A STUDY OF THE HEMICELLULOSES PRESENT IN THE WOOD OF ENGELMANN SPRUCE [*Picea engelmannii* (Parry) Engelm.]

by

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A STUDY OF THE HEMICELLULOSES PRESENT IN THE WOOD OF ENGELMANN SPRUCE [Picea engelmannii (Parry) Engelm.]

ABSTRACT

Three hemicelluloses have been isolated from the wood of Engelmann spruce, an important western pulpwood species. After removal of the lignin, fractional extractions and precipitations with aqueous alkali gave an arabinoglucuronoxylan (8% of the wood), a galactoglucomannan (2%) and a glucomannan (8%). Their structure was determined by the classical methylation method, by oxidation with periodate and by identification of the oligosaccharides obtained on partial acid hydrolysis of the polysaccharides. The xylan consisted of β-(1→4)-linked D-xylose residues carrying α-(1→2)-linked 4-O-methylglucuronic acid and α-(1→3)-linked L-arabinofuranose side chains. The two hexosans contained a framework of β-(1→4)-linked D-glucose and D-mannose residues to which were attached α-(1→6)-linked D-galactopyranose residues. Both hemicelluloses contained glucose and mannose in a ratio of 1:3 but the galactoglucomannan possessed ten times more galactose side chains than the glucomannan. An oligosaccharide giving equimolar amounts of galactose, glucose, and mannose on hydrolysis was obtained from the galactoglucomannan. This constituted the first unequivocal evidence for the triheteropolymer nature of this type of wood polysaccharide.

ENGELMANN SPRUCE

The commercial wood producing trees can be divided into two classes, the angiosperms and the gymnosperms. In the angiosperm class, usually referred to as the hardwoods, most of the important deciduous trees are dicotyledons. In the gymnosperm class the sub-order, Coniferales, usually referred to as the conifers or softwoods, contains the only genera of importance. The most important of these genera are: Pinus, the pines; Abies, the firs; and Picea, the spruces.

Engelmann spruce (Picea engelmannii (Parry) Engelm.), typically a mountain species, is found in the Rocky Mountains of the Western United States and Canada. It is a tolerant species, which occurs in large pure stands or associated with other conifers from an elevation of 1500 ft. up to the timberline (about (12,000 ft.). The species has good seed production and germination (up to 97%), but its growth is usually limited by a short growing season; under suitable conditions the species can attain dimensions of 165 ft. by 6 ft. The tree has a light, soft, non-porous wood which is a source of softwood and pulpwood lumber in British Columbia and the United States.

INTRODUCTION

The mechanical strength of any wood is due to three components; cellulose, hemicellulose and lignin. The cellulose and the hemicelluloses are deposited in the secondary wall of the cells, cellulose in the form of long part-crystalline microfibrils surrounded by the amorphous hemicellulose; and the whole being embedded in a matrix of lignin. Lignin, a three-dimensional polymer based on phenyl-propane units linked directly or by oxygen atoms, has a complex structure, all details of which are still not known. Cellulose, the chief constituent of the cell wall of all land plants is a linear polymer composed of D-glucose units linked by β-glycosidic bonds and in its native state has a weight-average degree of polymerisation in the region of 10,000. In most cases, unlike cellulose, the hemicelluloses are hetero-polymers containing D-xylose, D-glucose, D-mannose, D-galactose, L-arabinose, 4-O-methyl-D-glucuronic acid and occasionally a few other sugars. The more abundant hemicelluloses usually contain xylose or mannose and glucose as the main components and closely resemble cellulose in the manner of linkage, although they have a much lower degree of polymerisation (about 50-400).

In hardwoods the main hemicellulose present is usually a β(1→4)-linked xylan, containing side chains of 4-O-methyl-D-glucuronic acid, whereas in softwoods the predominant hemicellulose is usually a glucomannan, a linear polymer of (1→4)-β-linked glucose and mannose. In this thesis the investigation of the chemical constitution of three hemicelluloses from the wood of Engelmann spruce is discussed.

HISTORICAL INTRODUCTION

The name hemicellulose was first introduced in 1891 by Schulze,[1] who suggested the term to define the class of plant polysaccharides, which were soluble in aqueous alkali, and which were more easily hydrolysed by mineral acids than cellulose. The name is still accepted in polysaccharide chemistry, although some compounds classed as hemicelluloses, do not exactly conform to the original definition. The individual hemicelluloses are named according to the system proposed by Karrer[2] in 1925. In this system a polysaccharide is named after its constituent sugars, such as xylan, glucomannan and arabinogalactan.

The methods of investigation into hemicellulose chemistry have been reviewed by E.L. Hirst[3] and very recently by Bouveng and Lindberg.[4] The more important methods are; complete hydrolysis and identification of the component sugars, partial hydrolysis and identification of the oligosaccharides obtained, methylation and oxidation studies, including oxidation with sodium meta-periodate. The successful application of these methods is very largely dependent on the techniques of chromatography, including the more recent use of gas-liquid partition chromatography.[5]

Among the physical methods frequently applied in the study of hemicelluloses for the determination of the average molecular weights, are isothermal distillation, osmometry, viscometry, and ultra-centrifuge and light-scattering measurements.

The application of these techniques usually leads to a reasonable interpretation of the structure of a polysaccharide. However, the application is useful only if the polymer is in a pure state, thus purification is still one of the main problems in polysaccharide chemistry. Polysaccharides often occur in Nature as complex mixtures, consequently insufficient purification could well explain some of the conflicting results reported in the literature.[23] The methods for the isolation of a wide group of polysaccharides have been discussed in detail by Whistler and Smart,[6] and briefly by Bouveng and Lindberg.[4]

In favourable cases fairly pure polysaccharides can be obtained directly from the raw materials by suitable extraction methods.[6,7] Dimethyl sulphoxide, has been used for the isolation of polysaccharides containing O-acetyl groups,[8,9] which appear to be attached to the main hemicellulose only.[9] Polysaccharides usually possess high molecular weights, therefore precipitation procedures are sometimes the only method available for purification, and these procedures are of only limited application because of the tendency towards coprecipitation and because of possible fractionation by molecular weight due to polymolecularity of the polymers.

Various methods of precipitation have been used; such as changing solvent composition, the use of quaternary ammonium salts, reviewed recently by Scott,[10] and the formation of heavy metal complexes.[12,13] Even these methods can be dependent on the nature of the material the polysaccharide is associated with; it has often been observed that hemicelluloses containing lignin could not be separated into the pure components without further delignification.[4]

Chemical analysis of the component sugars, determination of the optical rotation and electrophoretic tests are used to give information about the homogeneity of the polysaccharides. Unfortunately, however, these are not unambiguous criteria of the purity of a polysaccharide and each biological material sets its own specific problem of purification.

The work of O'Dwyer from 1923 onwards made many important contributions to the field of hemicellulose chemistry.[13] Her analysis of wood hemicelluloses, indicated the presence of a high proportion of D-xylose residues associated with those of a methyl hexuronic acid. However, more than 25 years were to elapse before the introduction of chromatographic techniques for the separation of sugars[14] led to the first complete determination of the structure of a hemicellulose by Hirst and co-workers,[15] who obtained a linear xylan from esparto grass, which consisted entirely of β-(1→4)—linked xylose residues. Further evidence for the chemical structure of such xylans came from the isolation of xylobiose and xylotriose by Jones and Wise[16] and the isolation of the complete series of oligosaccharides from xylobiose to xyloheptose by Whistler and his co-workers provided proof for the presence of the β-(1→4)- linkage in a xylan molecule.

Early analysis of the hemicelluloses of wood had indicated the presence of a methyl ether of a hexuronic acid.[13] This observation was later confirmed by the work of Jones and Wise, who proved that some hexuronic acid residues were associated with a xylan by the isolation of an aldobiuronic acid from the partial hydrolysate of aspen wood.[18] Wood xylans are characterised by the presence of 4-O-methyl glucuronic acid linked to the 2-position of a D-xylose residue in the main chain, consequently the

aldobiuronic acid first characterised by Jones and Wise,[18] as O-(4-O-methyl-α-D-glucosyluronic acid)-(1→2)-D-xylose, is often isolated from the partial hydrolysates of such xylans. The partial hydrolysis of Monterey pine[19] also gave a minor yield of the (1→3)-linked aldobiuronic acid, but the role of this acid in the polysaccharide is not known. In addition to the uronic acid side groups, some wood xylans, especially those from the soft-wood hemicelluloses, contain varying proportions of L-arabinose residues, which are probably attached to the main chain by (1→3)-linkages.[20]

Because of the labile nature of the arabinofuranose ring structure, Bishop and Whitaker[21] had to use enzymic hydrolysis in order to isolate oligosaccharides containing both arabinose and xylose, thus demonstrating the presence of arabinose linked to a xylan. Recently Aspinall and co-workers[22] proved that the arabinose side chains in an arabinoxylan were present in the furanose form; they subjected the xylan to catalytic oxidation which oxidized the primary hydroxyl groups to a carboxyl group. The presence of the latter group stabilised the previously labile xylopyranose-arabofuranose linkage, enabling them to obtain a low yield of an aldobiuronic acid consisting of an oxidized arabofuranose, and a xylose residue.

Thus the principal hemicellulosic component of the grasses, cereal straws and hardwoods, and the minor hemicellulosic components of the softwoods form a family of xylans,[23] the members of which possess a main chain of β-(1→4)-linked xylose residues, a true xylan, to which are attached various proportions of L-arabinofuranose, D-glucuronic acid or 4-O-methyl-D-glucuronic acid units. The main chain of β-(1→4)-linked xylose residues which is characteristic of the xylans from all the land plants so far

examined contrasts with the xylan from the red alga Rhodymenia palmata,[23] which contains chains of (1→3)- and (1→4)-linked xylose, and the xylan from the green alga Caulerpa filiformis, in which the chain is composed entirely of (1→3)-linked xylose.[24]

The fractionation of the mannose containing polysaccharides can be very troublesome.[25,26] The report in 1956 by Hamilton and co-workers, represented the first isolation and characterisation of a wood glucomannan. It was formerly believed that the mannose residues found in the analysis of plant products were mainly derived from mannan polysaccharides. For example, the polysaccharide from Amorphallus, the so-called Iles mannan was thought to be a mannan until 1956, when Smith and co-workers established the structure as a glucomannan. The only true mannans (i.e. containing more than 95% of D-mannose residues) so far isolated from land plants are those from vegetable ivory,[23] and it is probable that glucomannans are present, to account for most of the mannose content of plant hemicellulose.

In 1923 one of the earliest attempts to isolate a mannose containing polysaccharide was reported by Sherrard and Bianco.[27] Other early reports[28,29] were not substantiated by the data recorded, but in 1937 Brauns and co-workers appear to have isolated a glucomannan from wood pulp.[30,31] Nishida and Hashima used methylation and acetolysis to investigate the structure of a glucomannan,[32] but because chromatography was not then available the structure could not be completely established. Many years later Leech[33] and Anthis[34] provided the first unequivocal evidence for the presence of a glucomannan in wood by the isolation of a disaccharide containing both glucose and mannose which was later confirmed by Jones and co-workers.[35]

In 1951 Jones and Painter[36] reported and characterised several oligosaccharides containing mannose and glucose; glucosyl mannose from a crude extract of loblolly pine sawdust and mannosyl glucose, glucosyl mannose, mannobiose and mannotriose from a hemicellulose fraction of loblolly pine. The data from methylation and periodate oxidation studies, and from the above partial hydrolyses, led the authors to postulate that the glucomannan from loblolly pine is composed of linear chains of β-(1→4)-linked D-mannose and D-glucose residues.

Similar work led Hamilton and co-workers to formulate the structure of a glucomannan isolated from the woodpulp of western hemlock (Tsuga heterophyllia) to be a linear polymer of β-(1→4)-linked D-mannose and D-glucose residues, having an average chain length of 137 hexose units.

Since the above formulation by Hamilton, the isolation and structural determination of many other wood glucomannans have been reported. Since glucomannans are more abundant in the coniferous woods the earlier studies were carried out on conifers, thus glucomannans have been isolated from the wood of Sitka spruce,[37] Norwegian spruce,[38] and white spruce,[39] (see Table IV) Recently, however, several glucomannans have been isolated in small yield from deciduous woods.[40,41]

In 1960 Hamilton and co-workers reported the isolation of a polysaccharide from a mixture of slash pine (Pinus elliottii) and long leaf pine (P. palustris),[42] which they identified as a galactoglucomannan. Since this time, work has proceeded in the authors laboratories on the isolation and characterisation of several polysaccharides, tentatively

identified as galactoglucomannans.[43] A review of the general characteristics of the glucomannans so far isolated shows that, with two exceptions, all contain a small percentage of galactose,[43] which was usually explained as an impurity, although careful purification failed to remove it. In view of the recent isolation of galactosyl mannobiose and galactosyl mannose from the partial hydrolysates of a glucomannan,[52] and the isolation, after methylation, of half of the galactose residues as tetra-O-methyl galactose,[53] it seems probable that the softwoods contain two types of galactoglucomannans, one previously known as a "glucomannan" and containing less than 5% galactose, and the other, the very recently isolated galactoglucomannan, containing more than 16% galactose. These two types will be discussed later, in conjunction with the "glucomannan", and galactoglucomannan isolated by the author.

The number of plant hemicelluloses now characterised indicate a possible relationship between molecular structure and botanical origin. Thus closely related plants usually have similar hemicellulosic constituents, whereas the properties of hemicelluloses from widely differing origins show that marked differences can occur.[23,24]

The only other types of hemicellulose which occur to any extent in wood, are the arabinogalactans present in many softwoods,[23] the largest proportion occurring in the larches.[44,45] A β-(1→4)-linked galactan has been isolated from spruce compression wood by Bouveng and Meier.[46] Pectic polysaccharides (mainly composed of D-galacturonic acid and L-arabinose) also occur widely in Nature, but in wood these are evenly distributed and are not usually isolated in the hemicellulose fraction.

The structure of a polysaccharide cannot be established until it is obtained in a pure state, which usually entails the delignification (completely or in part) of the wood. The methods available, the use of chlorine,[47] acidified sodium chlorite[48] or the industrial methods, are known to effect some degradation of the material.[23,49] It is therefore often impossible to isolate the hemicellulosic material in an unchanged (chemical) form, especially as the possibility of physical or chemical bonding to the lignin is still unresolved.[50] However, since the extent of the degradation is usually small,[49] the determination of the structures of the hemicelluloses is still of considerable significance.

The biosynthesis of the hemicelluloses, is presently of great interest, most of the work reported being on xylans,[51] where radio-active tracer studies have shown that the precursor of xylose in a xylan chain is glucose.

The structures of the hemicelluloses from many deciduous and coniferous trees have been reported. From these studies, certain generalisations have emerged, one of the most notable being that hemicelluloses from coniferous trees have a high mannose content, whereas those from the deciduous trees are low in this sugar and possess a higher xylose content. Part I of this thesis will describe the arabino-4-O-methylglucuronoxylan, and Part II will describe the glucomannan and galactoglucomannan hemicelluloses present in the wood of Engelmann spruce (Picea engelmannii (Parry) Engelm).

RESULTS AND DISCUSSION

PART I

WOOD XYLANS

WOOD XYLANS.

