CONSTITUTION OF A GLUCOMANNAN FROM WHITE SPRUCE

by

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GENERAL INTRODUCTION

Besides the extractives, which in certain species of woods can be very considerable, the three main components of wood are cellulose, hemicellulose and lignin. Cellulose is a linear polymer consisting of β-D-glucopyranose residues linked together by (1→ 4) glycosidic bonds. In native celluloses, no less than 5,000 such residues are probably present in an average cellulose macromolecule, corresponding to a weight-average degree of polymerization of 10,000 if the ratio between number- and weight-average values is assumed to be the usual 1:2. Lignin is believed to be a three-dimensional polymer of phenyl propane residues, many structural details of which are known with reasonable certainty while others are still rather obscure. The hemicelluloses constitute the alkali-soluble, non-cellulosic polysaccharides of wood. They are composed of many different sugar residues such as D-galactose, D-glucose, D-mannose, L-arabinose, D-xylose, L-rhamnose, L-fucose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid etc. The hemicelluloses are invariably of a much smaller molecular size than the cellulose, most of them containing only 100-200 units in an average molecule. While many of them, for example the hardwood xylans, appear to be linear, others are undoubtedly branched, such as the softwood glucomannans.

Another class of wood polysaccharides which is often included in the hemicelluloses is the so-called water-soluble polysaccharides. These are products obtained on extraction of the wood with water and constitute extra-cellulose polysaccharides, usually so-called arabogalactans, highly branched molecules with a framework of anhydrogalactose units. In most

hardwoods and softwoods the content of water-soluble arabogalactans is very small but they form a considerable part of the wood of the deciduous conifers belonging to the larch (Larix) family.

The woody angiosperms contain as a predominating hemicellulose a partially acetylated 4-O-methylglucuronoxylan, which has been extensively studied in recent years. A minor component is a glucomannan containing approximately equal numbers of glucose and mannose residues and which could therefore also be characterized as a mannoglucan. In the (botanically much older) conifers the glucomannan constitutes the main hemicellulose component with the xylan taking second place. The latter almost invariably appears to be a partially acetylated arabino-4-O-methylglucuronoxylan although some polysaccharides of this type have also been isolated which do not carry any arabinose side chains.

The softwood glucomannans have been studied only within the last few years in spite of their great abundance in nature. At the time when the present study was initiated (October 1955) the mannose residues in wood were generally believed to be derived from a mannan polymer similar to the one known to be present in vegetable ivory. The first report of the isolation of a wood glucomannan appeared a year later (20). Since that time, softwood glucomannans have been isolated and studied from a number of different species as can be seen from the following section.

The aim of the present study was to ascertain whether or not all, or at least most, of the mannose residues in a typical softwood were derived from a true glucomannan and, if such a product could be isolated

in a reasonable yield, to establish its structure and molecular properties. As a typical coniferous species, white spruce (Picea glauca) was chosen. The first of the above objectives was rapidly attained and a preliminary account of the results was given in Tappi 40:519 (1957). This, to the best of our knowledge, represented the first reasonably quantitative isolation of a wood glucomannan.

Since the initiation of the present study the structure of a water-soluble arabogalactan from white spruce has been reported, as well as the constitution of an aldotrio- and an aldobiouronic acid isolated from the same species of wood (88).

HISTORICAL INTRODUCTION

Plant polysaccharides that were soluble in alkali but insoluble in water in their original condition were designated "hemicelluloses" by Schulze (1). This term was employed to express the presumed chemical and structural relationship of this class of materials to cellulose. Schulze also directed attention to the two main differences between cellulose and hemicellulose, namely the solubility in alkali and the susceptibility to acid hydrolysis of the latter as compared to the former. Many years later, Norman (2) suggested that the non-cellulosic polysaccharides extracted from wood by alkali be divided into two classes, designated "polyuronide hemicelluloses" and "cellulosans." The former were believed to originate from the encrusting cell wall material (middle lamella) and to contain uronic acid groups. The cellulosans were believed to be more closely associated with the cellulose and to be free of uronic acids. Polyuronide hemicelluloses and cellulosans were separated by preparing Cross and Bevan cellulose (3). Apparently, the polyuronide hemicelluloses were closely associated with lignin (4). Sand and Nutter (5) advanced evidence for the occurrence of a lignin-carbohydrate complex.

The close association between lignin and polyuronide hemicellulose in plant materials presents an obstacle to the removal and subsequent purification of the polysaccharides, and the same evidently applies also to the cellulosans. If pure polysaccharides are to be obtained, lignin must therefore usually be removed from

plant tissues prior to extraction with alkali. Such a procedure also improves the yield of hemicellulose considerably, especially in the case of softwoods.

Schmidt and coworkers developed time-consuming methods (6,7) of delignification, involving the use of chlorine dioxide in a solution of pyridine and water. A more practical procedure was devised by Ritter and Kurth (8) who treated maple wood alternatively with gaseous chlorine and with a solution of pyridine in ethanol. Complete removal of lignin was not achieved by this method, but residual lignin could be removed by treatment with calcium hypochlorite. The residue, which was termed "holocellulose", retained nearly all the polysaccharides and associated groups present in the original wood. This procedure was later considerably improved by Van Beckum and Ritter (96) who substituted a 3% solution of monoethanolamine in ethanol for the pyridine-alcohol. Unfortunately, some of the ethanolamine is retained by the holocellulose and cannot be removed.

A third method of delignification utilizing sodium chlorite and acetic acid was developed by Jayme (9). Wood meals were alternatively treated with alkaline solutions and digested with sodium chlorite in acetic acid solution. This method was later improved by Wise, Murphy and D'Addieco who treated wood sawdust with a hot (70-80°C) solution of sodium chlorite and acetic acid for various lengths of time, fresh reagents being added every hour. It was found that the delignification should be stopped at a 2-3% lignin level since otherwise serious losses of hemicelluloses occurred.

Holocelluloses prepared by the chlorine or the acid chlorite

method were originally believed to represent a quantitative yield of carbohydrate material. Later, however, both hemicelluloses and free sugars were detected in chlorite liquors from slash pine (10) and spruce (11). Timell and Jahn (12) found that the Van Beckum and Ritter method resulted in less degradation and loss of carbohydrates than did the chlorite procedure of Wise and coworkers. Similar results were later reported by others (13,14).

The development and subsequent improvement of various methods for delignification of plant tissues with a minimum of change in the carbohydrate portion represented a great advance in hemicellulose chemistry. Before these methods were known, scientists extracted wood with cold and hot alkali alone, obtaining impure preparations in usually low yields. Even now, with a relatively small amount of lignin present in the residual holocellulose, the presence of lignin presents a serious problem in the fractionation of hemicellulose mixtures.

Some of the earliest investigations on wood hemicelluloses were concerned with the polyuronides of hardwoods. O'Dwyer, studying white oak wood (15-19), resolved the alkali-soluble hemicellulose into two components, one (A) which precipitated when the extract was neutralized with acetic acid and another (B) which was precipitated by addition of alcohol. Fraction A was an almost pure acidic xylan. Similar methods were later used to isolate glucomannans from western hemlock (20) and sitka spruce (21). Anderson and coworkers (22) investigated softwood hemicelluloses by similar methods. A fraction A from pine sapwood hemicellulose contained 36-46% mannose, 50-44%

xylose and 15-11% methyl uronic acid residues, and also gave positive qualitative tests for glucose. The first of the above values referred to material obtained from wood meal, the second from holocellulose. The other hemicellulose fraction (B) consisted of 89% mannose, 7.7% xylose and 3% methyl uronic acid.

Mannose Containing Polysaccharides from Plant Materials Other than Wood

The difficulty in obtaining mannose containing polysaccharides from softwoods in a reasonable state of purity turned the attention of early workers to other sources from which mannans could be obtained more readily.

The endosperms of certain seeds, such as the ivory nut and the date palm, have been found to contain a mannan polysaccharide as a reserve material which disappears on germination. As early as 1896 (23) mannose was shown by Johnson to be present in the hydrolyzate of material extracted from vegetable ivory with 10% alkali. Later, Baker and Pope (24) isolated a similar polysaccharide from the same source which contained 90% mannose and 10% fructose. Lüdtke (25) reported that ivory nuts contain two different mannans; one (A) was extracted with 5% sodium hydroxide from ivory nuts pretreated with chlorine dioxide, the other (B) being obtained from the residue by extraction with 10% sodium hydroxide. More recently, Aspinall (26) et al. found that mannans A and B both contained mostly mannose residues in addition to smaller quantities of galactose and glucose. Both polysaccharides were linear but the less soluble mannan B

apparently had a greater chain length. The majority of the mannose residues are linked through positions 1 and 4 by a β-glycosidic linkage. A later investigation showed, however, (27) that 1,6-linkages as well as α-glycosidic linkages are also present to a limited extent. Mannans A and B both contained two types of macromolecules, one terminated by a D-mannopyranose and the other by a D-galactopyranose residue.

Meier (28), studying the properties of mannans A and B, found that the former is crystalline both in its native state and after dissolution and reprecipitation, whereas B is amorphous and paracrystalline. Curiously enough, the crystalline material is the one most easily extracted, which is rather unusual. Another striking feature of this mannan B is that it is present in the plant in the form of microfibrils, similar to those formed by cellulose. Evidence was obtained for the presence of a chemical linkage between mannose and glucose in the ivory nut.

A mannan similar in structure to that obtained from endosperms was prepared from salep tubers by Pringsheim and Genin (29). Husemann (30) isolated a similar polysaccharide by precipitating an aqueous extract of alcohol-treated orchid bulbs. Rebers and Smith (31) isolated and determined the detailed structure of a glucomannan from the Amorphophallus oncophyllus and Amorphophallus variabilis plants which are native to Indonesia. The so-called "Iles mannan" meal was insoluble in water of aqueous alkali and only after preswelling in a 50% solution of xylene sulfonate was solubility in alkali attained. Treatment of the dilute alkaline solution with

Fehling solution (32) effected a partial separation of the crude product into a glucomannan which precipitated and a glucan which remained in solution. A resolution of the polysaccharide mixture was also achieved by fractional precipitation of the methylated material. The glucomannan was a linear polymer, composed of D-glucopyranose and D-mannopyranose residues in a ratio of 1:2, linked together by β-1,4-glycosidic bonds. The hydrolyzate of the fully methylated product contained large quantities of 2,3,6-tri-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-mannose which could not be separated very well on the paper chromatogram. Resolution was instead achieved through the preferential furanoside formation of the glucose derivative. In later work it has been found that these two isomers can be separated by column chromatography on either charcoal (33) or cellulose (21). Partial hydrolysis of the Iles glucomannan (34) gave further information concerning the sequence of the sugars in the polymer chain. Three oligosaccharides were identified, namely 4-O-β-D-glucopyranosyl-α-D-mannopyranose, 4-O-β-D-mannopyranosyl-α-D-glucopyranose and cellobiose. On the basis of this evidence the authors suggest the following tentative structure:

$$-\!-4\ \beta\text{-D-Gp 1}-\!-4\ \beta\text{-D-Gp 1}-\!-(\ 4\ \beta\text{-D-Man p 1}\ )-\!-_{4}$$

Similar glucomannans occur in the bulbs of Lily umbelatum, L. henryi, L. candidum (35) but in these cases the glucomannan is not associated with a glucan and can be extracted with cold water.

Here too, there are twice as many mannose as glucose residues present. Methylation studies on the L. umbelatum glucomannan indicated that it contains D-glucopyranose and D-mannopyranose residues linked together by β-1,4-glycosidic bonds and with an average number of 27 hexose residues present per non-reducing end group, all of which were glucopyranose in nature. A small number of the anhydroglucose units were linked through C_1, C_3 and C_6 and since the ratio of tetra-O-methyl glucose to di-O-methyl glucose was 1:0.65, approximately two branching points were present per molecule. Similar studies on the L. henryi glucomannan suggested the presence of β-1,4-linked hexopyranose residues with approximately 75 units present per glucose end group. In this case the tri-O-methyl mannose and tri-O-methyl glucose were separated on cellulose through their corresponding lactones.

Guaran is the principal component of guar seeds, consisting of approximately 36% galactose and 64% mannose residues. It has been extensively studied by Whistler and his coworkers (36, 37). Partial hydrolysis of the product yielded mannobiose and mannotriose for the first time, both containing β-1,4 linkages, as well as a 6-O-α-D-galactopyranosyl-β-D-mannopyranose. Guaran is composed of a straight chain of 1,4-linked β-D-mannopyranose residues with every second unit having an α-D-galactopyranose residue attached by a 1,6-glycosidic bond.

