HEMICELLULOSES FROM THE BARK OF ENGELMANN SPRUCE (*Picea engelmannii*)

by

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Identification of 2,3-Di-O-methyl-D-xylose

The syrup obtained (160 mg.) had $[\alpha]_D$ + 29.6° (C, 1.4) and on demethylation gave xylose and glucose in the approximate ratio of 4:1. The syrup was dissolved in a small amount of water and seeded with an authentic crystal of 2,3-di-O-methyl-D-xylose. On standing for more than a month large, white crystals were formed. The small amount of adhering syrup was quickly rinsed out by ethanol. The crystals had m.p. of 86-88°. On refluxing the crystals with alcoholic aniline, the corresponding aniline derivative was obtained which was crystallized from ethylacetate-petroleum ether. Recrystallization from ethylacetate gave crystals which had m.p. of 125-127° and $[\alpha]_D$ + 182.4° (C, 0.3 in ethylacetate).

ENGELMANN SPRUCE (Picea engelmannii)

The common pulpwoods are grouped into two general classes namely gymnosperms and woody angiosperms. All commercially important gymnosperms are conifers and are often referred to as "softwoods". In the same way, the arborescent angiosperms are termed "hardwoods".

Engelmann spruce (Picea engelmannii) is a well known tree belonging to the gymnosperm class. This species is found in Western Canada in the interior mountain ranges of British Columbia and on the eastern slope of the Rocky mountains. It is usually from 1 1/2 - 3 feet in diameter and 80 - 120 feet in height but may attain a diameter of 6 feet and a height of 180 feet under favourable situations. The wood is quite light in colour.

The bark of Engelmann spruce is thin, scaly and with a dull reddish brown colour. The total thickness of the bark is about 0.3 inch. The outer surface of the bark is rather rough and shows resin canals.

GENERAL INTRODUCTION

Bark - The term "bark" is applied in common parlance to denote the material that surrounds the woody substance (xylem) in a mature tree. It is thus situated outside the cambium which is the living tissue of a growing tree. The anatomical structure of bark is rather complex but in general it can be said that the bark tissue is composed of different kinds of cells, such as sieve, longitudinal parenchyma, ray parenchyma, fiber cells, etc., some of which are also typical of the wood tissue. The physiological function of these tissue elements in the bark is mainly concerned with the translocation and storage of solutes. As the tree matures, the outermost layers of the bark are broken and die by exposure and especially by the stoppage of food and water supplies. This layer is commonly referred to as the outer bark as opposed to the active tissue beneath it which is termed, inner bark or phloem.

Chemical Composition of Wood Barks - Work on the chemical nature of wood barks has progressed fairly steadily during the last three or four decades. These studies essentially follow the pattern developed for wood chemistry. Although little is known at present concerning the distribution of the chemical constituents over the various botanical structures in the bark, the overall chemical composition of various wood barks taken in their entirety has been established (1,2). The chemical constituents of the bark can thus be classified into four main groups, namely, lignin, cork, polysaccharides, and extraneous materials.

Lignin is a three-dimensional polymer based on phenylpropane

units. This is the xylem material which remains insoluble in 72% sulphuric acid and in the case of bark may include other materials like tannins, phlobaphenes and suberin. Consequently the material that occurs as "lignin" in barks varies in amount over a wide range unlike the woods where they occur within narrow limits. Bark lignin is characterized by a much lower methoxyl content than wood lignin.

The cork layer is deposited outside the phloem and inside the epidermis. It contains mostly suberin which is an ester condensation polymer of saturated and unsaturated hydroxylated fatty acids. It is insoluble in 72% sulphuric acid and thus interferes with lignin determinations in bark. The other component of cork is cutose which is again a mixture of high molecular weight acids.

The polysaccharides in bark are based predominantly on glucose, with galactose, mannose, arabinose, xylose and rhamnose constituting the other building units. Often one or the other of the latter groups are absent. Bark polysaccharides can be divided into three groups, namely, pectic substances which are probably extracellular and generally removed by neutral or acid extraction of the bark "holocellulose", the hemicelluloses which are removed from the remainder by extraction with alkali and cellulose which is the residue left after all the extractions. The cellulose content of phloem is lower than that of xylem. Gums and mucilages present in some barks are also polysaccharides. Bark, especially from autumn-felled trees, also contains appreciable amounts of starch which acts as a reserve food material.

Among the extraneous materials are found compounds that can be

extracted from the bark with neutral organic solvents like ether, benzene, alcohol, etc. They include tannins, phlobaphenes, colouring matters, resins, waxes, essential oils, hydrocarbons, fats and fatty acids, free organic acids, alkaloids, and other materials. Bark is also rich in mineral matters.

Studies in Bark - Bark represents about 10-20% of the weight of a tree and in the past very little use has been made of it. Most of the interest in commercial barks has so far been focused on the chemical nature of bark lignin and bark extractives, the latter with a view to develop compounds of commercial applications. Thus, among the extractives could be found several compounds of commercial value in medicine, tanning preparations for the leather industry and resins such as Canada balsam. Bark polysaccharides other than cellulose have not received any detailed examination in the past years, apart from the work of Anderson and Pigman on the nature of some bark pectins (3-5) and that of Hirst and co-workers on the chemical constitution of the mucilage which occurs in the inner bark of slippery elm (Ulmus fulva) (6-8). A systematic study of the chemical nature of carbohydrate constituents of wood barks was, therefore, started in this laboratory a few years ago. The present work forms part of this study and embodies the chemical nature of some of the hemicelluloses present in the bark of Engelmann spruce.

WOOD AND BARK HEMICELLULOSES

WOOD HEMICELLULOSES

The hemicelluloses rank next in abundance to cellulose as a naturally occurring organic material in the plant kingdom. They are a group of polysaccharides which vary in amount and kind from plant to plant and even from tissue to tissue within the same plant. The monomeric sugars that constitute these polysaccharides are usually D-galactose, D-glucose, D-mannose, L-arabinose, D-xylose, L-rhamnose and D-glucuronic acid and its 4-O-methyl ether. Unlike cellulose which consists of both crystalline and amorphous regions and has a high degree of polymerization in the range of 5,000-10,000, the hemicelluloses are invariably amorphous materials with a considerably lower degree of polymerization, usually of the order of 50-200. These polysaccharides may contain only one kind of polymerized sugar unit (homoglycans) or they may be constituted of two or more sugar units (heteroglycans). With increasing number of sugar residues the structure becomes more and more complicated. A linear or branched structure is possible for a hemicellulose, but never a three-dimensional network like that of lignin.

Although it has been recognized for a long time that the cell walls of plant materials contain hemicelluloses in addition to cellulose, progress in their structural characterization was first slow, largely due to difficulties encountered in the separation of closely related sugar derivatives. The advent of chromatographic methods 15 years ago was, therefore, the real beginning of structural hemicellulose chemistry.

Since then, progress has been remarkably rapid and an enormous amount of literature has accumulated on the chemical nature of hemicelluloses of multifarious origin. The subject has been recently reviewed by Aspinal (9).

Isolation and Purification of Hemicelluloses - The hemicelluloses occur in close association with cellulose and are held in the complex cellulose matrix by mechanical entanglements and numerous secondary valence forces. They are usually solubilized by alkaline solutions which swell the cellulose but do not dissolve it. When wood and especially softwood, is used as the starting material, a considerable portion of the hemicelluloses is not extractable by alkali except after prior delignification of the wood. This may be the consequence of mechanical obstruction imposed by the lignin due to its close association with the hemicelluloses and cellulose, thus inhibiting swelling of the structure to a degree whereby extraction is rendered facile or may be due to some chemical bonding, as yet unidentified, between lignin and hemicelluloses.

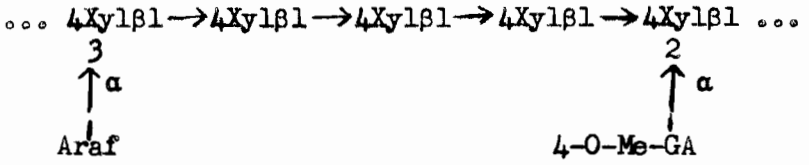
When delignified wood (holocellulose) is extracted with different alkaline reagents or one particular alkali of increasing concentration, a certain resolution of the hemicelluloses takes place. Quite often, however, such extracts are mixtures of polysaccharides and further purification is necessary to secure homogeneous materials, suitable for structural work. The most useful methods employed for purification of hemicellulose mixtures are fractional precipitation, salt formation with high molecular weight quarternary ammonium compounds, complex formation with Fehling's solution or barium hydroxide, and chromatography on columns using inert materials like carbon, celite, etc., or ion-exchange materials derived

from cellulose like diethylaminoethylcellulose. Such procedures have been recently reviewed by Bouveng and Lindberg (10) and also by Smith (11).

Structural Evaluation - The most widely used methods in structural hemicellulose chemistry are complete methylation of the polysaccharide and subsequent examination of the compounds obtained on hydrolysis of the methylated product, partial fragmentation of the polysaccharide and identification of the resulting oligosaccharides and oxidation with periodate and examination of the oxidized product by Smith's degradation scheme (borohydride reduction of the oxidized product followed by mild hydrolysis). All these methods have also been extensively reviewed by Bouveng and Lindberg (10).

In view of the fact that the study of bark polysaccharides has been immensely facilitated by the recent advances made in the structural chemistry of wood hemicelluloses, it is only appropriate to include here a brief review of the latter materials. The major hemicelluloses so far encountered in woody substances can be classified into three distinct groups, namely, xylans, glucomannans and arabinoglactans (9,12).

Wood Xylans - All xylans from wood investigated so far are characterized by the presence of a main chain of $\beta(1\rightarrow4)$-linked D-xylopyranose units to which are attached randomly or in ordered sequences single unit side chains of L-arabinofuranose residues glycosidically linked $(1\rightarrow3)$- to the main chain and 4-O-methyl-D-glucuronic acid units by $(1\rightarrow 2)$-linkage. The acid groups are attached by an α-glycosidic bond and indirect evidence (13) indicates that the arabinose residues are probably linked by an α-glycosidic bond, as shown below (Fig. I).



Xyl = D-xylopyranose

Araf = L-arabinofuranose

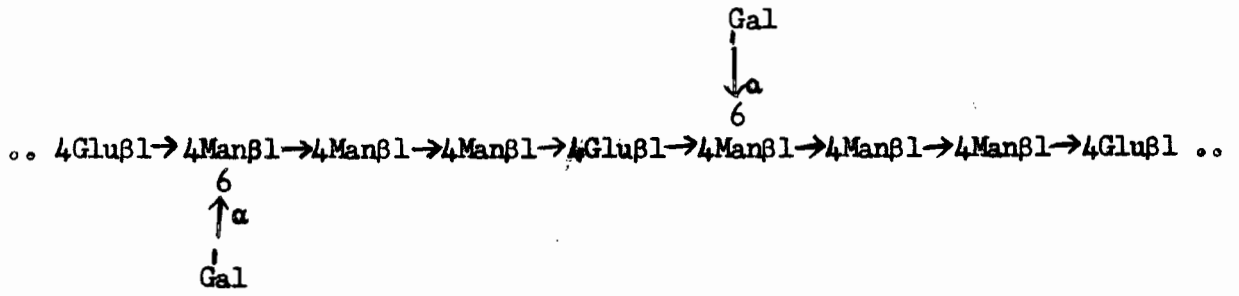
4-O-Me-GA = 4-O-methyl-D-glucuronic acid

FIG. I Schematic structure of an arabino-4-O-methylglucuronoxylan

The various xylans differ only in the proportions of these side chains and molecular size, the main structural features remaining the same. Some xylans, especially those derived from hardwoods are devoid of arabinose residues. Although the question of branching in the main xylan chain is still undecided in several cases the available evidence, admittedly not unequivocal, points to a linear framework.

Glucomannans from Wood - The glucomannans are present to a large extent in softwoods and in hardwoods make up only a very small percentage of the wood. They are on the whole less readily extracted from wood than the xylans but use of boric acid in conjunction with alkali facilitates their ready extraction (14). Borate apparently forms strong complexes with cyclic α-cis-glycol groupings such as are present in the mannose residues in a glucomannan. Because of the negative charge of these complexes they are readily soluble in the alkali. All glucumannans examined so far have revealed a striking similarity in their basic molecular structure. The glucose to mannose ratio is approximately 1:3 to 1:4. A very small percentage of galactose residues present in

all these polymers have been shown to be integral parts of the molecule (15). The main structural features are a chain of glucose and mannose residues distributed at random and united by β-(1→4)-glycosidic linkages. The galactose residues are considered to be present as non-reducing, terminal groups attached directly to the main chain by an α-(1→6)-glycosidic linkage, as shown below (Fig. II).



Glu = D-glucopyranose

Man = D-mannopyranose

Gal = D-galactopyranose

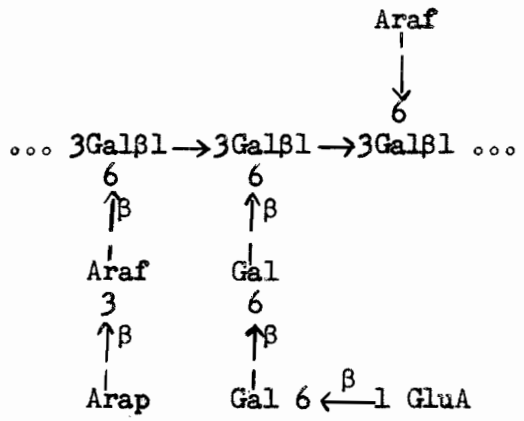
FIG. II - Schematic structure of a "glucomannan"

As in the case of xylans, the different glucomannans vary within themselves in possessing different chain lengths, glucose to mannose ratio and proportion of terminal galactose residues as integral parts of the structure. Whether or not branching occurs in these polymers has not been unequivocally established.

The mannose-containing polysaccharides of softwoods appear to include yet another type of polymer which is very similar to the one discussed above. Unlike the former ones which are soluble only in aqueous alkali, these polymers are water soluble and are readily extracted from wood even in the

absence of added borate. These polysaccharides are designated as galactoglucomannans. They generally contain a higher proportion of galactose residues than the "glucomannans". Most of the galactoglucomannans thus far studied contain galactose, glucose and mannose residues in an approximate ratio of 1:1:3 (15,16). Their structural features are similar to those of "glucomannans". The ready solubility of galactoglucomannans in water is apparently due to the large proportion of galactose side chains in the polymer which reduce the possibility of intermolecular forces between adjacent chains so that ready access to water molecules is favoured. There thus occurs in softwoods two closely related families of polymers, namely, "glucomannans" and galactoglucomannans, each with its own spectrum of subtle changes in the molecular architecture.

Arabinogalactans from Wood - Members of the genus Larix have been found to contain this polysaccharide in appreciable amounts. They are highly branched and hence water-soluble. The D-galactose residues are joined predominantly by (1→3)- and (1→6)-linkages with the possibility that some (1→4)-linkages may also be present (17-19). The arabinose units are integral parts of the polymer some of which occur as end groups in the furanose form and the rest as 3-O-β-L-arabinopyranosyl-L-arabinofuranose units. Recently it has been shown by Bishop and co-workers (62) that the arabinogalactan from tamarack (Larix laricina) contains D-glucuronic acid residues as integral parts of the molecule. A schematic representation of a glucuronoarabinogalactan is shown below (Fig. III).



Gal = D-galactopyranose

Araf = L-arabinofuranose

Arap = L-arabinopyranose

GluA = D-glucuronic acid

FIG. III - Schematic structure of a glucurono-arabinogalactan

BARK HEMICELLULOSES

As mentioned earlier, inner bark (phloem) contains significant amounts of polysaccharides other than cellulose. Although this fact has been recognized for a long time, progress in the structural chemistry of bark hemicelluloses did not keep pace with that of wood hemicelluloses. One reason for this is undoubtedly the fact that barks are not of any commercial value unlike woods which form the basic raw material for the paper and lumber industry. Another reason may presumably be due to the complicated nature of bark, which contains non-carbohydrate materials, such as suberin, cutose, tannins, phlobaphenes and various other phenolic compounds, all of which render the isolation of homogeneous polysaccharides from bark a rather difficult task. Lewis and co-workers (20) found that a commercial sulphite liquor cook of redwood bark fiber

gave a pulp with 31.8% of lignin and a re-cooked soda pulp from this bark retained 28.1% of lignin. This indicates the great resistance to delignification of barks, which is undoubtedly due to the presence in bark of the extraneous materials mentioned above. Whereas some of the tannins and phlobaphenes can be removed by extraction with organic solvents, others remain as contaminants in the fibrous residue and are subsequently extracted along with the polysaccharides. These phenolic substances undergo ready oxidation on exposure to atmosphere and in presence of acids and alkalis polymerize to produce tarry substances which are adsorbed on the polysaccharides. The experience gained with wood hemicelluloses has shown that acid sodium chlorite is a powerful oxidizing agent and very effective for removal of lignin in wood. This reagent is also excellent for destruction of most of the non-carbohydrate constituents of bark.

Much of the early investigations on bark hemicelluloses were concerned with general information as to the nature of the constituent sugar residues. Schwalbe and Neumann (21) in 1930 first recognized the presence in bark of a well-defined hemicellulose fraction, when they detected appreciable amounts of readily hydrolyzable hexosans and pentosans in the inner barks of spruce, pine and red beech. Buston and Hopf (22) in 1938 reported that ash bark contained approximately 7% pectic substances and 20% hemicelluloses. In the hydrolysis products of the latter, mannose, galactose, arabinose and galacturonic acid were identified. In 1947 Cram and co-workers (23), in a study of the chemical composition of western red cedar bark observed that the largest carbohydrate component of the outer bark was glucose, corresponding to 37.3% of the bark; xylose represented 7.4%, mannose 2.4% and galactose 0.7%. In a pulp from the outer bark obtained by cooking with

5% sodium hydroxide at 166-170°, they observed 78.3% glucose, 8.9% xylose, and 1.1% mannose while galactose was absent. From the yield of pulp obtained they reported that approximately one-third of the glucose had originated from a hemicellulose fraction. Recently Chang and Mitchell (24) determined the chemical composition of many common North American pulpwood barks. All of them contained residues of glucose, galactose, mannose, arabinose and xylose. Glucose was the major component among the reducing sugars, its amount varying from 50 to 70%. The proportions of arabinose and xylose in barks were found to differ somewhat from the patterns observed for woods.

The first attempt aimed at a systematic investigation of the structural features of bark polysaccharides was made by Painter and Purves (25) in 1960, who isolated six chemically distinct polysaccharides from the inner bark of white spruce (Picea glauca), some of which were submitted to structural analysis with a view to compare them with those present in the wood of the same species.

Maceration of the extractive-free bark with cold water gave granules of starch $[\alpha]_D$ + 190° identified by its blue stain with iodine and ready hydrolysis by α-amylase. Extraction of the bark with hot water gave a mixture of polysaccharides in a yield of 14%. Fractionation of this mixture with cupric acetate yielded a polysaccharide which was rapidly degraded by pectinase to give mainly D-galacturonic acid with smaller amounts of arabinose and galactose. This polysaccharide $[\alpha]_D$ + 210° was undoubtedly a pectic acid. From the supernatant solution, after removal of residual starch by α-amylase, a mixture of polysaccharides was isolated which on repeated fractionation with cetyltrimethylammonium hydroxide (CTA-OH) in

presence of added boric acid gave a galactan $[\alpha]_D$ - 22° containing residues of galactose and arabinose in the approximate ratio of 10:1. Examination of the methylated galactan suggested that a major part of the molecules were highly branched similar to the water-soluble arabinogalactan of white spruce wood (26). The identification of 2,3,6-tri-O-methyl-D-galactose suggested the presence of a small amount of β-(1→4)-linked galactan of the type usually associated with pectic materials. The bark remaining at this stage was oxidized with acidified sodium chlorite to remove non-carbohydrate materials. The holocellulose was subsequently extracted with increasing concentrations of sodium hydroxide (5,10 and 18%). Fractionation of the combined extracts by means of Fehling's solution yielded a polysaccharide containing residues of galactose, glucose, mannose and xylose in the approximate ratio of 1:2:2:1 together with traces of arabinose and 4-O-methylglucuronic acid. The fraction recovered from the filtrate of Fehling's precipitation contained residues of galactose, glucose and arabinose and on repeated precipitation with CTA-OH and boric acid gave an araban with $[\alpha]_D$ - 70° which was not further examined.

The hemicellulose fraction precipitated with Fehling's solution was remarkable in that it had a high content of glucose residues. Barium hydroxide, which has been used to selectively precipitate wood glucomannans (27) failed to resolve this polysaccharide mixture. However, by repeated precipitation with CTA-OH-boric acid, two fractions were obtained in small yields. One of these $[\alpha]_D$ - 12°, contained residues of galactose, glucose and mannose in the ratio 1:2:9. Methylation data suggested a linear chain of β-(1→4)-linked glucose and mannose residues. Although the authors assumed that these galactose residues originated from a contaminating galactan, Timell (33) in a subsequent communication pointed to the possibility

that these galactose residues might be integral parts of the polymer forming a triheteropolymer galactoglucomannan. A polysaccharide similar to the one above has been obtained also from white spruce wood (28,29.15).