Polysaccharides composed mainly of xylose residues are widely distributed in Nature. The xylans occur in the cell wall of many land plants and also in some water plants and as they often predominate,[10,11,12] they can be isolated in a fairly pure state by simple extraction procedures.[6,7] Thus Hägglund and co-workers isolated a xylan containing acetyl groups using dimethyl sulphoxide as the solvent.[8]

Several methods have been used to elucidate the structures of the xylans. These are (i) partial acid or enzymic hydrolysis of the pentosan and isolation of oligosaccharides containing β-(1→4)-linked D-xylopyranose.[16,17] D-xylose and L-arabinose[21] and D-xylose and 4-O-methyl-D-glucuronic acid.[18,57] (ii) methylation of the pentosan, hydrolysis of the methylated product, and identification of the methylated sugars obtained[20,54,68,70] and (iii) quantitative oxidation of the polysaccharides by periodate and identification of the products obtained by reduction and hydrolysis of the periodate-oxidized polysaccharide. The results from the application of the above methods show that the main feature of all xylans is a main chain composed of β-(1→4)-linked D-xylopyranose units possessing in general, side groups of hexuronic acid and/or L-arabofuranose residues. In the case of the Gramineae the xylan backbone can be branched,[23] but with two exceptions,[54,71] the main chains of the wood xylans have been reported to be linear. Further evidence in support of the β-(1→4)-type linkage of the D-xylose residues is

derived from the action of specific enzymes, the negative specific rotations of the various xylans $[\alpha]_D$ (usually about -30 to -100°) and their resistance to acid hydrolysis.

Two xylans have been reported which contain xylose residues only, namely those from esparto grass[15] and tamarind seed,[69] but xylans are usually heteropolymers which contain varying proportions of other sugar residues. Cereal straws and grasses usually contain hexuronic acid residues and the softwoods have intermediate compositions containing both hexuronic acid and L-arabinose residues.

The xylans from woods differ in average molecular size and in the content of the non-xylose residues; generally the proportion of the hexuronic acid units (4-O-methyl-D-glucuronic acid) is higher in the softwood xylans (15-20%) than in the hardwoods (8-15%).[23] The structure of many hardwood hemicelluloses have been investigated and some pertinent data is recorded in Table I. More recently the softwood xylans have been studied in some detail. Examples of these arabino-4-O-methyl-glucuronoxylans are those from western hemlock,[72] Norway spruce,[73] European larch,[74] loblolly pine,[54] eastern white pine,[20] amabilis fir,[68] and Engelmann spruce[60] which are collected in Table II. Although there is no unique structural division between the xylans from the different groups of land plants, the sugar residues in all the groups show the same preferred modes of linkage to xylan backbone; that is the hexuronic acids are linked by α-(1→2) bonds[18,76] and the L-arabofuranose is probably linked by α-(1→3) bonds since the rotation of the xylan does not change appreciably with the arabinose content.

TABLE I

The Composition of Hardwood Xylans

Species	Uronic acid/ Xylose	DPn	Ref.
European beech (*Fagus sylvatica*)	9	80	90
American beech (*Fagus grandifolia*)	8	54	71
Finnish birch (*Betula verrucosa*)	15	22	91
White birch (*Betula papyrifera*)	10	200	92
White birch (Bark)	10	230	77
White elm (*Ulmus americana*)	7	..	94
Trembling aspen (*Populus tremuloides*)	9	212	70
Apple wood (*Malus pumila*)	6	120	78
Cherry wood (*Prunus avium*)	7	110	79
Sugar maple (*Acer saccharum*)	10	..	93

TABLE II

The Composition of Softwood Xylans

Species	Xylose/ Arabinose	Xylose/ Uronic acid	$\overline{DP}n$	Ref.
European larch (*Larix decidua*)	..	6	100	74
Tamarack (*Larix laricina*)	17	6	20	102
Amabilis fir (*Abies amabilis*)	7	6	95	68
Amabilis fir (Bark)	10	6	..	67
Norwegian spruce (*Picea abies*)	6	5	85	73,85
Sitka spruce (*Picea sitchensis*)	High	5	..	89
Engelmann spruce (*Picea engelmannii*)	7	6	130	..
White pine (*Pinus strobus*)	7	7	101	20
Monterey pine (*Pinus radiata*)	8	6	..	19
Maritime pine (*Pinus palustris*)	10	7	..	20
Loblolly pine (*Pinus taeda*)	..	6	..	54
Scots pine (*Pinus silvestris*)	7	6	121	75
Western hemlock (*Tsuga heterophylla*)	12	4	..	72

The uronic acid content of the wood xylans (see Tables I and II) is due to the presence of 4-O-methyl-D-glucuronic acid, however, in other xylans D-glucuronic acid can also be present. L-arabinose is usually present in the furanose form, which is very susceptible to hydrolysis, consequently the arabinose content of any xylan may depend on the method of extraction and purification of the material. The lability of this linkage could also explain some of the wide variations reported for the content of this sugar in softwood xylans, which is usually about 12%.

It is interesting to note that xylans have been recently isolated from the bark of two species of wood bark [67,77] and that these xylans are remarquably similar to the xylan present in the parent wood inspite of the considerable differences in morphology between the wood and the bark.

Hemicelluloses are composed of polymer molecules, which vary in molecular weight (M) or degree of polymerisation (DP). i.e. they are polymolecular. Several methods are commonly in use for the determination of degrees of polymerisation; isothermal distillation and osmometry, which give a number-average molecular weight ($\overline{M}n$) and light scattering, which gives a weight-average molecular weight ($\overline{M}w$).

The values reported for the $\overline{DP}n$ of xylans (where $\overline{DP}n = \overline{M}n$/weight of polymer unit) have usually been determined on the acetylated or the methylated hemicellulose and are in the region of 70 to 130 (see Tables I and II). A recent study indicates that about 30% degradation occurs during the preparation of these derivatives. [87] This corresponds to initial $\overline{DP}n$ values of 100 to 175. However, most xylan polysaccharides are

isolated after delignification of the wood by acid chlorite, which has already caused depolymerisation.[88] Glaudemans and Timell using similar acid chlorite conditions[49] reported that the $\overline{DP}n$ of a 4-O-methylglucurono-xylan dropped from a value of 215 to 120. This indicates that the $\overline{DP}n$ of native wood xylans is probably in the range 200 to 220 with the $\overline{DP}w$ being about twice this value[20] and the corresponding values for bark xylans being even higher.[77] When the ratio of the weight- to the number-average molecular weight lies in the region of 2 the polymers possess a Flory distribution of molecular weights.[8,20,77]

ENGELMANN SPRUCE ARABINO-4-O-METHYLGLUCURONO-XYLAN

RESULTS AND DISCUSSION

Finely ground spruce wood was extracted with hot alcohol-benzene solution and then with hot water to give an "extractive free" wood. Acid hydrolysis of the wood was followed by de-ionisation and separation into neutral and acidic fractions. Qualitative analysis by paper chromatography indicated the presence of xylose, glucose, mannose, arabinose and galactose in the neutral fraction and 4-O-methyl glucuronic acid, an aldobiuronic acid and higher homologues in the acid fraction.

The acid sugars were resolved on a charcoal-Celite column by gradient elution.[80,95] (See Table IX). The 4-O-methyl-D-glucopyranosyl uronic acid was identified as its crystalline methyl ester methyl glycoside and as 4-O-methyl-D-glucopyranose obtained by the reduction of the above ester. The aldobiuronic acid was identified as methyl 2-O-[methyl(2,3-di-O-acetyl-4-O-methyl-α-D-glucopyranosyl)uronate]-3,4-di-O-acetyl-αβ-D-xylopyranoside,[76] which was obtained by acetylation of the methyl ester methyl glycoside.

Delignification of the extractive-free wood meal using acetic acid and sodium chlorite gave a holocellulose in 72% yield. Extraction of the holocellulose with 24% potassium hydroxide solution and addition of the filtrate to four volumes of acidified ethanol gave a precipitate, which after washing and drying gave crude Hemicellulose A. (Yield: 14.5% of the dry wood). The portion insoluble in potassium hydroxide solution

was retained for further extraction. Analysis of the crude Hemicellulose A (see Table X) indicated that it contained predominantly xylose and 4-O-methyl glucuronic acid together with smaller amounts of the sugars present in the wood. Two treatments of the crude polysaccharide with barium hydroxide solution gave the purified Hemicellulose A and the insoluble barium complex of the hexosan impurities, which was removed by centrifugation and which contained Hemicellulose C (see Part II of this discussion). Analysis of the purified Hemicellulose A (see Table X) indicated the presence of xylose, arabinose and 4-O-methyl glucuronic acid in the ratio of 6.3:1:0.9 and a trace of glucose.

Hemicellulose A, an arabino-4-O-methylglucuronoxylan was methylated and cleaved by methanolysis. After saponification of the esters of the acidic components by barium hydroxide solution, the glycosides were separated into neutral and acidic components by the use of an ion-exchange resin. Analysis of the acidic fraction by paper chromatography indicated that it contained one aldobiuronic acid only, although other aldobiuronic acids have been reported to occur in small amounts.[19,78] The acidic fraction was esterified, reduced by lithium aluminium hydride and then identified as the crystalline methyl 2-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyl)-3-O-methyl-αβ-D-xylopyranoside,[20] and by chromatographic identification of its hydrolysis products, namely 2-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose.

The neutral fraction was hydrolysed and the resulting sugars separated as before on a charcoal-Celite column,[20,80] and then identified by chromatography. The principal component was 2,3-di-O-methyl-D-xylose

together with smaller amounts of 2,3,4-tri-, 2-O- and 3-O-methyl-D-xyloses and 2,3,5-tri-O-methyl-L-arabinose. (See Table III).

The xylan was treated with hydrochloric acid at pH 2.3. This mild acid hydrolysis gave a 4-O-methylglucuronoxylan, virtually free from arabinose, which was also methylated, cleaved and resolved into acidic and neutral portions to give sugars similar to those from the methylated arabinose containing xylan (See Table III). If the arabinose residues terminate branched chains of xylose residues [Structure (i)] rather than be attached to the xylose residues of the main chains [Structure (ii)], then methylation after removal of the arabinose units should result in a ten-fold increase in the amount of tri-O-methyl xylose (xylose end-groups) obtained per average molecule of the polysaccharide.[20] Determination of the number-average molecular weights of the fully methylated xylan hemicellulose, the arabinose-free xylan, and the fully acetylated original xylan by osmometry[81] gave respective values for $\overline{M}n$ of 20,600, 19,200 and 26,200 (See Figure 2). These values correspond to degrees of polymerisation of 129, 120, and 130, and to the presence of 96, 99 and 97 xylose residues in the main chain of the average molecule.[20,68,85] Similar values have been reported for other softwood xylans.

The methylation data in Table III indicates that one xylose end-group occurs for every 73 xylose residues in the methylated arabinose-free xylan and one for every 70 in the methylated, unhydrolysed xylan. These values correspond to 1.37 and 1.36 non-reducing end-groups in the average molecules, i.e., there is no increase in the amount of end-groups after the removal of the arabinose residues. Therefore the L-arabofuranose residues must be linked directly to the main chain of the xylan; Structure (ii)

TABLE III

The Composition of the Methylated Xylans

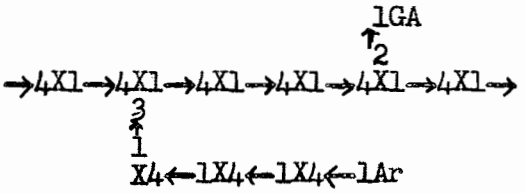
Component	Xylan Weight (mg.)	Xylan Mole Ratio	Arabinose-free Xylan Weight (mg.)	Arabinose-free Xylan Mole Ratio
2,3,5-Tri-O-methyl-L-arabinose	276 (419)[b]	6 (9)[b]	6	0.1
2,3,4-Tri-O-methyl-D-xylose	47	1	55	1
2,3-Di-O-methyl-D-xylose	2148	49	2790	55
2-O-Methyl-D-xylose	315	8	95	2
3-O-Methyl-D-xylose [a]	66	2	192	4
2-O-(2,3,4-Tri-O-methyl-D-gluco-pyranosyluronic acid)-3-O-methyl-D-xylose	830	10	1298	12

(a) Does not include the 3-O-methyl-D-xylose present in the aldobiuronic acid.

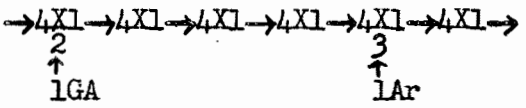
(b) Calculated from the composition of the hemicellulose.

Alternative Types of Structure for Engelmann Spruce

Arabino-4-O-methylglucurono-xylan



STRUCTURE (i)



STRUCTURE (ii)

Ar = L-arabinofuranose

X = D-xylopyranose

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

The volatility of the glycoside of 2,3,5-tri-O-methyl arabinose, may explain that the amount obtained (6 moles) is less than that calculated (9 moles) from the original xylose arabinose ratio of 7:1. These quantities of tri-O-methyl arabinose correspond approximately to the quantity of 2-O-methyl xylose (8 moles) obtained after methanolysis. This fact indicates that most of the arabinofuranose residues are linked to the main chain by (1→3)-bonds, which must also have the α-L-configuration since the negative rotation of the xylan is not appreciably altered by removal of the arabinose.

The quantity of branch points in the average molecule (mono-O-methyl xyloses) is greater than the number of side groups by one mole (20 less 19). This fact could indicate a small degree of branching or be due to demethylation during the methanolysis of the methylated polysaccharide. A comparison of the methylation data and the osmometry data shows that the number of end-groups per average molecule is approximately 1.4 corresponding to 0.4 branch points per average molecule. However very recent work in this laboratory has indicated that the values of $\overline{M}n$ determined in chloroform-ethanol solution (9:1 may be about 25 to 30% too high. The number of end groups per average molecule then becomes 1.0 to 0 96, which would be proof of the linear nature of Engelmann spruce xylan.

On oxidation with periodate the hemicellulose consumed 0.775 moles of oxidant per anhydropentose unit. Sodium borohydride reduction of the oxidized pentosan followed by chromatographic analysis of the hydrolysis products, indicated the presence of some unoxidized xylose

residues. This evidence is support for a main chain of (1→4)-linked xylose residues some of which are substituted in either the 2- or 3-positions.

The xylose:arabinose:4-O-methyl glucuronic acid ratio of 6.3:1.0:0.9 present in the arabino-4-O-methylglucurono-xylan requires a substituent of one out of every 3.32 xylose residues and a consumption of 0.780 moles of periodate per anhydropentose unit in good agreement with the experimentally determined value (0.775).

The stability of the main xylose chain of the hemicellulose to acid hydrolysis and the negative rotation of the xylan indicate the presence of β-linkages. The arabino-4-O-methylglucurono-xylan from the wood of Engelmann spruce accordingly contains at least 100 β-(1→4)-linked D-xylopyranose residues forming an essentially linear molecule, and to these xylose residues are attached in a random manner one side unit of β-(1→2)-linked 4-O-methyl-D-glucuronic acid per 6.3 xylose residues and one side unit of α-(1→3)-linked L-arabofuranose per 7 xylose units.

From a comparison of the ratios of L-arabinose and 4-O-methyl-D-glucuronic acid present in other softwood xylans contained in Table II it can be seen that the xylan from Engelmann spruce is similar to these acidic xylans. The evidence presented does not exclude the possibility that the xylan hemicellulose from Engelmann spruce could be a mixture of a 4-O-methylglucuronoxylan and an arabinoxylan. However, since no such mixture of softwood xylans has been reported its presence here is deemed very unlikely.[20]

RESULTS AND DISCUSSION

PART II

WOOD GLUCOMANNANS

It was formerly believed that coniferous woods contained a mannan. However, Leech[33] and Anthis[34] were able to show the presence of a glucomannan by the isolation of disaccharides containing both glucose and mannose from a partial hydrolysate enriched in the mannose-containing material, even before a glucomannan was isolated in a state of purity by Jones and co-workers.[35,36] Since this time the structures of many wood glucomannans have been elucidated. The material contained in three very recent reports[52,54] and the reconsideration of earlier results[43] (see Table IV), indicate that probably all softwood glucomannans contain up to 5% galactose residues.