The endosperm from fenugreek and lucerne seeds (38) contains a galactomannan similar to guaran. Results obtained by methylation and by periodate oxidation were consistent with a

structure involving a backbone of 1,4-linked β-D-mannopyranose residues with D-galactopyranose residues attached through C_1 to C_6 of the anhydromannose units. Mannobiose, mannotriose and 6-O-α-D-galactopyranosyl-D-mannopyranose were obtained on partial acid hydrolysis of the polysaccharide.

Several other glucomannans have been isolated from various plants such as the tubers of Amorphophallus konjak (39, 40), the leaves of Aloe vera (41), the roots of Cremastea variabilis (42) and Bletilla striata (42) and the bulbs of Narcissus tazetta (43). None of these glucomannans appeared to contain more glucose than mannose residues, but, since physical homogeneity was not established, their constitution has not been established in detail.

Wood Glucomannans

One of the first attempts to isolate a mannose-containing polysaccharide from wood was made by Sherrard and Bianco (44) who expected to obtain a mannan similar to that present in ivory nuts by subjecting wood to an analogous treatment involving the copper complex method. They observed that an amount of glucose, equal to or even larger than that of mannose was present in the hydrolyzate of the "mannan" and they concluded that these glucose residues might originate from a glucan or a mannoglucan. Since they found mannose in an alpha-cellulose which had been exhaustively extracted with alkali they also concluded that mannose residues must be incorporated in the cellulose chains or be associated with glucose in a polysaccharide as resistant to alkaline extraction as cellulose itself.

Hess and Lüdtke (45) and, later, Husemann (46) claimed the isolation of a wood mannan on the basis of the similarity in specific rotation and X-ray diffraction pattern of the product with those of an ivory nut mannan. No other data were given by these authors and it is now abundantly clear that their "mannans" were anything but pure and presumably consisted of more or less impure glucomannans.

Yundt, trying to isolate a wood mannan by a similar procedure (47), found that even after thorough purification and crystallization of the polysaccharide, only 50% of it consisted of mannose residues, no attempt being made to account for the other half.

Simons (48) was of the opinion that one extraction of an alpha-cellulose with alkali was not sufficient to remove all hemicelluloses but claimed that repeated extractions of bleached softwood pulps did free the residual alpha-cellulose from contaminating xylan and mannan. These results indicated that part of the hemicelluloses was closely associated with the alpha-cellulose and less readily soluble in alkali than other hemicelluloses.

Wise and Ratliff (49) subjected an alpha-cellulose from slash pine to eight successive treatments with 5% sulfuric acid. Even after this severe treatment, however, the pulp still contained 40% of the mannose residues originally present in the wood. The authors considered this as an evidence that mannan in wood was closely associated with the alpha-cellulose and probably formed an integral part of the cellulose, being chemically linked to glucose residues to form a "mannocellulose."

In an investigation of the alkali-insoluble wood "mannan" which is also relatively stable to acid hydrolysis, Leech (50) subjected a slash pine alpha-cellulose to acetolysis and resolved the mixture of oligosaccharides formed by column chromatography. The mother liquor of a cellobiose octa-acetate fraction contained a disaccharide consisting of glucose and mannose residues and a similar disaccharide containing only mannose residues was also isolated in the same investigation.

Anthis (51) continued the investigation of Leech and fully characterized three disaccharides, namely 4-O-β-D-mannopyranosyl-D-mannose, 4-O-β-D-glucopyranosyl-D-mannose and 4-O-β-D-mannopyranosyl-D-glucose. This evidence constituted the first proof that mannose residues in wood are chemically linked to glucose. Glucose has often been found in hydrolyzates of hemicelluloses from softwoods and prior to the above study it was commonly assumed that it was derived from low-molecular weight cellulose. Anthis considered three possible reasons why the mannose residues in the wood alpha-cellulose are so difficult to eliminate. First, they might exist in polysaccharides that are rendered insoluble by physical occlusion in the insoluble cellulose fibers. Second, they are insoluble because they form integral parts of polymers of higher degree of polymerization than the alkali-soluble hemicelluloses. Third, and finally, the mannose containing polysaccharides might be cross-linked to other, similar molecules or to cellulose itself.

In earlier investigations dealing with these polysaccharides the classical methods for isolation of alpha-, beta- and gamma-celluloses

were used. Immergut and Rånby (52) isolated several fractions of gamma-celluloses, considered by these workers to be hemicelluloses. The pentosan content varied from 92 to 5% and the glucose to mannose ratio in the most pure hexosan fraction was 1 : 3.5. In an earlier investigation by Dymling, Giertz and Rånby (53) it was pointed out that the resistant cellulose framework comprises approximately 42-43% of the dry, extractive-free spruce wood. The total glucan content of the same wood is reported by Björkqvist and coworkers (54) as 48-49%. From the data presented by Immergut and Rånby, it is evident that the main part of the non-cellulosic glucose residues in this wood (5-6%) is included in the isolated hexosans. Dymling, Giertz and Rånby also found that the mannose containing polysaccharides could be removed from a nitrated sulfite spruce pulp by fractional extraction with ethyl acetate-ethanol. This makes it improbable that even the resistant mannose residues could be integral parts of the cellulose. The general conclusion of Immergut and Rånby is that most, if not all, mannose residues in spruce occur in the form of a glucomannan. It should, however, perhaps be noted that their investigation was not published until 1957, long after the pioneering studies of Anthis (51), and Hamilton and his coworkers (20) had made it definitely clear that softwoods do contain a glucomannan as the preponderant hemicellulose constituent.

Much of the earlier evidence for the presence of mannose or xylose residues in the cellulose macromolecules in wood was rendered doubtful by the fractionation experiments of Snyder and Timell (55). These workers were unable to resolve an artificial

mixture of nitrated cellulose and ivory nut mannan by extensive fractional precipitation, mannose residues persisting in all the fractions, from the highest to the lowest. Similar difficulties were encountered in an attempt to resolve a mixture of cellulose and mannan by extraction with alkali. Failure to achieve separation in this way cannot accordingly be adduced as an evidence for the presence of foreign sugar residues within the cellulose macromolecules. Similar results have more recently been obtained by Ward and his coworkers (56, 57).

The real reason why some of the wood glucomannan is so difficult to remove from the cellulose is probably to be found in the strong adsorptive forces holding these two similar polysaccharides together. In the case of a decarboxylated xylan, Yllner and Enström (68) have recently shown how strong the adsorptive forces are that hold such a polysaccharide and cotton or wood celluloses together. This phenomenon is encountered in alkaline pulping and what happens is apparently that once the alkali concentration of the liquor has decreased during the later stages of the cook a portion of the wood xylan which is still in solution in the liquor becomes irreversibly readsorbed onto the cellulose microfibrils. There is no reason why the same could not happen with a glucomannan were it not for the fact that in this particular case the glucomannan of the wood is rapidly destroyed once it has gone into solution in the alkaline liquor.

Hamilton, Kircher and Thompson (20) extracted sulfite pulp from western hemlock (Tsuga heterophylla) with 18% sodium hydroxide. Neutralization of the alkaline extract yielded a fraction (so-called

beta-cellulose) which contained glucose and mannose residues in a ratio of 1 : 3.8 and which was slightly contaminated with xylose. A gamma-fraction had the same composition. The authors attempted to resolve the polysaccharide material into a mannan and a glucan by a number of methods, including the use of cuprammonium, the copper complex method, fractionation with borax solutions, etc. None of these methods were successful in markedly changing the ratio of glucose to mannose. Extraction with water changed the glucose to mannose ratio for all fractions. It is not stated which of the fractions is to be considered a pure and typical glucomannan. In a later publication from the same laboratory (58) it is stated that the ratio between glucose and mannose is 1 : 3 and that we are dealing with a mixture of several glucomannans. By fractional precipitation of the acetylated polysaccharide fractions were obtained with a glucose to mannose ratio of 2:1, 1:1 and 1:2, respectively. By the use of a method involving precipitation with barium hydroxide it was found possible, however, to remove any contaminating glucan from the glucomannan. The method used by Hamilton and coworkers for isolating a pure hemlock glucomannan cannot be considered to be the best possible.

One methylation by the method of Falconer and Adams (59) of the acetylated glucomannan sufficed to produce a fully methylated product. Hydrolysis of this material and separation by paper chromatography gave mostly 2,3,6-tri-O-methyl-D-mannose together with smaller quantities of 2,3,6-tri-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,4,6-tetra-O-methyl-D-glucose. Partial hydrolysis of the glucomannan with dilute acid gave 4-O-β-D-

-glucopyranosyl-D-mannose, 4-O-β-D-mannopyranosyl-D-glucose, 4-O-β-D-mannopyranosyl-D-mannose, 4-O-β-D-glucopyranosyl-D-glucose and mannotriose (β-1,4). From this and other evidence,the authors conclude that the glucomannan in western hemlock consists of a straight chain of 1,4-linked β-D-mannopyranose and β-D-glucopyranose residues and with the non-reducing end groups consisting of both glucose and mannose. Mention is also made of the presence of a highly branched galactoglucomannan.

A more conclusive investigation was carried out by Jones and Painter (60, 61, 62) who studied the glucomannan and 4-O-methylglucuronoxylan present in the wood of loblolly pine (Pinus taeda) wood. Alkaline extraction of the chlorite holocellulose gave a mixture of polysaccharides which could be resolved into four fractions via their acetate derivatives. Two acidic fractions contained xylose and 4-O-methyl-D-glucuronic acid (A), and xylose and glucuronic (or galacturonic acid) residues (B). Two neutral fractions consisted of galactose and arabinose (C), and mannose, glucose and galactose (D). The last fraction can to all intents and purposes be considered as a glucomannan. Another glucomannan was obtained from the holocellulose by extraction with 24% potassium hydroxide containing 4% of boric acid. The alkaline washings and filtrate were treated with Fehling solution, the insoluble copper complex was collected, washed by repeated dispersion in water and recovered by centrifugation. It was finally acidified with acetic acid, precipitated into ethanol, washed with acetone, again acidified with hydrochloric acid, and finally washed with ethanol and ethyl

ether and dried. The yield of 5% of the original wood and the hydrolyzate contained mostly glucose and mannose together with smaller quantities of galactose, xylose and 4-O-methyl glucuronic acid.

The glucomannan thus obtained was subjected to partial hydrolysis with 45% formic acid, formate esters were eliminated with dilute sulfuric acid and the mixture of sugars thus obtained was added to the top of a charcoal-Celite column. Elution with increasing concentrations of aqueous ethanol yielded a series of oligosaccharides which were further purified by paper chromatography. Each of the oligosaccharides was fully characterized by standard methods. The compounds obtained included 4-O-β-D-mannopyranosyl-α-D-mannopyranose (mannobiose), 4-O-β-D-mannopyranosyl-α-D-glucopyranose and a β-1,4-linked mannotriose. Peculiarly enough, no cellobiose or glucose-containing trisaccharides were observed.

Another glucomannan was isolated by the same authors by direct alkaline extraction of pine wood followed by a purification procedure similar to the one described above. The polysaccharide obtained contained galactose, glucose and mannose in a ratio of 1:7:19. In this case the yield was only 1% of the weight of the wood. Hydrolysis of the fully methylated derivative gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-mannose and the corresponding glucose derivative as well as a di-O-methyl hexose in a ratio of 1:1:30:1.

The authors conclude that the polysaccharide consists of essentially linear chains of 1,4-linked β-D-glucopyranose and β-D-mannopyranose residues, containing on the average two non-reducing

end groups for every 27-33 anhydrohexose units. One of these is a D-mannopyranose and the other a D-galactopyranose residue. The possibility that the chains contain a single branching point cannot be entirely excluded. The electrophoretic behavior of the glucomannan suggested that it contains a mixture of different, yet closely related polymers. It is evident that heteropolymer glucomannans must represent a major part of the material. The manner in which the galactose end groups are distributed is not known. The apparent absence of cellobiose in the partial hydrolyzate would suggest the presence of few contiguous glucose residues.

Lindberg and Meier (63) have studied a glucomannan from Norway spruce (Picea abies). They isolated a number of pure glucomannans from a chlorite holocellulose by extracting with various solvents in the following sequence: dimethyl sulfoxide, hot water, 14% potassium hydroxide, the same with 3% boric acid, 24% potassium hydroxide containing 3% of boric acid. The proportion of glucose to mannose in all fractions was throughout approximately 1:3.5 to 1:4. The most pure fractions were free from xylose but traces of galactose were always present, though whether as a constituent or an impurity was not decided. The fact that a glucomannan with the same composition was obtained from all the fractions indicates that this is the principal, or even the only, mannose-containing polysaccharide in Norway spruce. Reprecipitation from cuprammonium did not affect the carbohydrate composition.