The other fraction, $[\alpha]_D$ + 45°, contained residues of glucose, xylose and arabinose in the ratio 15:9:1. This was not attacked by α-amylase but was digested rather slowly by takadiastase, liberating glucose. The final enzyme-resistant residue $[\alpha]_D$ - 5°, contained residues of glucose, xylose and arabinose in the ratio 5:20:1. It was remarkable that only traces of acid groups were present in this xylan. Although methylation data indicated the presence of a (1→4)-linked xylan chain, the origin of glucose residues remained obscure. The arabinose residues were considered to be linked to the chain or originating from an araban contaminant.

The presence of appreciable amounts of glucose residues in conjunction with xylose led these workers to believe that a heteropolymeric glucoxylan is present in the inner bark of white spruce. Alternatively they visualized the existence of a separate "glucan", which is different from starch, contaminating the xylan fragment.

Jabbar Mian and Timell (30) in a subsequent investigation succeeded in isolating several polysaccharides in fairly good yields from the inner bark of white birch (Betula papyrifera). Ammonium oxalate extraction of the bark yielded a pectic material in 4.2% yield which was shown to consist predominantly of a polygalacturonic acid (31). The bark remaining after removal of pectin was freed of its non-carbohydrate constituents by treatment with acid chlorite. Extraction of the holocellulose with aqueous potassium hydroxide gave a pure 4-O-methylglucuronoxylan in a yield of 27%.

The hemicellulose, $[\alpha]_D$ - 68°, on methylation and linkage analysis revealed the presence of a linear backbone of 230 (1→4)-linked β-D-xylopyranose residues with approximately one (1→2)-linked 4-O-methylglucuronic acid side chain for every ten xylose residues. It was pointed out that this hemicellulose bore very close resemblance to the xylan occurring in the wood of the same species. The material remaining after removal of xylan, on extraction with sodium hydroxide in the presence of added boric acid (14) gave 3.8% of a polysaccharide mixture one of which was apparently a glucomannan. The material remaining after all the extraction was shown to be a pure cellulose (32).

The investigation of Painter and Purves on the inner bark of white spruce (gymnosperm) and that of Jabbar Mian and Timell on the inner bark of white birch (angiosperm) revealed that while the predominant hemicellulose in the latter was a 4-O-methylglucuronoxylan, the former apparently consisted of a complicated mixture of polysaccharides.

A detailed investigation of the polysaccharides occurring in the bark of several gymnosperm species was started by Timell (33). Four species were selected for this study, representing the four genera, Abies, Picea, Pinus and Ginkgo. They were respectively Amabilis fir (Abies amabilis), Engelmann spruce (Picea engelmannii), lodgepole pine (Pinus contorta) and Ginkgo biloba, the latter probably the world's botanically oldest, still living tree.

The hemicelluloses present in amabilis fir bark were extensively investigated. Extractive-free bark was oxidized with acid chlorite to remove non-carbohydrate material after which, the holocellulose was

successivley extracted with hot water, 0.5% aqueous ammonium oxalate, 24% potassium hydroxide and finally with 17.5% sodium hydroxide containing 4% boric acid. Such a sequence of fractional extractions has been previously used for gymnosperm woods with considerable success (15). From the hot water extract and potassium hydroxide extract two apparently similar galactoglucomannans (A and B) were isolated by aqueous barium hydroxide precipitation. The material remaining in solution, after removal of galactoglucomannan B from the potassium hydroxide extract, on further repeated precipitations with aqueous barium hydroxide gave a pure arabinomethylglucurono-xylan. The ammonium oxalate extract on fractionation with calcium acetate gave a calcium pectate from which a pectic acid containing 87% galacturonic acid was isolated. The sodium hydroxide-borate extract on treatment with aqueous barium hydroxide gave an alkali-soluble glucomannan containing minor amounts of galactose residues. The general characteristics of the polysaccharides isolated from fir bark are summarized in Table 1.

Structural analysis of the xylan (34) revealed that the polysaccharide consisted of a framework of at least 124 (1→4)-linked xylose residues, every sixth of which carrying a single, terminal (1→2)-linked 4-O-methyl-α-D-glucuronic acid unit and every tenth xylose residue carrying a (1→3)-linked L-arabinofuranose group attached as a single unit side chain.

The water-soluble galactoglucomannans A and B had similar properties and a structural investigation was carried out with a mixture of them (35). It was found that this polysaccharide consisted of a slightly branched framework of at least 80 β-(1→4)-linked D-mannose and

Table 1 Polysaccharide Components of Amabilis Fir Bark

Polysaccharide	Yield %	$[\alpha]_D$ degrees	Sugar residues in relative per cent					
			Galacturonic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Galactoglucomannan A	1.6	-14.4	Trace	10	23	67	Nil	Nil
Pectic Acid	3.5	+177	87	3	Nil	10	Trace	Nil
Arabinomethylglucuronoxylan	2.1	-57.4	Nil	Nil	Trace	Nil	7	74[1]
Galactoglucomannan B	1.2	-15.3	Trace	10	25	65	Nil	Trace
Glucomannan (alkali-soluble)	3.1	-35.4[2]	Nil	2	28	70	Nil	Trace

[1] In addition to 19 percent residues of 4-O-methylglucuronic acid

[2] In 10 per cent aqueous sodium hydroxide

D-glucose residues, every tenth of which on the average carried a (1→6)-linked D-galactopyranose unit. The alkali-soluble glucomannan possessed a linear structure of 70 randomly distributed β-(1→4)-linked glucose and mannose residues, with a few galactose residues existing as single unit side chains and thus forming an integral part of the molecule (36).

The above detailed investigation of the amabilis fir bark revealed that the component hemicelluloses were strikingly similar to those present in the wood of the same species (37) as well as other gymnosperm woods. A preliminary examination was also made with the barks from the other three species referred to earlier. It was noted that with the exception of the fir, the number of mannose residues in these barks were much lower than in gymnosperm woods. These barks also contained large amounts of glucose residues probably not derived from cellulose or galactoglucomannans, a situation which was noted earlier by Painter and Purves (25) for the inner bark of white spruce. The pine bark had an exceptionally high arabinose content which was noted earlier also by other workers (24).

Recently Thornber and Northcote, in a study of the changes in the chemical composition of a cambial cell during its development into xylem (wood) and phloem (bark) tissue in trees, (38-40), have estimated the carbohydrate composition of several polysaccharides for samples obtained from different regions in a tree. Some of their results are summarized in Tables 2 and 3. It is evident that the bark tissue is very rich in pectic substances. During the secondary thickening of the cambial cell wall, α-cellulose, hemicellulose and lignin are formed while little change occurs in the weight of pectic substances. From Table 3, it can be seen that the

Table 2 Composition of the Samples Taken from Each Tree

All values in per cent

Species	Fraction	Region of Plug			
		Phloem	Cambium	Sapwood	Heartwood
Birch (*Betula platyphylla*)	Pectic Substances	4.4	18.0	2.6	0.3
	Lignin	53.1	10.0	23.0	22.5
	α-cellulose	22.3	35.2	40.0	43.4
	Hemicellulose	19.2	36.5	35.0	33.8
Pine (*Pinus ponderosa*)	Pectic Substances	10.0	8.5	1.3	1.0
	Lignin	52.8	20.0	30.4	29.5
	α-cellulose	22.1	36.1	45.0	43.4
	Hemicellulose	15.3	35.0	23.0	25.0

Table 3 Carbohydrate Composition of the Hemicellulose Preparations from Each Tree

All values in per cent

Species	Anhydrosugar	Region of Plug			
		Phloem	Cambium	Sapwood	Heartwood
Birch	Galactan	4.1	12.2	4.5	2.8
	Glucan	13.0	11.5	11.3	9.6
	Mannan	0.9	Trace	1.0	1.0
	Araban	6.8	3.9	1.6	1.8
	Xylan	62.0	53.0	63.0	65.0
	Rhamnan	0.2	0.5	0.2	Trace
	Uronic Anhydride	12.5	19.5	19.0	19.0
Pine	Galactan	9.0	18.0	6.7	5.5
	Glucan	34.0	24.0	10.3	8.0
	Mannan	3.3	7.6	18.0	16.0
	Araban	11.5	13.0	5.7	6.0
	Xylan	27.0	25.0	38.0	44.0
	Rhamnan	Trace	0.2	Trace	Trace
	Uronic Anhydride	16.0	12.0	21.0	20.5

hemicellulose portion from the cambial region of pine contained a higher percentage of glucans, mannans and arabans with a correspondingly lower percentage of the other constituents compared to birch. The hemicellulose from the phloem and cambium of pine contained more glucans than the wood tissue which was also shown to be different from starch. Also the mannose content for the phloem tissue of pine was much smaller than that for sapwood. These results agree with those obtained earlier by Timell (33) and by Painter and Purves (25). It is possible that some of the glucose residues may originate from a β-glucan or a heteropolymeric "glucan".

The present investigation is concerned with the hemicellulose components of Engelmann spruce. Prior to this, a study of the hemicelluloses present in the wood of Engelmann spruce has been completed (41). The three major hemicelluloses in this wood were an arabino-4-O-methylglucurono-xylan, a water-soluble galactoglucomannan and an alkali-soluble glucomannan.

This study was particularly directed with a view to establish the origin of large amounts of glucose residues associated with the carbohydrate polymers, only part of which could have been derived from galactoglucomannans and glucomannans.

RESULTS AND DISCUSSION

Preliminary Treatments of the Bark

The spruce bark was first freed of the remaining xylem and cambium, after which it was exhaustively extracted with the azeotrope of benzene and ethanol to remove extraneous materials. The removal of lignin and the remaining tannins, phlobaphenes and other phenolic materials was accomplished by treatment of the bark with acidified sodium chlorite (chlorous acid) at elevated temperatures. Although the vigorous nature of such oxidation reactions can bring about glycosidic cleavage (42) and dissolution of significant amounts of carbohydrate materials (43,44), in general practice it has been observed that the extraction of polysaccharides is not complete without prior delignification (33). A certain amount of sacrifice is, therefore, necessary at this stage to attain the desired objective, namely, complete extraction of the polysaccharides. The products remaining after the oxidation may not correspond to the materials present in their native state. Nevertheless, their significance will not be lost if only they are considered as fragments of possibly more complex polymers. The treatment with chlorous acid was carried out in stages, resulting in a holocellulose. The yield of this material was approximately 50%, based on the original, extractive-free bark.

Isolation of Crude Hemicelluloses

For preliminary experiments, the extraction procedure suggested by Timell (15) was employed. Accordingly, the holocellulose was successively extracted with hot water, 0.5% hot aqueous ammonium

oxalate, 24% (w/w) potassium hydroxide and 17.5% (w/w) sodium hydroxide containing 4% boric acid. The sugar composition of the various extracts is given in Table 4. The water extract was a mixture of neutral and acidic polysaccharides while the ammonium oxalate extract consisted of pectic substances. These two extracts were not examined further. Attention was instead directed towards the two fractions obtained on extraction with aqueous alkali.

For the sake of convenience the following discussion has been divided into two parts. The first part deals with the attempts made with a view of isolating as many chemically homogeneous polysaccharides as possible from the alkaline extracts. Accordingly it concerns itself with the application on the crude extracts, of various fractionation procedures recorded in the literature. In the second part, after making a brief reference to the large-scale isolation of these crude polysaccharide mixtures, and their fractionation into three homogeneous components, their structural features employing the well-established techniques in carbohydrate chemistry, are discussed. This part is subdivided into three sections A, B and C, each dealing with one hemicellulose.

Table 4 Sugar Composition of the Various Extracts from Holocellulose of Engelmann Spruce Bark

Extract	Percent of extractive-free bark	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Hot water	2.8	14.0	16.0	18.0	16.0	30.8	5.2
Ammonium Oxalate	8.8	77.8	5.3	3.0	2.0	11.9	Nil
24% Potassium hydroxide	7.6	8.5	11.3	24.6	Trace	15.0	40.7
17.5% Sodium hydroxide + 4.0% boric acid	4.5	3.2	7.4	26.0	41.4	4.6	17.4

PART I - FRACTIONATION STUDIES

FRACTIONATION OF THE CRUDE POTASSIUM HYDROXIDE EXTRACT

1. Treatment with aqueous Barium hydroxide

The crude potassium hydroxide extract was remarkable in that it contained large amounts of glucose and only trace amounts of mannose (Table 4). This suggested the possible absence of significant amounts of the water-soluble galactoglucomannan which has been shown to be present in many coniferous woods (15, 16,37,41,45,46) and also in the bark of amabilis fir (35). In most of these studies it has been observed that this polymer is generally extracted along with the acidic xylan by aqueous potassium hydroxide, the fractionation of such a mixture being effected by repeated precipitation with aqueous barium hydroxide (27). However, in the present study barium hydroxide failed to produce a significant amount of precipitate, due presumably to presence of only trace amounts of mannose residues in the polysaccharide mixture. It is noteworthy that Engelmann spruce wood investigated by Mills and Timell (41) contained 1.8% of the water-soluble galactoglucomannan, an amount much smaller than that present in the wood of amabilis fir, 4.0% (37) or eastern hemlock, 4.8% (45). These preliminary observationsstrongly suggested the possibility that a predominantly glucose-based polysaccharide might be present in the alkaline extract, in addition to an arabino-glucurono-xylan of the general type encountered in most gymnosperm species. In this respect the present bark appeared to be different from the wood of the same species.

2. Electrophoresis of the Extract

Free boundary electrophoresis of the crude extract in borate buffer by the Tiselius method gave the results shown in Fig. IV. It is evident that in addition to the stationary salt boundary there was a slow-moving major boundary and a fast-moving minor one. The latter probably arose from a contaminating pectic impurity. Electrophoresis in sodium chloride (0.1N), however, indicated the presence of at least two major boundaries in addition to the fast-moving pectic contaminant. Although definite conclusions could not be drawn from these observations, it appeared likely that the crude extract might be a mixture of at least two major polysaccharides.

3. Enzymatic Hydrolysis of the Extract

The potassium hydroxide extract was digested with α-amylase at room temperature for 24 hours. The recovered, enzyme-resistant polysaccharide, on hydrolysis and quantitative paper chromatography was found to have a sugar composition identical to the starting material. Since a sample of starch was hydrolyzed completely to glucose under the same conditions, it was concluded that the glucose residues in the extract did not originate from starch.

4. Chromatography on Diethylaminoethylcellulose

Deuel and co-workers used a column of diethylaminoethylcellulose (cellulose $-OCH_2 \cdot CH_2 \cdot N(C_2H_5)_2$) for separation of neutral and acidic polysaccharides from their mixtures (47). Later it was successfully employed by Meier (48) to resolve a mixture of an

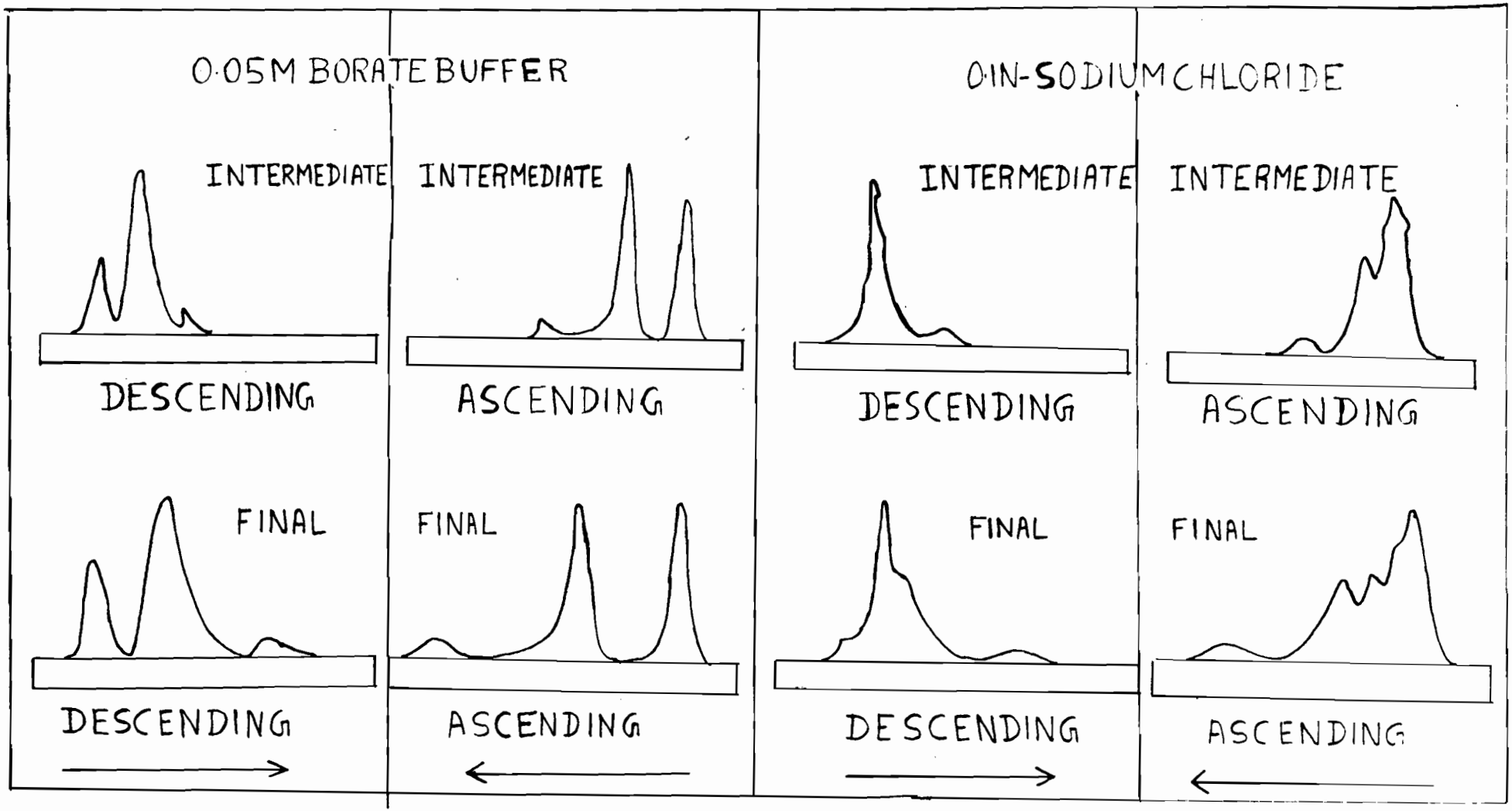


FIG. IV FREE BOUNDARY ELECTROPHORESIS OF KOH EXTRACT

O-acetyl-galactoglucomannan and an arabino-glucurono-xylan. Meier found that the acidic xylan was adsorbed on a column of diethylaminoethylcellulose in the phosphate form while the neutral galactoglucomannan was eluted. This neutral polysaccharide contained all the acetyl groups. In the present case, elution of the diethylaminoethylcellulose phosphate was carried out successively with water, 0.05M sodium dihydrogen phosphate (pH, 5.5), 0.5M sodium dihydrogen phosphate (pH, 5.5) and 0.1N sodium hydroxide. The polysaccharide fractions recovered from the various eluates had the sugar composition given in Table 5. Fig. V shows the typical fractionation curve that was obtained when the separation was followed by spectrophotometric analysis of the eluate. The data in Table 5 show that the aqueous eluate did not contain any acid residues and that glucose dominated among the neutral sugars. The 0.05M sodium dihydrogen phosphate eluate gave two separate fractions, one of which was rich in glucose, but also contained small amounts of uronic acid and arabinose in addition to xylose and galactose. This fraction was probably a mixture of a major neutral component contaminated with some acidic impurities. The second fraction was very likely a mixture of polysaccharides. The 0.5M sodium dihydrogen phosphate eluate was rich in xylose and probably contained an acidic arabinoxylan, while the sodium hydroxide eluate appeared to have a similar composition as the starting material. It should be noted that the total recovery of the polysaccharides was only about 55%. Meier (48) reports a total recovery of 69% in his investigation. These preliminary fractionation results indicate the possible

Table 5 Fractions Obtained on Chromatography of Potassium hydroxide Extract on Diethylaminoethylcellulose in the Phosphate Form

Eluate	Per cent of crude extract	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Water	6	Nil	11.8	50.0	Trace	Trace	38.2
0.05M Sodium dihydrogen phosphate Peak (a)	3	5.3	11.2	54.9	Trace	5.6	23.0
0.05M Sodium dihydrogen phosphate Peak (b)	6	5.7	7.6	34.0	Nil	17.1	35.5
0.5M Sodium dihydrogen phosphate	20	17.2	4.3	8.1	Nil	11.9	58.6
0.1N Sodium hydroxide	20	7.7	10.2	33.6	Trace	5.1	43.4