The very recent studies of Hamilton and co-workers,[42] Timell[43] and the author report the isolation of a new group of hemicelluloses, which contain glucose, mannose and 10% or more galactose residues, i.e., galactoglucomannans. In keeping with convention, the hemicelluloses originally classified as glucomannans could be renamed galactoglucomannans, however, as the name glucomannan is still used in the literature and to avoid complicating the following discussion, the original name of glucomannan will be retained for those hemicelluloses which contain less than 5% galactose, and the name galactoglucomannan will be used for the recently reported glucomannans, which contain more than 10% galactose.[42,43]

Glucomannans of the same structural type occur widely in Nature namely in the seeds of various land plants, and in close association with cellulose in softwoods and to a lesser extent in the hardwoods. They account for more than half the hemicellulose fraction of the coniferous woods, and generally are less readily extracted with alkali than are the xylans.

TABLE IV

The General Characteristics of Softwood Glucomannans

Species	Galactose (%)	Mannose/ Glucose	$[\alpha]_D$	Pn	Ref.
European larch (*Larix decidua*)	7.5	2.0	-29°	105	58
Tamarack (*Larix laricina*)	3.1	2.8	-30°	88	59
Norway spruce (*Picea abies*)	Present	3.5-4	-39°	68-100	52
Engelmann spruce (*Picea engelmannii*)	3.5	3.2	-40°	111	...
White spruce (*Picea glauca*)	3.0	3.1	-34°	107	121
Sitka spruce (*Picea sitchensis*)	Nil	2.5	-33°	..	123
Sitka spruce	Present	9-15	-28°	..	37
Jack pine (*Pinus banksiana*)	2.9	2.9	-26°	..	53
White pine (*Pinus strobus*)	1.8-3.0	2.7-3.8	-32°	95	86
Scots pine (*Pinus sylvestris*)	4.0	3.5	-31°	97	26
Loblolly pine (*Pinus taeda*)	3.7	2.7	-20°	..	54
Western hemlock (*Tsuga heterophylla*)	Nil	3.0	-38°	130	122
Eastern hemlock (*Tsuga canadensis*)	4.0	3.0	-40°	..	43
Western red cedar (*Thuja plicata*)	1.5	2.5	-37°	..	56
Amabilis fir (*Abies amabilis*)	3.0	3.3	-40°	130	68
Amabilis fir (Bark)	2.8	2.5	-34°	70	100
Ginkgo bilboa	4.2	3.6	-36°	96	62

Application of fractionation methods to the glucomannans so far isolated indicates that these hexosans are structurally homogenous,[38,39] also different glucomannans from the same source, show only minor structural differances with no particular variation in degree of polymerisation.[38]

The main structural feature of all glucomannans is the presence of a chain of β-(1→4)-linked D-mannopyranose and D-glucopyranose units, which are probably distributed at random. The ratio mannose to glucose varies from species to species, but generally in the softwoods the ratio lies between 2.5 to 4, and in the hardwoods the ratio is about 1 for the birchwoods and 1.5 to 2 for the remaining woods investigated.[40]

The presence of randomly distributed mannose and glucose residues in the main chain of glucomannans and galactoglucomannans, precludes that, providing there is not too great a difference in the stability of the glucose-mannose, and mannose-glucose and the mannose-mannose linkages, oligosaccharides containing both glucose and mannose should be isolated after partial hydrolysis. Of these oligosaccharides, mannobiose and mannotriose should predominate if the glucomannan contains more mannose than glucose. Where the isolation of the mixed disaccharides 4-O-β-D-mannopyranosyl-D-glucose (mannosyl glucose) and 4-O-β-D-glucopyranosyl-D-mannose (glucosyl mannose) or trisaccharides containing both glucose and mannose have been reported as products of partial hydrolysis, it is known that true glucomannans are present, rather than a mixture of closely related mannans and glucans, although some degree of heterogeneity cannot

be excluded. The isolation and characterisation of trisaccharides containing both glucose and mannose residues, was very recently reported by Perila and Bishop,[55] who isolated O-mannosyl-O-glucosyl-glucose and O-glucosyl-O-mannosyl-mannose from an enzymic hydrolysate of jack pine glucomannan.

The oligosaccharides, mannosyl glucose, glucosyl mannose, mannobiose, mannotriose and cellobiose are usually present in the partial hydrolysate of a glucomannan,[23] however, the absence of cellobiose in the mixture of oligosaccharides from loblolly pine, suggests that this glucomannan may contain only isolated glucose residues, and therefore does not have a statistical distribution of the hexose residues.

Analysis of the hydrolysis products of methylated glucomannans has shown the presence of 2,3,4,6-tetra-O-methyl-D-glucose and/or D-mannose, 2,3,6-tri-O-methyl D-glucose and D-mannose and small quantities of mixed di-O-methyl sugars.[34,37,38,41,44,54,56,58,59] The quantities of these compounds prove the presence of linear chains of β-(1→4)-linked units. The small quantity of di-O-methylated sugars could either be due to branching of some chains or be artifacts caused by demethylation or incomplete methylation of the hemicellulose. However, the majority of the workers believe that glucomannans are essentially of a linear nature.[59]

Recently, more attention has been given to the galactose content of glucomannans,[43] including an investigation of the structure of loblolly pine glucomannan by Jones and Painter.[54] The isolation of tetra-O-methyl galactose from the hydrolysate of the methylated glucomannan has also been reported by Hamilton and Partlow from western red cedar,[56] Dutton and

Hunt from Sitka spruce,[37] Aspinall and co-workers from European larch,[58] Bishop and Cooper from jack pine,[53] and Kooiman and Adams from tamarack.[59] From the isolation of tetra-O-methyl galactose (in the absence of appreciable quantities of tri- or di-O-methyl galactose) it follows that the galactose is attached to the main chain of the glucomannan as non-reducing end-groups and is not derived from a contaminating galactan.

In 1960 Meier reported the isolation of two oligosaccharides containing galactose and mannose,[52] namely 6-O-α-D-galactopyranosyl-D-mannose and O-α-D-galatopyranosyl (1→6)-O-β-D-mannopyranosyl-(1→4)-D-mannose, from the partial hydrolysate of a Norway spruce glucomannan. The isolation of these oligosaccharides provides the first conclusive proof that mannose and galactose are chemically combined in wood. Although these oligosaccharides could easily have come from a galactoglucomannan Meier believes them to be derived from a mixture of a glucomannan and a galactomannan. Conclusive proof will be obtained only when tri- or higher-saccharides containing both glucose, galactose and mannose have been isolated. This is probably an exacting task due to the lability of (1→6) galactose link and could perhaps only be achieved by the use of suitable enzymes.

Recently, Perila and Bishop[55] isolated a glucomannan from jack pine, which contained glucose, mannose, galactose and xylose in the ratio 10:25:1:1. The first instance of xylose residues being attached to a glucomannan was proved by the isolation of two oligosaccharides containing xylose from the enzymic hydrolysate; the sugars were 6-O-(α-D-xylopyranosyl)-D-glucopyranose and O-α-D-xylopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-D-glucopyranose. However, absolute proof that the xylose residues are

attached to the glucomannan must await the isolation of an oligosaccharide containing mannose, glucose and xylose.

The general characteristics of the softwood glucomannans are summarised in Table IV, from which it can be seen that Sitka spruce is a possible exception to the mannose glucose ratio. The molecular weights reported range from a number average degree of polymerisation ($\overline{Pn}$) of 68 to 130; however during isolation degradation occurs to a varying degree and it is probable that the native glucomannans have a higher molecular weight ($\overline{Pn}$ about 200). The specific rotations of the glucomannans are consistent with the presence of an essentially linear structure of β-(1→4)-linked residues, but the existence of a minor degree of branching cannot be excluded by the methods available.

Although the mannose content of the hardwood angiosperms is small, there is evidence [41,64-66] for the presence of glucomannans of the same type as those isolated from the softwoods. Recently the application of a general method of isolation of hardwood glucomannans to seven species, [40] has confirmed the above similarity between hardwood and softwood glucomannans, except for the proportion of mannose and glucose residues. In some cases the glucomannan could not be isolated free from xylose residues, [40,41,64] but in view of the recent isolation of a glucomannan from jack pine, [55] which contained xylose residues. It is possible therefore that the small proportions of xylose residues, where present, could be chemically combined in the hardwood glucomannans. Hardwood glucomannans are entirely devoid of galactose residues.

It is worthy of note, that the very recent investigations of the bark of several species [67,99,100] and also the wood and bark of ginkgo wood [62,67] (Ginkgo bilboa L.), geologically one of the oldest trees, [63] indicate that the bark and wood from these species contain glucomannans very similar to those isolated from softwoods [63].

In addition to the foregoing discussion, evidence has accumulated during the last three years for the existence in wood of water-soluble galactoglucomannans [43,43,60]. These sometimes form a considerable portion of the hemicellulose content of gymnosperm wood. Most of the galactoglucomannans reported to date were isolated by Hamilton and Timell and their co-workers [43,60,68]. In one investigation wood meal was extracted with 24% potassium hydroxide solution, and the resulting mixture of xylan and galactoglucomannan was separated by precipitation of the barium or copper complex of the hexosan from an alkaline solution. The latter was purified by further precipitation. The galactoglucomannans usually contained galactose, glucose and mannose in a ratio of 1:1:3 and had specific rotations of -5° to -10°. (See Table V). The belief that these hexosans are triheteropolymers is supported by their chemical properties and by electrophoresis and sedimentation studies [43].

The galactoglucomannans are very similar to the glucomannans, being composed of linear chains of β-(1→4)-linked D-mannose and D-glucose residues; differing mainly in galactose content and in degree of polymerisation, although the presence of the galactose side groups confers greater solubility in water or alkali. Because of this similarity between the two groups of hexosans, the application of the standard methods of analysis gives very

TABLE V

The Yield and Composition of the Galactoglucomannans from Softwoods

Species	Yield (%)	$[\alpha]_D^{19}$	Galactose	Glucose	Mannose	Ref.
Eastern hemlock (*Tsuga canadensis*)	4.2	-8.2°	1	1	3	43
Amabilis fir (*Abies amabilis*)	4.0	-5.6°	1	1	3	68
Engelmann spruce (*Picea engelmannii*)	2.0	-7.3°	1	1	3	...
White pine (*Pinus strobus*)	1.6	-5.5°	1	1	1.8	43
Southern pine (mixture)	1.0	+7.4°	1	1	3	42
Eastern white cedar (*Thuja occidentalis*)	0.90	-8°	1	2.4	3.3	43
Ginkgo bilboa	0.75	-7°	1	1	1.4	43

The specific rotations were determined in a 1% water solution.

similar results; thus partial hydrolysis of glucomannans and galactoglucomannans give similar yields of the oligosaccharides glucosyl mannose, mannosyl glucose, mannobiose, mannotriose and cellobiose,[43,60,61] most of the galactose appearing in the hydrolysate as the free sugar.

Analysis of the cleavage products of the methylated galactoglucomannans shows the presence of 2,3,4,6-tetra-O-methyl galactose, 2,3,6-tri-O-methyl mannose and glucose, and a small quantity of di-O-methyl sugars. From the amount of tetra-O-methyl galactose isolated it is evident that D-galactose is mainly present as end-groups. These results and the results of other chemical and physical studies,[43] indicate that galactoglucomannans are homogenous triheteropolymers.

THE GLUCOMANNAN FROM ENGELMANN SPRUCE

RESULTS AND DISCUSSION.

The crude polysaccharide (Hemicellulose B) was obtained in 12.5% yield (based on the dry extractive free wood) from Engelmann spruce holocellulose prepared by the acid chlorite method.[49,88] It was purified by precipitation of its barium complex. The purified glucomannan $[\alpha]D_{5}$ -40° (8.8% of the dry wood) contained glucose, mannose, and galactose in the ratio of 20:63:3 and a trace of xylose. Although glucomannans containing xylose have been recently reported[41,55] no evidence was obtained for the presence of xylose residues in this hemicellulose.

The O-methyl ethers of the sugars which were obtained by methanolysis and hydrolysis of the fully methylated glucomannan were separated by paper chromatography. Melting points and specific rotations were determined for the sugars or suitable derivatives. The methylated sugars obtained were 2,3,4,6-tetra-O-methyl-D-galactose and D-mannose, 2,3,6-tri-O-methyl-D-glucose and D-mannose in a ratio of 20:61 and a mixture of several di-O-methyl-derivatives of D-glucose and D-mannose (see Table VI). The amounts of the tri-O-methyl glucose and mannose correspond closely to the amounts present in the original polysaccharide. Since these tri-O-methyl sugars constitute the major portion of the O-methylated sugars obtained, the glucomannan must be linked by (1→4)-glycosidic bonds, which from the negative rotation of the glucomannan

TABLE VI

Composition of the Methylated Hexosans

Component	Glucomannan		Galactoglucomannan	
	Weight (mg.)	Mole ratio	Weight (mg.)	Mole ratio
2,3,4,6-Tetra-O-methyl-D-galactose	29.7	2	67.2	8
2,3,4,6-Tetra-O-methyl-D-mannose	16.3	1	8.8	1
2,3,6-Tri-O-methyl-D-mannose	858.5	56	205.6	25
2,3,6-Tri-O-methyl-D-glucose	281.5	18	64.0	8
2,3-Di-O-methyl-D-mannose	17.4	1.2		
2,3-Di-O-methyl-D-glucose	10.5	0.7	29.3	4
Di-O-methyl sugars A			29.7	4

($[\alpha]_D$, -47°) and of the methylated glucomannan ($[\alpha]_D$, -38°) are presumably of the β-type. The tetra-O-methylated sugars, D-mannose and D-galactose must be derived from non-reducing end groups. The 2,3,4,6-tetra-O-methyl-D-galactose was the only methylated galactose isolated and since it accounted for 73% of the galactose residues originally present, it was probably an integral part of the glucomannan molecule. The presence of di-O-methyl hexoses in the hydrolysate indicates some degree of branching. However, within experimental error the quantity of these di-O-methyl hexoses corresponds to the amount of tetra-O-methyl galactose present. The polysaccharide was therefore probably linear.

The glucomannan was subjected to partial hydrolysis and the sugars produced were separated on a charcoal-Celite column by gradient elution and by paper chromatography. The monosaccharides glucose and mannose and smaller amounts of galactose and xylose were identified by paper chromatography.

The oligosaccharides obtained were 4-O-β-D-mannopyranosyl-D-mannose (mannobiose), 4-O-β-D-mannopyranosyl-D-glucose (mannosyl glucose), 4-O-β-D-glucopyranosyl-D-mannose (glucosyl mannose), 4-O-β-D-glucopyranosyl-D-glucose (cellobiose), O-β-D-mannopyranosyl-(1→4)-O-β-D-mannosyl-(1→4)-D-mannose (mannotriose) and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannosyl-(1→4)-O-β-D-mannosyl-(1→4)-D-mannose (mannotetrose). These were identified by paper chromatography and by comparison of the rotations and melting points of the sugars and of their derivatives with those of authentic specimens. In addition one other triose was tentatively identified by hydrolysis, hydrolysis after sodium borohydride reduction and by its rotation to be O-mannosyl-O-glucosyl-mannose. Providing the

glycosidic bonds have a similar resistance to hydrolysis the relative quantities of these sugars obtained (see Table VII) and in particular the preponderance of mannobiose and mannotriose would be expected in the partial hydrolysate of a (1→4)-linked glucomannan (mannose predominating) which possessed a random distribution. The previous isolation of these oligosaccharides from the partial hydrolysates of many wood glucomannans[23,34,36,54,56,58,86] and the isolation of similar oligosaccharides by enzymic hydrolysis of glucomannans[52,55] give excellent support for the β-(1→4)-linked structure of Engelmann spruce glucomannan, a conclusion which is in agreement with the data from the methylation studies.

