7	85.0
Material removed, per cent	8.5	14.5	23.0	17.5	13.3	15.0

Second Treatment.

The water extracts consist of a mixture of pectic material, arabogalactan, and mannan, and were not completely purified by the preliminary treatment. They were futner purified as follows. The material was dissolved in approximately 20 times its weight of cold one per cent potassium hydroxide by rubbing in a large mortar. A viscous reddish brown solution was obtained containing a small amount of undissolved substance which was centrifuged and filtered off at the pump. A small amount of liquid bromine was added to the filtrate after making it slightly acid with dilute hydrochloric acid, and the mixture was allowed to stand for one hour with occasional shaking. The bromine at first decolorizes the solution, but on longer standing causes it to become more opaque. Therefore treatments with bromine at this point were shortened to one half hour. The solution was decanted from the excess bromine and approximately one half volume of alcohol was added to remove the free bromine. If a small amount of precipitate formed upon the addition of this alcohol it was removed by centrifuging and filtering at the pump. When the bromine color had disappeared the solution was made slightly alkaline with ammonium hydroxide to neutralize any hydrochloric and hydrobromic acids present. It was then made slightly acid with acetic acid, and a twenty per cent solution of calcium chloride was added slowly with stirring until on standing no more of the gelatinous precipitate

of calcium pectate formed. This was centrifuged, filtered off at the pump, and washed with 25 per cent alcohol. The arabogalactan remained in the filtrate and was afterward precipitated by addition of 8 to 10 volumes of alcohol. The supernatant solution was siphoned off and the arabogalactan centrifuged and filtered from the remaining mixture. It was washed with 95 per cent alcohol and ether, dried in vacuo and weighed. The yield of partially purified arabogalactan was 67 per cent of the crude material. In later experiments it was found that a one per cent solution of ammonium hydroxide dissolved the material more readily than the one per cent solution of potassium hydroxide described. The yields of material after the first purification are given in table 3.

TABLE 3

YIELDS OF WATER EXTRACTS AFTER FIRST PURIFICATION

Extraction	1	2	3	4	5	Total
Material treated, gs	13	5.26	14.3	20.0	20.0	72.76
Material from first treatment, gs	11.9	4.5	11.0	16.5	17.5	61.4
Material from second treatment, gs	10.3	14.5		11.8	12.1	48.7
Material remaining per cent	79.5	74.0		59	60	67

The treatment of the arabogalactan with bromine and calcium chloride solution was repeated essentially as described above. The yield from this second treatment was 59.5 per cent of the original extract. The dried material was a white powder having a specific rotation of + 11.7°, and was used in the analysis. Table 4 summarizes the results of the purification.

TABLE 4

PURIFICATION OF THE ARABOGALACTAN

Extraction	1	2	3	4	5	Total
Wood Extracted, gs	1500	1500	1500	1500	1000	4000
Material extracted, gs	13	5.26	14.3	20.0	20.2	72.76
Material after $HCl-Br_2$ treatment, gs	11.9	4.5	11.0	16.5	17.5	61.4
Material remaining per cent	91.5	85.5	77	82.5	86.3	85
Material after first $KOH-CaCl_2$ treatment, gs	10.3	14.5	[illegible]	11.8	12.1	48.7
Material remaining per cent	79.5	74.0		59	60	67
Material after second $KOH-CaCl_2$ treatment, gs	9.3		22		———	31.3
Material remaining percent	71.5		55.6		———	59.5
Specific rotation	+11.7°		+11.6°		+25.3°*	———

* This material was purified only once.

Hydrolysis of the Arabogalactan and Identification of Its Components

Hydrolysis of the arabogalactan was carried out in a two per cent sulfuric acid solution, containing fifty ml. of two per cent sulfuric acid for each gram of material. This solution was heated in a boiling water bath under an air reflux for 5 to 15 hours. The acid solution was neutralized by addition of barium hydroxide solution with constant stirring until only a slight acidity remained. The neutralization was completed by the addition of barium carbonate. The solution was then heated to 80° and allowed to stand over night. The barium sulfate and excess barium carbonate were filtered off and washed. The filtrate was concentrated in vacuo to a small volume, and the main part of the d-galactose was crystallized from alcohol. The d-galactose obtained showed a specific rotation of + 77.8°, and when heated with nitric acid gave large amounts of mucic acid melting at 216°. The solution filtered from the d-galactose was concentrated in vacuo to a sirup. L-arabinose was identified in this sirup by conversion to the alpha benzylphenylhydrazone with $[\alpha]_D$ - 12.8°, and a melting point of 173° to 174°. This melting point remained unchanged when the hydrazone was mixed with a known sample of l-arabinose alpha benzylphenylhydrazone. The literature gives