The number average degrees of polymerization of the fractions, as determined by osmotic pressure measurements on the nitrate derivatives,

varied between 70 and 140. It is striking how the ease of extraction was affected by the molecular weight of the glucomannans, those of lowest D.P. being removed first. After mild hydrolysis with sulfuric acid, most of the resistant glucomannan could be removed with alkali-borate. It had earlier been shown by Meier and Yllner (64) that in prehydrolyzed sulfate pulp from soft wood the mannose content is only 1.6%, as compared to a value of 9.6% for an ordinary, unhydrolyzed pulp.

The fact that a considerable amount of the glucomannan could be extracted with hot water after dimethyl sulfoxide treatment or with 14% potassium hydroxide strongly suggested that the glucomannans could not be integral parts of the cellulose macromolecules. It is more probable that they are situated on the surface of the cellulose microfibrils or within the non-crystalline regions. Another alternative would involve the presence of glucomannan microfibrils within the cell wall, similar to those formed by mannan B in vegetable ivory (28). So far, however, no experimental evidence has been obtained for the presence of such fibrils in wood.

Further studies on the same glucomannan from Norway spruce were carried out by Croon and Lindberg (33). Two polysaccharides were obtained, one (A) by extraction of a chlorite holocellulose with hot water, the other (B) by extraction with alkaline borate. The ratio between the glucose, mannose and xylose residues was 21.8 : 76.9 : 1.3 for sample A and 19.4 : 79.9 : 0.7 for B. The homogeneity of the glucomannans was ascertained by electrophoresis in a borate buffer. Methylation was carried out once according to

Haworth and three times according to the excellent method of Kuhn and coworkers (66) which entails the use of silver oxide and methyl iodide in the presence of dimethyl formamide. Resolution of the mixture of sugars obtained on hydrolysis of the fully methylated product was carried out on a column of charcoal-Celite and by the gradient elution technique originally developed by Alm (67) and later successfully used for separating methylated hexoses by Lindberg and Wickberg (65). Minor quantities were obtained of a di-O-methyl mannose and an unknown tri-O-methyl glucose in addition to 2,3,4,6-tetra-O-methyl-D-mannose. The preponderant sugars were a tri-O-methyl glucose and a tri-O-methyl mannose which were easily separated on the charcoal column and identified as 2,3,6-tri-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-mannose, respectively. The latter sugar was converted to its 1,4-di-O-acetyl derivative and chromatographed on paper impregnated with dimethyl sulfoxide using isopropyl ether as an eluant according to a technique developed by Wickberg (97). The result showed the fraction to be chromatographically homogeneous. Finally, a 2,6-di-O-methyl-D-glucose was fully characterized. The ratios between the 2,6-di-O-methyl-D-glucose, the 2,3,6-tri-O-methyl-D-mannose, the 2,3,6-tri-O-methyl-D-glucose and the 2,3,4,6-tetra-O-methyl-D-mannose were for glucomannan A 6.4 : 66.6 : 17.7 : 5.8 and for B 4.4 : 72.2 : 18.6 : 3.6.

The results indicated that both glucomannans contained chains of 1,4-linked β-D-glucopyranose and β-D-mannopyranose residues. The presence of glucose and mannose residues linked to C_3 of the glucose was not excluded, however, and 3-4 branch points were

probably present per 68 anhydrohexose units. The nature of the branches, the distribution of the glucose and mannose residues and of the branching points as well as the exact value of its weight-average molecular weight remained undetermined. It was finally pointed out that the polysaccharide in its "native" state is amorphous but that it yields a crystalline polymer on mild acid hydrolysis, similar to that of ivory nut mannan A. During this hydrolysis the ratio of glucose to mannose changed from 1 : 3.6 to 1 : 2.9. This is explained by the authors from the well-known fact that mannosidic linkages are more labile under conditions of acid hydrolysis than are glucosidic linkages.

Aspinall, Laidlaw and Rashbrook (27) isolated several fractions from Sitka spruce (Picea sitchensis) wood by alkaline extraction of the holocellulose, all of which contained glucose and mannose residues. One of these fractions had a ratio of glucose to mannose of 1 : 2.5. In addition to a glucomannan it probably also contained a contaminating, degraded cellulose, as pointed out by Croon and Lindberg (33) and, later, by Dutton and Hunt (21). A β-1,4-linked glucan was, actually, isolated from this fraction. Hydrolysis of the fully methylated glucomannan portion gave a mixture of tetra-O-methyl hexoses, 2,3,6-tri-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-mannose. The number-average degree of polymerization of the polysaccharides, as determined by the isothermal distillation method, was 47-51 whereas the number of non-reducing end groups indicated a value of approximately 35. It was concluded from these results that the polysaccharide is an essentially linear polymer of

β-1,4-linked D-glucopyranose and D-mannopyranose residues. In view of the doubtful purity of the material, however, the possibility of branching cannot be excluded. Partial hydrolysis of the product yielded cellobiose, mannobiose and a mannosylglucose, none of which, however, were completely identified.

Dutton and Hunt (21) somewhat later reported results obtained in a similar study of the same glucomannan from Sitka spruce. They extracted the chlorite holocellulose with 10% potassium hydroxide and subsequently treated the residue with 18% sodium hydroxide. The latter extract, after neutralization with acetic acid, yielded a precipitate which on hydrolysis gave glucose and mannose in a ratio of 1:9. The exceptionally high mannose content is in contrast to that found by Aspinall and coworkers. It should be remembered, however, that the two materials had not been isolated by similar methods. Hydrolysis of the methylated polysaccharide gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-mannose and (possibly) 2,3-di-O-methyl-D-mannose. The authors conclude that the glucomannan consists of 40-60 β-1,4-linked D-mannopyranose and D-glucopyranose residues and that no branching points are present. D-galactopyranose occurred as an end group in some cases.

Hamilton and Partlow (69) isolated a glucomannan from western red cedar (Thuja plicata) by extraction with 18% sodium hydroxide of a chlorite holocellulose which had previously been treated with 4% sodium hydroxide. When the alkaline extract was acidified, dialyzed and concentrated, no precipitation occurred and

the material was isolated only after addition of methanol. The hemicellulose was at this stage bleached with chlorine dioxide but the authors do not, unfortunately, discuss either the reason for or the result of this treatment. The product at this stage had a ratio between mannose, glucose, xylose and galactose of 46.0 : 18.3 : 8.1 : 1.0. The authors consider that the galactose arose from a contaminating galactoglucomannan and has no structural significance in this case, but the nature of the xylose residues is not discussed at all. Presumably, they constituted a strongly adsorbed xylan impurity. Partial hydrolysis yielded mannobiose, glucosyl mannose, mannosyl glucose and mannotriose, all of which were observed on the paper chromatogram. The methylated material on hydrolysis gave 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, 2,3,6-tri-O-methyl-D-mannose and a di-O-methyl galactose. It is concluded that the glucomannan is a short, linear polysaccharide, composed of 1,4-linked β-D-glucopyranose and β-D-mannopyranose residues with a ratio of glucose to mannose of 1 : 2.5.

A glucomannan isolated from unbleached Mitscherlich spruce pulp was investigated by Merler and Wise (73). The product was obtained by treating the pulp with cuprammonium when an insoluble copper complex was formed from which the glucomannan was isolated. The detailed structure of the product was not determined but certain data were obtained concerning its molecular properties. Electrophoresis showed the presence of at least three components and ultracentrifuge studies indicated that the material was polymolecular.

Graded acid hydrolysis gave a mixture of sugars from which several oligosaccharides were isolated, including 4-O-β-D-glucopyranosyl-D-mannose, 4-O-β-D-mannopyranosyl-D-mannopyranose, a mannotriose (β-1,4), a trisaccharide containing one reducing glucose residue, one glucose and one mannose residue and another containing one reducing glucose and two mannose residues.

Most recently Meier (74) has studied a glucomannan from Scots pine (Pinus sylvestris). It has been found in this laboratory (75) that pine glucomannans are more difficult to obtain free from contaminating xylans than are the corresponding polysaccharides from spruce wood, and the Scots pine wood was no exception in this respect. The chlorite holocellulose was successively extracted with dimethyl sulfoxide, hot water, 14% potassium hydroxide and potassium hydroxide containing boric acid. A sample of the final residue was dissolved in cupriethylenediamine, reprecipitated with acetic acid and extracted with potassium hydroxide-borate. Fractions rich in glucomannan contained 1.2 and 2.8% xylose, and, contrary to spruce glucomannans, also 2.8 and 4.7% galactose. The copper complex and the cetavlon (cetyltrimethylammonium bromide or hydroxide) methods did not reduce the xylose content. Application of the barium hydroxide technique, however, in one case lowered the xylan content of a fraction from 19.6 to 8.3% (74). The loss of polysaccharide, was, however, approximately 50%. The barium hydroxide method developed by Meier has proven extremely valuable in this laboratory and undoubtedly represents a great improvement over previously used procedures, for example the time-honored copper complex method.

The fraction which had been extracted with 14% potassium hydroxide contained 5% of lignin, 15.5% glucose/galactose, 28% mannose, 6.8% arabinose and 49.8% xylose. It was delignified by chlorination followed by extraction with alcoholic ethanolamine. When the thus delignified product was treated with barium hydroxide, the resulting polysaccharide contained 22.4% glucose, 5.2% galactose, 70% mannose, 2.4% xylose and a trace of arabinose. Both the glucomannan and the xylan were presumably chemically linked to the lignin and could be separated only after removal of the latter. Lindgren (69) has recently adduced evidence for the presence of a lignin-hemicellulose complex in wood. Difficulties encountered by many earlier workers in obtaining pure wood hemicelluloses can now accordingly be at least partly explained by the presence of residual lignin in the products under investigation.

The only reported work on a hardwood glucomannan is that of Jones, Merler and Wise (70) who investigated the hemicelluloses of trembling aspen (Populus tremuloides). After most of the acidic xylan had been removed, alkaline borate gave a fraction containing mostly glucose and mannose in a ratio of 1:2, which was quite different from the ratios of 1:3 to 1:4 usually encountered in soft-wood glucomannans. Methylation experiments indicated that the polysaccharide was composed of β-1,4-linked hexose residues.

Gillham and Timell (71) isolated a 4-O-β-D-glucopyranosyl-D-mannopyranose from a white birch alpha-cellulose. This indicated the presence of a glucomannan in this species of wood. It has now been isolated in this laboratory in a considerable quantity (50 g.)

and in a very pure state (only traces of xylose). The ratio of glucose to mannose is closer to 1:1 than to 1:2. Structural investigations on this polysaccharide are presently in progress (72). The yield is approximately 2% of the weight of the extractive-free wood.

RESULTS AND DISCUSSION

The composition of the sprucewood used is summarized in Table I.

Alpha-cellulose was determined by the standard chlorination procedure followed by exhaustive extraction with alkali.

Constituent sugars in the wood and the alpha-cellulose were estimated by a spectrophotometric method after hydrolysis and separation on paper chromatograms. The percentages of carbohydrate constituents were calculated assuming that lignin, acetyl, and ash constituted the remainder of the wood. The mannan content, which was 11.6%, was of the same order of magnitude as that found previously for other conifers (17).

Holocellulose was prepared from the extractive-free wood meal by the chlorite method (76) in a yield of 78.6%. A portion of the holocellulose was extracted at room temperature with 17.5% NaOH to which had been added 4% of H_3BO_3 (77). Fehling's solution (78) was added to the clear extract and the precipitated copper complex was washed with water and decomposed with a mixture of dilute hydrochloric and acetic acid.

A crude polysaccharide was precipitated from the acid solution of hemicelluloses by addition of alcohol, the yield amounting to 11.2% on the basis of the original wood. The combined washings, on addition of alcohol, gave an additional precipitate which, after decomposition of the

TABLE I

COMPOSITION OF WHITE SPRUCEWOOD

ALL VALUES IN PER CENT OF EXTRACTIVE-FREE WOOD

Component	Per cent
Alpha-cellulose	48.5
Alpha-cellulose, corr.	44.8[a]
Pentosan	9.8
Lignin	27.1
Acetyl	1.3
Ash	0.3
Uronic anhydride	3.6
Galactan	1.2
Glucan	46.5
Mannan	11.6
Araban	1.6
Xylan	6.8

[a] Corrected for non-glucan material.

copper complex, yielded a polysaccharide which was partially insoluble in dilute acids and gave mostly glucose and mannose on hydrolysis.