On oxidation with periodate, the glucomannan consumed 0.988 moles of oxidant per anhydro-hexose unit, a close agreement with the value of 1.0 moles per anhydro-hexose unit calculated for a (1→4)-linked glucomannan. The presence of the (1→4)-linkage and the absence of any unoxidized sugars in the hydrolysate of the reduced oxidation product[101] show that the hexose units are not substituted in either the 2- or 3-positions.

The number-average molecular weight of the fully methylated glucomannan was determined by osmometry[81] (see Figure 3.) giving a value for $\overline{M}n$ of 22,700. This value corresponds to a degree of polymerisation of 111 which is similar to the values obtained for other softwood glucomannans (see Table IV). The data from the methanolysis of the polysaccharide indicates that a minimum of 77 hexose residues are present in the average molecule per end group. Therefore there are 111/77 end groups which correspond to 0.5 branch points per average molecule.

TABLE VII

The Oligosaccharides Obtained by Hydrolysis of the Hexosans

Oligosaccharide	Galactoglucomannan Weight (%)	Glucomannan Weight (%)	$R_{cellobiose}$ (System B)
Mannobiose	6.4	3.8	1.15
Cellobiose	0.2	0.2	1.00
Glucosyl mannose	1.3	1.3	1.65
Mannosyl glucose	1.3	0.6	0.71
Mannotriose	2.8	3.0	0.33
Mannotetrose	...	0.3	~0.16
Mannosyl-glucosyl-mannose	...	1.0	0.46
Galactosyl-mannosyl-mannose	0.5	...	0.45
Glucosyl-mannosyl-mannose	0.8	0.1	0.23
A trisaccharide containing glucose, mannose and galactose	0.2	...	0.63

The evidence presented clearly indicates that the glucomannan from Engelmann spruce contains not less than 107 β-(1→4)-linked residues of D-glucose and D-mannose in a ratio of 20:63 forming a diheteropolymer to which is attached on the average 3.2% of galactose residues. The galactose residues are probably attached to the 6-position of both D-glucose and D-mannose residues. Proof of the type and position of linkage of the galactose residues would be obtained by the isolation of an oligosaccharide containing glucose, mannose and galactose. Recently two oligosaccharides containing D-galactose α-(1→6)-linked to mannose and mannobiose were reported by Meier but these were postulated to be derived from a galactomannan.[52] It is also possible that they could have arisen from a galactose-containing glucomannan or galactoglucomannan.[43]

ENGELMANN SPRUCE GALACTOGLUCOMANNAN

RESULTS AND DISCUSSION

The crude galactoglucomannan (Hemicellulose C) was obtained as an insoluble barium complex during the purification of the potassium hydroxide extract of Engelmann spruce holocellulose. Treatment of the latter extract directly with barium hydroxide solution and one further purification via the barium complex were sufficient to produce a suitably purified galactoglucomannan $[\alpha]_D$ - 7.3° in 1.8% yield (based on the dry wood).

Methanolysis of the fully methylated hemicellulose gave a mixture of O-methyl sugars similar to those obtained from the methylated glucomannan. Separation and identification by the methods outlined for the glucomannan sugars gave approximately equimolar amounts of 2,3-di-O-methylated D-glucose and D-mannose, 2,3,6-tri-O-methylated D-glucose and D-mannose in a ratio of 1:3.2 and 2,3,4,6-tetra-O-methylated D-galactose and D-mannose in a ratio of 7.6:1. (See Table IV). The tetra-O-methyl D-galactose made up about 16% of the O-methyl hexoses, which is in agreement with the galactose content of the galactoglucomannan and contrasts with the 3% present in the glucomannan. The quantity of tetra-O-methyl mannose corresponds to about one end group per 40 hexose units, making a total of about 8 end groups per 40 units. This indicates either a lower degree of polymerisation than the glucomannan or one branch point more per average molecule. The preponderance of the 2,3,6-tri-O-methyl sugars

again indicates a (1→4)-linked polysaccharide. The total quantity of di-O-methyl sugars corresponds closely to the quantity of tetra-O-methyl galactose obtained, thus indicating that the galactose residues are (1→6)-linked.

Partial hydrolysis of the galactoglucomannan followed by a separation and identification of the sugars by methods similar to those outlined for the glucomannan gave the monosaccharides glucose, mannose, galactose and a trace of xylose and the oligosaccharides cellobiose, mannobiose, mannotriose, glucosyl mannose, mannosyl glucose and three trioses which were tentatively identified as O-glucosyl-O-mannosyl-mannose, O-galactosyl-O-mannosyl-mannose and a trisaccharide which contained glucose, galactose and mannose. Many of these oligosaccharides have been obtained in similar proportions from the hydrolysis of glucomannans.[23,34,36,52,56,58,86] (See Table VII). Therefore it seems reasonable to assume that the galactoglucomannan also contains β-(1→4)-linked chains of D-glucose and D-mannose to which must be linked the D-galactose residues; a structure which is in agreement with the methylation data (see Table VI).

On oxidation with periodate the galactoglucomannan consumed 1.16 moles of oxidant per anhydro-hexose unit, which closely agrees with the value of 1.20 moles per anhydro-hexose unit calculated for a chain of (1→4)-linked residues in the pyranose form, which has on the average, every fourth residue substituted in the 6-position by D-galactose. Since no sugars were detected in the hydrolysate of the reduced oxidation product,[41] substitution cannot occur at either the 2- or 3-position of the hexose units.

Two types of structure can be derived from the evidence available; namely a highly branched network of β-(1→4)- and (1→6)-linked D-glucose and D-mannose residues terminated by (1→4)-linked D-galactose, Structure (iii) or an unbranched chain of β-(1→4)-linked D-glucose and D-mannose residues with (1→6)-linked side groups of D-galactose, Structure (iv). Since Structure (iii) is very highly branched it should have different physical properties and give different products upon partial hydrolysis than would a straight chain polysaccharide. However the products of partial hydrolysis were similar to those from most glucomannans.[59] The galactoglucomannan must therefore possess an essentially straight chain of β-(1→4)-linked residues thus alternative (iv) is the more probable structure of Engelmann spruce galactoglucomannan.

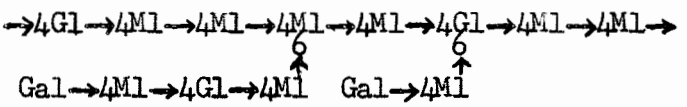
The specific rotation of the galactoglucomannan ([α]D, -7.3°) is lower than that of glucomannans ($[\alpha]_D$, is usually about -40°). The galactose is therefore probably linked by an α-type bond, giving the polysaccharide as a whole a more positive rotation.

The number-average molecular weight of the fully methylated glucomannan was determined by osmometry[81] (see Figure 3.) giving a value for $\bar{M}n$ of 23,800. This value corresponds to a degree of polymerisation of 117, which is similar to the values obtained for other softwood glucomannans. (See Table IV) The data from methanolysis of the methylated polysaccharide indicates that a minimum of one mannose end group per 40 hexose residues is present per average molecule. Therefore there are 117/40 end groups corresponding to 1.5 branch points per average molecule.

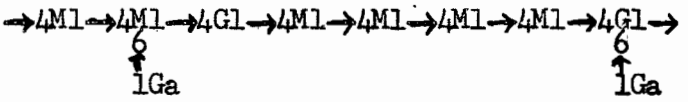
Alternative Structures for Engelmann Spruce

Galactoglucomannan

STRUCTURE (iii)



STRUCTURE (iv)



M = D-mannopyranose

G = D-glucopyranose

Ga = D-galactopyranose

The evidence presented indicates that the galactoglucomannan from Engelmann spruce contains not less than 100 β-linked residues of D-glucose and D-mannose in a ratio of 1:3 with an average of one D-galactose residue being attached per four hexose residues. The linkage is probably α-(1→6) to equimolar quantities of glucose and mannose (see Structure iv).

The isolation of a small quantity of a triose R_c, 0.63 and $[\alpha]_D$, + 30° which contained equimolar quantities of glucose and mannose, is the first evidence reported for the linkage of all three sugar residues present in the galactoglucomannans and in some glucomannans. Unfortunately the small quantity available did not allow unequivocal determination of the positions and types of linkage present.

EXPERIMENTAL

All evaporations were carried out under reduced pressure and below 45°C. and all drying was carried out *in vacuo* unless otherwise stated. Specific rotations were determined at 20°C. and were equilibrium values and all melting points were corrected.

Paper Chromatography

The chromatograms were developed at room temperature by the descending method on Whatman No. 1 or (for preparative purposes) on No. 3 MM paper using the following solvent systems (v/v).

(A) ethyl acetate:acetic acid:water (9:2:2)

(B) butan-1-ol:pyridine:water (10:3:3)

(C) ethyl acetate:pyridine:water (8:2:1)

(D) butanone:water (89:11) containing 2% ammonia

(E) butanone:water:formic acid (89:8:5)

(F) butanone:water:ethanol (89:11:2)

(G) ethanol:benzene:water (47:200:15)

(H) butan-1-ol:pyridine:water (6:4:4)

Electrophoresis

Electrophoresis was carried out in a 0.05M borate buffer (pH 9.4) at 500 volts using Whatman No. 3MM paper.

The reducing sugars on the chromatograms were detected by the use of *o*-aminodiphenyl spray reagent.[103]

Darco G-60 charcoal was used throughout this work for the

preparation of charcoal-Celite columns using gradient elution with ethanol-water mixtures and increasing ethanol gradients.

The logs of Engelmann spruce were soaked in boiling ethanol, and the bark removed. The logs were then cut into chips and ground in a Wiley Mill (40-60 mesh). The finely ground wood was extracted with alcohol-benzene 1:2 in a Soxhlet Apparatus and then with hot water and dried. This procedure gave an extractive free wood having a moisture content of 10.8%.

TABLE VIII

Composition of the Extractive Free Wood (%)

Component	Per cent
Lignin	28.0
Pentosan	9.2 (uncorr.)
Ash	0.30
Uronic anhydride	3.3
Alpha cellulose	48.7
Moisture	10.8

The methods used for the above determinations of pentosan, ash and moisture were standard TAPPI methods. Uronic acid was determined by the procedure of Browning[104] and the methoxyl contents were determined by the procedure of Timell and Purves.[105]

Hydrolysis of the Extractive Free Wood Meal (40-60 mesh)

Wood meal (111 g.) was gradually added to cold 72% sulphuric acid (150 ml.) and mixed to a paste. After standing for 12 hours the mixture was diluted to 4 litres and refluxed for 8 hours. After neutralisation with warm barium hydroxide (570 g. in 4 litres of water)

the mixture was filtered and the filtrate concentrated to 200 ml. *in vacuo*. The barium ions were removed with Amberlite IR-120 exchange resin. The solution was passed down a column of Dowex IX-4 resin (acetate form) and water was added until the eluate gave a negative Molisch test (6 litres). The eluate was evaporated to give a syrup which was analysed for constituent sugars. Found: glucose, mannose, xylose, and smaller amounts of arabinose and galactose. The acidic components which were retained by the column, were eluted by 30% acetic acid (2 litres). Concentration of the eluate gave a mixture of acidic sugars (5.9 g.), an aqueous solution (30 ml.) of which was added to the top of a charcoal-Celite column and fractionated by gradient elution using mixtures of ethanol and water, (*vide infra*). Four main fractions were obtained (see Table IX) together with smaller quantities of higher uronic acids.

TABLE IX

Separation of the Uronic Acids

Fraction	Weight (g.)	Component
I	0.25	4-O-methyl glucuronic acid
II	0.95	impure I
III	1.4	an aldobiuronic acid
IV	1.1	impure III
V	Smaller quantities of higher homologues	

Identification of the Uronic Acids

4-O-methyl-D-glucuronic acid (Fraction I)

Found: OMe, 15%, Equiv. 203 $[\alpha]_D$ + 50° (c, 1.7 water). The hexuronic acid (0.1 g.) was dissolved in methanolic hydrogen chloride (12 ml., 0.7N) and refluxed for 8 hours in the presence of a little

Drierite to remove any water formed and to facilitate boiling. The solution was neutralised with silver carbonate, filtered (Celite), treated with hydrogen sulphide, filtered again and evaporated to a syrup. The methyl ester-glycoside was dissolved in methanol, filtered and allowed to stand. White needles were obtained (3.8 mg. m.p. 119°). Evaporation of the methanol gave a syrup (70 mg.), which was dried and then reduced [112] using lithium aluminium hydride (0.5 g.) in tetrahydrofuran (50 ml.). After hydrolysis the product of the reduction was indistinguishable chromatographically from 4-O-methyl-D-glucose $[\alpha]_D$ + 47° (c, 2.0 water). Found: OMe, 16.1; calc. for $C_7H_{14}O_6$: OMe, 16.0%.

2-O-(4-O-methyl-α-D-glucopyranosyl uronic acid)-D-xylose (Fraction III)

The syrup $[\alpha]_D$ + 84° (c, 1.8 water) was set aside under methanol for 2 days, and crystals were obtained (1.4 g.) having no m.p. up to 300° $[\alpha]_D$ + 89.4° (c, 0.85 water). The acid (850 mg.) was refluxed with methanolic hydrogen chloride (100 ml. 0.7N), using the procedure outlined above to give a syrup (0.90 g.) of the aldobiuronic acid methyl ester methyl glycoside. The methyl-ester-glycoside (900 mg.) was dissolved in dry pyridine (50 ml.) and redistilled acetic anhydride (15 ml.) was added. After 24 hr. at 25° the mixture was poured into iced water (600 ml.) and the whole was extracted with chloroform (4 x 150 ml.). The chloroform extract was washed with cold 10% hydrochloric acid (0°C) (3 x 150 ml.), saturated sodium bicarbonate solution (3 x 100 ml.) and water (100 ml.), dried over anhydrous sodium sulphate and then evaporated to dryness leaving a syrup (0.95 g.)

The syrup was dissolved in dry ether (40 ml.) and after standing overnight white crystals were obtained (0.47 g. m.p. 198-200°). Recrystallisation from dry methanol gave crystals (250 mg.) having m.p. 201-202°. The product also gave an infrared spectrum and a mixed m.p. identical with those from an authentic specimen of methyl 2-O-(2,3-di-O-acetyl-4-O-methyl-α-D-glucopyranosyl)uronate-3,4-di-O-acetyl-D-xylopyranoside.[76] $[\alpha]_D$ + 96° (c, 1.0 chloroform). Calc. for $C_{22}H_{32}O_{15}$: OMe, 17.3; Found: OMe, 17.3%.

Preparation of the Holocellulose

The extractive-free Englemann spruce wood (1200 g. moisture 10.8%) was added to hot water (14 litres 80-90°). With constant stirring and maintaining the temperature between 75 and 90°, acetic acid (120 ml.) was added. Sodium chlorite (360 gm.) was gradually added during a period of 40 minutes. The addition of acetic acid and sodium chlorite was repeated three times at hourly intervals. After standing for one hour the insoluble holocellulose was washed thrice by decantation, filtered, washed on the filter with water (6 litres) and ethanol, and then air dried. Yield 73% (on dry wood).