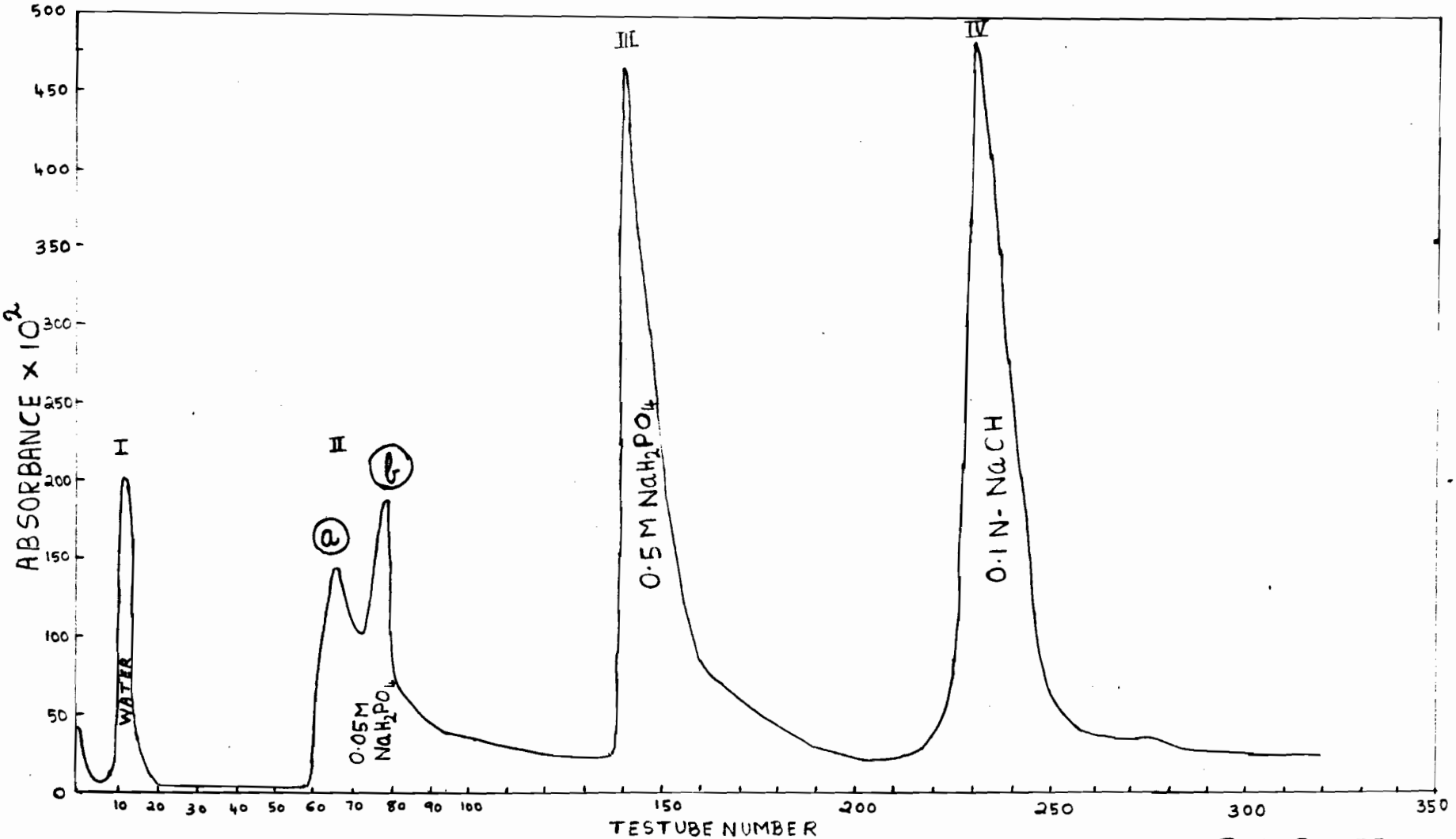


FIG. V CRUDE KOH EXTRACT - CHROMATOGRAPHY ON DEAE CELLULOSE PHOSPHATE

presence of a glucose-rich polysaccharide (a "glucan") and a xylose-rich polysaccharide (a "xylan") in the mixture.

5. Fractional Precipitation

Fractional precipitation of polysaccharide mixtures from aqueous solutions by ethanol or other organic liquids has been successfully employed by many workers, especially in the field of gums and mucilages (11). Although efficient fractionation is often impeded by co-precipitation, satisfactory results have been obtained when the solubilities of the components in the mixture vary widely from one another. Fractional precipitation of the potassium hydroxide extract from an aqueous solution by means of ethanol gave four fractions, the sugar composition of which is presented in Table 6(a). Evidently, the fractionation was not very satisfactory. The first fraction appeared to be a mixture, while the later fractions were rich in xylose. Refractionation of the first two fractions in the same manner gave four fractions each, the composition and specific rotation of which are presented in Tables 6(b) and 6(c). Most of the glucose-yielding material appeared in the first fraction (I-1, Table 6(b)), although probably contaminated by a pectic araban. The subsequent fractions were undoubtedly mixtures. The high arabinose content of the last fraction is particularly noteworthy. Refractionation of fraction II gave four fractions having more or less the same sugar composition and specific rotations (Table 6(c)). This would suggest either that fraction II was homogeneous or that the fractionation was not entirely satisfactory. The presence of galactose and glucose residues might be due to a contamination of

Table 6(a) Fractional Precipitation of Potassium hydroxide Extract

Fraction number	Per cent of crude extract	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
I	45	4.7	11.1	38.6	1.5	9.2	34.9
II	20	10.8	3.9	9.4	Trace	12.8	63.1
III	10	13.1	3.7	11.6	Nil	19.0	52.6
IV	5	14.8	7.7	19.5	Nil	15.8	42.2

Table 6(b) Refractionation of Fraction I of Table 6(a)

Fraction number	Per cent of fraction I	Specific rotation, $[\alpha]_D$ degrees	Sugar residues in relative per cent					
			Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
I-1	25	+27.7 (C, 2.0%)	4.4	11.4	45.7	Trace	9.3	29.2
II-1	23	+21.2 (C, 2.0%)	5.2	9.3	34.7	Trace	13.5	37.2
III-1	23	+2.9 (C, 2.0%)	7.6	8.6	32.9	Trace	12.5	38.4
IV-1	10	-2.8 (C, 1.0%)	11.9	13.8	30.0	Trace	20.0	24.3

Table 6(c) Refractionation of Fraction II of Table 6(a)

Fraction number	Per cent of fraction II	Specific rotation, $[\alpha]_D$ degrees	Sugar residues in relative per cent					
			Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
I-2	22	-45.6 (C, 1.0%)	11.1	3.7	12.5	Nil	12.2	60.5
II-2	22	-47.6 (C, 1.0%)	13.0	4.9	8.9	Nil	13.2	59.9
III-2	22	-45.6 (C, 1.0%)	9.2	3.7	8.2	Nil	14.1	64.8
IV-2	15	-46.2 (C, 1.0%)	10.1	4.3	11.1	Nil	14.0	60.5

the "xylan" by a glucose-rich polysaccharide. The per cent yield given in the above tables were those actually obtained from these experiments. Since no attempts were made to achieve quantitative yields, the total recovery in all cases was less than 100%. This is true also for subsequent fractionation experiments.

6. Fractionation with Fehling's Solution

Treatment of the potassium hydroxide extract with Fehling's solution gave a precipitate from which a polysaccharide fraction was recovered which had the composition shown in Table 7. Two more successive precipitations of this fraction with Fehling's solution eliminated most of the arabinose and some uronic acid residues. This fraction, $[\alpha]_D$ + 46°, was rich in glucose and was similar to the glucose-rich fractions encountered in the earlier fractionation experiments. The component recovered from the filtrate of the first precipitation, contained mostly xylose together with some galactose and glucose residues. This fraction undoubtedly contained the "xylan" portion of the original mixture.

7. Homogeneity of the Fraction Precipitated with Fehling's Solution

The polysaccharide fraction recovered from the precipitated copper complex of the potassium hydroxide extract appeared to be homogeneous on fractional precipitation of an aqueous solution by ethanol. All four fractions obtained had similar sugar composition and specific rotation as can be seen from Table 8.

Chromatography of this material on a diethylamino-ethylcellulose column in the phosphate form gave the fractionation

Table 7 Fractionation of the Potassium hydroxide Extract with Fehling's Solution

Fraction	Per cent of crude extract	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
First precipitate	8	8.3	11.8	42.0	Trace	5.0	32.9
Fraction recovered after two more precipitations	6	3.2	12.8	50.2	Trace	Trace	33.7
Fraction recovered from the filtrate of the first precipitation	75	11.5	8.0	21.7	Trace	12.5	46.4

Table 8 Fractional Precipitation of the Precipitated Copper Complex from Potassium hydroxide Extract

Fraction number	Percent of copper complex fraction	Specific rotation, $[\alpha]_D$ degrees	Sugar residues in relative per cent					
			Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
I	20	+42.0 (C, 1.5%)	3.1	12.0	57.3	Trace	Nil	27.6
II	50	+41.4 (C, 1.2%)	2.8	12.5	58.7	Trace	Trace	25.9
III	20	+48.0 (C, 1.8%)	3.0	12.0	55.3	Trace	Trace	29.7
IV	5	-	3.4	13.9	49.5	2.0	Trace	31.2

curve presented in Fig. VI. Four fractions were obtained all of which contained mostly glucose and xylose. (Table 9). It is interesting to note that fractions having more or less similar sugar composition were eluted with different eluants. The possibility cannot be excluded that fractionation occurred also according to molecular weight.

8. Filtrate Remaining after Addition of Fehling's Solution

As can be seen from Table 7, the polysaccharide fraction recovered from the filtrate remaining after addition of Fehling's solution to potassium hydroxide extract, contained predominantly xylose residues. In order to test its homogeneity a fractional precipitation* was carried out from an aqueous solution by addition of ethanol. Ten fractions were obtained, the first four of which were undoubtedly acidic "xylans" having similar sugar composition (see Table 10). The presence of galactose and glucose residues was again noticeable. The last six fractions exhibited a pronounced increase in arabinose content with a corresponding lowering of the proportion of xylose residues, suggesting that a pectic araban was perhaps present in the crude extract. Similar fractions with high content of arabinose were also obtained in some of the earlier fractionation experiments.

Chromatography of the material on a column of

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* The material used for fractional precipitation had a slightly different sugar composition from that given in Table 7, because it was obtained from another batch of holocellulose.

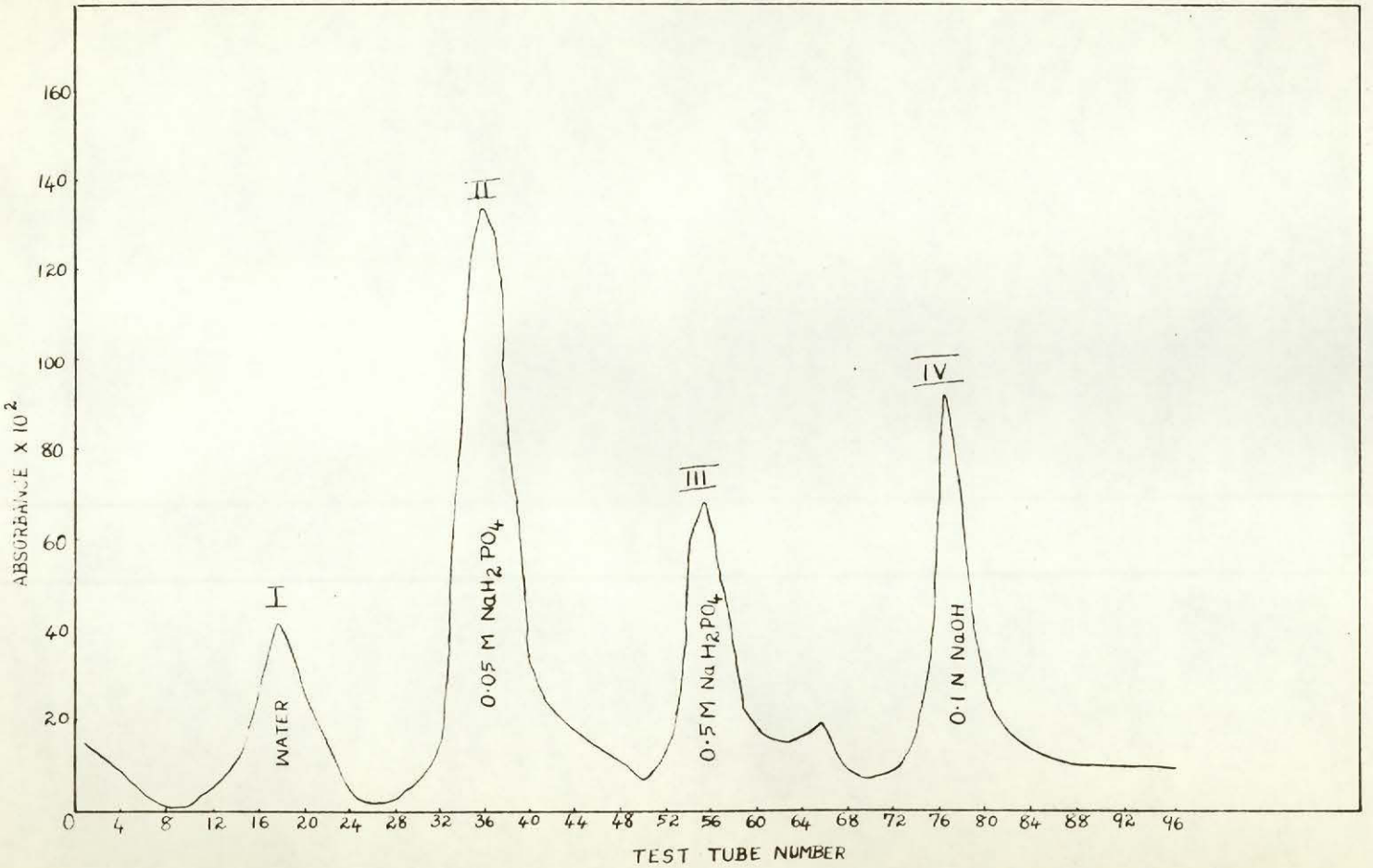


FIG. VI INSOLUBLE COPPER COMPLEX OF KOH EXTRACT-CHROMATOGRAPHY ON DEAE CELLULOSE PHOSPHATE

Table 9 Chromatography of the Copper Complex Fraction on Diethylaminoethylcellulose

Eluate	Per cent of copper complex fraction	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Water	5	Nil	11.1	55.9	Trace	Trace	33.0
0.05M Sodium dihydrogen phosphate	20	2.4	13.0	58.7	Nil	Nil	26.0
0.5M Sodium dihydrogen phosphate	13	5.9	12.0	55.1	Trace	Trace	27.0
0.1N Sodium hydroxide	20	4.9	12.2	46.4	Trace	Trace	36.5

Table 10 Fractional Precipitation of the Material Recovered from the Filtrate Remaining after Addition of Fehling's Solution to Potassium hydroxide Extract *

Fraction number	Yield %	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
1	18	9.9	4.3	14.0	Trace	10.4	61.4
2	12	7.3	7.2	16.2	Trace	11.5	57.8
3	11	6.3	5.2	18.0	Nil	11.5	59.0
4	15	6.8	5.9	20.2	Nil	12.0	55.1
5	6	4.0	9.4	28.3	Nil	20.8	37.5
6	6	4.1	9.6	24.8	Nil	22.8	38.7
7	5	5.6	8.3	21.1	Nil	23.3	41.7
8	3	5.0	9.1	22.0	Nil	23.8	40.1
9	4	5.0	6.8	23.2	Nil	20.2	44.7
10	2	5.0	3.0	26.4	Nil	20.6	45.0

* The material used for fractional precipitation had the following per cent composition:

Uronic acid	- 7.9	Galactose	- 9.1	Glucose	- 25.1
Mannose	- Nil	Arabinose	- 14.3	Xylose	- 43.6

Being obtained from a different batch of holocellulose, the composition is slightly different from that of similar material shown in Table 7.

diethylaminoethylcellulose in the phosphate form (Fig. VII and Table 11) revealed that an acidic arabinoxylan formed the main constituent. No fraction was eluted with water and the one eluted with 0.05M sodium dihydrogen phosphate was very small. The xyaln was mainly found in the 0.5M sodium dihydrogen phosphate eluate. It is remarkable that the material eluted with 0.1N sodium hydroxide had a similar sugar composition as the corresponding fraction obtained from the crude extract (see Table 5). The presence of large amounts of glucose residues in the fraction could not be satisfactorily explained.

9. Fractionation with Cetyltrimethylammonium hydroxide (CTA-OH)

Quarternary ammonium salts have been used to separate acidic from neutral polysaccharides (10). The most commonly used salt is cetyltrimethylammonium bromide ($C_{16}H_{31}\overset{+}{N}(CH_3)_3\overline{Br}$) often referred to as "Cetavlon". The acidic polysaccharide combines with the base to give a precipitate. Occasionally, the free, quarternary base obtained by exchanging the bromide ion with hydroxide ion on an anion exchange resin, is used. In this way the ionic strength of the solution is kept low. The precipitate obtained may be dissolved by increasing the acidity, when the carboxyl ions are transformed into undissociated carboxyl groups.

Treatment with cetyltrimethylammonium hydroxide of the fraction recovered from the filtrate remaining after addition of Fehling's solution to the potassium hydroxide extract, resulted in the formation of a precipitate in about 11% yield. This

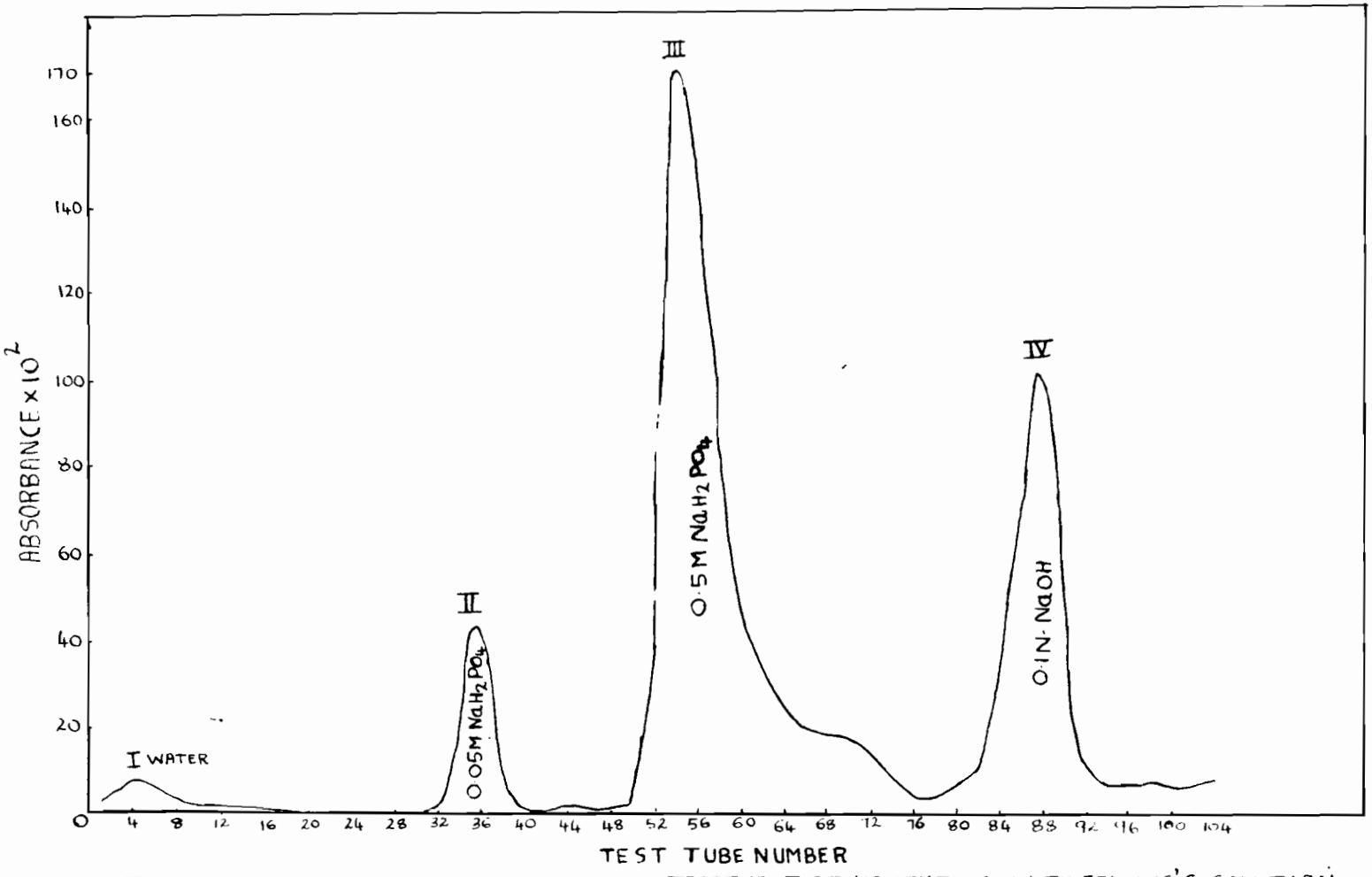


FIG VIII PRECIPITATE FROM THE FILTRATE AFTER FIRST TREATMENT OF KOH EXTRACT WITH FEHLING'S SOLUTION-CHROMATOGRAPHY ON DEAE CELLULOSE PHOSPHATE

Table 11 Diethylaminoethylcellulose Chromatography of the Fraction Recovered from the Filtrate on Precipitation with Fehling's Solution of the Potassium hydroxide Extract

Eluate	Per cent of filtrate fraction	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
0.05M Sodium dihydrogen phosphate	5	4.1	11.9	52.0	Trace	5.3	26.7
0.5M Sodium dihydrogen phosphate	40	16.1	5.0	9.1	Nil	12.2	57.6
0.1N Sodium hydroxide	20	8.6	8.4	31.1	Trace	3.9	47.9

fraction contained large amounts of arabinose (Table 12) in addition to appreciable quantities of glucose and xylose suggesting that it was a mixture. The precipitate recovered from the filtrate appeared to be the acidic "xylan". It is noteworthy that this fraction as well as other xylose-rich fractions obtained earlier contained appreciable amount of galactose and glucose residues.