The crude polymer was purified by dissolution in alkali and reprecipitation as the copper complex. After treatment with acids, glucomannan was again precipitated by addition of alcohol and the series of purifications was repeated once more. The final product thus obtained represented 6.7% of the original wood. The sugar composition of the crude and purified materials is presented in Table II. The crude polysaccharide apparently still contained minor amounts of galactan, araban, and xylan, in addition to some uronic acids, which were not determined. In the purified product all these sugars had disappeared except for a faint trace of xylan. The ratio of glucose to mannose remained essentially the same thoughout and approximated 1:3. The specific rotation of the purified material, $[\alpha]_D^{23}$, was -33.5°(c. 1.0 in 10% NaOH). The intrinsic viscosity in cupriethylenediamine was 0.24, corresponding to a degree of polymerization of 35 to 40. All these data are in agreement with the corresponding values found for the glucomannan from western hemlock (20).

A chemically homogeneous polysaccharide should, on further fractionation, yield portions with chemical properties identical to those of the unfractionated polymer (80). This requirement was fulfilled by the present hemicellulose as far as specific rotation and the ratio of glucose to mannose was concerned. It can therefore be concluded that the material was probably chemically homogeneous and should be regarded as a true glucomannan heteropolymer, composed of anhydroglucose and anhydromannose units.

TABLE II

COMPOSITION OF GLUCOMANNAN IN PER CENT

A and B: Crude Glucomannan from Duplicate Experiments

C: Pure Glucomannan

Component	Glucomannan		
	A	B	C
Galactan	1.1	1.0	Nil
Glucan	22.8	21.6	25.0
Mannan	72.6	73.7	75.0
Araban	1.7	Nil	Nil
Xylan	1.8	3.7	Trace
Glucan:mannan	1:3.2	1:3.4	1:3.0

It is interesting in this connection to note the different behavior toward alkali of the crude glucomannan and the material constituting the endosperm of the vegetable ivory, which on hydrolysis gives 10% glucose and 86% mannose in addition to minor amounts of other sugars. Extraction with 10% NaOH of the latter product gives a high yield of a material containing only 2% glucose units and more than 97% mannose.

The amount of mannose residues present in the crude glucomannan was 72% of the total number of these sugar units present in the wood. If the acid-insoluble fraction referred to above is also included, this figure is raised to 82%. The pure glucomannan contained 43.2% of the mannose units in the wood. It is evident from these figures that the major portion of the mannose residues in white sprucewood form a part of a glucomannan hemicellulose. The composition of the white spruce alpha-cellulose is given in Table III, the assumption being that 1 mole of glucose is associated with 3 moles of mannose.

If the amount of anhydroglucose present as cellulose, namely 43.9%, is deducted from the total glucan content, 46.5%, a remainder of 2.6% is obtained. The ratio between this "extra" glucan and the total mannan content is 1:4.3, or somewhat higher than the ratio of 1:3.0 found for the glucomannan, but almost the same as that observed for a glucomannan from western hemlock (20).

This discrepancy between the glucan to mannan ratio and the composition of the glucomannan suggests two possibilities, namely either that there might be present another kind of glucomannan, which contains

TABLE III

COMPOSITION OF ALPHA-CELLULOSE FROM SPRUCEWOOD

VALUES IN PER CENT OF EXTRACTIVE-FREE WOOD

Component	Per cent
Cellulose	43.9
Mannan	3.0
Glucan	1.0
Araban	0.2
Xylan	0.5

more mannose residues or that mannose residues may be present in a polysaccharide which does not contain any glucose at all, for example, in a galactomannan.

An attempt to solve this problem was made by fractionating the glucomannan by copper complex formation. By this method the crude glucomannan could be separated into three fractions:

About 1.5% of the original wood was a glucomannan which did not precipitate as a copper complex at pH 12.5, or higher. It could be precipitated from the solution by the addition of ethanol (10%). After decomposition of the copper complex, a white polysaccharide was easily obtained which was insoluble in an acidic medium.

This product was exceptionally resistant to acid hydrolysis, was neither soluble nor swelled in cuprammonium solution and was contaminated by xylan polyuronides. The glucose to mannose ratio was difficult to determine but seemed to be only slightly different from 1:3. The solubility of this product in alkaline solution is abnormal. This type of glucomannan represents 80% of the glucomannan extractable from highly bleached spruce pulp. In this case, it represents 2.6% of the original wood. It may be that it is a fraction of degraded and oxidized glucomannan, possibly adulterated by chemical agents.

The second fraction is the glucomannan which precipitates as a copper complex at pH 12.5 or higher, but dissolves at pH 11.5. This product is lost during purification of the bulk of the glucomannan. In all aspects except its pentosan content it has the same properties as the glucomannan in the third fraction.

The third fraction was a glucomannan which formed a copper complex at pH 11.5. This is the only kind of glucomannan which could be successfully purified by the copper complex method.

It was entirely soluble in cuprammonium solution and ethylenediamine as well as in sodium or potassium hydroxide solutions. This product remained in solution when its copper complex was decomposed by acetic acid and its Klason lignin content was 2%. It was slightly colored and partially soluble in water, the less soluble fraction being darker. The glucose to mannose ratio was 1:3 and the product corresponded to 6.7% of the original wood.

Paper chromatography of the hydrolysate obtained from the three glucomannan fractions showed that the difference between the three groups depended more upon the purity of the fractions than on the mannose to glucose ratio.

A second attempt to differentiate or fractionate the white spruce glucomannan was made by selective extraction of the holocellulose, combined with barium complex precipitation in conjunction with direct chlorination. Selective extraction was suggested by Hamilton and Quimby (86) and the barium complex method with chlorination was used by Meier (74).

The holocellulose was prepared by a modified method to attain a higher degree of delignification than is possible by the usual (76) chlorite procedure. After three delignifications in the usual way, more treatments were carried out in a 33% dioxane-water medium.

A holocellulose was obtained in 64% yield after the six treatments compared to a 78% yield after seven treatments by the standard technique.

Of the original wood hemicellulose and lignin, another 14% was thus solubilized by the use of dioxane.

A preliminary extraction of the holocellulose with 1% sodium hydroxide solution showed, again, that mostly galactan, mannan and pentosans were removed. However, much less glucose-yielding material was extracted with the dilute alkali. It was to be expected that a glucomannan would be harder to extract than a galactomannan, which is characterized by water solubility.

Extraction of the holocellulose with 2.5% sodium hydroxide lowered the yield from 64 to 48%. The resulting extract was mixed with barium hydroxide solution, but no precipitation occurred until a substantial amount of ethanol had been added. The barium complex was removed on the centrifuge and by addition of more ethanol (over 60%), another polysaccharide fraction was separated which apparently did not form a barium complex. Both fractions were hydrolyzed and chromatographed. Only the barium complex showed a substantial amount of hexoses, whereas the second fraction contained almost exclusively pentoses and uronic acids.

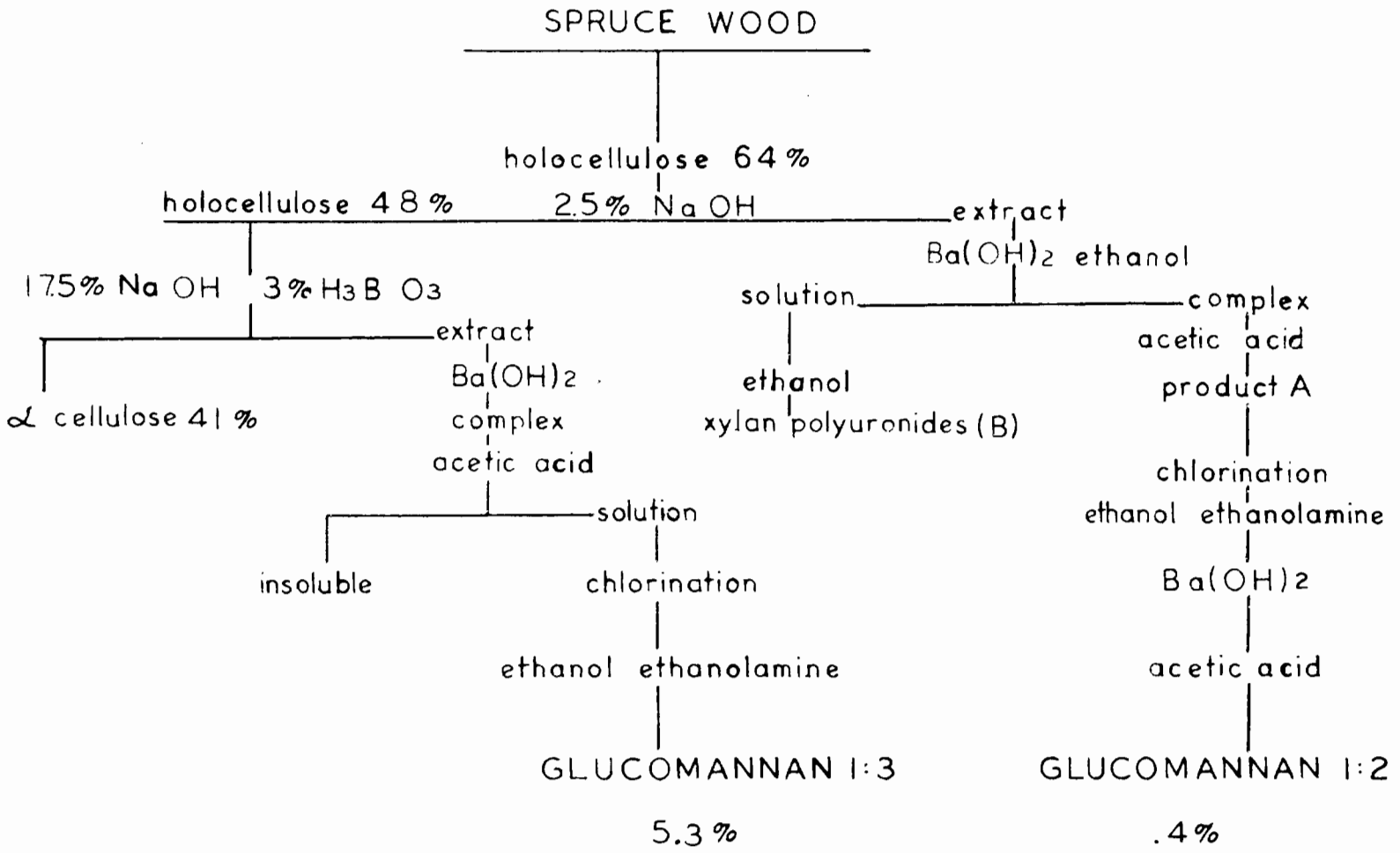
The barium-precipitatable material (product A) resisted chlorination at 5°C i.e., no purification was effected, but at room temperature chlorination yielded a pure glucomannan, soluble in alkali and water. This glucomannan was completely free of galactose and xylose residues. The polysaccharide showed a glucose to mannose ratio of 1:2. It was precipitated by barium hydroxide in the same way as product A. The product thus contained fewer mannose residues than the bulk of the glucomannan. It seems possible that in white spruce there exists a polysaccharide responsible for the excess of mannose, probably a short chain, which cannot be isolated as a copper or barium complex.

The 1:2 glucomannan needs drastic treatment to be isolated and cannot be directly extracted with water; it represented only 0.4-1.0% of the original wood and its influence on the total mannose balance in the white spruce seems to be negligible. Certainly it does not originate from the 1:3 glucomannan and it is possible that even its morphological origin

FIGURE 1

SUCCESSIVE EXTRACTION OF WHITE SPRUCE HOLOCELLULOSE

WITH 2.5 and 17.5% SODIUM HYDROXIDE SOLUTION


SPRUCE WOOD
holocellulose 64 %
2.5 % Na OH
holocellulose 48 %
extract
Ba(OH)2 ethanol
17.5 % Na OH
3 % H3 B O3
solution
complex
acetic acid
extract
Ba(OH)2
ethanol
product A
α cellulose 41 %
complex
xylan polyuronides (B)
acetic acid
chlorination
ethanol ethanolamine
solution
Ba(OH)2
insoluble
chlorination
acetic acid
ethanol ethanolamine
GLUCOMANNAN 1:3
5.3 %
GLUCOMANNAN 1:2
.4 %

is different, similar to the A and B mannans from ivory nut (28). The existence of a water-soluble galactoglucomannan, 0.08% of the original wood, in white spruce has recently been reported by Adams (87). The glucose to mannose ratio was shown to be 1:3.7, but again because of the low yield of this polysaccharide, it contributes little to the overall mannose content of the wood. This polysaccharide exists in a very small amount in 78% spruce holocellulose and can be isolated by fractionation of the copper complex. Isolation of this product by the barium complex method from 64% white spruce holocellulose failed.