Isolation of the Hemicelluloses

Holocellulose (890 gm. moisture 6%) was shaken for 12 hr. with 24% potassium hydroxide solution (w/w) (8 litres). The resulting slurry was filtered and the first 8 litres of filtrate and washings was poured into 24 litres of ethanol containing acetic acid, (4 litres) adding more acid if necessary to bring the pH to 5 to 6. After allowing the precipitate formed to stand overnight, the supernatant liquid

was poured off and the remaining slurry filtered. The precipitate was washed with 80% ethanol until the filtrate was clear (6 litres) and then ethanol 6 litres and petroleum ether (30-60°) taking care not to let the precipitate dry. The residual petroleum was removed by aspiration, giving a mixture of hemicelluloses A and C (157 g., 14.5% of the dry wood). The product, insoluble in the potassium hydroxide solution, was well washed with water, partially dried by suction and then shaken with 17.5% sodium hydroxide solution containing 4% boric acid (6 litres) for 12 hours. The slurry was filtered and the first six litres of filtrate and washings were collected, poured into 3 volumes of ethanol which contained acetic acid (2 litres) and allowed to stand overnight. The supernatant liquid was decanted and the remaining slurry collected by filtration or centrifugation. The precipitate was washed with 80% ethanol, ethanol, light petroleum (30-60°) and the product was dried over phosphorous pentoxide to give 107 g. of crude hemicellulose B (10% of the dry wood). The residue which was insoluble in the sodium hydroxide solution was well washed with distilled water and a small portion was analysed for sugars. Only glucose was found and the residue was thus pure cellulose.

ENGELMANN SPRUCE XYLAN

Separation of Hemicelluloses A and C

Analysis of the crude potassium hydroxide extract showed the presence of xylose and glucuronic acid as the major component and lesser amounts of glucose, mannose, galactose and arabinose. (See Table X). For purification purposes it was found that potassium rather than sodium

TABLE X

Composition of the Xylan Fractions

Fraction	Galactose	Glucose	Mannose	Arabinose	Xylose	X/Ar
Crude xylan	..	..	..	..	..	9
1. $Ba(OH)_2$ Treatment		17	17	9	57	6.3
2. $Ba(OH)_2$ Treatments		Trace	..	12	88	7.2
A repeat purification (2. treatments)		Trace	..	12	88	7.2

X = D-xylose

Ar = L-arabinose

hydroxide solution was a more effective solvent for the crude product, therefore the hemicellulose (100 g.) was stirred for one hour with water (1 litre) and then potassium hydroxide solution (1 litre 20%) was added and the mixture stirred for 2 hours. Barium hydroxide solution (5%, 4 litres) was added dropwise with stirring. After standing overnight the precipitate was collected (by centrifugation) and the supernatant liquid was retained. The clear supernatant solution was poured into 3 volumes of ethanol and the precipitate collected after standing overnight. The product was washed with 80% ethanol, ethanol, with light petroleum, and dried in vacuo. Repetition of the above procedure gave a purified hemicellulose A, (8% yield). Hydrolysis and chromatographic analysis showed the presence of xylose, arabinose, 4-O-methylglucuronic acid and a slight trace of glucose. Found: OMe, 2.8%. (See Table X).

The precipitate of the barium complex was washed three times with water and five times each with 80% ethanol, ethanol, and light petroleum (30-60°) whilst still in the centrifuge. The product was collected to yield crude hemicellulose C (1.5%), whose composition is given in Table XIII. An improved method of isolation and purification is described under the isolation of the galactoglucomannan (Hemicellulose C).

Methylation of the Arabino-4-O-methylglucuronoxylan (Hemicellulose A)

The purified hemicellulose A (25 g.) was dissolved in 40% sodium hydroxide solution (500 ml. w/w) by constant stirring in an atmosphere of nitrogen. Dimethyl sulphate (300 ml.) was slowly added with constant stirring at 30°. The addition of sodium hydroxide and dimethyl sulphate was repeated four times. The products were neutralised

with dilute sulphuric acid, made alkaline with a little sodium hydroxide and then acidified to pH 4 with acetic acid. The mixture was heated to 90° and the precipitated, partially methylated products collected and washed with hot water, dried and dissolved in chloroform. The chloroform solution was further dried over sodium sulphate, evaporated to dryness and dried over phosphorous pentoxide. The dry, partially methylated xylan (28.5 g.) was dissolved in dry dimethyl formamide (400 ml.). Silver oxide (50 g.) and methyl iodide (50 ml.) were added and the mixture was shaken for 48 hours in the dark. Two similar additions of silver oxide and methyl iodide were made. Chloroform (500 ml.) was added and the insoluble silver salts removed by centrifugation. The salts were washed with chloroform (4 x 250 ml.) and the washings combined with the chloroform solution, which was extracted with 10% potassium cyanide solution (2 x 500 ml.) and water (2 x 500 ml.) and dried over sodium sulphate. The dry solution was filtered and concentrated to about 200 ml., which was then added dropwise to light petroleum (2 litres). The precipitated product was collected by filtration, washed with a little petroleum, and dried by aspiration over phosphorous pentoxide. Yield 8.8 g. OMe, 39.2% $[\alpha]_D$ + 56° (chloroform). Calc. for the fully methylated hemicellulose: OMe, 39.6%. The infrared spectrum of the product indicated the absence of any hydroxyl groups.

Methanolysis of the Methylated Xylan

The methylated xylan (7.7 g.) was refluxed with methanolic hydrogen chloride (500 ml. 0.7N) for 8 hours. After neutralisation with silver carbonate and evaporation, the product was treated with 5% barium

hydroxide solution (200 ml.) for 2 hours at 60°. Solid carbon dioxide was added and the barium carbonate removed by filtration through Celite. After treatment with Amberlite IR-120 the mixture was added to the top of a column of Dowex 1-X4 resin (acetate form) and the neutral glycosides were eluted by water (4 litres), evaporation of which gave a pale yellow syrup (5.6 g.). The acidic sugars were eluted by 30° acetic acid (2 litres), which on evaporation gave a pale yellow syrup of an acid glycoside (1.71 g.).

Identification of the Acidic Fraction

A small portion of the acidic sugar syrup was hydrolysed for 7 hours with normal sulphuric acid, neutralised, treated with Amberlite IR-120, and examined by paper chromatography in systems (A) and (E), which indicated that the syrup contained only one component. The remaining portion of the syrup was converted to the methyl ester using methanolic hydrogen chloride. After neutralisation (Ag_2CO_3) the methanol was removed and the product carefully dried (1.78 g.). The ester syrup (0.85 g.) was dissolved in dry tetrahydrofuran, which was added to lithium aluminium hydride (1.5 g.) in dry tetrahydrofuran (150 ml.) and refluxed for 2 hours. The excess of lithium aluminium hydride was decomposed with ethyl acetate and then with water. The insoluble salts were removed by centrifugation and washed twice with 50% ethanol. The combined centrifugate and washings were de-ionized with Amberlite IR-120 and Dowex 1-X4 (acetate form) and evaporated to dryness. Two recrystallisations of the product (0.64 g. syrup) from ethyl acetate gave white needles having m.p. 167-168° and $[\alpha]_D$ + 84°. The melting point was undepressed when mixed

with an authentic sample of methyl 2-O-(2,3,4-tri-O-methyl-α-D-gluco-pyranosyl)-3-O-methyl-αβ-D-xylopyranoside, which also had the same infrared spectrum. Calc. for $C_{16}H_{30}O_{10}$: OMe, 40.6; Found: OMe, 40.7%. A portion of the product was hydrolysed to give a mixture of 3-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose.

Separation of the Neutral Sugars from the Methanolysis of the Methylated Xylan

The mixture of neutral glycosides (5.6g.) was hydrolysed by refluxing with N-sulphuric acid (200ml.) for 8 hours. Neutralisation with barium carbonate, filtration through Celite, and evaporation of the filtrate after de-ionisation with a little Amberlite IR-120 and Dowex-1 X4 gave a syrup (5.2g.). A portion (4.5g.) was added to the top of a charcoal-Celite column,[95] (4.5 x 60 cm.). Resolution was effected by gradient elution using the following solvents: 2 and 15% ethanol, and 15 and 30% ethanol, 3 litres each followed by 1 litre each of 30 and 40% ethanol. Twentyfive-ml. fractions were collected and a portion from every third was examined by paper chromatography (system F). (See Table XI).

Identification of the Methylated sugars.

3-O-methyl-D-xylose (Fractions 15-17)

De-ionisation of the fraction gave a product (35mg.) $[\alpha]_D$, + 18° (c, 1.2 water), which had the same electrophoretic mobility as an authentic psecimen of 3-O-methyl-D-xylose. Calc. for $C_6H_{15}O_5$: OMe, 18.9; Found: OMe, 18.6%. The derived 3-O-methyl-N-phenyl-D-xylosamine[107] had m.p. 134°.

TABLE XI

Resolution of the Methylated Sugars

Component	Methylated Xylan		Methylated Arabinose-free Xylan	
	Weight (mg.)	Fraction (3x25ml.)	Weight (mg.)	Fraction (3x25ml.)
3-O-Methyl-D-xylose	44	15-17		
3- and 2-O-Methyl-D-xylose	398	18-25	270	4-8
2-O-Methyl-D-xylose	61	26-29		
2-O- and 2,3-Di-O-methyl-D-xylose			70	9-10
2,3-Di-O-methyl-D-xylose	2078	30-75	2755	11-60
2,3-Di-O-methyl-D-xylose plus impurity	127	76-100		
2,3,5-Tri-O-methyl-L-arabinose plus impurity	161	101-117		
2,3,5-Tri-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose	196	118-133		
2,3,4-Tri-O-methyl-D-xylose	65	134-156	50[(a)]	67-83

(a) Contains a trace of tri-O-methyl arabinose.

Fraction 18-25

The syrup was shown by electrophoresis and chromatography (System F) to be a mixture of 2- and 3-O-methyl-D-xylose. An electrophoretic separation gave 2-methylxylose (270 mg.) and 3-methylxylose (31 mg.).

2-O-methyl-D-xylose (Fraction 26-29)

Purification of the crude material gave a product (45 mg.) $[\alpha]_D^{20}$ + 30°, (c, 1.2 methanol), which was crystallised from ethyl acetate and had m.p. 134°. Calc. for $C_6H_{12}O_5$: OMe, 18.9%. Found: OMe, 18.5%.

2,3-di-O-methyl-D-xylose (Fraction 30-75)

Paper electrophoresis indicated the absence of any 3,4-di-O-methylxylose in this fraction. Crystallisation of the product from ethanol gave white crystals (1.5 g.) $[\alpha]_D^{20}$ + 24° (c, 1.7 water), having m.p. 98-100°. Calc. for $C_7H_{14}O_5$: OMe, 34.8%. Found: OMe, 34.7%.

2,3-Dimethyl xylose (208 mg.) was dissolved in ethanol (5 ml.) and aniline (0.3 ml.) was added. After standing overnight at 60° the ethanol was removed and the residue kept *in vacuo* for 3 days to remove excess aniline. Recrystallisation from ethyl acetate gave a crystalline solid (71 mg.) $[\alpha]_D^{108}$ + 197° (c, 2.3 ethyl acetate), having m.p. 140-142°. Calc. for $C_{13}H_{20}O_5N$: OMe, 23.2%. Found: OMe, 22.5%.

2,3,5-tri-O-methyl-L-arabinose (Fraction 101-117)

Purification of the material from the column gave a syrup $[\alpha]_D$ - 27° (c, 0.7 water) (71 mg.), which gave only arabinose on demethylation with boron trichloride[109] and had an R_F value slightly higher than that of 2,3,4-tri-O-methyl-D-xylose in systems F and G. Calc. for

for $C_8H_{16}O_5$: OMe, 48.4%. Found: OMe, 48.0.

The trimethyl arabinose (50 mg.) was dissolved in water and barium carbonate (50 mg.) and then bromine (5 drops) was added. The mixture was kept in the dark for 72 hours. The excess bromine was removed by aeration, the solution acidified with dilute hydrochloric acid (pH 2), and then extracted with chloroform. The dried chloroform extract was placed in a cold finger distillation apparatus and after removal of the chloroform the arabinolactone was distilled (bath temp. 120°, pressure 0.07 mm. Hg.). The purified lactone was dissolved in methanol (4 ml.), treated with anhydrous ammonia and kept for 24 hours at room temperature. After evaporation the dried product was recrystallised from isopropyl ether. The product (22 mg.) had m.p. 123-7°.[72] Recrystallisation gave a small quantity of material (1.5 mg.) having m.p. 129-131°.

2,3,4-tri-O-methyl-D-xylose (Fraction 134-156)

Extraction of the crude product with benzene and evaporation gave a semi-crystallised product (35 mg.). Recrystallisation from benzene gave colourless crystals (18 mg.) $[\alpha]_D$ +17° (c, 0.9 ethanol) having m.p. 89-90° and a mixed m.p. with an authentic specimen 90-91°.[110] Calc. for $C_8H_{16}O_5$: OMe, 48.4%. Found: OMe, 48.2.

Fraction 118-133

This fraction was extracted with hot benzene and the residue after evaporation was separated by paper chromatography (system D) to give trimethyl xylose (17 mg.) and trimethyl arabinose (55 mg.).

Selective Removal of the L-Arabinofuranose Residues from the Xylan

The purified arabinoglucuronoxylan (Hemicellulose A) (20 g.) was dissolved in hydrochloric acid (350 ml., 0.02N) and the pH adjusted to 2.3 (about 60 ml., 0.1 N HCL). The solution was then heated to 90° for 4 hours, poured into ethanol (4 litres) and allowed to stand for 2 hours. The precipitate was collected by filtration, washed by solvent exchange (75% ethanol, ethanol, and light petroleum) and dried over $CaCl_2/KOH$. Yield 14.2 g. Hydrolysis and chromatographic analysis (Systems A and C) indicated the presence of xylose and uronic acid residues only.

Methylation of the Arabinose-free Xylan

The arabinose-free xylan (14 g.) was methylated using the same procedure as for hemicellulose A, giving a fully methylated 4-O-methylglucuronoxylan (7.37 g.) (OMe, 39.83%), whose infrared spectrum indicated the absence of any hydroxyl groups.

Methanolysis of the Methylated Arabinose-free Xylan

The methylated hemicellulose (7.3 g.) was refluxed with methanolic hydrogen chloride (350 ml. 0.7N) and the acidic and neutral sugars (4.73 g.) obtained as before.

Resolution of the Mixture of Methylated Sugars

A portion of the methylated neutral sugars (3.73 g.) were separated on a charcoal-Celite column as previously described, using gradient elution (2 litres of each solvent).

Fraction 4-8

Paper electrophoresis indicated this fraction to be a mixture

of 2-O- and 3-O-methyl-D-xylose in a ratio of 1:3. These were separated by chromatography (System D) giving 65 mg. of the 2-isomer and 195 mg. of the 3-isomer.

Fraction 9-10

This fraction was separated into 2-O-methyl (30 mg.) and 2,3-di-O-methyl-D-xylose (35 mg.) in system D.

Identification of 2,3-di-O-methyl-D-xylose (Fraction 11-60)

The material was crystallised from ethyl acetate. Yield: 1.7 g., m.p. 98-100° $[\alpha]_D$ + 25° (c, 1.6 water). Calc. for $C_7H_{14}O_5$: OMe, 34.8%. Found OMe, 34.4. This material did not depress the m.p. of an authentic sample of 2,3-di-O-methyl xylose.[75] The aniline derivative was prepared, had m.p. and mixed m.p. 140-2°.