10. Treatment of the Potassium hydroxide Extract with CTA-OH

CTA-OH treatment of the crude potassium hydroxide extract itself did not give satisfactory results as can be seen from the results in Table 13. The yield of the precipitate was only 2%. Fractional precipitation of the filtrate by addition of ethanol gave two xylose-rich fractions, one of which contained a higher proportion of uronic acid and arabinose residues than the other with a correspondingly lower galactose and glucose content. The presence of more arabinose in the more soluble fraction (Fraction II) is noteworthy, because similar arabinose-rich fractions were also found in the more soluble portions obtained in fractional precipitation of both the crude extract (Table 6(a)) and of the filtrate remaining after copper complexing of the extract (Table 10).

Both fractions I and II were resolved on a column of diethylaminoethylcellulose phosphate. The results are summarized in Tables 14(a) and 14(b). These results were in full accordance with the earlier observations. The fractionation curve

Table 12 Treatment with CTA-OH of the Fraction Recovered from the Filtrate Remaining after Addition of Fehling's Solution to Potassium hydroxide Extract

Fraction	Yield %	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Precipitate	11	9.7	9.7	20.7	Trace	30.5	29.3
Fraction recovered from the filtrate	70	5.3	5.3	18.6	Trace	8.2	62.5

Table 13 CTA-OH Fractionation of Potassium hydroxide Extract

Fraction	Yield %	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Precipitate	2	16.6	5.8	9.6	Trace	8.1	59.9
Precipitate from filtrate of above (Fraction I)	55	5.7	10.5	31.9	Trace	8.3	43.6
Precipitate from filtrate of above (Fraction II)	22	11.5	6.5	20.1	Nil	17.2	44.7

Table 14a

Column Chromatography on Diethylaminoethylcellulose phosphate of Fraction I of Table 13

Eluate	Yield %	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Water	3	Nil	12.6	49.3	Trace	Nil	38.2
0.05M Sodium dihydrogen phosphate Peak (a)	20	1.8	9.3	52.4	Trace	4.6	31.9
0.05M Sodium dihydrogen phosphate Peak (b)	6	2.9	9.2	51.1	Nil	7.1	29.8
0.5M Sodium dihydrogen phosphate	20	7.9	5.1	18.0	Nil	11.5	57.5
0.1N Sodium hydroxide	15	4.2	10.1	37.4	Trace	3.1	45.2

Table 14b

Column Chromatography on Diethylaminoethylcellulose phosphate of Fraction II of Table 13

Eluate	Yield %	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
0.05M Sodium dihydrogen phosphate	5	6.6	10.6	41.4	Nil	11.2	30.1
0.5M Sodium dihydrogen phosphate	35	10.7	5.3	15.3	Nil	12.1	56.7
0.1N Sodium hydroxide	15	8.2	8.1	31.5	Nil	5.1	47.1

for fraction I (Fig. VIII) bore a very close resemblance to that of the crude extract itself (Fig. V).

11. Acetylation of the Crude Potassium Hydroxide Extract

One of the methods used for separating a neutral glucomannan from an acidic xylan is to acetylate the mixture and then separate the acetylated derivatives by taking advantage of their different solubilities in organic solvents. Jones and Painter (49) found that the xylan acetate formed an emulsion in chloroform with aqueous sodium carbonate while the neutral glucomannan acetate was found in the clear chloroform solution. Hamilton, Partlow and Thompson (16) acetylated a mixture of an acidic arabinoxylan and a neutral galactoglucomannan. Acetone extraction of the acetylated mixture gave the galactoglucomannan, the xylan acetate being far less soluble than the hexosan acetate. In view of the fact that the fractionation experiments in the present case suggested the presence of a glucose-based polysaccharide and an acidic "xylan" in the crude potassium hydroxide extract, an attempt was made to achieve a resolution via the acetylated mixture. The acetylated product (yield - 80%, acetyl - 38.2%) was extracted with acetone. Most of the product went into solution. The acetone-insoluble residue was unexpectedly found to be rich in glucose (Table 15). Because this method offered no special advantages compared to some of the fractionation methods discussed earlier, it was not used any further.

The various fractionation results discussed above indicated

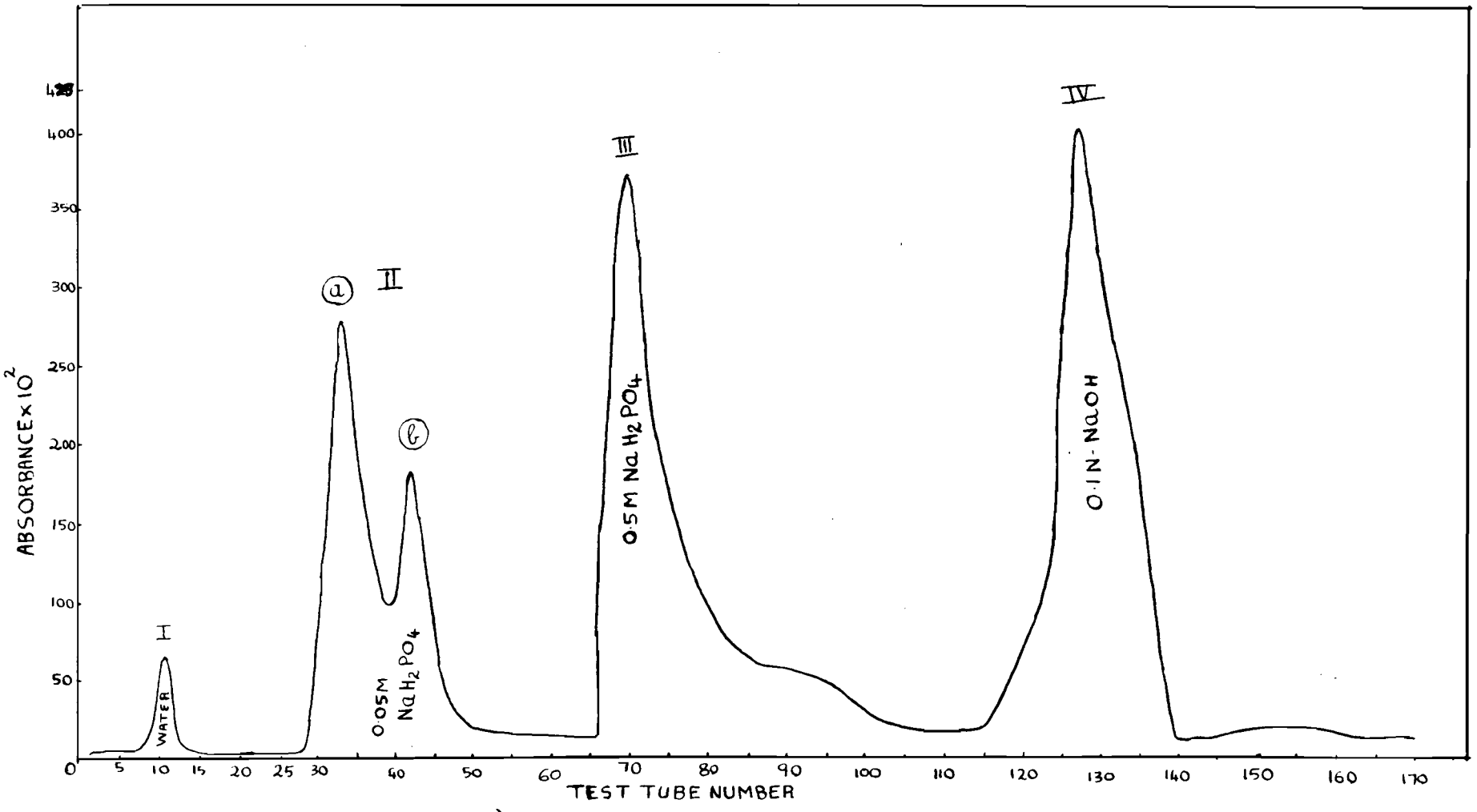


FIG. VIII PRECIPITATE (FRACTION I) FROM THE FILTRATE AFTER TREATMENT OF KOH EXTRACT WITH CTA-OH- CHROMATOGRAPHY ON DEAE CELLULOSE PHOSPHATE

Table 15 Fractionation of the Acetylated Potassium hydroxide Extract

Fraction	Per cent of acetylated product	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Acetone-soluble fraction	75	6.2	7.5	30.1	Trace	11.9	44.2
Acetone-insoluble fraction	10	7.3	7.3	57.3	Trace	12.2	15.9

the presence in the potassium hydroxide extract of two major polysaccharides. One was an acidic arabinoxylan of the type commonly found in all gymnosperm species and the other a polysaccharide based predominantly on glucose, xylose and galactose residues. Although the galactose and xylose might arise from a "galactan" and a "xylan" respectively, consistency in composition of the various fractions containing these residues in association with glucose suggested that a triheteropolymeric polysaccharide might be present in the extract.

STEP-WISE EXTRACTION OF THE HOLOCELLULOSE WITH AIKALI

In the isolation of hemicelluloses from wood, several workers have used the technique of step-wise extraction with alkali of increasing concentration (50). Some fractionation occurs in these extractions. The reason for applying a sequential extraction in the present case was that there existed a possibility that one type of polymer might be more accessible than the other to alkali of a particular concentration. Accordingly, in the present case, the holocellulose was first extracted with hot aqueous ammonium oxalate to remove most of the pectic substances and other water-soluble polysaccharides. It was then successively extracted with 10% aqueous sodium carbonate, 1, 5, 10, 24% (w/w) aqueous potassium hydroxide and finally with 24% aqueous potassium hydroxide (w/w) containing 4% borate. The sugar composition of the various fractions is given in Table 16.

The aqueous sodium carbonate and 1% potassium hydroxide

Table 16 Step-wise Extraction of Holocellulose with Increasing Alkali Concentration

Reagent for extraction	Per cent of extractive-free bark	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
10 per cent sodium carbonate	4.5	34.4	10.8	Trace	Trace	20.3	34.4
1 per cent potassium hydroxide	2.0	34.1	5.7	12.7	Trace	13.7	33.7
5 per cent potassium hydroxide	4.6	8.7	7.2	20.2	2.1	10.5	51.3
10 per cent potassium hydroxide	3.0	5.7	8.3	27.9	5.4	10.9	41.8
24 per cent potassium hydroxide	1.0	3.2	8.8	30.8	22.8	6.8	27.7
24 per cent potassium hydroxide + 4 per cent boric acid	1.0	2.6	7.5	25.3	52.0	4.6	7.9

extracts were apparently mixtures of pectic materials and acidic xylan. The former was especially interesting in view of the virtual absence of both glucose and mannose residues and the presence of appreciable amounts of arabinose. When this extract was treated with aqueous calcium chloride solution, a precipitate was formed which contained mostly arabinose residues (Table 17) suggesting the possible presence of an araban. The fraction recovered from the filtrate was rich in xylose but also contained appreciable amount of arabinose, only part of which could have been associated with the acidic xylan. When the precipitate obtained with calcium chloride was treated with ammonium oxalate, the corresponding soluble ammonium salt was formed, which on repeated alternate treatments with aqueous calcium chloride and ammonium oxalate, finally yielded a polysaccharide (ammonium salt) in low yield (last column, Table 17), whose composition was substantially similar to the original calcium salt precipitated from the extract. The low yield was to be attributed mainly to difficulties experienced in the recovery of the precipitates because of their tendency to form colloidal solutions.

The 5 and 10% potassium hydroxide extracts of the holocellulose contained mostly the acidic xylan, although contaminated to some extent by the "glucan". The last two fractions contained mainly the "glucan" and the glucomannan, the latter being the main component of the sodium hydroxide-borate extract of the holocellulose (see later).

From these results it might be concluded that successive extraction with increasing alkali concentration did not offer any advantages over the use of the different reagents described previously.

Table 17 Fractionation of 10% Sodium carbonate Extract with Aqueous Calcium Chloride

Fraction	Per cent of extract	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Precipitate obtained with aqueous calcium chloride (calcium salt)	60	28.5	22.5	Trace	Nil	39.3	9.8
Fraction received from the filtrate	25	16.1	12.0	Trace	Trace	26.4	45.5
Three alternate treatments of the calcium salt above with ammonium oxalate and calcium chloride - the final ammonium salt of the polysaccharide	5	21.8	24.4	Trace	Nil	44.9	8.9

It did, however, point to the beneficial effects that could be obtained by the interposition of an aqueous sodium carbonate extraction prior to the use of a stronger alkali. As noted above, such an extraction removed some arabinose-based polysaccharide presumably of pectic nature. In the subsequent large-scale isolation of these hemicelluloses, a prior extraction with aqueous sodium carbonate was carried out.

FRACTIONATION OF THE CRUDE SODIUM HYDROXIDE-BORATE EXTRACT

The sodium hydroxide-borate extract had the sugar composition given in Table 4. Three successive precipitations of the extract with aqueous barium hydroxide (27) gave a pure alkali-soluble glucomannan containing 4% galactose residues (Table 18). The glucose to mannose ratio was approximately 1:2.8, very close to the ratio of 1:3 common for most softwood glucomannans. It is noteworthy that the fraction recovered from the filtrate of the first barium hydroxide precipitation (Table 18) had the same sugar composition as the 24% potassium hydroxide extract (see Table 4). This might indicate that the extraction with potassium hydroxide was incomplete.

Table 18 Fractionation of the Sodium hydroxide-Borate Extract - Barium hydroxide Treatment

Fraction	Percent of the extract	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
Material precipitated twice with barium hydroxide	50	2.3	5.2	25.3	63.6	Trace	3.6
Material precipitated three times with barium hydroxide	35	Trace	4.1	25.3	70.6	Nil	Trace
Precipitate recovered from the filtrate after the first treatment	25	11.3	5.8	25.0	Trace	11.8	46.1

PART II - STRUCTURAL STUDIES

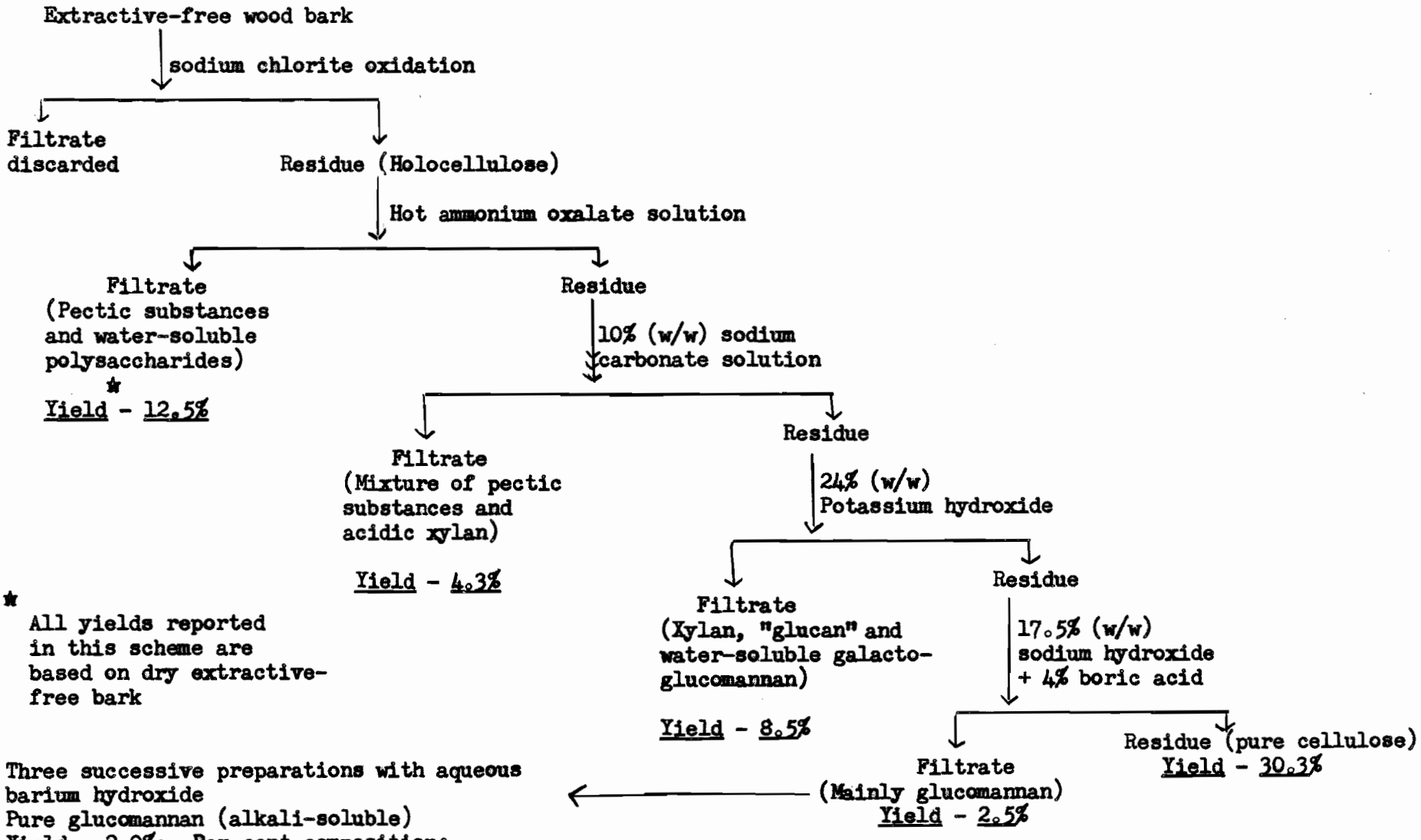
LARGE-SCALE ISOLATION OF HEMICELLULOSES

The three major hemicelluloses present in the bark of Engelmann spruce, as indicated by the fractionation studies, were an acidic arabinoxylan, a heteropolymeric "glucan" and an alkali-soluble glucomannan. For the isolation of these polysaccharides in larger amounts for structural studies, the fractionation scheme presented in Figs. IX(a), (b) and (c) was used. The pectic material not removed with aqueous ammonium oxalate was eliminated by extraction with aqueous sodium carbonate. A certain amount of acidic xylan was also lost in this extraction.

Unlike the earlier fraction used in the preliminary studies, the 24% potassium hydroxide extract in the present case was found to contain a small proportion (3.3%) of mannose residues. Precipitation of this fraction with Fehling's solution increased the mannose content to 10.8% (see Fig. IX(b)). The presence of mannose residues at this stage suggested that a small proportion of water-soluble galactoglucomannan was present in admixture with the "glucan". The elimination of this polymer was achieved by precipitation with aqueous barium hydroxide. The resulting heteropolymeric "glucan" still contained about 2% mannose which could not be eliminated by further barium hydroxide treatments.