Partial Hydrolysis of the Glucomannan

White spruce glucomannan is relatively easy to hydrolyze. Four methods were used to check the yields and relative proportions of the oligosaccharides formed. The acetolysis used to hydrolyze an Iles mannan glucomannan (34) seems to be too drastic and the yield was poor, especially for higher oligosaccharides. The partial hydrolysis with N-sulphuric acid at 80°C has a similar disadvantage. Hydrochloric acid has a tendency to cause the formation of a suspension which is hard to centrifuge even after addition of methanol. The best method was found to be the formic acid technique used by Jones and Painter (61,62). This method can be repeated several times to effect an almost complete hydrolysis of the glucomannan. It seems to be much milder than the methods employing mineral acids and apparently it is selective for glucomannans or mannans, whereas cellulose fragments are resistant. An experiment with ivory nut meal showed that after a number of formic acid treatments the remaining material contained glucose only.

The optical rotation of the glucomannan was followed during hydrolysis with 45% formic acid at 98°C to give the following results.

Time hrs.	$[\alpha]_D$
0	-32
1	-25
3	-19
7	0
10	+10
24	+24

The sirup obtained after the first hydrolysis was dissolved in 0.5N sulphuric acid to hydrolyze any formate ethers formed, but as there was no chromatographic evidence for their presence at any time, this treatment was later omitted. During the hydrolysis, and especially the sulphuric acid treatment, substantial amounts of a grayish insoluble precipitate was formed. No differences were observed in the proportion of oligosaccharides obtained during the five consecutive hydrolyses, which suggested that the oligosaccharides originated from a homogeneous heteropolymer. The resulting sirup was resolved on a charcoal column and five oligosaccharides were fully identified.

(A) 4-O-β-D-Mannopyranosyl-D-mannose

This compound (1070 mg.) moved on the paper chromatogram (solvent C) at the same rate as an authentic sample of (1→4)-β-D-mannobiose. Chromatographic examination of the hydrolysate indicated the presence of mannose only. The disaccharide crystallized from methanol, m.p. and mixed

m.p. 203-204°, $[\alpha]_D$ -7° (c 1.0 in water). The X-ray diffraction pattern and infrared absorption diagram were identical with those of the authentic specimen.

(B) 4-O-β-D-Mannopyranosyl-D-glucose

This disaccharide (82 mg.) was chromatographically identical with a sample of an authentic (1→4)-β-D-mannosyl glucose. Paper chromatographic analysis indicated equal amounts of mannose and glucose in the hydrolyzate. Crystals were obtained from moist ethanol, m.p. and mixed m.p. 201-202°, $[\alpha]_D$ +18° (c 1.0 in water).

(C) O-β-D-Mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannose

This compound (481 mg.) was chromatographically identical to an authentic specimen of a (1→4)-β-linked mannotriose. On complete hydrolysis, only mannose was obtained while partial hydrolysis yielded both mannose and mannobiose. After seeding with an authentic crystal, the product crystallized from ethanol, m.p. and mixed m.p. 165-169°, $[\alpha]_D$ -25° (c 2.5 in water).

(D) 4-O-β-D-Glucopyranosyl-D-mannose

The disaccharide (383 mg.) was chromatographically identical with an authentic specimen of a (1→4)-β-linked mannosyl glucose. Equal amounts of mannose and glucose were formed on hydrolysis. The compound crystallized from ethanol, m.p. and mixed m.p. 135-137°, $[\alpha]_D$ +16° (c 0.8 in water). The

derived octaacetate, prepared with acetic anhydride and zinc chloride, had m.p. 204-205°, unchanged on admixture with an authentic specimen (35), $[\alpha]_D$ +36° (c 1.0 in chloroform).

(E) O-β-D-Mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannose

Complete hydrolysis of this compound (100 mg.) indicated the presence of mannose only in the hydrolyzate, while partial hydrolysis gave rise to mannose, mannobiose and mannotriose on the paper chromatogram. Application of the method of Peat, Whelan and Roberts (83) suggested the presence of a tetrasaccharide, while oxidation with hypoiodite indicated an equivalent weight of 700 (calcd. 666) (95). The product crystallized spontaneously from methanol-water, m.p. 212-214°, raised to 232-234° after recrystallization from aqueous methanol, $[\alpha]_D$ -31° (c 1.6 in water) (82).

Oligosaccharide F

When hydrolyzed, this oligosaccharide (50 mg.) gave rise to glucose and mannose in a ratio of 1:2. The presumed trisaccharide moved faster on the paper chromatogram (solvents A and B) than mannotriose. Partial hydrolysis with formic acid yielded mannose, glucosyl mannose, glucose and mannosyl glucose, with the former two sugars predominating. The hydrolysis of the residual material did not show any substantial change in the glucose to mannose ratio. The yields of the identified oligosaccharides are summarized in Table IV.

TABLE IV

YIELD OF OLIGOSACCHARIDES

(A) Mannobiose	1,070 mg.
(C) Mannotriose	481 mg.
(H) Mannotetraose	113 mg.
(B) Mannosyl-glucose	82 mg.
(D) Glucosyl-mannose	383 mg.
Presumed oligosaccharides	
(E) Cellobiose	27 mg.
(I) Mannosyl-glucosyl-mannose	108 mg.

Fig. 2 presents percentage of alcohol and amount of eluent used to wash every oligosaccharide from the charcoal column.

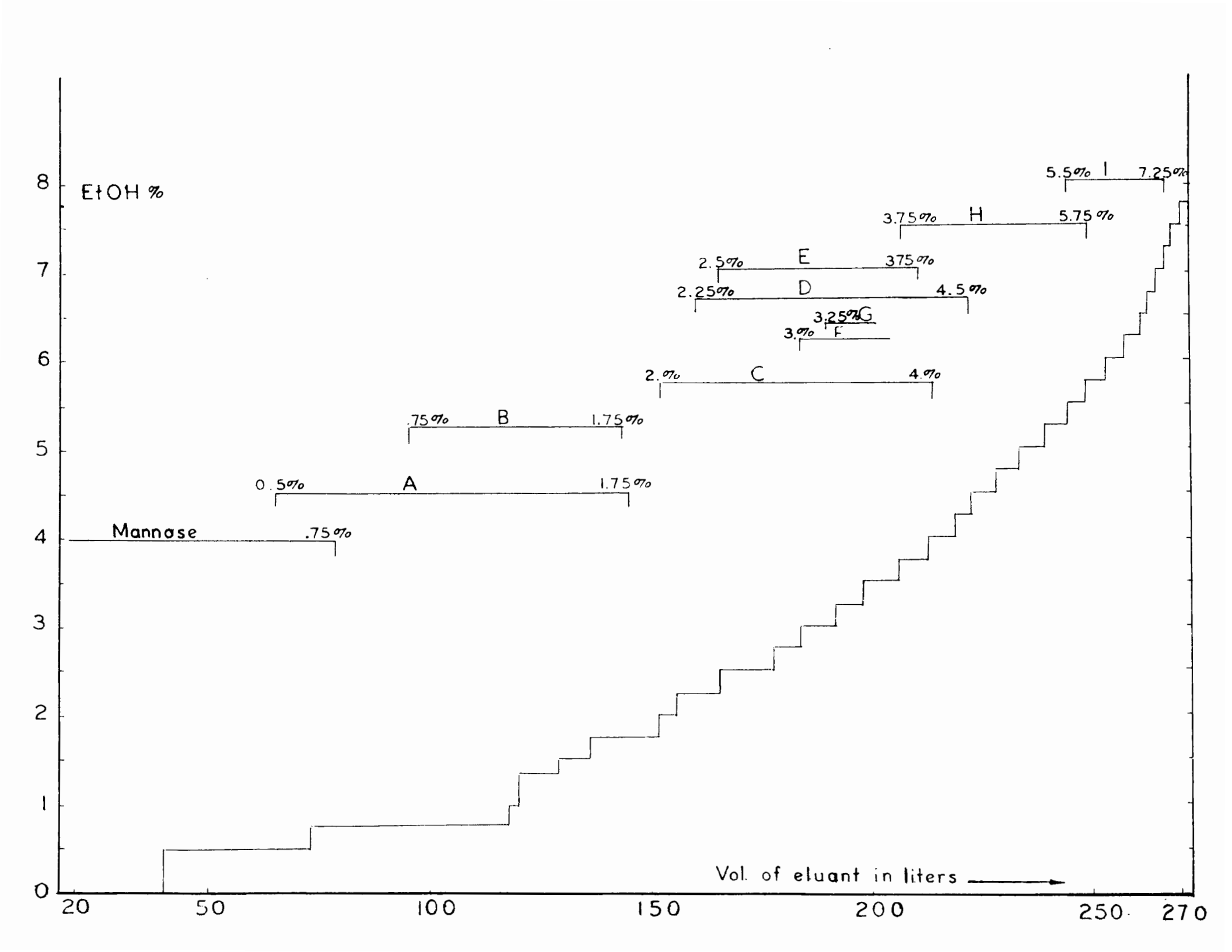
As was found in previous investigations (58,62,73,79), the yield of mannosyl-glucose and cellobiose is several times smaller than the yield of other oligosaccharides. It is independent of the methods of partial hydrolysis. There are no apparent reasons why the yields of glucosyl-mannose and mannosyl-glucose should differ by more than 1 to 2%, unless we assume that mannosyl-glucose is 4 to 5 times easier to hydrolyze than glucosyl-mannose.

The explanation for the small number of cellobiose units is different. Certainly cellobiose is the most stable oligosaccharide formed by partial hydrolysis of the glucomannan. In the series of trisaccharides only a trace of a product was present which could correspond to cellotriose. Three other trisaccharides contained more mannose than glucose. There was

FIGURE 2

SEPARATION OF OLIGOSACCHARIDES ON

NUCHAR CHARCOAL COLUMN


EtOH %
8
7
6
5
4
3
2
1
0
5.5% I 7.25%
3.75% H 5.75%
2.5% E 3.75%
2.25% D 4.5%
3.25% G
3.% F
2.% C 4.%
.75% B 1.75%
0.5% A 1.75%
Mannose .75%
20
50
100
150
200
250
270
Vol. of eluant in liters

no evidence that glucose was accumulated in the higher oligosaccharides or in the unhydrolyzed residue.

Thus it can be concluded that very few of the glucose residues in the glucomannan were contiguous. The high proportion of mannotetraose and mannotriose shows that contrary to glucose, mannose forms contiguous chains of four and maybe more anhydromannose units.

Methylation of the Glucomannan

The glucomannan was first methylated with dimethyl sulphate and sodium hydroxide. Complete methylation was achieved by the Kuhn method (66). The final product showed no free hydroxyl groups in the infrared absorption diagram. A portion of the fully methylated product was hydrolyzed and the resulting sirup was resolved on a charcoal column yielding three principal components.

Ethanol, %	Amount, g.	Component
2-5	1.870	2,3,6-Tri-O-methyl-D-mannose
5-7	.373	2,3,6-Tri-O-methyl-D-glucose
19-20	.063	Tri-O-methyl glucose chromatographically and electrophoretically identical to 2,3,4-tri-O-methyl-D-glucose.

2,3,6-Tri-O-methyl-D-mannose, a slightly colored sirup $[\alpha]_D$ -12° (c 1.0 in water) was characterized as its crystalline di-p-nitrobenzoyl ester, showing m.p. 187-188° (31). On the demethylation of this product, mannose was obtained. It was chromatographically identical to an authentic specimen of 2,3,6-tri-O-methyl-mannose obtained after hydrolysis of a fully methyl-

ated ivory nut mannan. A second tri-O-methyl-hexose moved a little faster than the first on the paper chromatogram at a rate identical to an authentic specimen of 2,3,6-tri-O-methyl glucose. The product crystallized spontaneously and after recrystallization it had m.p. and mixed m.p. 119-120°. The sugar was thus identified as 2,3,6-tri-O-methyl-D-glucose.

The third methylated sugar obtained in a small amount was chromatographically identical to an authentic sample of 2,3,4-tri-O-methyl-D-glucose. An attempt to prepare the crystalline derivative failed and so this methylated sugar could not be completely identified. A 2,3,4-tri-O-methyl-glucose has previously been isolated from a spruce glucomannan by Croon and Lindberg (33).

In addition to the above sugars, a small quantity (23 mg.) of dimethylhexose was also isolated, probably a demethylation product during the hydrolysis. The amount isolated is considered too insignificant to prove or disprove the existence of branching points. In the region on chromatogram, corresponding to tetra-O-methyl hexoses, a faint spot was observed on elution with 11.5% ethanol and with 13.5-14% ethanol two spots occurred immediately above this position. The first of these spots was reddish in color, when sprayed with o-amino-biphenyl and was considered to be a tri-O-methyl-xylose.