Identification of 2,3,4-tri-O-methyl-D-xylopyranose (Fraction 67-83)

Benzene extraction of this fraction gave a part-crystalline residue (46 mg.), which was recrystallised from benzene giving colorless needles (26 mg.) $[\alpha]_D$ + 17° (c, 1.2 ethanol), which had m.p. and mixed m.p. 91-92°. Calc. for $C_8H_{16}O_5$: OMe, 48.4%. Found: OMe, 48.3. Chromatographic examination of the residue (19 mg.) from the recrystallisation showed the presence of 33% 2,3,5-tri-O-methyl-L-arabinose.[77]

Acetylation of Engelmann Spruce Xylan

Dry hemicellulose A (2.0 g.) was dissolved in dry formamide (40 ml.) and redistilled acetic anhydride (20 ml.) dissolved in freshly distilled dry pyridine was added. The mixture was shaken and cooled for the first 20 min. and occasionally thereafter. After shaking for 3 hours acetic anhydride (20 ml.) was again added, the addition

being repeated after a further 3 hours. Throughout the shaking process the pressure inside the reaction vessel was released periodically. After the reaction mixture had stood at 4°C for 3 days it was poured into a stirred mixture of 4% hydrochloric acid (1 litre) and ice (1.5 kg.). After standing for 30 min. the suspension was collected by filtration and the product washed with ice cold water and then with water until the washings were neutral. After washing with ethanol and light petroleum the product was dried over $CaCl_2$/KOH. Yield: 1.5 g. of greyish powder.

Periodate Oxidation of the Xylan [111]

Ash-free xylan (1.412 g.) was placed in a 250 ml. volumetric flask, and dissolved in the water and periodate solution (100 ml. 0.1781M) which were added to the solution to make up the volume. Aliquots of 10 ml. were withdrawn from time to time and titrated against sodium arsenite solution (0.06013M) and the titre subtracted from a predetermined blank titre of the periodate solution thus giving the moles of oxidant consumed. These values are plotted against time (See Figure 1) and extrapolated to zero time. The value obtained (3.15×10^{-4} moles oxidant per 10 ml. aliquot was equivalent to a consumption of 0.775 moles per anhydroxylose unit. Reduction, with sodium borohydride, hydrolysis of the oxidized polysaccharide and chromatographic analysis (Systems A and C) indicated the presence of some xylose residues.

Preparation of the Ash-free Xylan

The purified xylan (0.5 g.) was treated with 15% sulphuric acid and burned in a platinum crucible and was found to contain 12% ash. A

portion of the xylan (10g.) was dissolved in 10% hydrochloric acid (500ml.) and after filtration and standing for 30 minutes the solution was poured into 4 volumes of ethanol. The product (8.3g.) had a low ash content (0.15%). Found: OMe, 3.2%, $[\alpha]_D$, -48° (1% in water).

ENGELMANN SPRUCE GLUCOMANNAN

Isolation of the Glucomannan

The isolation of the crude glucomannan (Hemicellulose B) is described on page 47.

Purification of the Glucomannan

After trial purifications on a small scale (5.0g.), by precipitation of the barium and copper complexes it was found that the use of the barium complex gave better removal of the uronic acid containing impurities.

The crude glucomannan (Hemicellulose B) (75g.) was suspended in water (1.5 litres) by stirring for 3 hours. Solution was completed by the addition of 20% sodium hydroxide solution (1.5 litres) and stirring for 2 hours. The solution was filtered (glass wool) and then 5% barium hydroxide solution (3 litres) was added dropwise with stirring. After standing overnight the precipitate was collected by centrifugation, washed three times with 6% potassium hydroxide solution, once with 2.5% sodium hydroxide solution and dissolved in 50% acetic acid, which had been cooled to -10°. The acetic acid solution was poured into 4 volumes of ethanol. After standing overnight, the precipitate was collected, washed by solvent exchange (70% ethanol, ethanol and light petroleum 30-60°) and dried. Yield: 62.9g., $[\alpha]_D$, -40° (1% solution in 10% NaOH)

Hydrolysis and chromatographic analysis (systems A, B and C) showed the presence of glucose, mannose, a little galactose and a trace of xylose. (See Table XII)

Methylation of the Glucomannan

The purified glucomannan (21g.) was methylated using the same procedure outlined for Hemicellulose A. This gave a pale yellow powder (11.5g.) having a methoxyl content of 42.5%. This product was methylated using the method of Purdie and Irvine;[113] The partly methylated polysaccharide was dissolved in methyl iodide (200ml.) and freshly prepared silver oxide (20g.) was added and the mixture shaken for 24 hours. Two similar additions were made. After a final addition of silver oxide (40g.) the reaction mixture was shaken for a further 48 hours and chloroform was added. The silver salts were removed by filtration and thoroughly washed with chloroform. The chloroform solution was concentrated (250ml.) and added dropwise to vigorously stirred light petroleum (3 litres 30-60°). The precipitate was collected, washed with light petroleum and dried. The yield of an almost white powder was 6.9g. Calc. for a fully methylated hexosan: OMe, 45.6; Found: OMe, 44.8%.

Methanolysis of the Methylated Glucomannan and resolution of the Hydrolysate

The fully methylated glucomannan (4g.) was refluxed with 2.3% methanolic hydrogen chloride (200ml.) for 8 hours. Most of the methanol was removedby a stream of air, then normal hydrochloric acid (200ml.) was added and the solution refluxed for 8 hours. The solution was neutralised with silver carbonate, filtered (Celite), treated with a little hydrogen sulphide and filtered again. The filtrate gave a light brown partially crystalline material on drying. Yield: 4.26g.

TABLE XII

The Composition of the Glucomannan Fractions

Fractions	Galactose	Glucose	Mannose	Xylose	Ma/Gl
Crude glucomannan	..	23	74	3	3.2
1. $Ba(OH)_2$ Treatment	6	26	68	..	2.8
2. $Ba(OH)_2$ Treatments	3.6	22.4	74.1	..	3.3
A repeat purification (2. treatments)	3.5	23	73.5	..	3.2

Ma = D-mannose

Gl = D-glucose

Chromatographic analysis of the filtrate (system G) indicated the presence of di- tri- and tetra-O-methyl hexoses. Part of the product was added to the top of a charcoal-Celite column and resolved by gradient elution using mixtures of ethanol and water. (5 and 15% ethanol 2 litres each, 15 and 30% ethanol, then 30 and 50% ethanol 4 litres in each case. Two hundred and fifty fractions (75ml.) were collected and examined by paper chromatography (system G). However, a correspondance between the fractions collected and the sugars expected could not be obtained, nor could any tetra-O-methyl galactose be detected. Methanolysis was again carried out, using a further portion of the product (1.6g.) and the methylated sugars (94% yield) were separated by paper chromatography using system D. The sugars obtained and their respective yields are given in Table VI.

Identification of the Methylated Sugars

2,3,4,6-Tetra-O-methyl-D-galactose

The syrup $[\alpha]_D$, + 95° (c, 0.7 water) was chromatographically identical (system D) to an authentic sample of 2,3,4,6-tetra-O-methyl-D-galactose and demethylation gave galactose only. The sugar (20mg.) was dissolved in ethanol (2ml.) and after the addition of aniline (20mg.) the solution was kept at 60° overnight. Removal of the solvent by a current of air and any excess aniline *in vacuo* gave a residue, which was crystallised from isopropyl ether to yield white needles (12mg.) having m.p. and mixedm.p. 192-193° and $[\alpha]_D$, -135° (c, 0.7 pyridine).

2,3,4,6-Tetra-O-methyl-D-mannose

The syrup $[\alpha]_D$, + 25° (c, 0.5 water) was chromatographically pure (system D) and demethylation of a small portion (2mg.) with boron trichloride indicated the presence of mannose only.[109)]

2,3,6-Tri-O-methyl-D-mannose

The syrup $[\alpha]_D$, -10° (c, 2.3 water) was chromatographically identical with an authentic sample of 2,3,6-tri-O-methyl-D-mannose (systems D and E). The di-p-nitrobenzoate was prepared using the method of Rebers and Smith[114] and had m.p. and mixed m.p. 189°, $[\alpha]_D$, + 33° (c, 0.9 chloroform) after recrystallisation from ethyl acetate.[115]

2,3,6-Tri-O-methyl-D-glucose

The syrup $[\alpha]_D$, + 69° (c, 1.3 water) was crystallised from isopropyl ether and had m.p. and mixed m.p. 116°, $[\alpha]_D$, + 70° (c, 1.7 water).[116] Demethylation gave glucose only.

2,3-Di-O-methyl-D-mannose

This fraction had an R_f value slightly less than that of the other di-O-methyl sugar. Demethylation of a portion of the syrup showed the presence of mannose only, and electrophoresis indicated that the sugar was identical with an authentic sample of 2,3,di-O-methyl-D-mannose.

2,3-Di-O-methyl-D-glucose

Demethylation of a portion of the syrup gave only glucose and electrophoresis indicated that the sugar was similar to 2,3-di-O-methyl-D-glucose.

Partial Hydrolysis of the Glucomannan and Resolution of the Hydrolysate

The purified glucomannan (20g.) was dissolved in 90% formic acid (200ml.) and water (200ml.) was added. After heating to 100° for 3 hours the formic acid was removed by repeated evaporations from water. Formate esters were hydrolysed by treatment with sulphuric acid (500ml., 0.5 N) at 100° for 5 minutes. Neutralisation of the filtrate with

barium carbonate followed by deionisation (Amberlite IR 120 and Dowex 1X-4, acetate form) and evaporation gave a pale yellow syrup. Yield: 13.9g. A portion of the hydrolysate (10g.) was added to the top of a charcoal-Celite column and resolved using mixtures of ethanol and water. (4 litres each of water and 10% ethanol, 10 and 20% ethanol, followed by 2 litres each of 20 and 40% ethanol). The eluate was collected in 25-ml. fractions.

Identification of the Sugars

All sugars were chromatographically identified with authentic specimens in systems A,B and H.

Monnosaccharides

The monosaccharides were separated by paper chromatography (system C) and identified as mannose, glucose and smaller amounts of galactose and xylose.

Oligosaccharides

The fractions obtained from the column were usually not pure components and were further purified by paper chromatography (system B). They are given here in order of elution.

M = mannopyranose; G = glucopyranose; X = xylopyranose.

Mannobiose ($Ml^{\beta}\rightarrow 4M$)

Hydrolysis of a portion of this fraction (378mg.) indicated the presence of mannose only. Crystallisation from methanol gave a product (345mg.) $[\alpha]_D$, -9° (c, 2.2 water) having m.p. 210-211°, which was undepressed when admixed with an authentic specimen of 4-O-β-D-manno-pyranosyl-D-mannose.[55,117]

Mannosyl glucose ($Ml^{\beta}\rightarrow 4G$)

Hydrolysis of a portion of the syrup (45mg.) gave equal amounts

of glucose and mannose and hydrolysis after reduction with sodium borohydride indicated the presence of mannose. Crystallisation from methanol containing a little butan-1-ol gave a product (17mg.) $[\alpha]_D$, + 22° (c, 2.1 water) having m.p. 168-170°, which was not depressed by admixture with authentic 4-O-β-D-mannopyranosyl-D-glucopyranose.[34]

Xylobiose

Hydrolysis of the syrup (20mg.) gave xylose only. This disaccharide was not investigated further.

Mannotriose (Ml$\xrightarrow{\beta}$4Ml$\xrightarrow{\beta}$4M)

The syrup (303mg.), which on hydrolysis gave mannose and on partial hydrolysis gave mannose and mannobiose was crystallised from methanol containing a little propan-1-ol. The crystals (235mg.) $[\alpha]_D$, -21° (c, 1.7 water) had m.p. 170-171° which was not depressed when admixed with an authentic specimen of O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose.[55,118]

Cellobiose (Gl$\xrightarrow{\beta}$4G)

Hydrolysis of a portion of the solid (15mg.) $[\alpha]_D$, + 34° (c, 0.4 water) indicated the presence of glucose only. A small quantity of crystals were obtained by treatment of the residue with methanol. These had m.p. and mixed m.p. of 231-232° with an authentic specimen of 4-O-β-D-glucopyranosyl-D-glucose.[58,119]

Glucosyl mannose (Gl$\xrightarrow{\beta}$4M)

Hydrolysis of a portion of the syrup (124mg.) gave equal amounts of glucose and mannose and hydrolysis after reduction with sodium borohydride gave glucose. Crystallisation from methanol containing a little butan-1-ol gave crystals (95mg.) $[\alpha]_D$, + 8° (c, 3.0 water) having m.p. 188-190°[52] which was not depressed when admixed with an authentic

specimen of 4-O-β-D-glucopyranosyl-D-mannose.[55,120]

Mannotetrose ($Ml\xrightarrow{\beta}4Ml\xrightarrow{\beta}4Ml\xrightarrow{\beta}4M$)

Hydrolysis indicated the presence of mannose. Partial hydrolysis gave mannose, mannobiose and mannotriose. A small amount of crystals was obtained m.p. 230-232° which was not depressed on admixture with an authentic specimen of O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannose. The specific rotation of the residue (20mg.) was -30° (c, 1.9 water).[86]

O-mannosyl-O-glucosyl-mannose ($Ml\xrightarrow{\beta}4Gl\xrightarrow{\beta}4M$)

The syrup (92mg.) $[\alpha]_D$, -15° (c, 0.9 water), (R_c, 0.46 system B) could not be induced to crystallise. Hydrolysis indicated the presence of mannose and glucose in a ratio of 2:1 and partial hydrolysis indicated the presence of mannosyl glucose and glucosyl mannose.

Preparation of the Nitrate Derivative of the Glucomannan

The glucomannan (2.1g.) was treated with a nitric acid:phosphorus pentoxide:phosphoric acid mixture (64:10:26 w/w, 100ml.) for one hour at 17°[98] and the reaction mixture was poured into a 20% brine solution (2 litres) cooled to -20°. The glucomannan nitrate was collected by filtration and washed with water and methanol. Yield: 3.3g.

Periodate Oxidation of the Glucomannan

Portions of the glucomannan (50mg.) were dissolved in 0.03 M sodium metaperiodate solution (20ml.) and kept in the dark at 25° for various periods of time. The periodate consumed was determined by the excess arsenite method (see Figure 1.). Extrapolation to zero time gave a consumption of periodate of 0.988 moles per anhydro-hexose unit. Reduction with borohydride and then hydrolysis of the oxidized polysaccharide

followed by chromatographic analysis of the products (system C) did not indicate any unoxidised sugars were present.[59, 101)]

ENGELMANN SPRUCE GALACTOGLUCOMANNAN

Isolation of the Hemicellulose

The crude Hemicellulose C, which contained the galactoglucomannan was obtained during the purification of the xylan hemicellulose. Later a modified method of extraction was used which bypassed the collection of the crude xylan. Thus, the filtrate from a 10% potassium hydroxide extract (8l.) of the holocellulose (880g.) was treated directly with 5% barium hydroxide solution (8 litres). After standing overnight the precipitate was collected by centrifugation, washed twice with water, dissolved in cold (-20°) 50% acetic acid and the resulting solution poured into 4 volumes of ethanol. The precipitate of crude Hemicellulose C was washed with 70% ethanol, ethanol and light petroleum and dried. Yield: 44.6g. Analysis indicated the presence of galactose, glucose, mannose, arabinose and xylose. (See Table XIII) The supernatant solution from the barium hydroxide precipitation was poured into acidified ethanol and the product collected as before, giving the xylan. (Hemicellulose A 112g.)