the melting point of this hydrazone as 174°, and its specific rotation as - 12.8°. These observations establish the presence of d-galactose and l-arabinose in the arabogalactan.

Analysis of the Arabogalactan

Moisture and ash determination.

Accurately weighed samples of the material were dried in vacuo over phosphorous pentoxide at the temperature of boiling water for three to five hours, and reweighed. The loss in weight represents the amount of moisture contained. The purified arabogalactan washed with 95 per cent alcohol and ether, and dried in vacuo contained 0.785 per cent moisture.

The dried material was charred and ignited over a burner to constant weight. The ash formed was 1.10 per cent of the purified arabogalactan. The total moisture and ash content was 1.89 per cent.

Optical rotation.

An accurately weighed amount of the material was dissolved in a one per cent solution of ammonium hydroxide and made up to a known volume. This solution was poured into a one decimeter tube, and its rotation measured in a polariscope using sodium light. The results are given in table 6.

TABLE 5

OPTICAL ROTATION OF THE ARABOGALACTAN

Sample	1	2	3
Arabogalactan, gs	0.1920	0.0865	0.0494
Volume of solution, ml.	25	25	25
Material per 100 ml.	0.7680	0.3460	0.1976
Degree of rotation	+0.09°	+0.04°	+0.05°
Specific Rotation	+11.7°	+11.6°	+25.3°

The optical activity of the arabogalactan is to some extent a measure of its purity. Sample 3 had been purified only once, and rotated more strongly dextro than did samples 1 and 2. Evidently it still contained traces of pectic material which rotates very strongly dextro. When a small portion of the arabogalactan was purified a third time by treatment with bromine and calcium chloride, it had a specific rotation of between +11.9° and +12.3°. Schorger and Smith[1] reported that the e-galactan of western larch showed a specific rotation of +12.11°.

Uronic acid determination.

Substances containing uronic acid groups are decomposed upon heating with twelve per cent hydrochloric acid to give

carbon dioxide and furfural. The amount of furfural is less than the theoretical, but the amount of carbon dioxide formed from the carboxyl groups is quantitative, and is used in their determination. The carbon dioxide was determined gravimetrically by absorption in ascarite. A description of the method used is given in Methods of Analysis, The Institute of Paper Chemistry, Appleton, Wisconsin.[14] A more complete description is given in the thesis of Keith Taylor, 1947.[15] The results obtained in the uronic acid determination are given in table 6.

TABLE 6

DETERMINATION OF THE PER CENT CARBON DIOXIDE

Material	Purified once		Purified twice		
Sample	1	2	3	4	5
Weight of Sample, gs	0.1305	0.1010	0.1201	0.1349	0.1246
Carbon dioxide evolved, gs.	0.0023	0.0014	0.00066	0.0004	0.00051
Carbon dioxide present, Per cent	1.70	1.39	0.55	0.30	0.41
Averages, per cent	1.55		0.42		

The results of the uronic acid determination indicated that no more than a trace of uronic acid was present in the arabogalactan purified twice. Since pectic materials were

present in the crude water extract, the material purified only once contained a higher per cent of carbon dioxide than that purified twice. Uronic acid was assumed to be present as an impurity.

Mannose determination.

In previous work[16] a mannan was shown to be present in the water soluble portion of Douglas fir, but had never been determined quantitatively. Its quantitative determination was carried out on material purified twice by precipitating and weighing its phenylhydrazone. The d-galactose was removed from the hydrolysis solution of the arabogalactan by crystallizing from a cold concentrated alcohol solution. This was filtered off. A solution of acetic acid and phenylhydrazine was then added, and the phenylhydrazone of mannose precipitated in the cold. After standing in the cold, the precipitate was filtered off and weighed. Approximately one half of one per cent of mannose was calculated to be present. This was assumed to be present as an impurity, and not a constituent of the arabogalactan. Arabogalactan which had been purified a third time seemed to contain no mannose when treated as described above. Wise and Peterson[2] do not report the presence of mannose in the arabogalactan of western larch.