In order to obtain the xylan portion of the original extract, the fraction recovered from the filtrate after copper complexing was treated with cetyltrimethylammonium hydroxide in the presence of some

FIG. IX(e) Fractionation Scheme for Extraction of Hemicelluloses from Bark of Engelmann Spruce



* All yields reported in this scheme are based on dry extractive-free bark

FIG. IX(b) Fractionation Scheme for Extraction of Hemicelluloses from the Bark of Engelmann Spruce: Resolution of 24% Potassium hydroxide Extract

24% Potassium hydroxide Extract

Composition: (relative per cent) Uronic acid - 7.3; Galactose - 8.8; Glucose - 29.4; Mannose - 3.3; Arabinose - 8.8; Xylose - 42.5

↓ Three successive Precipitations with Fehling's Solution

↓ Precipitate
Yield - 2.2%

Composition: (relative per cent) Uronic acid - 3.3; Galactose - 11.4; Glucose - 46.6; Mannose - 10.8; Arabinose - Trace; Xylose - 27.9

↓ Aqueous Barium hydroxide (5%)

↓ Insoluble Portion
Yield - 0.3%

Composition: (relative per cent) Uronic acid - 2; Galactose - 10; Glucose - 38; Mannose - 40; Arabinose - Trace; Xylose - 10

(This fraction consists mainly of the water-soluble galactoglucomannan)

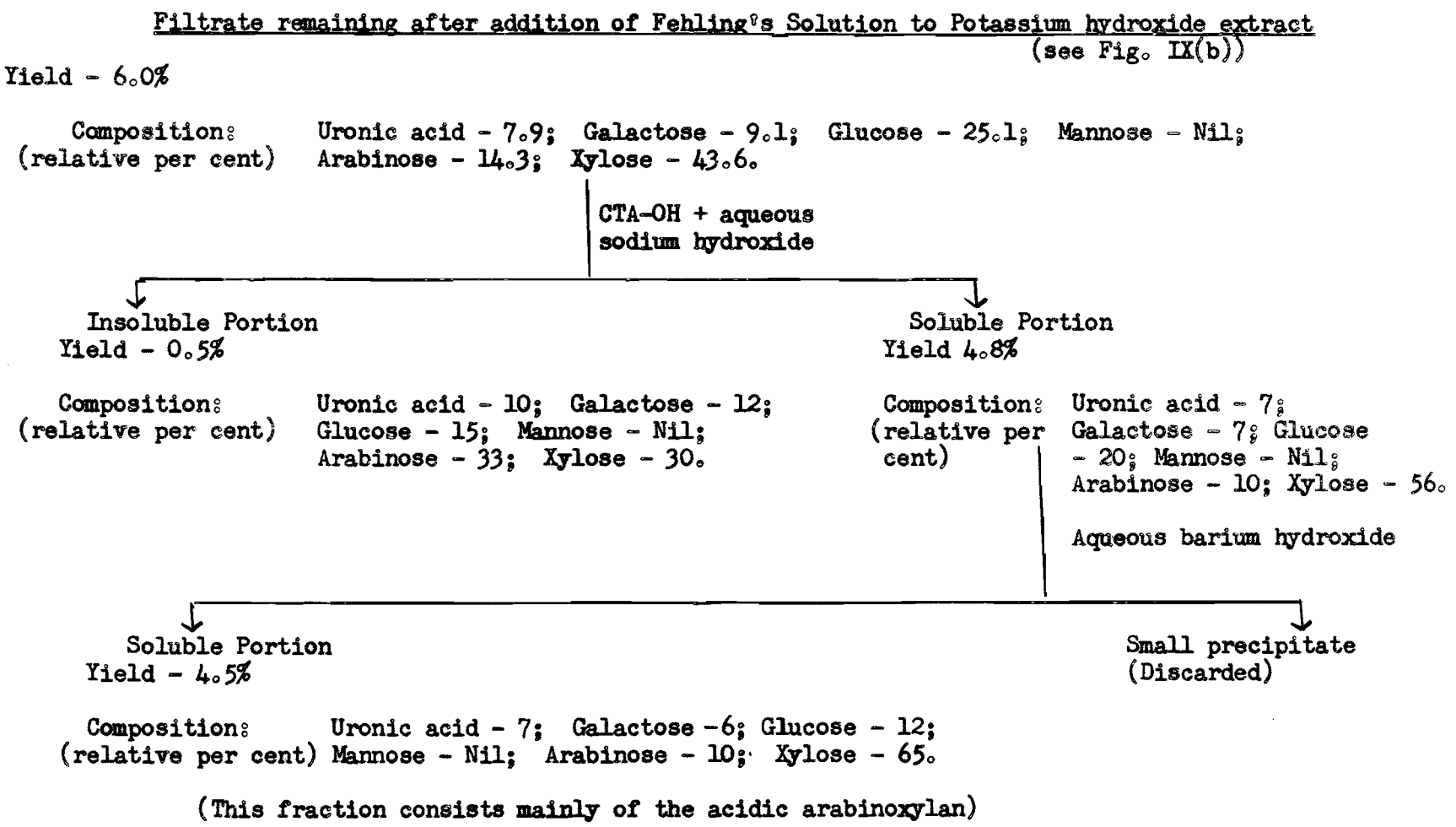
↓ Soluble Portion
Yield - 1.8%

Composition: (relative per cent) Uronic acid - 2; Galactose - 11; Glucose - 57; Mannose - 2; Arabinose - Trace; Xylose - 28.

(This fraction consists mostly of the heteropolymeric "glucan")

→ Filtrate (see Fig. IX(c))

FIG. IX(c) Fractionation Scheme for Extraction of Hemicelluloses from the Bark of Engelmann Spruce: Resolution of 24% Potassium hydroxide Extract (continued)



sodium hydroxide. The xylan was found in the filtrate. The glucose content of this fraction was 20%. Treatment of this fraction with aqueous barium hydroxide reduced the glucose content to 12%.

The glucomannan was obtained from the sodium hydroxide-borate extract by repeated precipitation with aqueous barium hydroxide. The material had the same composition as that obtained earlier in the preliminary studies.

The total quantity of polysaccharides other than cellulose amounted, in the large-scale extraction, to about 28% of the extractive-free bark, compared to 24% in the earlier isolation (see Table 4). A comparison of the yields of various hemicelluloses in the bark and wood of Engelmann spruce and Amabilis fir is shown in Table 19.

The structural features of the glucomannan, the acidic xylan and the heteropolymer "glucan" are discussed in that order in the following.

Table 19 **Hemicelluloses from the** Bark and Wood of Engelmann Spruce and Amabilis Fir

Hemicellulose	Per cent yield[2] of the hemicellulose in			
	Engelmann spruce wood (41)	Engelmann spruce bark[1]	Amabilis fir wood (37)	Amabilis fir bark (34-36)
Arabino-4-O-methyl-glucurono-xylan	8.0	4.5	7.0	2.1
Water-soluble galactoglucomannan	1.8	0.3	4.1	2.8
Alkali-soluble glucomannan	8.1	2.0	8.1	3.0
Heteropolymeric "glucan"	-	1.8	-	-

[2] All yields based on dry extractive-free material

[1] This xylan also contained about 18% of galactose and glucose residues

A. GLUCOMANNAN

The glucomannan, $[\alpha]_D$ - 43°, which was obtained in a yield of 2.0% of the extractive-free bark, contained galactose, glucose and mannose residues in ratio of 1:6.3:17.8 and was chemically homogeneous on fractional precipitation of an alkaline solution with aqueous barium hydroxide (see Table 20). Osmotic pressure measurements on the nitrate derivative (51) gave a number-average degree of polymerization of 86.

Oxidation of the glucomannan with periodate resulted in the consumption of 1.02 moles of the oxidant per anhydro-hexose unit (Table 21), thus suggesting the presence of (1→4)-glycosidic bonds.

A portion of the glucomannan was partially hydrolyzed with aqueous formic acid (52,49). The negative specific rotation of the polysaccharide upon hydrolysis changed to a positive value, indicating that a predominant proportion of its glycosidic bonds were of the β-type. Further confirmation of this came from the paper chromatographic identification of a number of β-(1→4)-linked oligosaccharides in the partial hydrolyzate. The oligosaccharides identified by comparing their chromatographic mobilities with those of authentic specimens, were 4-O-β-mannopyranosyl-D-mannose (mannobiose), 4-O-β-D-mannopyranosyl-D-glucose (mannosyl glucose), 4-O-β-D-glucopyranosyl-D-mannose (glucosyl mannose), 4-O-β-D-glucopyranosyl-D-glucose (cellobiose), O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannose (mannotriose) and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannose (mannotetraose).

Table 20 Fractionation of the Glucomannan with Barium Hydroxide

Fraction	Yield %	Sugar residues in relative per cent					
		Uronic acid	Galactose	Glucose	Mannose	Arabinose	Xylose
I	25	Trace	3.9	24.9	71.2	Nil	Trace
II	40	Trace	3.8	25.3	70.9	Nil	Trace
III	15	Trace	4.1	25.0	70.9	Nil	Trace
IV	8	Trace	5.2	23.2	71.6	Nil	Trace

Table 21 Periodate Oxidation of the Glucomannan

Time in hours	24	48	72	96	120	144	168	216
Periodate consumption moles/anhydro-hexose unit	0.82	0.91	0.99	1.01	1.02	1.02	1.02	1.02

The glucomannan was methylated to completion by the methods of Haworth (53) and Kuhn (54). The methylated polysaccharide had a number-average degree of polymerization of 91. This value was slightly higher than that obtained for the original polysaccharide and suggested that low-molecular weight material was lost during the methylation. The fully methylated product ($[\alpha]_D$ - 20°), after methanolysis and subsequent hydrolysis, gave a mixture of reducing sugars which was resolved by preparative paper chromatography. The relative amounts of the various methylated monomers obtained are shown in Table 22. These sugars were characterized by their rate of movement on the paper chromatogram, by their behavior on paper electrophoresis along with authentic specimens, by the sugar formed on demethylation, by their specific rotation and by the melting point of either the sugar itself or one of its crystalline derivatives.

The large amounts of 2,3,6-tri-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose obtained on methylation and hydrolysis of the methylated polysaccharide indicated that these residues were united in the polymer by (1→4)-glycosidic bonds. The ratio of these two sugars was approximately 1:3 which corresponded to the ratio in which glucose and mannose residues were originally present in the polysaccharide. The negative rotation of the original and methylated polysaccharides indicated that the glycosidic linkages were of β-modification. The nature of the hexose oligosaccharides obtained on partial hydrolysis corroborated these conclusions.

The tetra-O-methylated mannose and glucose sugars undoubtedly

Table 22 Hydrolysis Products of Methylated Glucomannan

Spot number in chromatogram in order of increasing mobility	Component	R_G	Weight mg.	Mole per cent	Ratio by parts
1	2,3-Di-O-methyl-D-mannose 2,6-Di-O-methyl-D-glucose	0.18	15	2.1	1.0
2	2,3-Di-O-methyl-D-glucose	0.24	10	1.4	0.7
3	A Tri-O-methyl-D-galactose	0.32	4	0.5	0.2
4	2,3,6-Tri-O-methyl-D-mannose	0.50	530	68.0	32.4
5	2,3,6-Tri-O-methyl-D-glucose	0.60	180	23.1	11.0
6	2,3,4,6-Tetra-O-methyl-D-galactose	0.78	24	2.8	1.3
7	A mixture of 2,3,4,6-Tetra-O-methyl-D-mannose and 2,3,4,6-Tetra-O-methyl D-glucose	1.00	15	2.1	1.0

originated from the non-reducing end of the polysaccharide. Their ratio, as determined by demethylation was 1:1, which might indicate that half the non-reducing end groups of the polymeric backbone consisted of glucose and the other half of mannose residues. From the last column of results shown in Table 22, it followed that there was one end group for 47.6 glucose and mannose residues. Since the methylated polymer contained on the average 91 such sugar residues, it might be concluded that the average molecule contained approximately one branching point. Although a majority of glucomannans isolated from gymnosperm woods have been reported to be linear, a certain degree of branching has been found to occur in some (55,56).

All the galactose originally present in the polysaccharide appeared as 2,3,4,6-tetra-O-methyl-D-galactose. The very minor amount of a tri-O-methyl-D-galactose detected might very well be an artifact caused by accidental demethylation of the tetra-O-methyl-D-galactose. This suggested that all the galactose residues in the original hemicellulose were present as terminal, non-reducing end groups. If the galactose residues originated from a galactan, methylated galactose other than tetra-O-methyl-D-galactose would have been found in appreciable amounts among the hydrolysis products. Terminal galactose residues could, however, originate from a galactomannan or a galactoglucan in the original hemicellulose, a situation quite unlikely because of the homogeneity of the polysaccharide on fractional precipitation. Since the methylated galactose accounted for approximately 75% of the galactose residues originally present, it was probably an integral part of the polymer. The presence of 2,3- substituted glucose and mannose among the

di-O-methylhexoses made it probable that the galactose residues were attached to these units through (1→6)-galactosidic bonds. Within limits of experimental errors, the relative amounts of the tetra-O-methyl-D-galactose and 2,3-di-O-methylhexoses were similar. The galactosidic linkages were probably of the α-type because such a linkage was found to occur in a glucomannan isolated from Norway spruce by Meier (57). Partial hydrolysis of this glucomannan gave 6-O-α-D-galactopyranosyl-D-mannose and 6-O-α-D-galactopyranosyl-D-mannobiose.

Whether the galactose residues were linked directly to the backbone of the polysaccharide or whether they occurred at the end of side-chains containing β-(1→4)-linked mannose and glucose units, cannot be decided on the basis of available experimental evidence. However, several of the glucomannans from gymnosperm woods have been reported to contain the former arrangement.

Summarizing, the alkali-soluble glucomannan present in the bark of Engelmann spruce consisted of a minimum number of 85 glucose and mannose residues present in a ratio of 1:2.8 and linked together by β-(1→4)-glycosidic bonds to a slightly branched macromolecule. A few of the hexose residues carried (1→6)-linked D-galactosyl units, probably in the α-modification and attached directly to the main chain as non-reducing, terminal groups (see Fig. II). This glucomannan was thus very similar to the corresponding polysaccharides present in the wood of the Engelmann spruce (41) and other gymnosperm species.

B. ARABINO-4-O-METHYLGLUCURONO-XYLAN

The Engelmann spruce xyaln ($[\alpha]_D$- 35°) which amounted to 4.5% of the extractive-free bark, contained residues of uronic acid, galactose, glucose, arabinose and xylose in the ratio 7:6:12:10:65. The acidity of this xylan was found to be due to 4-O-methyl-D-glucuronic acid residues. The xylan isolated by Mills and Timell (41) from Engelmann spruce woood contained residues of uronic acid, arabinose and xylose in a ratio of 12:10:70 and a trace of glucose. It is noteworthy that the acidity of the bark xylan is much less than that of the wood. Thornber and Northcote (40), while comparing the xylose to 4-O-methyl-D-glucuronic acid ratios in xylan preparations from different regions of the tree, observed that the xylan fraction in the phloem of sycamore maple (Acer pseudoplatanus) contained twice the number of 4-O-methyl-D-glucuronic acid residues for a given xylose content compared to the xylan fraction of the xylem, whereas for a pine (Pinus ponderosa) the phleem xylan fraction contained only half as many uronic acid residues as that of the xylem. Earlier, Painter and Purves (25) also observed that the xylan from the inner bark of white spruce contained very few acid groups. Their xylan contained residues of glucose, xylose and arabinose in the ratio 19:77:4, with trace amounts of 4-O-methyl-D-glucuronic acid.

Preliminary investigations on the constitution of this polysaccharide were aimed at the isolation of identifyable oligosaccharides. Partial hydrolysis of the hemicellulose with aqueous formic acid gave a mixture of sugars, which was resolved into a neutral and an acid portion on a column of anion exchange resin. The acid portion was further resolved

by preparative paper chromatography into 9 oligosaccharides. One of them was identified as 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose (A_2). Chromatographic evidence suggested the presence of an aldotrio-(A_3), an aldotetrao-(A_4) and an aldopentaouronic acid (A_5). A_2, A_3, A_4 and A_5 were obtained in yields of 3.5, 2.0, 0.8 and 0.4% respectively based on the hemicellulose. A_2 was characterized by forming the crystalline tetraacetate derivative, methyl 2-O-[methyl (2,3-di-O-acetyl-4-O-methyl-α-D-glucopyranosyl) uronate]-3,4-di-O-acetyl-α,β-D-xylopyranoside (58,59) whose melting point and mixed melting point as well as the specific rotation agreed with those of an authentic specimen. The identification of these oligosaccharides in the partial hydrolyzates of the hemicellulose indicated that 4-O-methyl-D-glucuronic acid residues were integral parts of the xylan molecule.

From the neutral sugar mixture obtained above, four oligosaccharides were isolated by preparative paper chromatography. Due to paucity of material, detailed structural analysis of these oligosaccharides could not be carried out. The component sugars of the oligomers were determined and the results have been summarized in Table 23. Oligosaccharide D was tentatively characterized as β-(1→4)-linked xylobiose by comparing the equilibrium specific rotation and rate of movement on the paper chromatogram in different solvent systems with those of an authentic specimen. Chromatographic examination of oligosaccharides B and C in various solvents indicated the presence of minor amounts of cellobiose in each, in addition to the major oligosaccharide component. It is likely that the glucose residues obtained on hydrolysis of oligosaccharides B and C (see Table 23) originate from the cellobiose, the

Table 23 Neutral Oligosaccharides from Partial hydrolysis of the Xylan

Oligosaccharide	Per cent of the hemicellulose	$R_{Galactose}$	Specific rotation $[\alpha]_D$ degrees	Component sugars on complete hydrolysis and their approximate ratio
A	0.30	0.18	+ 2 (C, 1.4)	Galactose and xylose (1:2)
B	0.40	0.28	+ 1 (C, 1.4)	Galactose, glucose and xylose (4:1:2)
C	0.60	0.50	+ 10.2 (C, 1.6)	Galactose:glucose and xylose (6:1:6)
D	1.00	0.82	- 24 (C, 0.8)	Only xylose

galactose and xylose residues in each originating from the respective major component. There is thus a strong possibility that a chemical linkage exists between galactose and xylose residues in the present hemicellulose. Such a linkage has been shown to be present in a xylan isolated from corn hull (60). Mild acid hydrolysis of this hemicellulose gave in addition to xylobiose, 4-O-β-D-galactopyranosyl-β-D-xylopyranose which was reported to have $[\alpha]_D$ + 15°. The xylobiose isolated in this study probably represented a fragment of the xylan framework. It is noteworthy that in the present case no oligosaccharide containing only xylose and glucose could be isolated, although cellobiose was observed as a minor contaminant of oligosaccharides B and C. It appears very likely, therefore, that glucose residues in the original polysaccharide were not integral parts of the xylan, but probably arose from a contaminating "glucan".

A portion of the hemicellulose was methylated by the methods of Haworth (53) and Kuhn (54). The fully methylated product ($[\alpha]_D$ - 45°) was subjected to methanolysis and the mixture of glycosides was resolved into a neutral and an acid portion on an anion exchange resin. The acid fraction contained only one component, the ester glycoside of which was reduced with lithium aluminium hydride to give crystalline methyl 2-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyl)-3-O-methyl-D-xylopyranoside, identical with respect to melting point and specific rotation to an authentic specimen (59). Chromatographic examination of the hydrolysis products of the methylated disaccharide revealed the presence of 3-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose, both of which were tentatively identified by their relative rates of movement on the paper chromatogram

and sugars formed on demethylation. These results indicated that the original acid in the methanolysis products of the methylated hemicellulose was methyl 2-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyluronic acid)-3-O-methyl-D-xylopyranoside.

The hydrolyzate of the neutral O-methyl glycosides was resolved by preparative paper chromatography. A series of methylated sugars were obtained (see Table 24) which were qualitatively characterized by identifying the sugar(s) formed on demethylation and also by comparing their R_G values with those of authentic specimen in different solvents. Crystalline derivatives could not be prepared because most of them were obtained as mixtures and the rest in insufficient amounts. The two mono-O-methyl xyloses and the di-O-methyl glucose were also characterized by paper electrophoresis, comparing their relative positions in the electrophoretogram with those of authentic specimens.

The principal methylated product was 2,3-di-O-methyl-D-xylose (38%). This, coupled with the low rotation of the original and the methylated hemicellulose, indicated the presence of (1→4)-linked β-D-xylopyranose residues in the polysaccharide. The 2,3,4-tri-O-methyl-D-xylose (7.6%) was derived from the terminal D-xylopyranose units in the polysaccharide. The 2,3,5-tri-O-methyl-L-arabinose (3.0%) was obtained only in small amounts. This was probably due to the loss of this compound during evaporation due to its volatility (61). However, its presence indicated that the polysaccharide contained terminal, non-reducing residues of L-arabinofuranose. By analogy with other softwood xylans investigated thus far, it is likely that these arabinose residues

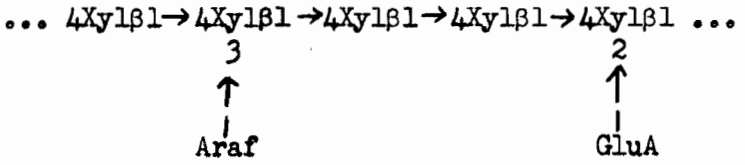
Table 24 Neutral Sugars Obtained on Hydrolysis of the Methylated Xylan

Methylated Sugar	R_G[1]	Relative yield %	Component sugar(s) on demethylation and their approximate ratio	Identity of the methylated sugar
a	0.13	1.2	only galactose	Probably a di-O-methyl D-galactose
b	0.18	5.9	only xylose	2-O-methyl-D-xylose
c	0.21	4.4	only xylose	3-O-methyl-D-xylose
d	0.24	8.8	only glucose	2,3-di-O-methyl-D-glucose
e	0.34	5.9	galactose:glucose 1 : 1	Probably contains 2,3,4-tri-O-methyl-D-galactose
f	0.46	5.9	glucose:arabinose 1 : 1	?
g	0.57	47.2	glucose:xylose 1 : 4	2,3-di-O-methyl-D-xylose and 2,3,6-tri-O-methyl-D-glucose
h	0.78	5.9	galactose	2,3,4,6-tetra-O-methyl-D-galactose
i	1.00	8.8	glucose:xylose 1 : 6	2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose
j	1.09	5.9	arabinose:xylose 1: 1	2,3,5-tri-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose

[1] R_G values refer to 2,3,4,6-tetra-O-methyl-D-glucose

were attached to the main xylan chain by (1→3)-glycosidic bonds. Such a linkage would account for the isolation of 2-O-methyl-D-xylose (5.9%). The earlier characterization of the partly methylated aldobiouronic acid obtained as the only acidic product on methanolysis of the methylated hemicellulose, proved that the 4-O-methyl-D-glucuronic acid residues were linked as single unit side chains to C-2 of the xylose residues in the main chain. In spite of the fact that no acid side groups were removed during methanolysis, some 3-O-methyl-D-xylose (4.4%) was isolated. This might indicate the possibility of branching in the main chain. It is also probable that, since most of the galactose originally present in the polysaccharide appeared as tetra-O-methyl-D-galactose (5.9%, some of the galactose units may be attached to the xylan chain as single unit side chains. The earlier isolation of some oligosaccharides containing galactose and xylose residues from the partial hydrolyzate of the xylan (see Table 23) might offer some justification for this suggestion. The glucose residues present in the original hemicellulose appeared as 2,3-di-O-methyl-D-glucose (8.8%), 2,3,6-tri-O-methyl-D-glucose (9.4%) and 2,3,4,6-tetra-O-methyl-D-glucose (1.3%). This undoubtedly indicated the presence of small amounts of a (1→4)-linked "glucan".