The rate of movement of the other two sugars was not identical to that of any known tetra-O-methyl-hexose. Hydrolysis of another portion of the methylated glucomannan and direct separation of the sugars on the paper chromatogram indicated the presence of a tetra-O-methyl hexose. Several chromatograms were obtained, the appropriate sections were excised

and the sugars eluted and weighed.

The amount of tetra-O-methyl hexose was only 0.1% of the total mixture.

It is possible that the nature of the non-reducing end groups would become more evident, if the methylation was carried out with a glucomannan which was more delignified than the present preparation (93). Periodate oxidation showed consumption of .97 mole of oxidant per hexose residue within the first 72 hours, thus suggesting the presence of an essentially linear polymer.

A determination of the number-average molecular weight of the nitrated glucomannan by osmometry was made with two samples, giving 28,400 and 30,700, respectively, corresponding to a D.P. range of 100-107. The corresponding weight-average values were 51,700 and 182, respectively, as determined by light-scattering measurements with n-butylacetate solutions of the nitrates.

From the above evidence a tentative structure can now be suggested for the white spruce glucomannan which contains glucose and mannose residues only in a ratio of 1:3. The isolation of 2,3,6-tri-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose from the fully methylated glucomannan shows that the polysaccharide is composed of mannose and glucose residues linked together by (1→4) glycosidic bonds. This conclusion is further corroborated by the consumption of .97 mole of oxidant per anhydrohexose unit on periodate oxidation of the glucomannan. The negative rotation of the latter indicates that the hexose residues are present in the β configuration. From the

yield of oligosaccharides it can be concluded that the glycosidic linkages are not of the same strength. This would perhaps explain why it is impossible to isolate a glucomannan from white spruce wood as a single homogeneous hemicellulose fraction instead of in the form of at least three fractions, which together represent only 82% of the total mannan content in the wood. It could also explain the considerable loss of glucomannan occurring during methylation. Contrary to the glucose, the mannose residues form longer contiguous arrangements.

The isolation in a relatively high yield of mannose-containing oligosaccharides shows that the glucomannan contains sections of adjacent mannose residues between which single glucose residues are inserted. It would suggest that the average single glucomannan repeating unit is $-\left[Gl-M-M-M\right]_n-$ as a result of the 1:3 glucose to mannose ratio. However, the relatively high yield of mannotetraose suggests that in reality a large number of anhydromannose units form chains longer than three anhydromannose units, thus postulating the existence of two different chain arrangements in the glucomannan molecule $-\left[M-M-M-M\right]_n-$ and $-\left[M-Gl-M-Gl\right]_n-$. This suggestion is further corroborated by the relatively high yield of mannosyl-glucosyl-mannose, the second most abundant trisaccharide. This oligosaccharide which contains two glycosidic bonds of apparently unequal strength should be very rare, if the arrangement of a glucose inserted between two mannoses were not quite common.

It might be that contiguous mannose chains which originated from glucomannan increase the ratio of mannose to glucose in crude glucomannan fractions.

EXPERIMENTAL

Material

Wood of white spruce (Picea glauca) was obtained through the courtesy of Mr. Sigmund Wang, Industrial Cellulose Research Limited, Hawkesbury, Ontario, in the form of a 40-year old, healthy specimen. The 40-60 mesh wood meal was exhaustively extracted with ethanol-benzene (1:2 v/v) and with cold water.

Analytical Methods

Alpha-cellulose, lignin, pentosan and uronic anhydride were determined by standard TAPPI methods. For estimation of the sugar content of the wood and the α-cellulose, 1-gram samples were hydrolyzed to the corresponding sugar mixtures according to the procedure of Saeman and co-workers (78). The sugars were separated on Whatman No. 1 filter papers, guide strips were cut out and the sugars were located by spraying with an acetic acid solution of phosphoric acid and o-aminodiphenyl (84). The appropriate areas of the paper chromatograms were excised and the sugars were eluted with water, diluted to a suitable volume and quantitatively determined by the o-aminodiphenyl method of Timell, Glaudemans and Currie (84).

Paper Chromatography

Chromatographic separation of sugars was carried out on Whatman

No. 1 or, for preparative purposes, on Whatman No. 3 MM filter papers. The solvents used included (A) ethyl acetate-acetic acid-water (9:2:2 v/v), (B) butan-1-ol-pyridine-water (10:3:3), (C) butan-1-ol-pyridine-water (6:4:3) and (D) butan-1-one-water (89:11).

All specific rotations were equilibrium values, unless otherwise stated, and were determined at a temperature of 21°C. Melting points were all corrected.

Isolation of the Glucomannan

Holocellulose was prepared in 78.6% yield from the extractive-free wood meal by seven treatments with acidified sodium chlorite at 70-75°C according to the procedure of Wise, Murphy and D'Addieco (76), fresh portions of sodium chlorite and acetic acid being added every hour. The holocellulose was recovered by filtration, washed with large quantities of distilled water, followed by ethanol and petroleum ether. The pentosan content of the product was 11.8% instead of the expected 12.5%, thus indicating some loss of hemicelluloses during the delignification.

Holocellulose, 89.7 g., was extracted in a nitrogen atmosphere at 30°C with 1,500 ml. of a solution containing 17.5% sodium hydroxide and 4% boric acid (77). The mixture was cooled to +5°C and the undissolved material, which amounted to 69% of the total holocellulose, was removed by filtration and washed rapidly with 1,500 ml. of cold, distilled water. Fehling's solution (32), 175 ml., was added to the combined filtrates,

when a thick, blue precipitate was formed within a few minutes. The precipitate was stirred with distilled water which was subsequently removed by centrifuging. This treatment was repeated twice and the combined washings were added to ethyl alcohol when a second precipitate of a copper complex was obtained. The main portion was suspended in cold water and the copper complex was destroyed by addition of an aqueous mixture of acetic and hydrochloric acids. The acid solution of hemicelluloses was centrifuged to yield a small amount of acid-insoluble material. The clear solution was added to an equal volume of ethanol, when a precipitate of crude glucomannan was formed. Yield: 12.73 g., corresponding to 11.2% of the original wood.

The second, minor precipitate of the copper complex was treated in a similar way. In this fraction, however, the glucomannan was found in the portion insoluble in dilute acids. The amount of this material was 1.73 g., or 1.5% of the wood.

Purification of the Glucomannan

The glucomannan was dissolved in the same alkaline solution. After centrifuging, Fehling's solution was added as before and the precipitate was washed four times with distilled water, the time of contact between the copper complex and the water being kept at 2 to 3 hours to ensure complete extraction. The copper was eliminated and the glucomannan was precipitated by addition of ethanol. This series of treatments was repeated once more with the number of washings extended to seven times. The final product was solvent-exchanged through ethanol and dried from

ethyl ether *in vacuo* at 50°C. The amount obtained was 7.60 g., corresponding to a yield of 6.7%, calculated on the original wood. The pure glucomannan was an almost white powder, easily soluble in 10% alkali.

The glucose component was identified chromatographically. Mannose was characterized through its phenylhydrazone, m.p. and mixed m.p. 198°C. The specific rotation of the glucomannan was -84.8° in 17.5% sodium hydroxide containing 4% boric acid (*c*, 1.0) and -33.5° (*c*, 1.0) in 10% sodium hydroxide. The intrinsic viscosity of the glucomannan in M cupri - ethylenediamine was 0.24 dl./g.

Preparation of Glucomannan by a Modified Procedure

Holocellulose was prepared in a yield of 64% by the method described above, involving the use of sodium chlorite. The partially delignified wood meal was recovered by filtration and was then subjected to three more treatments with acid chlorite in 33% aqueous dioxane. The holocellulose thus obtained was washed with water, dioxane and ethanol.

The holocellulose, 192 g., was first extracted with 2 liters of 2.5% aqueous sodium hydroxide in a nitrogen atmosphere at room temperature. The unextracted remainder amounted to 145 g., corresponding to 48.2% of the original wood. The extract did not yield a precipitate on addition of barium hydroxide solution. Subsequently, however, addition of 830 ml. of ethanol to 2,700 ml. of extract and washings yielded a precipitate which was separated on the centrifuge, dissolved with acetic acid and reprecipitated with ethanol. The product obtained (product A) amounted to 12 g.,

corresponding to 3.1% of the original wood.

The alkaline solution remaining after removal of the barium hydroxide complex was poured into ethanol when a precipitate was formed. The recovered polysaccharide (fraction B) consisted almost exclusively of short-chain acidic xylan molecules and was not further studied.

A portion of product A, 10 g., was dissolved in 250 ml. of water and treated at room temperature with chlorine gas for five minutes. It was then extracted with an equal volume of ethanol containing 3% of ethanolamine for five minutes at 95°C. The solution was cooled and 150 ml. of aqueous barium hydroxide was added slowly with stirring. The precipitate formed was separated, washed with 40% alcohol and decomposed with acetic acid (the insoluble portion was discarded.). The polysaccharide was precipitated by addition of alcohol to the acidic solution. After washing with 70% ethanol, anhydrous ethanol and ethyl ether, a pure, white glucomannan was obtained, corresponding to 13% of fraction A. The approximate ratio between glucose and mannose residues in this product was 1:2.

The residual holocellulose, 145 g., was extracted with 2.5 liters of 17.5% (w/w) aqueous sodium hydroxide containing 3% of boric acid in an atmosphere of nitrogen at +5°C. After shaking for two hours, the remaining undissolved α-cellulose was removed by filtration on sintered glass. Acetic acid was added to the combined filtrate and washings until a pH of 13.2 had been reached. Aqueous barium hydroxide was then added and the precipitate formed was separated, washed with 25% ethanol and dissolved by

addition of ice-cold acetic acid. The insoluble portion was recovered on the centrifuge.

The acidic glucomannan solution was diluted to 1,500 ml., cooled to +5°C and chlorinated for five minutes with gaseous chlorine. Alcoholic ethanolamine, 1,000 ml., was added and the mixture was heated for five minutes at 60°C. The precipitate formed on addition of ethanol was removed by centrifuging and washed free of barium ions with 30% ethanol. The yield of glucomannan thus obtained was 5.3%, while the loss in weight in this extraction was 7.2%. The final α-cellulose represented 41% of the weight of the original wood.

Partial Hydrolysis of the Glucomannan and Isolation of the Hydrolysate

The glucomannan as obtained by the first method, 12 g., was dissolved in 150 ml. of formic acid and diluted with water to 300 ml. The solution thus formed was heated at 96-98°C for four hours. The solution was cooled and the insoluble portion was discarded. On evaporation of the solution a thick, brown sirup was obtained. Repeated evaporation from methanol removed most of the formic acid. The sirup was diluted with 50 ml. of water followed by addition of 200 ml. of methanol. The precipitate formed was recovered on the centrifuge, dried from methanol, dissolved in formic acid and again subjected to partial hydrolysis as above. This treatment was repeated five times. After the fifth hydrolysis the residual solids weighed less than 1.5 g. and were discarded. The mixture of partially hydrolyzed material amounted to 9.5 g.

Resolution of the Partial Hydrolyzate

The hydrolyzate (9.5 g.) was placed on the top of a column (4.5 x 46 cm.) containing Nuchar carbon. Washing with water, 40 liters, followed by 25 liters of 0.5% aqueous ethanol removed all monosaccharides in addition to a portion of an oligosaccharide (A). Eight liters of 0.5%, followed by six liters of 0.75%, ethanol gave the pure oligosaccharide, free from any traces of mannose. An additional quantity of 16 liters of .75% ethanol gave a further quantity of the same, chromatographically pure oligosaccharide. Washing with 48 liters of 1.75% ethanol removed the remaining portion of product A together with a slower moving oligosaccharide (B). This mixture was further resolved by paper sheet chromatography. Altogether, 1,070 g. of product A was collected and .082 g. of oligosaccharide B.

Sixty liters of 2-4% ethanol eluted 481 mg. of a chromatographically pure oligosaccharide (C). Elution with sixty-five liters of 2.25% ethanol gave a mixture of product C and another oligosaccharide (D) which was resolved by paper chromatography, yielding 0.383 g. of the latter. Elution with 2.5-2.7% ethanol yielded oligosaccharide E, 25 mg., while elution with 3.0-3.25% alcohol gave two additional oligosaccharides (F and G). In the same way, washing with 45 liters of 3.75-5.5% ethanol yielded oligosaccharide H. Another portion of this compound was desorbed with 5.25-7.25% ethanol together with another oligosaccharide (I) which was obtained pure after paper sheet chromatography.