Purification of the Hemicellulose C

The method was similar to that used for the purification of the glucomannan, except that the precipitate of the barium complex was washed twice only with water before solution in 50% acetic acid. Yield: 21.5g. Analysis indicated the presence of glucose, mannose and galactose. $[\alpha]_D$, -7.3° (1% in water) (See Table XIII)

TABLE XIII

The Composition of the Galactoglucomannan Fractions

Fraction	Galactose	Glucose	Mannose	Ar-X	Gl/Ma
Crude galactoglucoamnnan (first preparation)	20.9	12.8	31.5	16.8	0.41
Crude galactoglucomannan (second preparation)	18.6	17.5	47.7	16.5	0.37
Purified by 2 $Ba(OH)_2$ treatments.	20.4	20.0	59.8	....	0.33
Purified, then treated with Fehling solution	14.7	22.5	62.8	....	0.36

Ar = L-arabinose

Gl = D-glucose

Ma = D-mannose

X = D-xylose

Methylation of the Galactoglucomannan

The galactglucomannan (20g.) was methylated by a procedure similar to that used for methylation of the glucomannan to give a product (4.5g.) which had a methoxyl content of 42.2%. A portion of this (1.5g.) was subjected to a second methylation according to Purdie[113] giving a product (1.35g.) having a methoxyl content of 44.9%.

Methanolysis of the Methylated Galactoglucomannan

The fully methylated galactoglucomannan (0.61g.) was treated with 2.3% methanolic hydrogen chloride using a procedure similar to that used for the methylated glucomannan, except that product was resolved into constituent sugars by paper chromatography (system D). (See Table VI)

Identification of the Methylated Sugars

The methylated sugars were identified by procedures similar to those used for the hydrolysis products of the methylated glucomannan. Therefore only yields, melting points and rotations will be recorded. (See Table VI).

2,3,4,6-Tetra-O-methyl-D-galactose

(67mg.) $[\alpha]_D$, + 109° (c, 2.3 water) The aniline derivative (6mg.) had m.p. and mixed m.p. 190-191° and $[\alpha]_D$, -135° (c, 0.3 pyridine).

2,3,4,6-Tetra-O-methyl-D-mannose

(8.8mg.) $[\alpha]_D$, + 17° (c, 0.9 water) Demethylation gave mannose only.

2,3,6-Tri-O-methyl-D-mannose

(205mg.) $[\alpha]_D$, -7° (c, 1.1 water) The di-p-nitrobenzoate had m.p. and mixed m.p. 187-188°.

2,3,6-Tri-O-methyl-D-glucose

(64mg.) $[\alpha]_D$, + 72° (c, 1.0 water) m.p. and mixed m.p. 119°.

2,3-Di-O-methyl-D-glucose

Demethylation of a portion of the syrup (29mg.) indicated the presence of mannose only and electrophoresis indicated that the sugar was identical with an authentic specimen of 2,3-di-O-methyl-D-glucose.

Di-O-methyl sugars (fraction A)

Demethylation of a portion of the syrup indicated the presence of mannose and glucose in the approximate ratio of 2:1 and electrophoresis indicated the presence of 2,3,-di-O-methyl-D-glucose and D-mannose.

Partial Hydrolysis of the Galactoglucomannan

The purified galactoglucomannan (20g.) was subjected to a hydrolysis procedure similar to that used for the glucomannan except the time of hydrolysis was reduced from 3 to 2 hours. Yield: 9.35g. of which 7.5g was resolved on the charcoal-Celite column.

The oligosaccharides were identified by procedures similar to those used for the corresponding sugars obtained by hydrolysis of the glucomannan. Therefore only the melting points, yields and rotations are recorded.

Mannobiose

(483mg.) $[\alpha]_D$, -9° (c, 3.6 water) m.p. and mixed m.p. 200-202°.

Mannosyl glucose

(100mg.) Only a small amount of crystalline material (7.5mg.) was obtained having m.p. and mixed m.p. 197-198° and $[\alpha]_D$, + 24° (c, 0.7 water)

Mannotriose

(211mg.) $[\alpha]_D$, -20° (c, 1.9 water) m.p. and mixed m.p. 168-170°.

Cellobiose

(15mg.) $[\alpha]_D$, + 32° (c, 1.1 water) m.p. 231-233° Cellobiose (13mg.) was refluxed with acetic anhydride (2ml.) and anhydrous sodium acetate (50mg.) for 3 hours. The excess anhydride was removed in a current of air and the residue neutralised with sodium bicarbonate solution. Extraction of the mixture with chloroform and evaporation of the dried chloroform solution gave a residue, which was crystallised from methanol. m.p. and mixed m.p. 219-220°.

Glucosyl mannose

(91mg.) $[\alpha]_D$, + 12° (c, 2.7 water) m.p. and mixed m.p. 180-182°[52)]

A triose

A small amount of sugar (9mg.) was obtained having R_c, 0.22 (system B) and the degree of polymerisation of the compound was 2.8[96)] Partial hydrolysis with formic acid gave unchanged triose, but hydrolysis indicated the presence of glucose and mannose in a ratio of 1:2.

O-galactosyl-O-mannosyl-mannose

The sugar (38mg.) $[\alpha]_D$, + 21° (c, 0.9 water) and R_c, 0.45 (system B) could not be crystallised. Analysis indicated the presence of galactose and mannose and partial hydrolysis gave galactosyl mannose and mannobiose.

O-glucosyl-O-mannosyl-mannose

The sugar (61mg.) $[\alpha]_D$, -6° (c, 1.7 water) and R_c, 0.29 (system B) could not be induced to crystallise. Hydrolysis indicated the presence of glucose and mannose in the ratio of 1:2 and partial hydrolysis gave glucosyl mannose and a trace of mannobiose.

A triose

The sugar (15mg.) $[\alpha]_D$, + 30° (c, 0.6 water) and R_c, 0.63

(system B) could not be induced to crystallise. Hydrolysis indicated the presence of galactose, glucose and mannose in equimolar proportions, and partial hydrolysis gave the same sugars and a disaccharide which was probably an O-galactosyl glucose. Hydrolysis after reduction with sodium borohydride gave galactose and a trace of glucose.

Preparation of the Nitrate Derivative of the Galactoglucomannan

The galactoglucomannan (1.3g.) was nitrated using the procedure outlined for the glucomannan. Yield: 0.98g.

Periodate Oxidation of the Galactoglucomannan

The galactoglucomannan (0.612g.) was oxidized using the procedure outlined for the xylan except that less periodate was used (50ml., 0.1781M). Extrapolation gave a value (0.175) which was equivalent to the consumption of 1.16 moles of oxidant per anhydrohexose unit. (See Figure 1.)

Determination of the Molecular Weight of the Hemicellulose Derivatives

The osmotic pressures were determined using Zimm-Myerson osmometers[97] as modified by Stabin and Immergut.[98] Gel cellophane membranes were used, which had never been allowed to dry. The temperature was 30 $\pm$ 0.01° and the static method was used to measure the osmotic height. The solvent used for the methylated and acetylated hemicelluloses was chloroform-ethanol (9:1 v/v) and n-butyl acetate was used for the nitrated hemicelluloses. (See Figure 2. for the xylan, Figure 3. for the glucomannan and Figure 4. for the galactoglucomannan)

PERIODATE OXIDATION DATA FOR THE THREE HEMICELLULOSES

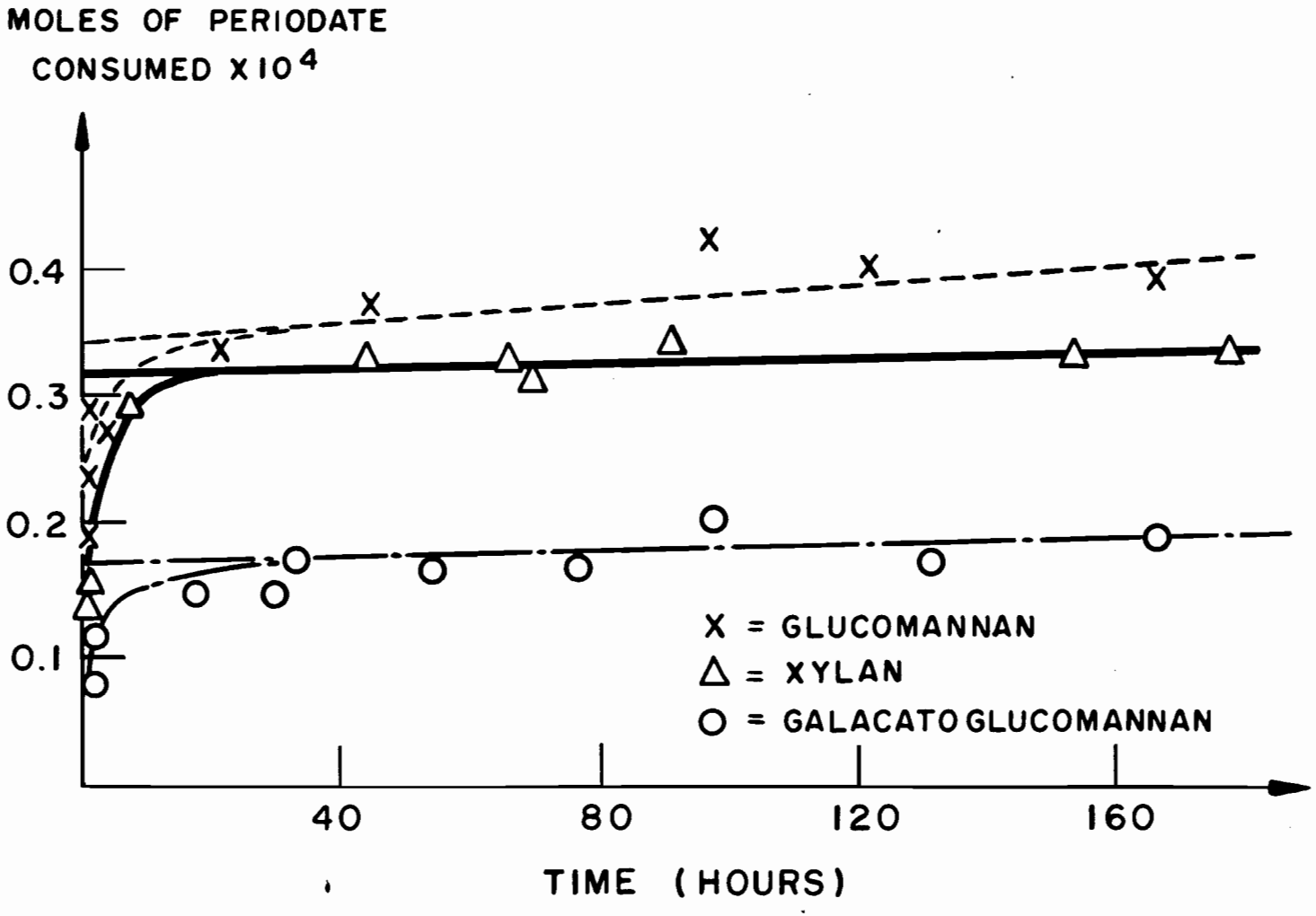

MOLES OF PERIODATE
CONSUMED X 10 4
0.4
0.3
0.2
0.1
X = GLUCOMANNAN
△ = XYLAN
O = GALACATO GLUCOMANNAN
40
80
120
160
TIME (HOURS)

FIGURE 1.

OSMOMETRY DATA FOR THE HEMICELLULOSE DERIVATIVES

h = The height of solution (cm.)

w = Concentration (g./Kg.)

Extrapolation to zero concentration gives values for h/w from which the number-average molecular weight ($\bar{M}n$) is given by the relationship $25,700 = \bar{M}n \times (h/w)_{w=0}$.

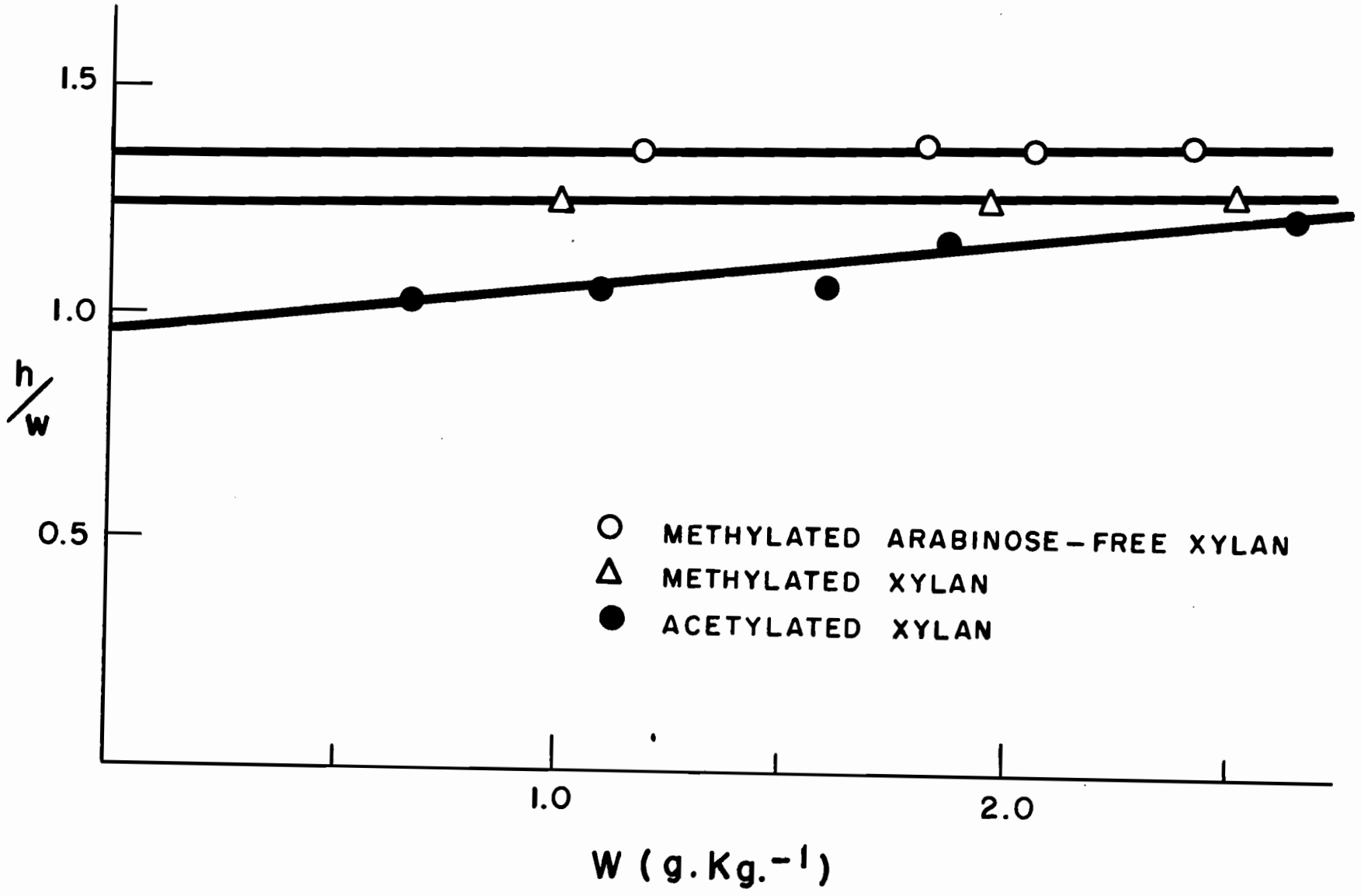

1.5
1.0
0.5
h/w
METHYLATED ARABINOSE-FREE XYLAN
METHYLATED XYLAN
ACETYLATED XYLAN
1.0
2.0
W (g.Kg.⁻¹)

FIGURE 2.

FIGURE 3.