The results obtained indicated that the xylan consisted of a backbone of β-(1→4)-linked xylopyranose residues to which were directly attached (1→2)-linked 4-O-methyl α-D-glucuronic acid residues. Whether the L-arabinofuranose residues were also attached directly to the xylan chain (Fig. Xa) or were linked through interposed xylose residues (Fig. Xb) could not be ascertained on the basis of the available evidence.



Xyl - D-xylopyranose

GluA - 4-O-methyl-D-glucuronic acid

Araf - L-arabinofuranose

FIG. X(a)

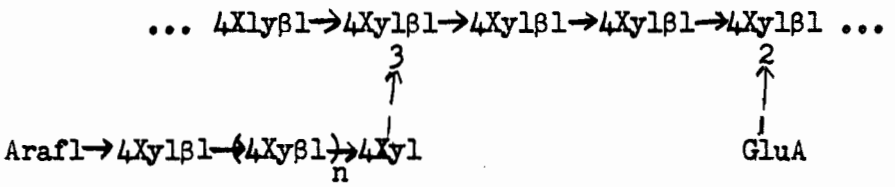


FIG. X(b)

FIGS. X(a) and X(b) - Alternative structures for the xylan

Furthermore, while the glucose residues in the original hemicellulose were not integral parts of the xylan, some of the galactose residues might be attached to the xylan main chain, although the evidence for such a linkage is admittedly insufficient.

C. "GLUCAN"

This polysaccharide ($[\alpha]_D$ + 35°) was isolated in a yield of 1.8% on extractive-free bark and contained residues of uronic acid, galactose, glucose, mannose and xylose in a ratio of 2:11:57:2:28. It was shown to be essentially homogeneous by fractional precipitation and chromatography on diethylaminoethylcellulose phosphate.

The hemicellulose was partially hydrolyzed with aqueous formic acid. The hydrolyzate, on standing overnight, deposited a small residue in a yield of 0.8%. Complete hydrolysis of this material gave mostly glucose with minor amounts of galactose and xylose. No uronic acid was detected. Partial hydrolysis of the material with dilute sulfuric acid yielded, in addition to galactose, glucose and xylose, also three oligosaccharides which were qualitatively identified as cellobiose, cellotriose and cellotetraose by comparing their relative movement on the paper chromatogram in different solvents with those of authentic specimens.

The partial hydrolyzate, after removal of the above residue, was resolved on a column of anion exchange resin into a neutral and an acidic portion. From these fractions four acidic and two neutral oligosaccharides were isolated by preparative paper chromatography.

The first three of the acidic oligosaccharides (see Table 25) could not be resolved on paper chromatography in different solvent systems. The fourth compound (d), however, exhibited heterogeneity in some solvents, the contaminant amounting to about 20% and having a

Table 25 Acidic Oligosaccharides Obtained on Partial Hydrolysis of the "Glucan"

Oligosaccharides	Per cent of the hemicellulose	$R_{Glucuronic}$ acid	Specific rotation $[\alpha]_D$ Degrees	Component sugars on hydrolysis	Component sugars after prior reduction with sodium borohydride
(a)	0.60	0.28	- 11.7 (C, 0.9)	galactose, xylose and glucuronic acid	galactose and glucuronic acid
(b)	0.80	0.41	- 13.2 (C, 1.0)	galactose and glucuronic acid	glucuronic acid
(c)	0.24	0.56	very low (C, 1.2)	galactose and galacturonic acid	-
(d)	0.30	0.81	- 7.0 (C, 0.7)	galactose, xylose, galacturonic acid ? and glucuronic acid	-

slightly lower mobility than the main portion. The component sugars in all these compounds were determined and the reducing end group in some characterized by identifying the sugar components after reduction with sodium borohydride.

The nature of the acidic oligosaccharides (a) and (b) indicated that a chemical linkage existed between galactose and glucuronic acid. It is noteworthy that Bishop and co-workers (62) have isolated the aldobiouronic acid 6-O-(β-D-glucopyranosyluronic acid)-D-galactose from a hydrolyzate of an arabinogalactan from tamarack (Larix laricina). They pointed out that D-glucuronic acid is an integral part of the arabinogalactan. Their value for the specific rotation of the barium salt of the acid (-5°) corresponds fairly well with the specific rotation for the acid itself in the present case, -13°. Such an aldobiouronic acid has also been found in the hydrolyzates of many plant gum exudates (11) as well as in the acid hydrolyzate of maritime pine wood as reported by Roudier and Eberhard (63). They were able to isolate a glucuronoarabinogalactan from the hot water-soluble polysaccharides of that wood by fractionation. As will be seen later, methylation data also pointed to the existence of a linkage between galactose and glucuronic acid in the "glucan". It is very likely, therefore, that oligosaccharide (b) is a glucuronosylgalactose with galactose at the reducing end and (a), an aldotriouronic acid with xylose as the reducing end group. This would, of course, presuppose a linkage between xylose and galactose. Earlier, in connection with the study of the acidic arabinoxylan some evidence was presented, not entirely unequivocal, for the existence of a galactose-xylose linkage.

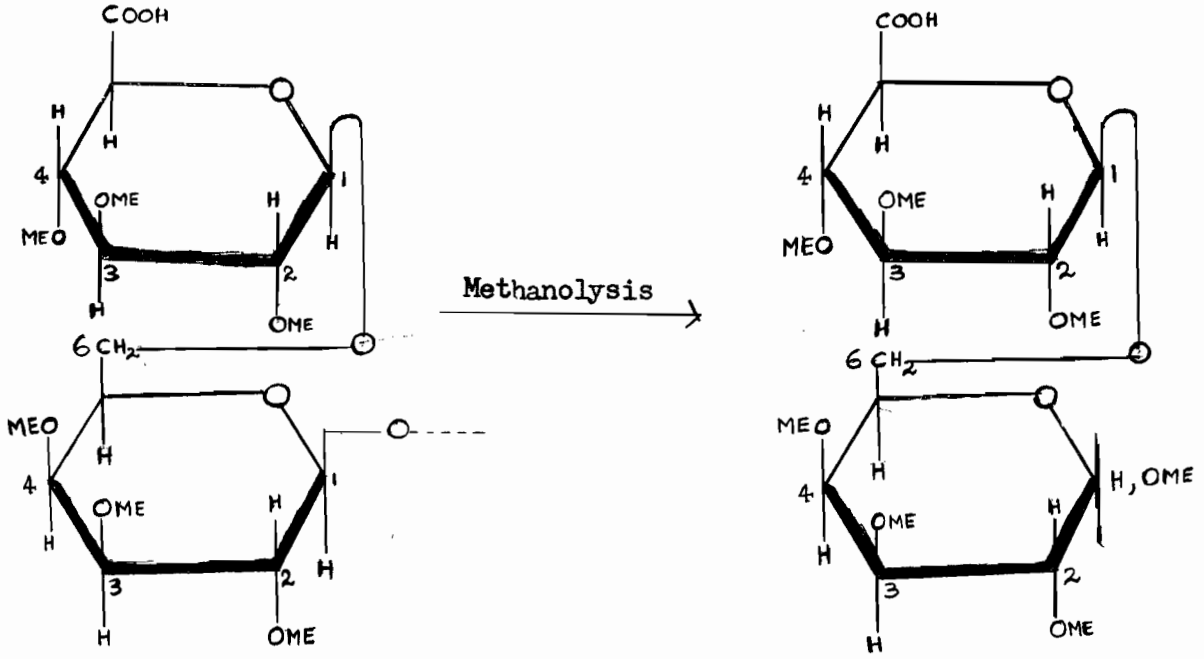
Preliminary examination of the two neutral oligosaccharides indicated the presence of galactose, glucose and mannose residues in one and galactose, glucose and xylose residues in the other (see Table 26). Chromatographic evidence was obtained, however, for the presence of a mannobiose contaminant in oligosaccharide (e). This might suggest the presence of a linkage between galactose and glucose residues. This component was quite probably a trisaccharide because it had a mobility lower than either cellobiose or mannobiose. Oligosaccharide (f), likewise, revealed heterogeneity when examined by paper chromatography. The major component moved slightly faster than mannobiose and the minor one slightly slower than xylobiose. It is indeed very difficult to draw definite conclusions from these observations. It might seem likely that an oligosaccharide (probably a disaccharide) of galactose is present. Meier isolated from tension wood of beech (Fagus silvatica) two disaccharides of galactose (64). One was 6-O-β-D-galactopyranosyl-D-galactose, ($[\alpha]_D$ + 26°) and the other 4-O-β-D-galactopyranosyl-D-galactose ($[\alpha]_D$ + 67°). The present oligosaccharide (f) had a specific rotation of + 19.6° which might suggest that such an oligosaccharide if present, was very likely the (1→6)-linked compound. As will be seen later, methylation data also suggested a (1→6)-linked galactose chain in the polymer.

The hemicellulose was methylated by the methods of Haworth and Kuhn. The methylated product ($[\alpha]_D$ + 15°) was methanolized and the acidic and neutral glycosides were separated in a column of anion exchange resin. The partially methylated acidic component was reduced with lithium aluminum hydride and hydrolyzed. The hydrolyzate, on

Table 26 Neutral Oligosaccharides Obtained on Partial Hydrolysis of the "Glucan"

Oligosaccharide	Per cent of the hemicellulose	$R_{Galactose}$	Specific rotation $[\alpha]_D$ degrees	Component sugars on hydrolysis and their approximate ratio
(e)	1.1	0.32	+ 6.2 (C, 1.2)	galactose:glucose:mannose 1 : 2 : 4
(f)	1.3	0.53	+ 19.6 (C, 1.0)	galactose:glucose:xylose 8 : 1 : 1

examination by paper chromatography in various solvents, revealed the presence of 3-O-methyl-D-xylose (7%), 2,3-di-O-methyl-D-glucose (14%), 2,3,4-tri-O-methyl-D-galactose (27%) and 2,3,4-tri-O-methyl-D-glucose (52%) (see Table 27). It was obvious from this that the partially methylated acidic component was a mixture of two or more compounds. The tentative identification of 2,3,4-tri-O-methyl-D-glucose indicated that glucuronic acid was the acid moiety in the partially methylated aldobiouronic acid mixture. This, coupled with the probable presence of 2,3,4-tri-O-methyl-D-galactose in the hydrolyzate, might suggest the existence in the mixture of a methylated glucuronosyl galactose linked (1→6)- as shown in Fig. XI. Such a compound could probably be derived from a galactan chain carrying terminal glucuronic acid group.



Methylated galactan chain (Partial structure)

Methylated glucuronosyl-(1→6)-D-galactose (methyl glycoside)

FIG. XI

Table 27 Partially Methylated Acidic Components from the Methylated "Glucan" - Sugar Composition After Reduction with Lithium Aluminum Hydride

Methylated sugar	Approximate relative amounts %	R_G1 (solvent F)	R_G (solvent G)	Sugars formed on demethylation	Probable identity of the sugar
(g)	7	0.21	-	xylose	3-O-methyl-D-xylose
(h)	14	0.25	0.65	glucose	2,3,Di-O-Methyl-D-glucose
(i)	27	0.34	0.72	galactose	2,3,4-Tri-O-methyl-D-galactose
(j)	52	0.68	0.85	glucose	2,3,4-Tri-O-methyl-D-glucose

1 R_G values refer to 2,3,4,6-tetra-methyl-D-glucose

As will be seen later, some evidence was obtained for the existence of a (1→6)-linked galactose chain in the "glucan". It was noted earlier that partial hydrolysis of the "glucan" gave rise to an acid oligosaccharide containing galactose and glucuronic acid (see Table 25) and a neutral oligosaccharide containing mainly galactose (see Table 26). The occurrence of 3-O-methyl-D-xylose and 2,3-di-O-methyl-D-glucose in the hydrolyzates, might suggest that some of the glucuronic acid residues were attached to xylose and glucose units. Earlier it was observed that partial hydrolysis of the "glucan" gave rise to an acidic oligosaccharide containing galactose, xylose and glucuronic acid (Table 25). However, no oligosaccharide containing glucose and glucuronic acid was detected. The presence of 2,3-di-O-methyl-D-glucose in the hydrolysis product of the partially methylated oligosaccharide mixture cannot therefore be satisfactorily explained.

The neutral O-methylated glycosides were hydrolyzed to the corresponding mixture of methylated sugars which was resolved by preparative paper chromatography (Table 28). Some of them were tentatively identified from their R_G values and the sugars obtained on demethylation, while others were also characterized through their crystalline derivatives.

The preponderance of 2,3-di-O-methyl-D-glucose (33.3%) suggested that a (1→4)-linked glucose chain was present which possessed branch points at C-6 of the glucose residues. The 2,3,6-tri-O-methyl-D-glucose (17.1%) undoubtedly arose from (1→4)-linked glucose units which had no branch point and the 2,3,4,6-tetra-O-methyl-D-glucose (2.4%) was derived from the terminal, non-reducing glucose residues in the chain. Except

Table 28 Neutral Methylated Sugars from the "Glucan"

Methylated sugar	R_G[1]	Relative yield %	Component sugar(s) on demethylation and their approximate ratio	Identity of the methylated sugar
a	0.13	1.4	Galactose	Probably a di-O-methyl-D-galactose
b	0.18	1.9	Glucose:Mannose 1 : 1	Probably 2,6-di-O-methyl-D-glucose and 2,3-di-O-methyl-D-mannose
c	0.24	33.3	Glucose	2,3-di-O-methyl-D-glucose
d	0.33	9.5	Galactose	2,3,4-tri-O-methyl-D-galactose
e	0.47	3.3	Glucose:Mannose 2 : 1	?
f	0.58	34.2	Glucose:Xylose 1 : 1	2,3,6-tri-O-methyl-D-glucose and 2,3-di-O-methyl-D-xylose
g	0.77	12.8	Galactose	2,3,4,6-tetra-O-methyl-D-galactose
h	1.00	3.6	Glucose:Xylose 2 : 1	2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose

[1] R_G value refers to 2,3,4,6-tetra-O-methyl-D-glucose

for a small amount of di-O-methyl-D-galactose (1.4%), all the galactose originally present appeared as 2,3,4-tri-O-methyl-D-galactose (9.5%) and 2,3,4,6-tetra-O-methyl-D-galactose (12.8%). The tetra-O-methyl-D-galactose unquestionably arose from terminal, non-reducing galactose units, while the identification of 2,3,4-tri-O-methyl-D-galactose suggested the presence of a (1→6)-linked galactose chain. Earlier some evidence was presented (see Table 26) for the occurrence of a galactobiose, probably 6-O-β-D-galactopyranosyl-D-galactose in the partial hydrolyzate of the "glucan". This might corroborate the presence of a (1→6)-linked galactose chain. The isolation of glucuronosyl galactose from the partial hydrolyzate of the "glucan" (see Table 25) and of the partially methylated glucuronosyl-(1→6)-D-galactose from the methanolysis product of the methylated "glucan" (See Fig. XI) suggested that the galactose chain was terminated by a glucuronic acid group. The 2,3,4,6-tetra-O-methyl-D-galactose, therefore, very probably arose from single unit side chains of galactose attached to a main backbone of glucose units or from terminal, non-reducing galactose residues of the (1→6)-linked galactose chains, which were devoid of glucuronic acid substituents. The formation of large amounts of 2,3-di-O-methyl-D-glucose, however, suggested that the former possibility is more likely. Earlier some meagre evidence was obtained for the presence of an oligosaccharide containing glucose and galactose (see Table 26). This could happen if there were terminal galactose residues attached to a main chain of glucose.

The xylose residues in the original polysaccharide appeared as 2,3-di-O-methyl-D-xylose (17.1%) and 2,3,4-tri-O-methyl-D-xylose (1.2%). The preponderance of 2,3-di-O-methyl-D-xylose suggested the presence of

a (1→4)-linked xylose chain, with the 2,3,4-tri-O-methyl-D-xylose arising from terminal xylose units. It is noteworthy that no mono-O-methylxyloses were detected. Whether or not there was a linkage between glucose and xylose cannot be ascertained from the available evidence. Such a linkage has previously been reported by Perila and Bishop (65). Enzymic hydrolysis of a Jack pine glucomannan gave two oligosaccharides in low yields. One was 6-O-(α-D-xylopyranosyl)-D-glucopyranose and the other O-α-D-xylopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-D-glucopyranose. They concluded that the xylose units might have been attached as single unit side chains to a glucose-containing polysaccharide, which could be either a xyloglucan or a xyloglucomannan. Later Adams (66) isolated 2,3,4-tri-O-methyl-D-xylose among the products of hydrolysis of a methylated glucomannan from sugar maple (Acer saccharum), indicating that D-xylose was an integral part of the glucomannan. It must be pointed out that several workers have observed the presence of trace amounts of xylose in glucomannans. This has usually been attributed to the presence of small amounts of the acidic xylans which occur in these woods and which had not been completely removed by the fractionation procedure used. It is therefore very likely that some of the xylose units in the present polysaccharide were attached to the main glucose chain by (1→6)-linkages. The identification of 2,3-di-O-methyl-D-xylose might, therefore, indicate that either a separate (1→4)-linked xylan macromolecule was present or that such a chain could be present as a long branch attached to the main glucose chain. It must be pointed out that partial hydrolysis of the "glucan" failed to yield any oligosaccharide containing xylose and glucose. Also, no

xylobiose was detected in the partial hydrolyzate of the "glucan", as was observed in the case of the arabino-4-O-methylglucuronoxylan.

Summarizing, it can be said that the "glucan" apparently consisted of a backbone of (1→4)-linked glucose units, which possessed branch points at C-6. Some of the galactose residues in the original hemicellulose were probably attached to the main chain as single unit side chains. The rest were present as (1→6)-linked galactan, the terminal unit of which was attached to a glucuronic acid substituent at C-6. Whether or not this galactan chain is an integral part of the main glucose chain is not certain. The xylose residues existed as a (1→4)-linked macromolecule which may or may not be attached to the (1→4)-linked glucose chain. The fractionation results, however, pointed to the heteropolymeric nature of the "glucan".

It appears from this study that the spectrum of glucose-containing polysaccharides in lignified materials, other than cellulose and starch, is much broader than the one encompassing the various glucomannans and galactoglucomannans. The "glucan" obtained from the bark of Engelmann spruce represents a type of hemicellulose which has not been isolated from woody tissues investigated so far. As pointed out earlier, Painter and Purves (25) suggested the possibility that the inner bark of white spruce might contain a xyloglucan. Later Thornber and Northcote (38-40) obtained evidence for the presence of glucose-based polysaccharides in the bark of gymnosperms.

A polysaccharide containing glucose and xylose in a ratio 1:36

has been isolated from the pith of Kerria japonica (67). Fractionation studies on this polymer revealed that the polysaccharide was homogeneous and that glucose did not originate from an impurity. Aspinall and co-workers (68) isolated a β(1→4)-linked glucan along with a glucomannan from Sitka spruce wood. They suggested the possibility that the glucan was a degraded cellulose, differing from normal cellulose only in molecular size. Morak and Ward Jr. (69), in an investigation of hemicelluloses from spruce wood and pulps therefrom, obtained some fractions which were rich in glucose. They suggested that these residues probably arose from a degraded cellulose. Alternately, they considered the possibility that a glucose-based hemicellulose was present in the wood holocellulose. Hamilton and Thompson (70), isolated from a neutral sulfite semi-chemical pulp of western red alder, a hemicellulose fraction containing galactose, glucose and xylose. They obtained no indication, however, that this polymer was a galactan, a glucogalactan or possibly some polymer incorporating xylose. Apart from these isolated investigations, other glucose-based polysaccharides referred to in literature are the water-soluble cereal gums from barley and oats, which contain as major components β-glucans, possessing both (1→4)- and (1→3)-linkages (9). Callose, which is a polysaccharide accumulating on the sieve tubes of wood barks has been shown to be a β-(1→3)-linked glucan (71). The amount of callose in bark is not very large, however, and especially not so in a spring-felled tree such as was used in the present investigation, since the polysaccharide appears to be enzymatically removed at the beginning of each growing season (72).

EXPERIMENTAL

All evaporations were carried out under diminished pressure at a bath temperature of 40°C or less. The specific rotations were equilibrium values measured at 23° ± 3°C at 546 and 578 mμ using a Zeiss photoelectric polarimeter and referred to 589 mμ (sodium-D-line) with the aid of the Drude equation. Unless otherwise stated water was used as the solvent. All melting points were corrected.

Paper Chromatography

Chromatographic separations were carried out at room temperature by the descending technique on Whatman No. 1 and No. 3MM paper, the latter being also used for preparative purposes. The following solvent systems (v/v) were employed

(A) ethylacetate:pyridine:water (8:2:1)

(B) ethylacetate:acetic acid:water (9:2:2)

(C) ethylacetate:acetic acid:water (18:7:8)

(D) ethylacetate:acetic acid:formic acid:water (18:3:1:4)

(E) n-butanol:pyridine:water (10:3:3)

(F) butanone:water:ammonia (90:8:2)
(the mixture was saturated for 24 hours at 4°C and the organic layer was used)

(G) n-butanol:ethanol:water (4:1:5) upper layer

Electrophoresis

Electrophoresis was carried out in a 0.05M borate buffer (pH 9.5) at 700 volts using Whatman No. 3MM paper.

Sugars were located on the chromatograms and electrophoretograms

by spraying with a solution of O-aminodiphenyl in glacial acetic acid (73).