Identification of the Oligosaccharides

Oligosaccharide A

This compound moved with solvent systems A and C at the same rate as an authentic sample of β-1,4-mannobiose isolated from guaran (36, 37) and obtained through the courtesy of Professor R.L. Whistler, Purdue University. Chromatographic examination of the hydrolyzate indicated the presence of mannose only. The oligosaccharide was crystallized from a methanol-butanol mixture. After recrystallization from methanol the melting point was 202-203°C, undepressed on admixture with the authentic sample, $[\alpha]_D$ -7° (c, 1.2 in H_2O). The X-ray diffraction pattern and infrared absorption diagram were identical with those of the authentic specimen. Oligosaccharide A was therefore 4-O-β-D-mannopyranosyl-D-mannose.

Oligosaccharide B

This compound was chromatographically identical (solvent A, B and C) with a sample of authentic β-1,4-mannosyl glucose kindly supplied by Professor J.K.N. Jones, Queen's University. Paper chromatographic analysis indicated equal amounts of mannose and glucose in the hydrolyzate. Fine crystals were obtained when a moist alcohol solution of the compound was seeded with a small crystal of the authentic sample. M.p. and mixed m.p. was 200-201°C and $[\alpha]_D$ was +34° +18° (1 hr., c 1.0 in water). Oligosaccharide B was thus 4-O-β-D-mannopyranosyl-D-glucose.

Oligosaccharide C

This compound was chromatographically indistinguishable from an authentic specimen of a β-1,4-linked mannotriose obtained through the courtesy of Professor J.K.N. Jones, (solvents A and C). On hydrolysis, only mannose was obtained on the paper chromatogram, whereas both mannose and mannobiose were obtained on partial hydrolysis. On seeding with a crystal of the authentic sample, the product crystallized from moist ethanol, m.p. and mixed m.p. 164-169°, $[\alpha]_D$ -21° → -25° (2 days, c 2.5 in water). Oligosaccharide C was therefore O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannose.

Oligosaccharide D

This compound was chromatographically identical (solvents A and C) with an authentic specimen of a β-1,4-glucosyl mannose, kindly donated by Professor J.K.N. Jones. Chromatographic analysis suggested the presence of equal amounts of glucose and mannose in the hydrolyzate. Rhombic crystals were obtained from the solution in moist ethanol, m.p. and mixed m.p. 135-137°, $[\alpha]_D$ + 16° (c 0.8 in methanol). Oligosaccharide D was accordingly 4-O-β-D-glucopyranosyl-D-mannose monohydrate.

When a portion of the oligosaccharide, 70 mg., was acetylated with acetic anhydride and zinc chloride (61), 135 mg. of an acetylated product was obtained which crystallized in needles on addition of water to its ethanol solution. Its m.p. was 204-205°C, undepressed on admixture with an authentic sample of an octaacetate of a β-1,4-linked glucosyl

mannose, kindly supplied by Dr. T.J. Painter of this Division, (61). The product was thus identified as the octaacetate of 4-O-β-D-glucopyranosyl-D-mannose, $[\alpha]_D$ +36° (c 1.2 in $CHCl_3$).

Oligosaccharide H

Complete hydrolysis of this compound indicated the presence of mannose only on the paper chromatogram while partial hydrolysis suggested formation of mannotriose, mannobiose and mannose (solvents A and C). The product crystallized spontaneously from aqueous methanol, m.p. 212-214°, raised to 232-234° after recrystallization from aqueous methanol, $[\alpha]_D$ -31.2° (c 1.6 in water). A determination of the degree of polymerization by the method of Peat and co-workers (83) indicated a value of 3.5. Oxidation with hypoiodite (95) indicated a molecular weight of 700. Oligosaccharide H was accordingly O-β-D-mannopyranosyl-(1→4)-O-β-D-mannosyl-(1→4)-O-β-D-mannosyl-(1→4)-D-mannose, identical with a similar compound previously obtained by partial hydrolysis of a mannan from vegetable ivory (82).

Only small quantities were isolated of the other oligosaccharides and these could therefore be only partially characterized.

Oligosaccharide E

This compound had the same rate of movement on the paper chromato-

gram (solvents A and C) as cellobiose and maltose. Hydrolysis yielded only one sugar, glucose. The product failed to crystallize.

Oligosaccharide F

This compound, which moved slightly slower on the paper chromatogram than mannotriose, on hydrolysis yielded glucose and mannose in an approximate ratio of 1:2. It could not be induced to crystallize.

Oligosaccharide G

Hydrolysis of this oligosaccharide gave mannose only. The paucity of material available prevented further investigations.

Oligosaccharide I

This compound was obtained in a larger quantity than the other three referred to above. When hydrolyzed, it gave rise to glucose and mannose in an approximate ratio of 1:2. This presumed trisaccharide moved faster on the paper chromatogram than mannotriose but was desorbed from the charcoal column together with or after mannotetraose. Partial hydrolysis, effected by heating the compound for 2 hours with 50% formic acid at 85-90°C, yielded mannose, glucosyl mannose, glucose and mannosyl glucose, with the former two sugars predominating.

Methylation of the Glucomannan

Purified glucomannan (23 g.), isolated by the first method, was

introduced into a 5-liter, three-necked, round-bottomed flask fitted with a mechanical stirrer, a dropping funnel and an inlet for nitrogen, which was purified by passage through alkaline pyrogallol. Water, 500 ml., was added and the mixture was stirred in the nitrogen atmosphere for 2 hours. The flask was then immersed in ice-water and 60 g. of solid sodium hydroxide was added slowly with vigorous stirring. An additional 300 ml. of water was added to lower the viscosity of the solution and stirring was continued overnight.

Dimethyl sulfate, 100 ml., was added dropwise to the cooled mixture over a period of 5 hours, followed by 60 g. of solid sodium hydroxide. This treatment was repeated in all nine times, a total amount of 920 g. of sodium hydroxide and 1,800 ml. of dimethyl sulfate being added, with half of the former in the form of a 40% (w/w) solution. The mixture was finally heated at 60°C in a water-bath for 30 minutes to ensure complete saponification of the dimethyl sulfate.

The cooled reaction mixture was neutralized with acetic acid and evaporated to 2.5 liters on a rotary evaporator. The resulting solution and the precipitated salts were extracted exhaustively at 40°C with chloroform (five times), and the combined extracts were evaporated to dryness. The residue thus obtained was dissolved in tetrahydrofuran, 500 ml., and precipitated by dropwise addition with stirring to 3 liters of petroleum ether (b.p. 30-60°). A grayish precipitate of partially methylated glucomannan was obtained, 16.8 g.

The above product, 16.5 g., was dissolved in 130 ml. of anhydrous

dimethyl formamide (dried and freshly distilled over barium oxide) and a small insoluble fraction was discarded after centrifugation. Methylation was carried out according to Kuhn and co-workers (66). Methyl iodide, 60 g., and dry, freshly prepared silver oxide, 61 g., were added to the solution in a round-bottomed flask, which was shaken at room temperature for 24 hours. After this time, the remaining methyl iodide was removed by evaporation in vacuo and the mixture was poured into 500 ml. of aqueous 5% potassium cyanide which was heated slightly to dissolve colloidal silver. The aqueous solution was extracted three times with 100 ml. portions of chloroform. The combined extracts were exhaustively extracted with water in a liquid-liquid extractor for 2 days. The chloroform solution was dried with anhydrous sodium sulfate and evaporated to dryness and the residue was redissolved in tetrahydrofuran, 100 ml., and reprecipitated in 2 liters of petroleum ether with vigorous stirring. No disubstituted sugars were obtained on hydrolysis of the product, which was thus considered to be fully methylated. Yield: 13.7 g. Methoxyl content: 41%. The infrared diagram of the product indicated the absence of any hydroxyl groups.

Hydrolysis of the Methylated Glucomannan

A portion of the fully methylated glucomannan, 5 g., was dissolved in 100 ml. of 90% formic acid, diluted with the same amount of water (to give a 45% solution) and finally heated at 97°C for 4 hours. After cooling, methanol was added to the solution followed by evaporation to dryness. This treatment was repeated twice. After the formic acid had been thus

removed, the alcoholic solution of the hydrolyzate was added to an excess of water when a precipitate was formed. The latter amounted to 7.5% of the original material and was discarded.

The tri-O-methyl and tetra-O-methyl hexoses were eluted from the excised paper strips with the aid of guide-strips and an attempt was made to determine their relative amounts by the hypoiodite method (Jones (95)). The results obtained indicated a very approximate molar ratio between the tetra-O- and tri-O-methyl hexoses of 1:1000.

Identification of the Methylated Hexoses

Tri-O-Methylmannose (I)

This sugar moved on the paper chromatogram (solvent system D) at a slightly slower rate than 2,3,6-tri-O-methyl-D-glucose and at a rate identical with that of an authentic specimen of 2,3,6-tri-O-methyl-D-mannose obtained after hydrolysis of a fully methylated ivory nut mannan. Demethylation (79) of the compound yielded D-mannose in addition to partially methylated sugars. The sugar formed a slightly colored sirup, which could not be induced to crystallize, α_D -12° (c 1.0 in water). The sugar was characterized as its di-p-nitrobenzoyl ester, prepared according to the directions of Smith and Rebers (31) by treating a portion of the sirup (173 mg.) with p-nitrobenzoyl chloride in anhydrous pyridine. The crystals, after recrystallization from methanol, showed m.p. 187-188°. The methylated sugar was accordingly identified as 2,3,6-tri-O-methyl-D-mannose.

Resolution of the Mixture of Methylated Sugars on a Charcoal Column

The water-soluble hydrolyzate, 4.6 g., was added to the top of a column (4 x 82 cm.) containing activated Nuchar charcoal. The column was first eluted with 2 liters of water and subsequently with 40 liters of .5% aqueous ethanol. Elution with 25 liters of 1.75% ethanol yielded small amounts of dimethylated sugars. Resolution by paper chromatography gave 23 mg. of a di-O-methyl hexose, which was, however, considered to be a result of incomplete methylation or demethylation during hydrolysis. Elution with 72 liters of 2-5% ethanol gave 1.87 g. of a tri-O-methyl hexose (I). Elution with 70 liters of 5-7% ethanol yielded another tri-O-methyl hexose which moved on the paper chromatogram (solvent system D) slightly below compound I and which amounted to 373 mg. (II). When the alcohol concentration was increased to 13-15%, traces of a tri-O-methyl xylose or tetra-O-methyl hexose were obtained.

Resolution of the Mixture of Methylated Sugars on the Paper Chromatogram

A portion, 300 mg., of the mixture of methylated sugars was also resolved by paper chromatography using solvent system D. Again, traces of sugars corresponding in color reactions and rate of movement to a di-O-methyl hexose and a tetra-O-methyl hexose were observed in addition to the three tri-O-methyl hexoses which constituted the main portion of the mixture. The latter could not be completely resolved in this way.

Tri-O-Methyl-D-glucose (II)

This sugar moved on the paper chromatogram (solvent D) at a rate identical to that of an authentic specimen of 2,3,6-tri-O-methyl-D-glucose obtained from a fully methylated cellulose. It crystallized spontaneously from an ethyl ether solution. The product, after recrystallization from a mixture of ethyl acetate and ethyl ether, showed m.p. and mixed m.p. 119-120°C, $[\alpha]_D$ +70° (c 0.7 in water). The sugar was thus identified as 2,3,6-tri-O-methyl-D-glucose.

Tri-O-Methyl-D-glucose (III)

This sugar was chromatographically identical to an authentic sample of 2,3,4-tri-O-methyl-D-glucose (solvent D). Demethylation (79) yielded D-glucose. An attempt to prepare the crystalline azoyl ester (85) failed and the sugar could thus not be unequivocally characterized.

Preparation of the Nitrate Ester Derivative of the Glucomannan

A pure glucomannan was isolated by method I above with every precaution taken to avoid unnecessary depolymerization. A portion of the product (1 g.) was nitrated for 2 hours at 17°C with 100 ml. of the nitrating mixture of Alexander and Mitchell (90) which contains nitric acid, phosphoric acid and phosphorus pentoxide in the weight ratio of 64:26:10. The product was recovered in the usual way and washed free of acid over several days. Yield:1.6 g. The nitrate content of the product was determined by the semi-micro Kjeldahl procedure as described by Timell and Purves (34). The average value of two determinations was 13.38% (I),

corresponding to a degree of substitution of 2.71 and an average molecular weight of each anhydroglucose unit of 284. The product was easily soluble in acetone, ethyl acetate and butyl acetate. A duplicate preparation gave a product containing 13.45% (II), corresponding to an average molecular weight of the repeating unit of 286.