Pentosan determination.

The per cent pentosan was determined by the method of Tollens

and Krøber.[17] A description of this method is given by Browne and Zerban.[18] The determination depends upon the conversion of pentose sugars and pentosans into furfural by distillation with hydrochloric acid, and the precipitation of the furfural as furfural phloroglucide. The results obtained are given in table 7.

TABLE 7

DETERMINATION OF THE PER CENT ARABAN

Sample	1	2	3
Weight of sample, gs	0.2875	0.2942	0.3101
Phloroglucide precipitate obtained, gs	0.0285	0.0295	0.0319
Pentose from tables, gs	0.0301	0.0309	0.0329
Pentosan, per cent	10.5	10.5	10.5
Araban from tables, gs	0.0328	0.0360	0.0356
Araban, per cent	11.4	11.4	11.5

Schorger and Smith reported 10.54 per cent pentosan, and Wise and Peterson reported 11.95 per cent anhydroarabinose in the arabogalactan of western larch.

Galactose determination.

Galactose may be determined gravimetrically by oxidizing it to insoluble mucic acid, but the yields are less than the theoretical. The weight of mucic acid formed is converted to the weight of d-galactose by reference to tables. With slight changes, the method of Van der Haar[19] was used in this determination. The arabogalactan to be treated was accurately weighed, and hydrolyzed with two per cent sulfuric or nitric acid. The acid solution was diluted to a known volume, and aliquots of this solution were used for analysis. Thirty ml. of 50 per cent nitric acid were added to thirty ml. of the hydrolyzed solution in a beaker six centimeters wide by twelve centimeters high. This was placed in a boiling water bath and allowed to evaporate with frequent shaking until the solution weighed twenty grams, plus or minus onetenth of a gram. After cooling, five tenths of a gram of pure mucic acid, accurately weighed was added and the mixture allowed to stand in the cold with shaking, as directed by Van der Haar.[19] The mucic acid was filtered off in a weighed Gooch crucible, dried and reweighed. The weight of mucic acid precipitated was determined by subtracting the weight of that which was added from the total amount present. The per cent d-galactose was calculated by reference to the tables of Van der Haar.[19] Results of this determination are given in table 8.

TABLE 8

DETERMINATION OF THE PER CENT GALACTOSE

PART A

MATERIAL HYDROLYZED IN TWO PER CENT SULFURIC ACID

Sample	1	2	3	4	5	6
Weight of sample, gs	.4280	.6430	.6430	.4280	.2370	.1810
Mucic acid formed, gs	.2180	.3701	.3920	.2412	.1310	.0971
Galactose from tables, gs	.320	.513	.540	.352	.200	.149
Galactose, per cent	75	80	84	82.2	84.4	82.5

PART B

MATERIAL HYDROLYZED IN TWO PER CENT NITRIC ACID

Sample	1	2
Weight of sample, gs	.591	.591
Mucic acid formed, gs	.3283	.3354
Galactose from tables, gs	.455	.465
Galactose per cent	77	79

Part A of table 8 gives the results of the mucic acid test when the arabogalactan was hydrolyzed with two per cent sulfuric acid, and part B, when it was hydrolyzed with two per cent nitric acid. Wise and Peterson[2] report the presence of 80 to 86 per cent galactose in the arabogalactan of western larch.

SUMMARY

Douglas fir heartwood was extracted with acetone, alcohol, and then water. Only the water extract was studied in this investigation. Two per cent of the wood was soluble in boiling water. The material extracted was precipitated from the concentrated aqueous solution by addition of alcohol. This crude water extract was shown to contain:

1. An arabogalactan.
2. A pectic material.
3. A mannan.

The arabogalactan was seperated from the pectic material and the mannan, and purified. By analysis it was shown to contain 11.4 per cent araban, and 84 per cent galactan. It has a specific rotation of + 12°.

This arabogalactan appears to be a homogeneous compound, and identical with the water soluble arabogalactan of western larch.

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