Hydrolysis of the Polysaccharide

Hydrolysis of all polysaccharide was carried out by the method of Saeman and co-workers (74). The polysaccharide when available only in small amounts was treated with enough 72% sulfuric acid to form a good paste. It was heated at 30°C for a time sufficient to obtain a more or less clear solution. After dilution with about 20 times the volume with water it was autoclaved (at 15 lb. pressure) for 75 minutes. After neutralization with barium carbonate and treatment of the filtrate with Amberlite IR-120 exchange resin, the solution was concentrated and stored in the cold.

Quantitative Sugar Estimation

Polysaccharide hydrolyzates were resolved by paper chromatography in solvent A. After elution with appropriate amounts of water, the various reducing sugars were estimated by the spectrophotometric method of Timell, Glaudemans and Currie (73).

Preparation of the Bark

Bark from a spring-felled tree of Engelmann spruce (Picea engelmannii) was kindly supplied by Columbia Cellulose Company, Ltd., Prince Rupert, B.C. The fresh and still wet bark was kept immersed in boiling ethanol for one hour. After drying in the air, the bark was manually freed from the remaining xylem and cambium. It was subsequently broken up to small pieces and converted to sawdust in a Wiley mill, the 20-80 mesh fraction being collected.

Extractive-free Bark

The bark was exhaustively extracted in a Soxhlet apparatus with the azeotrope of ethanol-benzene (1:2 v/v) and then air-dried. The extractive-free bark had a moisture content of 11.0% and a composition as given in Table 29. Lignin, ash and pentosan contents were determined by standard Tappi procedures for wood (75-77). Uronic anhydride was determined by the method of Browning (78) and O-acetyl by Timell's modification (79) of the procedure of Whistler and Jeanes (80). It is to be noted that the pentosan content is higher than the total of the arabinose and xylose. This is because the xylose occurring as unhydrolyzed methylglucuronosyl xylose in the hydrolyzates, was not accounted for in the sugar analyses.

Preparation of the Holocellulose

The holocellulose was prepared by the chlorite method of Wise and co-workers (81). Extractive-free bark (1150 g. on a dry basis) was suspended in water (18 litres) at 75-80°C. Glacial acetic acid (120 ml.) was added followed by sodium chlorite (360 g.) which was added portion-wise in a period of 1 hour with vigorous stirring of the suspension. Fresh reagents were added at hourly intervals and the process was repeated three times. The material was washed with water by decantation several times, after which the oxidation was repeated twice as before. The white holocellulose was washed first by decantation and then on a Büchner funnel with distilled water and ethanol and was finally air-dried. Yield, 575 g.; 50% of the dry extractive-free bark.

Table 29 Chemical Composition of the Extractive-free Bark

Component	Per cent of extractive-free bark
Summative data	
"Lignin"	39.4
Ash	4.0
Acetyl	0.5
Uronic anhydride	8.0
Residues of:	
Galactose	2.4
Glucose	35.7
Mannose	2.9
Arabinose	3.3
Xylose	3.8
Other data	
Pentosan	11.3
Material soluble in cold water	9.0
Material soluble in hot water	20.3
Material soluble in 1 per cent sodium hydroxide	51.6

Isolation of Pectic and other Water-soluble Materials

The bark holocellulose (565 g. moisture 11.6%) was suspended in 18 litres of an aqueous solution of ammonium oxalate (0.5%) at a temperature of 70-75°C. and stirred vigorously for 16 hours at that temperature. After allowing it to stand overnight the suspension was filtered through a large Büchner funnel and washed with hot water until the washings were colorless. The combined filtrate and washings were evaporated to a small volume and precipitated in four volumes of ethanol. The precipitate was washed successively with 70% ethanol, absolute ethanol and petroleum ether and dried *in vacuo*. Yield 128 g., 12.5% of the dry extractive-free bark.

Isolation of Crude Hemicelluloses

The residue from above was mechanically shaken with 5.5 litres of 10% (w/w) aqueous sodium carbonate at room temperature for 16 hours in an atmosphere of nitrogen. The resulting slurry was filtered through sintered glass (Pyrex coarse) and washed a few times with distilled water. The combined filtrate and washings(8 litres) were neutralized carefully with glacial acetic acid and then precipitated in three volumes of ethanol. After allowing the precipitate to settle overnight, it was filtered and recovered by solvent-exchange as before. Yield, 45.0 g., 4.3% of the dry extractive-free bark.

The residue from the above extraction was suspended in 5 litres of aqueous potassium hydroxide (24% w/w) and mechanically shaken as before. The alkaline solution was filtered and the solid residue washed with distilled water. The combined filtrate and washings (6 litres) were poured

into four volumes ethanol containing glacial acetic acid (3 litres). The recovered product weighed 87 g., 8.5% of the dry extractive-free bark.

The remaining solid material was extracted in the same manner with 17.5% (w/w) aqueous sodium hydroxide containing 4% borate. The recovered product weighed 26.0 g. Yield, 2.5% of the dry extractive-free bark.

The solid residue was acidified with dilute acetic acid, washed with water and ethanol and dried in the air. Yield, 312 g., 30.3% of the extractive-free bark.

Fractionation of the Crude Potassium Hydroxide Extract

Hydrolysis with α-amylase - The potassium hydroxide extract (500 mg.) was dissolved in water (100 ml.) and α-amylase (500 mg.) was added to the solution. After mechanically shaking the solution for 24 hours at room temperature, the contents were poured into acetone:ethanol (1:1) and the precipitated polysaccharide was recovered by filtration. It was then hydrolyzed with sulfuric acid (74) and quantitatively analysed for the reducing sugars (73). There was no change in the composition of the polysaccharide. A sample of water-soluble starch after a similar treatment, gave no precipitate on addition of the aqueous solution to acetone-ethanol and no coloration with iodine.

Chromatography on Diethylaminoethylcellulose - Diethylaminoethylcellulose was first digested with 0.5N hydrochloric acid. After centrifugation, the residue was suspended in 0.5N sodium hydroxide. This alternate

treatment with acid and alkali was repeated three times after which the cellulose derivative was washed with distilled water several times to remove the alkali. It was deaerated by maintaining it in vacuum overnight and subsequently immersed in 0.5M sodium dihydrogen phosphate (PH 4.3). The pH of the solution was adjusted to 5.5 by addition of disodium hydrogen phosphate. It was again deaerated overnight, after which the supernatant solution was decanted, and the residue was washed with distilled water until the washings were free of phosphate ions.

The diethylaminoethylcellulose (Phosphate form) as prepared above was packed in a column (2 1/2 cm. x 40 cm.). The polysaccharide to be chromatographed (200-500 mg.) was dissolved in a small amount of water and placed on top of the column. It was successively irrigated with water, 0.05M sodium dihydrogen phosphate (PH 5.5), 0.5M sodium dihydrogen phosphate (PH 5.5) and 0.1N sodium hydroxide. The fractions were collected in 20 ml. portions with the rate of flow maintained between 0.8 to 1 ml. per minute.

The chromatographic separation was followed by examination of each test tube for the presence of polysaccharides by hydrolyzing the content of the tube (1 ml.) with concentrated sulfuric acid (5 ml.) in the presence of phenol (1 ml.) (82). The orange-yellow color developed was estimated spectrophotometrically by measuring its absorbance at 480 mμ. A plot of the absorbance against the test tube number gave fractionation curves as presented in Figs. V, VI, VII and VIII.

The various fractions were recovered by collecting the contents of the test tubes that fell in the corresponding peak and evaporating

them under reduced pressure. The fractions eluted with aqueous phosphate and sodium hydroxide were also de-ionized with Amberlite IR-120 (H^+ form) and IR-4B (OH^- form).

Fractional Precipitation - To a 1% aqueous solution of the polysaccharide, ethanol was added dropwise from a burette, the solution being maintained at 25 ± 1°C. At the sign of turbidity, the addition of ethanol was stopped, the solution cooled to + 5°C and the precipitated polysaccharide recovered by ultracentrifugation at 19,000 r.p.m. The process was repeated to obtain as many fractions as was possible. A similar fractionation procedure was also employed for testing the homogeneity of some hemicellulose preparations.

Acetylation - The polysaccharide (2 g.) was dissolved in dry formamide (50 ml.) freshly distilled *in vacuo*. Pyridine (125 ml.), freshly distilled over barium oxide, was added portion-wise in half an hour followed by acetic anhydride (20 ml.). The mixture was cooled under tap water. The solution was mechanically shaken overnight, after which another 20 ml. of acetic anhydride was added. A total of three such additions was made, and after allowing for a reaction time of 2 days, the mixture was poured with vigorous stirring into 2 litres of ice-cold water containing 2% hydrochloric acid. The liquids were removed by filtration through a Büchner funnel and the residue was washed with ice-cold water until neutral. It was successively washed with methanol and petroleum ether and dried *in vacuo*. Yield, 2.7 g. (82%), acetyl, 38.2%.

Isolation of the Glucose-Rich Hemicellulose ("Glucan")

The crude potassium hydroxide extract (230 g.) was dissolved in water (4.5 litres). A small amount (3 g.) of insoluble material was removed by centrifugation. The clear, dark brown solution was treated with Fehling's solution (2600 ml.) whereupon the copper complex precipitated. It was allowed to settle and the supernatant, clear liquid was decanted off. The remaining liquid was removed on the centrifuge. The residue was washed sparingly with water. The combined centrifugate and washings were used for the recovery of the xylan (see later).

The residue was dissolved in a minimum amount of ice-cold hydrochloric acid (1.5N) and the solution was poured into four volumes of ethanol to give a white flocculent precipitate. It was washed on a Büchner funnel with slightly acidified acetone:water (60:40) to remove copper completely and then with the solvent mixture for removal of the acid and finally with ethanol and petroleum ether. The dried product weighed 61 g. It was further purified by two more successive precipitations with Fehling's solution as before to yield a very white powder (41.0 g.), having the sugar composition given in Fig. IX(b).

Purification of the Copper Complex

In order to eliminate or reduce the mannose content in this fraction, it was subjected to a purification treatment with aqueous barium hydroxide. The polysaccharide (40 g.) was dissolved in 20% (w/w) potassium hydroxide (400 ml.). Water (400 ml.) was added and the solution was agitated with 5% aqueous barium hydroxide (2 litres) which was added slowly over a period of 2 1/2 hours. The precipitate formed was washed

on the centrifuge twice each with 5% sodium hydroxide and distilled water. It was then dissolved in the minimum amount of ice-cold 50% acetic acid. Addition of this solution to four volumes of ethanol gave a precipitate which was recovered by solvent-exchange in the usual way. Yield, 6.0 g. This was evidently a galactoglucomannan. The centrifugate and washings were combined and neutralized with ice-cold 50% acetic acid. The solution was concentrated and precipitated into ethanol. The recovered material (32 g.) had the composition given in Fig. IX(b) and was the "glucan" portion of the potassium hydroxide extract; $[\alpha]_D$ + 35° (C, 1.3).

Isolation of the Xylan

The xylan was recovered from the centrifugate and washings obtained on the first treatment of the potassium hydroxide extract with Fehling's solution. The soluble copper complex was added to four volumes of ethanol. The blue precipitate formed was allowed to settle. The supernatant liquid was decanted off and the residue was washed a few times with ethanol. It was then dissolved in ice-cold hydrochloric acid (1.5N) and the solution was poured into ethanol. The white precipitate obtained weighed 160 g. and had the sugar composition shown in Fig. IX(c).

Purification of the Xylan

The hemicellulose obtained above (50 g.) was dissolved in water (750 ml.). Cetyltrimethylammonium hydroxide (0.1N, 500 ml.), prepared by passing a 3.7% solution of the corresponding bromide ("cetavlon") through a column of Amberlite IR-45 (OH^- form) exchange resin, was gradually added to the solution with stirring. No significant precipitation occurred until after the addition of 1N sodium hydroxide (150 ml.). The sticky precipitate

then formed was recovered by ultracentrifugation at 19,000 r.p.m. and washed twice on the centrifuge with distilled water. The precipitate was dissolved in a minimum amount of ice-cold 6% acetic acid and poured into four volumes of ethanol. The recovered white substance weighed 4.1 g. and had the composition given in Fig. IX(c).

The combined centrifugate and washings were de-ionized with Amberlite IR-120 (H^+ form) exchange resin and precipitated into ethanol; yield 40 g. The polysaccharide had the composition given in Fig. IX(c). When this material was treated with aqueous barium hydroxide, a small amount of precipitate was formed which was discarded. The fraction recovered from the soluble portion was obtained in a yield of 37.5 g. and contained less amounts of glucose residues than before. The composition of this xylan fraction is given in Fig. IX(c); $[\alpha]_D$ - 35° (C, 1.4).

Isolation of the Glucomannan

The sodium hydroxide-boric acid extract (45 g.) was dissolved in 10% aqueous sodium hydroxide (900 ml.) and precipitated with 5% barium hydroxide solution (2.5 litres). The precipitate was recovered as described earlier. Two more precipitations on this material with aqueous barium hydroxide yielded a white powder. Yield = 35.5 g. The composition is given in Fig. IX(a); $[\alpha]_D$ - 43° (C, 1.0 in 10% NaOH).

GLUCOMANNAN

Partial Hydrolysis of the Glucomannan and Qualitative Identification of the Oligosaccharides

The glucomannan (1 g.) was dissolved in 90% formic acid (10 ml.) and diluted with water (10 ml.). The solution ($[\alpha]_D$ - 28.1°) was heated

in a boiling water bath (98°C) for 3.5 hours at the end of which time the specific rotation was + 19°. The formic acid was removed by repeated evaporations from water, after which the residue was boiled under reflux with 0.5N sulfuric acid (40 ml.) for 5-6 minutes for elimination of formate esters. The solution was neutralized with barium carbonate, filtered through Celite, de-ionized with Amberlite IR-120, filtered again and evaporated to yield a syrup (0.7 g.).

Chromatographic examination of the syrup in solvents A, B and E revealed the presence of six oligosaccharides in addition to galactose, glucose, mannose and a small amount of xylose. The oligosaccharides were separated on Whatman No. 3MM filter paper, using solvent E. Each compound was tentatively identified by comparing its mobility on the chromatogram (solvents A and E) with authentic specimens of oligosaccharides. The sugars identified were β(1→4)-linked mannobiose, mannosyl glucose, glucosyl-mannose, cellobiose, mannotriose and mannotetraose.

Methylation of the Glucomannan

The glucomannan (16 g.) was dissolved with stirring in 35% (w/w) sodium hydroxide (400 ml.) in an atmosphere of nitrogrn. Dimethyl sulfate (200 ml.) was added slowly over a period of 8 hours with constant stirring. the temperature was maintained at about 10°C. The addition of sodium hydroxide and dimethyl sulfate was repeated three times. Each time solid sodium hydroxide (200 g.), and dimethyl sulfate (400 ml.) was added. Good stirring throughout was maintained by adding enough water. After completed reaction, the mixture was carefully neutralized with concentrated sulfuric acid to pH 7 and then 50% acetic acid was added to bring the pH to about 4.

The solution was heated to boiling when the methylated product separated as a cake at the surface of the solution. It was recovered by filtration through a Büchner funnel, washed with a little hot water, and dried *in vacuo*. The combined filtrate and washings were extracted with chloroform. The chloroform extract was evaporated and the small residue obtained was added to the main portion. The total material was subsequently dissolved in chloroform, dried over anhydrous sodium sulfate, evaporated and dried over phosphorus pentoxide *in vacuo*. Yield 18.0 g. The partially methylated polysaccharide was dissolved in 200 ml. of dimethyl formamide (dried over barium oxide and distilled shortly before use). The solution was shaken in the dark for 24 hours with 75 g. of silver oxide (freshly prepared) and methyl iodide (75 ml.). Two more similar additions of silver oxide and methyl iodide were made, the solution being shaken for 24 hours each time. A semi-solid, grayish mass was obtained. This was centrifuged, and the residue was washed on the centrifuge with chloroform (4 x 300 ml). At this stage, the chloroform solution deposited a small amount of a crystalline precipitate, presumably an addition product of silver oxide-silver iodide-dimethylformamide, which was removed by filtration. The chloroform solution was washed with 5% potassium cyanide (2 x 300 ml.) and water (2 x 500 ml.) and dried over anhydrous sodium sulfate. The solution was filtered through folded paper and evaporated to about 200 ml. This was poured very slowly into vigorously agitated petroleum ether (1600 ml.) to give a pale yellow, fibrous precipitate. This was washed on the filter with petroleum ether and dried *in vacuo* over phosphorus pentoxide to yield a pale yellow powder (11 g.). OMe, calculated for a fully methylated hexosan 45.6%; Found: OMe, 43.6%. The infra-red spectrum of the methylated product (KBr pellet) showed only a

very slight band in the region of hydroxyl absorption; $[\alpha]_D$ - 20° (C, 2.6 in $CHCl_3$).

Methanolysis of the Methylated Glucomannan

The methylated glucomannan (4 g.) was refluxed with 0.7N anhydrous methanolic hydrogen chloride (120 ml.) for 8 hours. The solution was neutralized with silver carbonate, filtered through Celite, treated with hydrogen sulfide, again filtered through Celite and evaporated to dryness. The product was dissolved in 1N sulfuric acid (120 ml.) and boiled under reflux for 8 hours. The acid was neutralized with barium carbonate, filtered through Celite, washed with much distilled water (negative Molisch test), concentrated to a small volume, de-ionized with Amberlite IR-120, filtered, and again evaporated to give a pale yellow syrup. Yield, 3.6 g.

Separation of the Methylated Sugars

The syrupy mixture of methylated sugars revealed the presence of seven components on paper chromatography in solvent F. A portion of the mixture was spotted on 38 sheets (18 cm. wide) of Whatman No. 3MM filter paper, provided with a wick of Whatman No. 50 paper (16). The various fractions were eluted from the chromatograms with water, evaporated to a small volume and treated with a mixture of Amberlite IR-120 and IR-45 exchange resins and Darco G-60 charcoal. The solids were removed by filtration (Whatman No. 5 filter paper) and washed with alcohol:water (1:1) until the washings gave a negative Molisch test. After evaporation to dryness, the solid residue was extracted with ethyl acetate and filtered. Evaporation to dryness gave clear pale yellow syrups which were dried in

high vacuum over phosphorus pentoxide.

Identification of the Methylated Sugars

2,3,Di-O-methyl-D-mannose and 2,6-Di-O-methyl-D-glucose - These sugars were obtained as a syrupy mixture $[\alpha]_D$ - 6.3 (C, 1.5). Paper electrophoresis of the syrup gave two spots which had the same positions on the electrophoretogram as authentic specimens of 2,3-di-O-methyl-D-mannose and 2,6-di-O-methyl-D-glucose. Demethylation of thesyrup with borontrichloride (83) gave mannose and glucose in an approximate ratio of 4:1.

2,3-Di-O-methyl-D-glucose - This sugar was obtained as a syrup $[\alpha]_D$ + 43.1° (C, 1.0). It was chromatographically indistinguishable (solvent F) from an authentic specimen of 2,3-di-O-methyl-D-glucose. Paper electrophoresis also indicated their identical nature. Demethylation gave only glucose.

A Tri-O-methyl-D-galactose -The syrupy sugar $[\alpha]_D$ + 30° (C, 0.4) obtained in very small amounts, gave only galactose on demethylation. Its R_G value in solvent F was 0.32 which was much lower than that of 0.49 for an authentic specimen of 2,3,6-tri-O-methyl-D-galactose (84). It might very likely be 2,3,4-tri-O-methyl-D-galactose formed by accidental demethylation of the fully methylated product.

2,3,6-Tri-O-methyl-D-mannose - This syrup had $[\alpha]_D$ - 9° (C, 2.4) and gave only mannose on demethylation. The 1,4-di-p-nitrobenzoate derivative, prepared according to the method of Rebers and Smith (85) had m.p. and mixed m.p. 188-189°C and $[\alpha]_D$ + 32° (C, 0.8 in chloroform) after recrystallization from methanol.

2,3,6-Tri-O-methyl-D-glucose - The syrupy sugar gave only glucose on demethylation. It was crystallized from ethyl ether solution. After one recrystallization, the sugar had m.p. and mixed m.p. 119-120°C and $[\alpha]_D$ + 68° (C, 2.0).

2,3,4,6-Tetra-O-methyl-D-galactose - The syrup $[\alpha]_D$ + 86° (C, 0.8) was chromatographically indistinguishable from an authentic specimen of 2,3,4,6-tetra-O-methyl-D-galactose (solvent F). Demethylation gave only galactose. The aniline derivative (2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine) after two recrystallizations from ethylacetate-petroleum ether had m.p. and mixed m.p. 195-196°C.

2,3,4,6-Tetra-O-methylated D-mannose and D-glucose - The mixture of these two sugars had $[\alpha]_D$ + 49° (C, 2.1). Demethylation gave mannose and glucose in approximate ratio 1:1. Refluxing the mixture with alcoholic aniline produced a syrup which failed to crystallize in ethyl acetate-petroleum ether or ethyl ether-petroleum ether.

Periodate Oxidation of the Glucomannan

Portions of the glucomannan (100-150 mg.) were introduced into an aqueous solution of 0.05M sodium metaperiodate (50 ml.) and mechanically shaken in the dark for various lengths of time. At the end of each period the periodate consumption was determined by the Müller-Friedberger method (86). The consumption was found to attain a constant value of 1.02 moles per anhydrohexose unit after 5 days (see Table 21).