Osmotic Pressure Measurements

The osmometers used were of the original Zimm-Myerson type (91), as improved later by Stabin and Immergut (92). For easy filling of the osmometers, hypodermic syringes were used, fitted with long needles reaching to the bottom of the instrument. The latter were closed with mercury during the actual measurements. Stainless steel rods were employed for adjusting the level of the meniscus before each series of measurements. The solvent was n-butyl acetate, freshly distilled shortly before use. Gel cellophane membranes which had never been allowed to dry were used and in each case the membrane was "equilibrated" with the polymer solution for at least 18 hours before actual measurements were initiated with a fresh aliquot of the same solution. The temperature was kept at 30 $\pm$ 0.01°C. The static method of measurement was used and equilibrium was rapidly established within 2-3 hours.

The results obtained are given in Tables V and VI as well as in the following Figs. 3 and 4.

Assuming the weights given above for the repeating units in the two samples, the data obtained indicate a number-average molecular weight

TABLE V

Nitrate I — Nitrogen Content: 13.38%
D.S. = 2.71
M_R = 284

w*	h**	h/w
6.447	6.893	1.069
4.843	4.980	1.028
3.810	3.816	1.001
3.065	3.020	0.985
1.759	1.729	0.982
0	-	0.904

TABLE VI

Nitrate II — Nitrogen Content: 13.45%
D.S. = 2.75
M_R = 286

6.544	7.029	1.074
5.444	5.650	1.037
4.658	4.700	1.009
3.829	3.759	0.9817
3.345	3.325	0.994
2.944	2.833	0.962
1.873	1.805	0.963

*Concentration in g./kg. solution

**Osmotic height in cm. solvent.

FIGURE 3

MOLECULAR WEIGHT DETERMINATION BY OSMOMETRY

GLUCOMANNAN NITRATE I

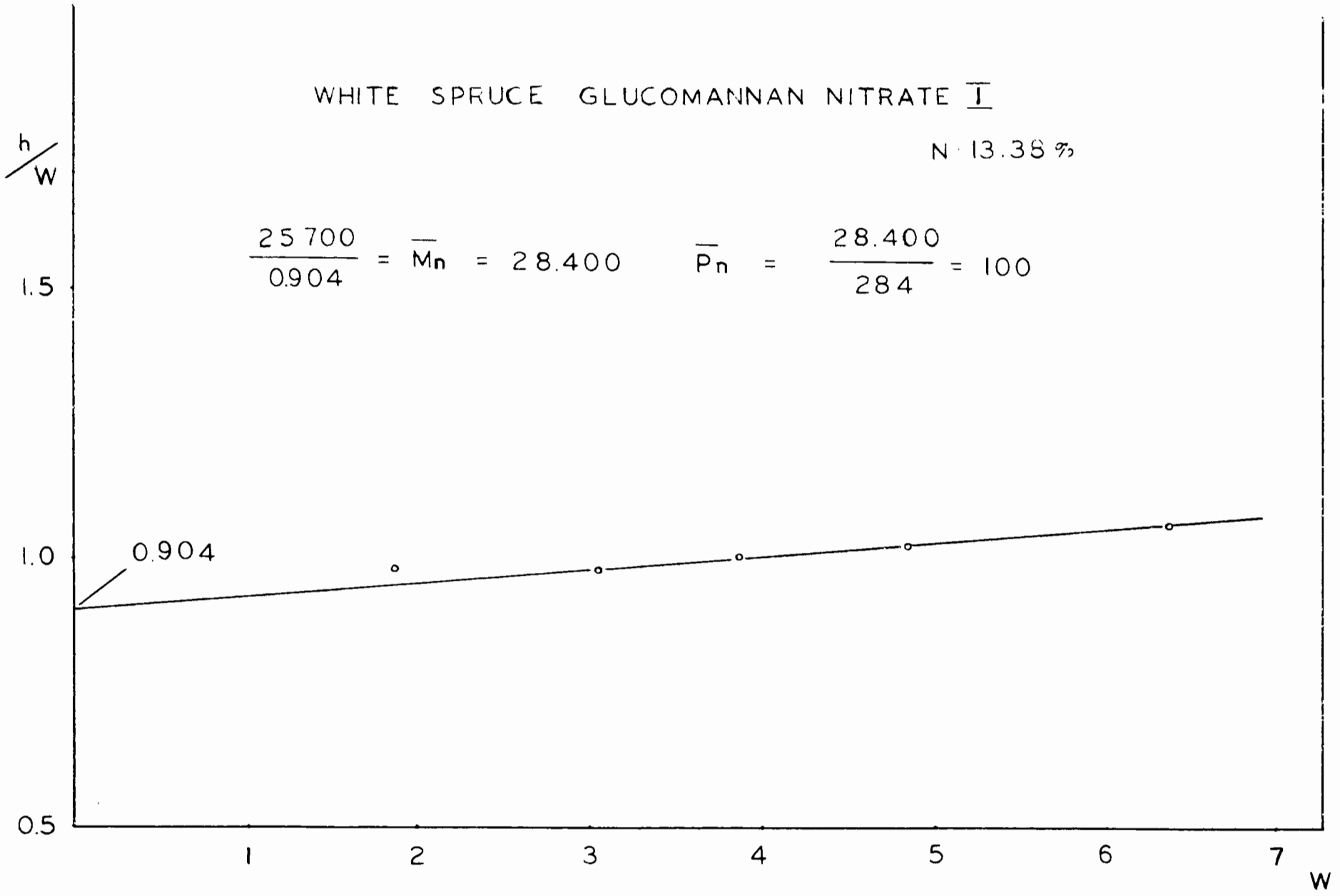
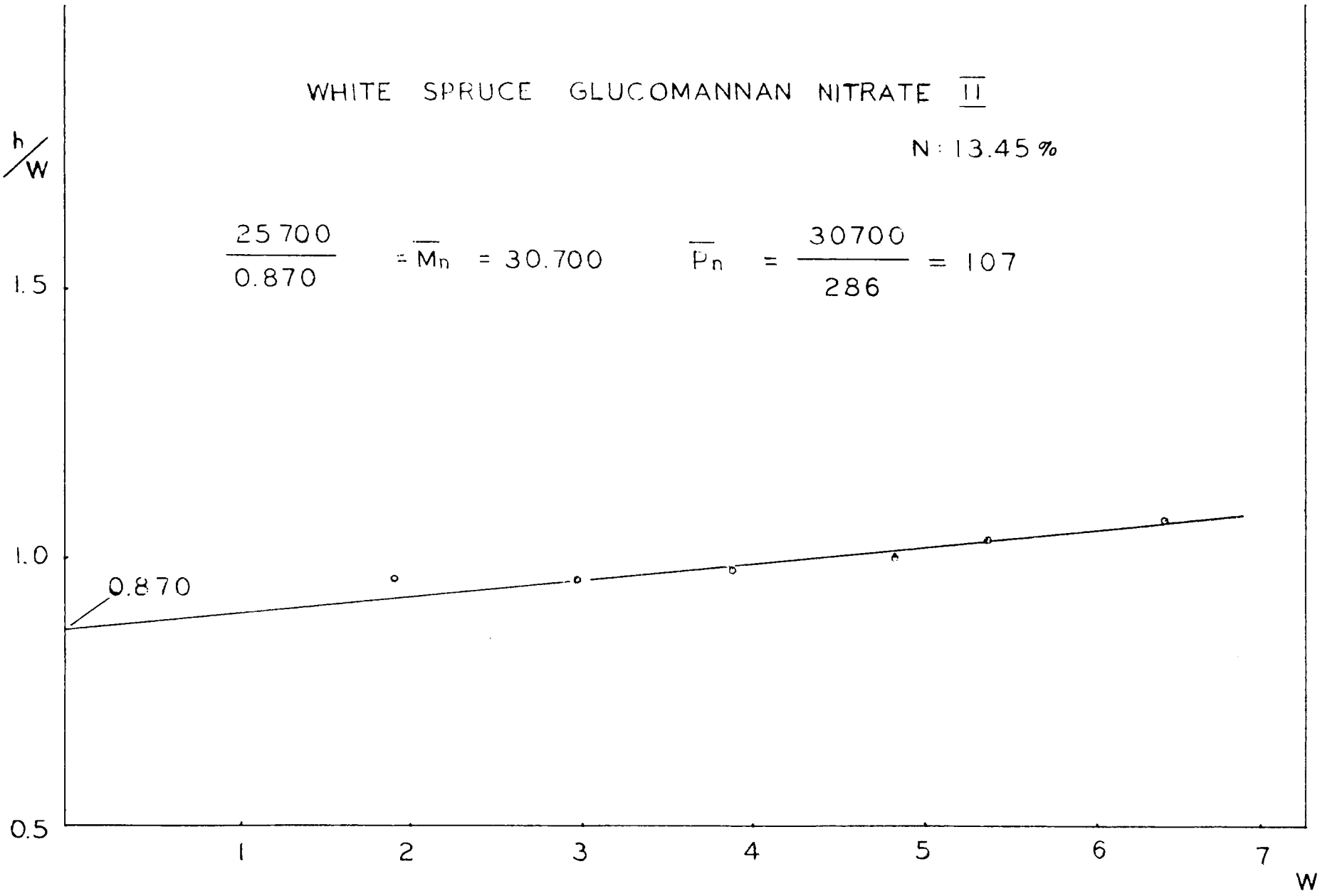

WHITE SPRUCE GLUCOMANNAN NITRATE I
N 13.38 %
25 700 / 0.904 = $\overline{M}_n$ = 28.400
$\overline{P}_n$ = 28.400 / 284 = 100
h/W
1.5
1.0
0.5
0.904
1
2
3
4
5
6
7
W

FIGURE 4

MOLECULAR WEIGHT DETERMINATION BY OSMOMETRY

GLUCOMANNAN NITRATE II


WHITE SPRUCE GLUCOMANNAN NITRATE II
N : 13.45 %
h/W
25 700 / 0.870 = $\overline{M_n}$ = 30.700
$\overline{P_n}$ = 30700 / 286 = 107
1.5
1.0
0.5
0.870
1
2
3
4
5
6
7
W

of 28,400 and 30,700, corresponding to a degree of polymerization of 100 and 107, respectively, for samples I and II. The higher value is probably the more correct one in view of the fact that depolymerization easily occurs during the isolation of the nitrate ester.

Periodate Oxidations

Samples, 150 mg., of the glucomannan, were oxidized for various lengths of time in the dark at 27°C and with continuous shaking with 50 ml. of 0.05 M sodium metaperiodate. The consumption of peridoate was measured by the usual sodium arsenite-iodine method. The results obtained suggested the consumption of 0.97 moles of periodate per hexose residue.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. A hemicellulose composed mainly of glucose and mannose residues has been isolated from the wood of white spruce (Picea glauca (Moench) Voss), its mannose component representing 82% of the anhydromannose units in this species. This product, on further purification, yielded a polysaccharide containing only glucose and mannose residues, with the latter representing 43% of the total amount present in the wood. This represents the first isolation of any wood glucomannan in a reasonably quantitative yield.

2. Since the ratio between glucose and mannose residues (1:3) remained essentially unchanged during the various stages of purification and as the product was electrophoretically homogeneous, it was concluded that the polysaccharide represented a true diheteropolymer.

3. No evidence could be adduced for the presence of any noticeable quantities of a galactoglucomannan in white spruce chlorite holocellulose.

4. Partial hydrolysis of the polysaccharide yielded the following oligosaccharides, all of which were obtained in crystalline form: 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), 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). The last compound has

not before been isolated from any wood. All compounds were obtained after resolution of the partial hydrolyzate on a Nuchar charcoal column by batchwise elution with aqueous ethanol of increasing concentration in conjunction with paper chromatography.

5. Resolution of the hydrolyzate obtained from the fully methylated glucomannan on a similar column yielded small amounts of tetra-O-methyl hexoses, 2,3,6-tri-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose in addition to a tri-O-methylglucose which was probably the 2,3,4-derivative.

6. Oxidation of the polysaccharide with sodium metaperiodate indicated the consumption of 0.97 mole of periodate per anhydrohexose unit.

7. The number-average degree of polymerization of the nitrate derivative of the glucomannan was 107 as determined by osmometry.

8. From the above evidence it is concluded that the glucomannan present in white spruce wood consists of glucose and mannose residues linked together by β-(1$\rightarrow$4) glycosidic bonds. Each macromolecule contains on the average slightly more than 100 hexose residues, very few of them consisting of contiguous anhydroglucose units. The degree of branching of the polysaccharide has still to be determined.

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