Preparation of the Nitrate Derivative of the Glucomannan

A nitrating mixture containing nitric acid, phosphoric acid

and phosphorus pentoxide in a weight ratio 64:26:10 (87, 88) was prepared. Dry glucomannan (3 g.) was treated with this mixture at + 17°C for 2 hours. At the end of this period, the reaction mixture was poured into a 20% brine solution (8 litres) cooled to -15°C. The glucomannan nitrate was recovered by filtration and washed with water and methanol. The dried product weighed 4.5 g., 86% yield. Theoretical nitrogen content, 14.1%. Found, 13.3%, corresponding to a base molecular weight of 280.

Determination of the Number-Average Molecular Weight of the Nitrated and Methylated Glucomannans

The osmometric measurements were carried out with modified (89) Zimm-Myerson type (90) osmometers provided with gel cellophane membranes and the solvent was n-butylacetate for the nitrated and a mixture of chloroform and ethanol (9:1 v/v) for the methylated glucomannan. The temperature of measurement was 30 ± 0.01°C and the static method of measuring the osmotic height was used. The results obtained are summarized in Table 30.

ARABINO-4-O-METHYLGLUCURONO-XYLAN

The isolation of this hemicellulose has been described earlier.

Partial Hydrolysis of the Xylan and Separation into Neutral and Acidic Components

Partial hydrolysis of the xylan was carried out as described for the glucomannan. An aqueous solution of the syrup obtained was added to the top of a column (3.7 cm. x 25 cm.) containing Dowex 1-X4 (acetate form) exchange resin. Neutral sugars were first eluted with

Table 30 Osmometry Data for Nitrated and Methylated Glucomannan

Glucomannan Nitrate		Methylated Glucomannan	
W	h/W	W	h/W
4.330	1.312	2.437	2.111
3.614	1.248	2.074	2.052
2.457	1.234	1.618	1.999
1.934	1.180	1.224	1.710
1.436	1.132	0.793	1.644
0	1.060	0	1.390

W = Concentration in grams/kilogram of solution

h/W = Osmotic height in centimeters of solution/concentration

water (3 litres). Evaporation of the eluate gave a clear syrup (2.6 g.). The acidic sugars were displaced from the column with 30% aqueous acetic acid (4 litres), which was evaporated to give a clear syrup (1.3 g.).

Preliminary examination of the neutral sugar mixture by paper chromatography (solvents A and E) revealed at least five oligosaccharides in addition to galactose, glucose, arabinose and xylose. For isolation of the oligosaccharides, solvent A was employed. Four sugars (see Table 23) were obtained.

Examination of the acidic syrup by paper chromatography (solvents B,C and D) revealed the presence of at least nine oligosaccharides in addition to traces of 4-O-methyl-D-glucuronic acid. For separation of these sugars on a preparative scale, solvent B was used. Since some of the oligosaccharides were present only in small amounts, the separation was directed with a view to isolate those oligosaccharides which were present in significant amounts. Four such sugars were isolated.

Preliminary Characterization of Neutral Oligosaccharides

Oligosaccharide (A) - This sugar (14 mg.) on hydrolysis with sulfuric acid gave galactose and xylose in an approximate ratio of 1:2 and had $[\alpha]_D$ + 2° (C, 1.4). It gave a single spot on the paper when chromatographed in solvents A, B and E, indicating that it was homogeneous. It had a mobility on the chromatogram very close to that of authentic β-(1→4)-xylotriose.

Oligosaccharide (B) - This compound (21 mg.) was obtained as an amorphous powder and had a very low specific rotation which could not be measured.

On hydrolysis it gave galactose, glucose and xylose in the approximate ratio of 4:1:2. Although it gave a single spot on the paper when chromatographed in solvent A, examination in solvent E revealed minor amounts of a contaminant which was identical in mobility to authentic cellobiose. The major oligosaccharide had a lower mobility than cellobiose. It is very likely that the major component in oligosaccharide (B) was composed of galactose and xylose residues.

Oligosaccharide (C) - This sugar (29 mg.) was an amorphous powder and had $[\alpha]_D$ + 10.2° (C, 1.6). On hydrolysis it gave rise to galactose, glucose and xylose in the approximate ratio 6:1:6. Paper chromatography in solvent E again revealed heterogeneity, but the contaminant was present only in traces and had a mobility similar to that of cellobiose. The major oligosaccharide moved slightly faster than cellobiose and it is likely that it was a disaccharide consisting of galactose and xylose residues.

Oligosaccharide (D) - This oligosaccharide (50 mg.) had $[\alpha]_D$ - 24° (C, 0.8) and gave only xylose on hydrolysis. Its chromatographic mobility (solvents A, B and E) as well as specific rotation were identical to those of an authentic specimen of a β-(1→4)-linked xylobiose.

Preliminary Characterization of Acidic Oligosaccharides from the Xylan

The four oligosaccharides obtained were chromatographed on paper (solvents B, C and D) along with authentic specimens of acidic oligosaccharides obtained from other softwood xylans. One of them had the same mobility on the chromatogram as 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose (A_2). The other three oligosaccharides had

the mobilities respectively of an aldotrio-(A_3), an aldotetrao-(A_4) and an aldopentaouronic acid (A_5).

Characterization of 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose

The aldobiouronic acid (140 mg.) was boiled under reflux with 0.7N methanolic hydrogen chloride (5 ml.) for 7 hours. After neutralization with silver carbonate, filtration through Celite, treatment with hydrogen sulfide, filtration and evaporation yielded a syrup (150 mg.). The methyl ester methyl glycoside of the aldobiouronic acid was dissolved in dry pyridine (40 ml.) and treated with acetic anhydride (15 ml.) and after a reaction time of 24 hours at 25°C, the solution was poured into ice-water (100 ml.). The aqueous solution was extracted with chloroform (3 x 100 ml.). The chloroform extract was washed with 10% ice-cold hydrochloric acid (3 x 100 ml.), saturated aqueous sodium bicarbonate (3 x 100 ml.), and water (100 ml.). The extract was dried over sodium sulfate and then evaporated to yield a syrup (160 mg.). The latter was dissolved in boiling ethyl ether. The crystals of the tetraacetate formed were recrystallized from anhydrous ethanol:ether (1:1) and had m.p. and mixed m.p. 199-200°C and $[\alpha]_D$ + 98° (C, 1.0 in chloroform) identical with those of an authentic specimen of methyl 2-O-[methyl (2,3-di-O-acetyl-4-O-methyl-α-D-glucopyranosyl) uronate]-3,4-di-O-acetyl-α,β-D-xylopyranoside.

Methylation of the Xylan

The xylan was methylated five times with dimethyl sulfate and sodium hydroxide and four times with silver oxide and methyl iodide as

described for the glucomannan. The methylated product had OMe, 38.4%; calculated for a fully methylated pentosan OMe, 39.6%; $[\alpha]_D$ - 45°(C, 1.8 in $CHCl_3$).

Methanolysis of the Methylated Xylan and Separation of Acidic and Neutral Components

Methylated hemicellulose (4 g.) was boiled under reflux with 2.5% methanolic hydrogen chloride (110 ml.) for 12 hours. After neutralization and purification in the usual way, the syrup obtained (4.5 g.) was heated at 60°C in 5% aqueous barium hydroxide (25 ml.) for 2 hours to saponify the methyl ester of the acidic component (91). Solid carbon dioxide was added, and barium carbonate was removed by filtration through Celite. The solution was de-ionized with Amberlite IR-120 exchange resin and evaporated to give a clear syrup (4.2 g.). The acidic glycoside mixture was added to the top of a column (3.4 cm. x 25 cm.) of Dowex-1X4 anion exchange resin (acetate form). Neutral glycosides were eluted with water to yield a syrup (3.4 g.) and acid glycosides were removed with 30% aqueous acetic acid to give a syrup (0.8 g.) which was dried in vacuo over phosphorus pentoxide for several days.

Preparation and Identification of Methyl 2-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyl)-3-O-methyl-α,β-D-xylopyranoside

The acid glycoside (0.8 g.) was converted to its methyl ester by refluxing with 2.5% methanolic hydrogen chloride for 7 hours. The ester was reduced with lithium aluminum hydride (2 g.) in dry ethyl ether (50 ml.) to yield a pale yellow syrup of the reduced

disaccharide, which on drying *in vacuo* crystallized spontaneously. After recrystallization from ethyl acetate, the crystals had m.p. and mixed m.p. 165-166°C, $[\alpha]_D$ + 89° (C, 1.5) identical with that of an authentic specimen.

Hydrolysis of the Partially Methylated Disaccharide and Identification of 3-O-Methyl-D-xylose and 2,3,4-Tri-O-methyl-D-glucose

The partially methylated disaccharide (5 mg.) was hydrolyzed with 1N sulfuric acid. The sugar mixture obtained, when examined by paper chromatography (solvent F) revealed two components having identical mobilities as authentic specimens of 3-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose respectively.

Hydrolysis of the Neutral Glycosides from the Methanolysis of the Methylated Xylan and Separation of the Methylated Monomers

The mixture of neutral glycosides (3.4 g.) was boiled under reflux with 1N sulfuric acid (75 ml.). Part of the recovered syrup (2.9 g.) was resolved by paper chromatography (solvlent F).

Preliminary Identification of the Methylated Sugars

The various sugars were tentatively characterized by their chromatographic mobilities, sugars formed on demethylation and electrophoretic behaviour (mono-O-methyl-D-xyloses, 2,3-di-O-methyl-D-xylose and 2,3-di-O-methyl-D-glucose). The results are summarized in Table 24.

THE "GLUCAN"

The isolation of this hemicellulose has been described earlier.

Partial Hydrolysis of the "Glucan", Separation of Neutral and Acidic Components and their Resolution

The procedure was the same as that used for the xylan. The neutral oligosaccharide mixture (2.6 g.) was resolved into two sugars by paper chromatography (solvent A) and the acid oligosaccharide mixture (0.6 g.) was resolved in solvent C into four fractions.

Preliminary Characterization of the Acidic Oligosaccharides

Oligosaccharide (a) - This compound (30 mg.) had $[\alpha]_D$ - 11.7° (C, 0.9) and gave galactose, glucuronic acid and xylose on hydrolysis. It was homogeneous when examined by paper chromatography (solvents B, C and D). The glucuronic acid was identified on the chromatogram by two characteristic spots, a slower moving one due to the acid itself and a faster moving one due to the glucurono-(6→3)-lactone.

Oligosaccharide (b) - This sugar (40 mg.) had $[\alpha]_D$ - 13.2° (C, 1.0) and gave galactose and glucuronic acid on hydrolysis. It was homogeneous when examined by paper chromatography (solvents B, C and D).

Oligosaccharide (c) - This compound (12 mg.) had a very low specific rotation and gave galactose and galacturonic acid on hydrolysis. Paper chromatography (solvents B, C and D) indicated homogeneity.

Oligosaccharide (d) - This coumpound (15 mg.) had $[\alpha]_D$ - 7.0° (C, 0.7) and gave galactose, xylose, galacturonic acid, and glucuronic acid on hydrolysis. Paper chromatography (solvents B, C and D) revealed that it contained two oligosaccharides of slightly differing mobilities.

Characterization of Reducing End Groups in Acidic Oligosaccharides from the "Glucan"

Ten mg. portions of oligosaccharides (a) and (b) were reduced with sodium borohydride (10 mg.) in aqueous solution. After allowing 4-6 hours reaction time, the excess borohydride was destroyed by addition of aqueous acetic acid. The solution was de-ionized with Amberlite IR-120 exchange resin and evaporated a few times from methanol. The product was subsequently hydrolyzed with 1N sulfuric acid and the constituent sugars were identified by paper chromatography (solvents A, B and D). Oligosaccharide (a) gave galactose and glucuronic acid and oligosaccharide (b) gave only galactose.

Preliminary Examination of Neutral Oligosaccharides

Oligosaccharide (e) - This compound (54 mg.) was obtained as an amorphous, grayish white powder and had $[\alpha]_D$ + 6.2° (C, 1.2). On hydrolysis, it gave rise to galactose, glucose and mannose in the approximate ratio of 1:2:4. Examination by paper chromatography (solvent E) indicated that the oligosaccharide was a mixture of two components, one of which had the same mobility as an authentic specimen of β(1→4)-linked mannobiose. The other component had a lower mobility than mannobiose.

Oligosaccharide (f) - This compound (67 mg.) was also obtained as a slightly yellowish amorphous powder and had $[\alpha]_D$ + 19.6° (C, 1.0). On hydrolysis it gave rise to mainly galactose with minor amounts of xylose and glucose. Chromatographic examination in solvent E revealed a trace of a product with a mobility slightly lower than that of β(1→4)-xylobiose. The major oligosaccharide was most likely a (1→6)-linked galactobiose.

Methylation of the "Glucan"

The procedure was similar to those described earlier. The product had OMe, 42.1% and $[\alpha]_D$ + 15° (C, 1.7 in $CHCl_3$). The infra-red spectrum (KBr pellet) showed only a very slight band in the hydroxyl absorption region.

Methanolysis of the Methylated "Glucan" and Separation of Acidic and Neutral Components

The procedure was the same as that outlined for the xylan.

Examination of the Methylated Acid Glycoside Component

The acid glycoside portion was obtained as a syrup (0.18 g.). It was converted to its methyl ester, and the ester was reduced with lithium aluminum hydride (0.5 g.) in dry ethyl ether (15 ml.). The recovered syrup (100 mg.) was hydrolyzed by refluxing with N sulfuric acid (4 ml.) for 6 hours. The product obtained (70 mg.) was resolved into its constituent sugars by paper chromatography (solvent F). Four methylated sugars were obtained.

Preliminary Examination of the Methylated Sugars

3-O-Methyl-D-xylose - This fraction (2.5 mg.) was chromatographically (solvent F) and electrophoretically identical with an authentic specimen of 3-O-methyl-D-xylose. Demethylation gave only xylose.

2,3,-Di-O-methyl-D-glucose - This fraction (5 mg.) was identical to an authentic specimen of 2,3-di-O-methyl-D-glucose in chromatographic (solvents F, G) and electrophoretic mobilities. Demethylation gave only glucose.

2,3,4-Tri-O-methyl-D-galactose - This sugar (10.5mg.) gave only galactose on demethylation. Its R_G-value in solvent F was 0.34 and that in solvent G was 0.72. Aspinall and co-workers (92, 93) reported a value of 0.72 for both 2,3,4-tri-O-methyl- and 2,3,6-tri-O-methyl-D-galactose. But the latter had an R_G-value of 0.49 in solvent F (84).

2,3,4-Tri-O-methyl-D-glucose - This fraction (20 mg.) gave only glucose on demethylation. Its chromatographic mobility in solvents F and G were identical with those of an authentic specimen of 2,3,4-tri-O-methyl-D-glucose.

Separation of the Neutral Components of the Methanolized Methylated "Glucan"

The neutral glycoside mixture was hydrolyzed with N sulfuric acid as described for the xylan. The pale yellow syrup obtained (2.9 g.) revealed the presence of di, tri and tetra-O-methylated sugars on examination by paper chromatography (solvent F). A part of this mixture was resolved in the same solvent to give a series of methylated sugars.

Preliminary Examination of the Methylated Sugars

A-Di-O-methylated-D-galactose - Demethylation of this fraction (6 mg.) gave only galactose. It was possibly 2,3-di-O-methyl-D-galactose since its R_G-value in solvent G (0.48) was identical with that reported for this sugar by Aspinall and co-workers (92,93).

A Mixture of Di-O-methylated Glucose and Mannose - This fraction (8 mg.), on demethylation gave glucose and mannose in the approximate ratio of 1:1. Its R_G-value (0.18 and 0.58 respectively in solvents F and G) suggested

that at least one component in the mixture was 2,6-di-O-methyl-D-glucose.

2,3-Di-O-methyl-D-glucose - This syrup (140 mg.) $[\alpha]_D$ + 60° (C, 1.9) gave only glucose on demethylation. Its R_G- value in solvents F and G (0.24 and 0.65 respectively) were identical with those of an authentic specimen of 2,3-di-O-methyl-D-glucose. On refluxing it in ethanol solution with aniline, the corresponding glycosylamine was obtained. Crystallization of this in ether twice, gave a product having m.p. 130°-132°C; reported value 134°C (94).

2,3,4-Tri-O-methyl-D-galactose - This fraction (40 mg.) $[\alpha]_D$ + 98° (C, 2.7) gave only galactose on demethylation. Its tentative characterization has been reported elsewhere. The aniline derivative failed to crystallize.

A Mixture of Tri-O-methylated D-glucose and Di-O-methylated D-xylose - This syrup (144 mg.) $[\alpha]_D$ + 45° (C, 1.9), on demethylation gave glucose and xylose in the approximate ratio of 1:1. The R_G-value (0.58, solvent F) suggested the presence of a mixture of 2,3,6-tri-O-methyl-D-glucose and 2,3-di-O-methyl-D-xylose. Paper electrophoresis indicated the absence of 3,4-di-O-methyl-D-xylose in the fraction.

2,3,4,6-Tetra-O-methyl-D-galactose - This compound (54 mg.) $[\alpha]_D$ + 80° (C, 2.1) on demethylation gave only galactose. The aniline derivative had m.p. and mixed m.p. 192-193°C.

A Mixture of Tetra-O-methylated-D-glucose and Tri-O-methylated D-xylose - This fraction (15 mg.) $[\alpha]_D$ + 26° (C, 0.6) gave glucose and xylose on demethylation in the approximate ratio of 2:1. The R_G-value (1.00, solvent F) suggested the presence of a mixture of 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose.

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SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. Three hemicelluloses have been isolated from the bark of Engelmann spruce (*Picea engelmannii*) based predominantly on residues of xylose, glucose and mannose respectively. The former two were present in the potassium hydroxide extract of the pectin-free holocellulose, from which they were fractionated by Fehling's solution. The latter was obtained from the sodium hydroxide-borate extract by precipitation with aqueous barium hydroxide.

2. The xylan, obtained in a yield of 4.5% of the extractive-free bark, contained residues of galactose, glucose, 4-O-methyl-glucuronic acid, arabinose and xylose in ratio of 6:12:7:10:65.

3. Partial hydrolysis of the xylan gave, in addition to β(1→4)-xylobiose and 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose, three oligosaccharides which were predominantly constituted of galactose and xylose units.

4. The methylated xylan on methanolysis and hydrolysis yielded a preponderance of 2,3-di-O-methyl-D-xylose. The other methylated sugars identified were 2,3,4-tri-O-methyl-D-xylose, 2-O-methyl-D-xylose, 3-O-methyl-D-xylose, 2,3,5-tri-O-methyl-L-arabinose and 2-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyluronic acid)-3-O-methyl-D-xylose.

5. The above data indicated the presence of a β(1→4)-linked xylose framework to which were directly attached by

(1→2)-glycosidic linkages, 4-O-methyl-α-D-glucuronic acid units. The arabinose residues occurred as terminal, non-reducing end groups.

6. The methylated xylan also gave rise to 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose and 2,3-di-O-methyl-D-glucose. It was concluded from these as well as partial hydrolysis results that some of the galactose residues in the original xylan might be integral parts of the molecule.

7. The glucose-based polysaccharide ("glucan") was obtained in a yield of 1.8% and contained residues of uronic acid, galactose, glucose, mannose and xylose in ratio of 2:11:57:2:28. Various fractionation studies on this polysaccharide gave strong evidence for its homogeneity.

8. Partial hydrolysis of this polysaccharide gave some evidence for the presence of a (1→6)-linked galactobiose and a glucuronosyl galactose in the hydrolyzate.

9. The methylated "glucan" on methanolysis and hydrolysis gave large amounts of 2,3-di-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-glucose along with 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-galactose and a methylated glucuronosyl-(1→6)-D-galactose. The xylose in the original polysaccharide appeared predominantly as 2,3-di-O-methyl-D-xylose with minor amounts of 2,3,4-tri-O-methyl-D-xylose.

10. The above data suggested that the "glucan" apparently consisted

of a backbone of (1→4)-linked glucose units, which possessed branch points at C-6. Some of these branch points presumably carried single unit side chains of galactose units. Some of the galactose was also present as a (1→6)-linked galactan, the terminal unit of which carried a glucuronic acid substituent at C-6. It was not known whether the galactose chain was an integral part of the glucose backbone or existed as a separate entity. The xylose residues originated from a (1→4)-linked xylan macromolecule which might or might not be attached to the main glucose chain.

11. The glucomannan was obtained in a yield of 2.0% and contained residues of galactose, glucose and mannose in ratio of 1:6.3:17.8. It had a number-average degree of polymerization of 86.

12. Periodate oxidation of the glucomannan resulted in a consumption of 1.02 moles of the oxidant per anhydro-hexose unit.

13. Partial hydrolysis of the glucomannan yielded the disaccharides mannobiose, mannosyl glucose, glucosyl mannose, cellobiose, the trisaccharide mannotriose and the tetrasaccharide mannotetraose, all of which were β(1→4)-linked.

14. The methylated glucomannan had a number-average degree of polymerization of 91. Hydrolysis of the material gave predominantly 2,3,6-tri-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose along with 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-mannose, 2,3-di-O-methyl-D-glucose, 2,6-di-O-methyl-D-glucose and 2,3-di-O-methyl-D-mannose.

15. The above data showed that the hemicellulose consisted of a backbone of (1→4)-linked β-D-glucopyranose and β-D-mannopyranose residues, some of which carried as a side chain an α-D-galactopyranose unit directly attached to their 6- positions.