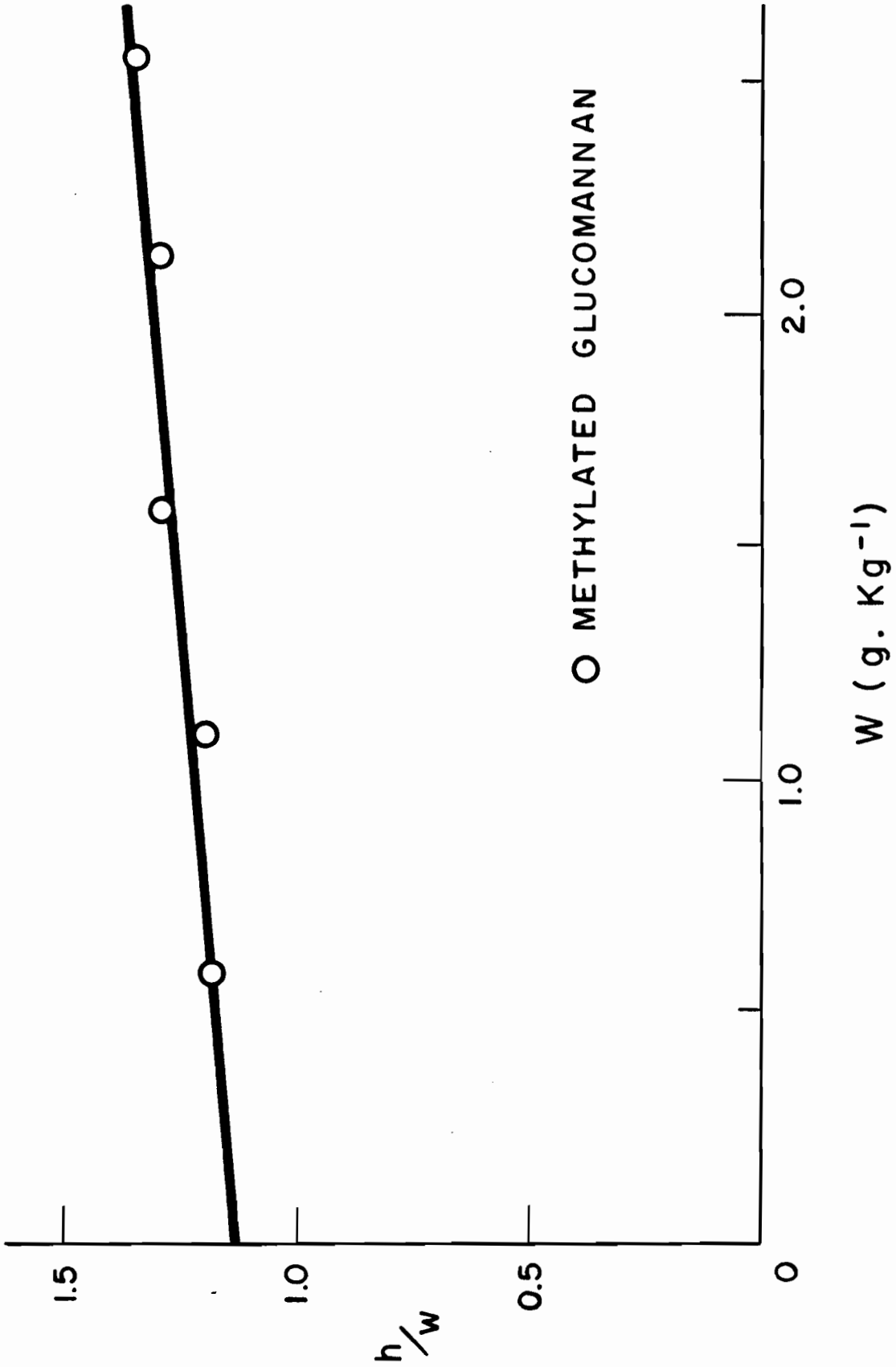
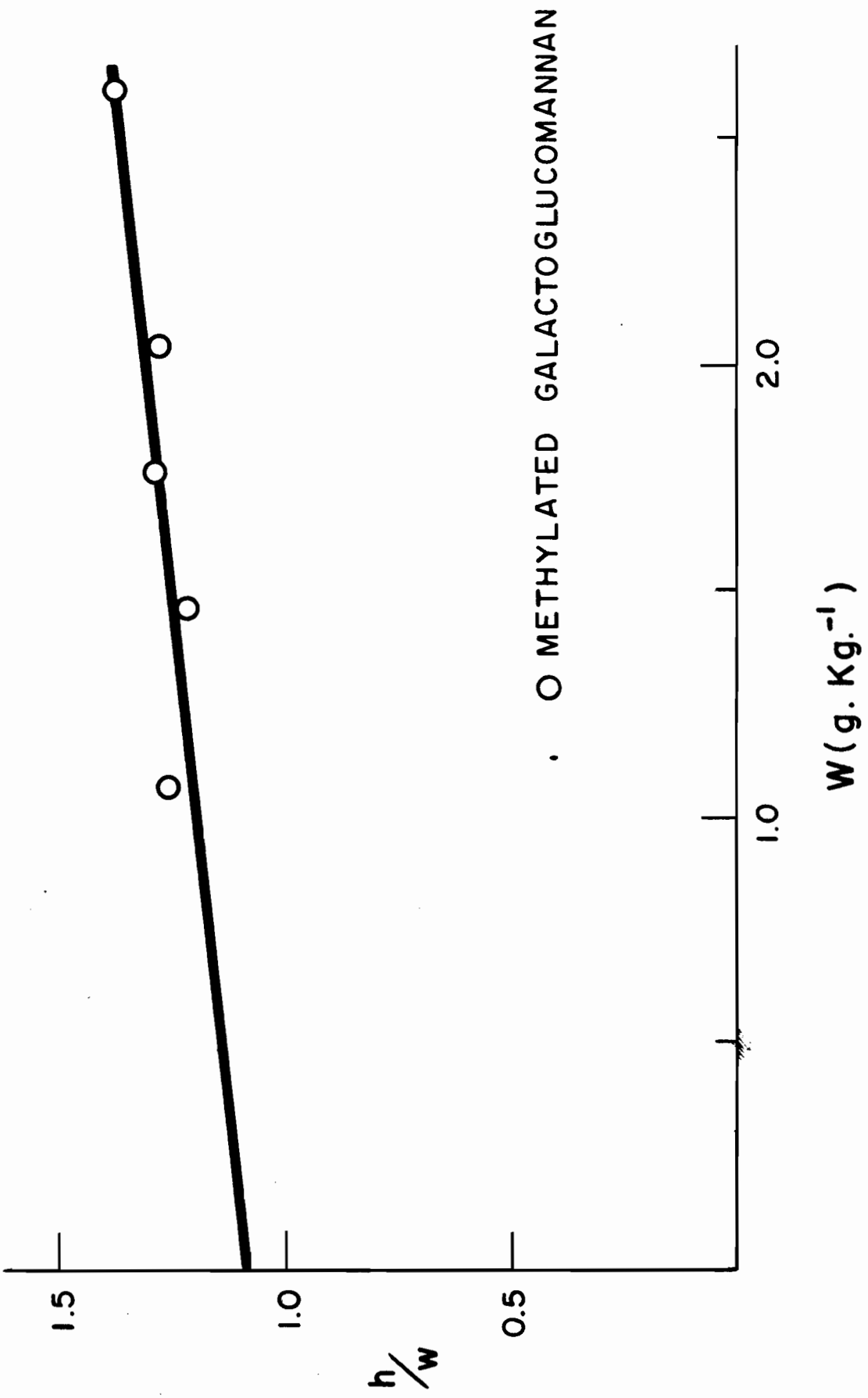


FIGURE 4.



REFERENCES

1. E. Schulze, Ber., 24, 2277 (1891)
2. P. Karrer, Polymere Kohlenhydrate, Leipzig, p.263 (1925)
3. E.L. Hirst, J. Chem. Soc., 2974 (1955); idem, Proc. Roy. Soc., A,252, 287-312 (1959)
4. H.O. Bouveng and Lindberg B., Advances in Carbohydrate Chem., 15, 52 (1960)
5. C.T. Bishop and F.P. Cooper, Can. J. Chem., 38, 388 (1960)
6. R.L. Whistler and C.L. Smart, Polysaccharide Chemistry, Academic Press Inc., New York, 1953
7. J.K.N. Jones, L.E. Wise and J.P. Jappe, Tappi, 39, 139 (1956)
8. B. Hägglund, B. Lindberg and J. McPherson, Acta Chem. Scand., 10, 1160 (1956)
9. H. Meier, ibid, 15, 1381 (1961)
10. J.E. Scott, Methods of Biochem. Anal., 8, 146 (1960)
11. H. Meier, Acta Chem. Scand., 12, 144 (1958)
12. A.J. Erskine and J.K.N. Jones, Can. J. Chem., 34, 821 (1956)
13. H.M. O'Dwyer., Biochem.J., 34, 149 (1940) and previous papers.
14. G.N. Kowkabany, Advances in Carbohydrate Chem., 9, 303 (1954)
15. G.O. Aspinall, E.L. Hirst, R.W. Moody and E.G.V. Percival, J. Chem. Soc., 1631 (1953); 1289 (1950)
16. J.K.N. Jones and L.E. Wise, ibid, 2750 (1950)
17. R.L. Whistler and C.C. Tu, J. Am. Chem. Soc., 74, 3609 (1952); 75, 645 (1953)

18. J.K.N. Jones and L.E. Wise, J. Chem. Soc., 3389 (1952)

19. D.J. Brasch and L.E. Wise, Tappi, 39, 581, 768 (1956)

20. S.K. Banerjee and T.E. Timell, ibid, 43, 489 (1960)

21. C.T. Bishop and D.R. Whitaker, Chem. and Ind., 119 (1955)

22. G.O. Aspinall, I.M. Cairncross and A. Nicholson, Proc. Roy. Soc., 270 (1959)

23. G.O. Aspinall, Advances in Carbohydrate Chem., 14, 429 (1959)

24. S. Peat, Gwen J. Thomas and W.J. Whelan, J. Chem. Soc., 456 (1952)

25. J.K. Hamilton and H.W. Kirchner, J. Am. Chem. Soc., 80, 4703 (1958)

26. I. Croon, B. Lindberg and H. Meier, Acta Chem. Scand., 13, 1299 (1959)

27. E.C. Sherrard and G.W. Bianco, J. Am. Chem. Soc., 45, 1008 (1923)

28. K. Hess and M. Lüdtke, Ann., 466, 18 (1928)

29. A.P. Yundt, Tappi, 34, 94 (1951)

30. F.E. Brauns and H.F. Lewis, Paper Trade J., 105, No. 10, 35 (1937)

31. H.F. Lewis, F.E. Brauns and M.A. Buchanan, ibid, 110, No. 5, 36 (1940)

32. K. Nishida and H. Hashima, J. Dept. Agric. Kyushu Imp. Univ., 2, 277 (1930)

33. J.G. Leech, Tappi, 35, 249 (1952)

34. A. Anthis, Tappi, 39, 401 (1956)

35. D.H. Ball, J.K.N. Jones, W.H. Nicholson and T.J. Painter, Tappi, 39, 438 (1956)

36. J.K.N. Jones and T.J. Painter, J. Chem. Soc., 669 (1957)

37. G.G.S. Dutton and K. Hunt, J. Am. Chem. Soc., 80, 5697 (1958)

38. I. Croon and B. Lindberg, Acta Chem. Scand., 12, 453 (1958)

39. T.E. Timell and A. Tyminski, Tappi, 40, 519 (1957)

40. T.E. Timell, Svensk Paperstidn., 63, 472 (1960)

41. G.A. Adams, Can. J. Chem., 39, 2423 (1961)

42. J.K. Hamilton, E.V. Partlow and N.S. Thompson, J. Am. Chem. Soc., 82, 451 (1960)

43. T.E. Timell, Tappi, 44., 88 (1961)

44. G.O. Aspinall, E.L. Hirst and J. Ramstad, J. Chem. Soc., 593 (1958): G.O. Aspinall and A. Nicholson, ibid, 2503 (1960)

45. H. Bouveng and B. Lindberg, Acta Chem. Scand., 12, 1977 (1958)

46. idem, ibid, 13, 1884 (1959)

47. W.G. Van Beckum and G.J. Ritter, Paper Trade J., 108, 1, 27 (1939)

48. L.E. Wise, M. Murphy and A.A. D'Addieco, ibid, 122, 35 (1946)

49. C.P.J. Glaudemans and T.E. Timell, Svensk Paperstidn., 60, 869 (1957)

50. P.W. Lange, Fundamentals of Paper Making Fibres; Tech. Sect. Brit. Paper and Board Makers Assoc., pp. 147-185 (1958)

51. A.C. Neish, Can. J. Biochem. and Physiol., 36, 187 (1958)

52. H. Meier, Acta Chem. Scand., 14, 749 (1960)

53. C.T. Cooper and C.T. Bishop, Can. J. Chem., 38, 793, (1960)

54. J.K.N. Jones and T.J. Painter, J. Chem. Soc., 573, (1959)

55. O. Perila and C.T. Bishop, Can. J. Chem., 39, 815 (1961)

56. J.K. Hamilton and E.V. Partlow, J. Am. Chem. Soc., 80, 4880 (1958)

57. T.E. Timell, Can. J. Chem., 40, 22 (1962)

58. G.O. Aspinall, R. Begbie and J.E. McKay, J. Chem. Soc., 214 (1962)

59. P. Kooiman and G.A. Adams, Can. J. Chem., 39, 889, (1961)

60. T.E. Timell, unpublished results.

61. A.R. Mills and T.E. Timell, unpublished results.

62. A. Jabbar Mian and T.E. Timell, Svensk Paperstidn., 63, 884, (1960)

63. T.E. Timell, ibid, 63, 652 (1960)

64. J.K.N. Jones, E. Merler and L.E. Wise, Can.J. Chem., 35, 634 (1957)

65. J.K. Hamilton and N.S. Thompson, Pulp Paper Mag. Canada, 59, 233 (1958); ibid, Tappi, 42, 752 (1959)

66. T.E. Timell, Chem. and Ind., 905 (1958)

67. idem, Svensk Paperstidn., 64, 651 (1961)

68. E.C.A. Schwarz and T.E. Timell, unpublished results.

69. G.R. Savur, J. Chem. Soc., 2600 (1956)

70. J.K.N. Jones, C.B. Purves and T.E. Timell, Can. J. Chem., 39, 1059 (1961)

71. G.A. Adams, ibid, 35, 556 (1957)

72. G.G.S. Dutton and F. Smith, J. Am. Chem. Soc., 78, 3744 (1956)

73. G.O. Aspinall and M.E. Carter, J. Chem. Soc., 3744 (1956)

74. G.O. Aspinall and J.E. McKay, ibid, 1059, 1958)

75. H. Meier, Acta Chem. Scand., 12, 1911 (1958)

76. T.E. Timell, Can. J. Chem., 37, 827 (1959)

77. A. Jabbar Mian and T.E. Timell, Tappi, 43, 775 (1960)

78. G.G.S. Dutton and G.T. Murata, Can. J. Chem., 39, 1995 (1961)

79. G.G.S. Dutton and Shirley A. McKelvey, ibid, 39, 2583 (1961)

80. B. Lindberg and B. Wickberg, Acta Chem. Scand., 8, 569, (1954)

81. C.P.J. Glaudemans and T.E. Timell, Svensk Paperstidn., 61, 1 (1958)

82. H.O. Bouveng, Acta Chem. Scand., 13, 1887 (1959)

83. F.W. Barth and T.E. Timell, J. Am. Chem. Soc., 80, 6320 (1958)

84. I. Croon and T.E. Timell, Can. J. Chem., 38, 720 (1960)

85. J. Saarnio, Suomen Kemistilehti B29, 35 (1956)

86. M.O. Gyaw and T.E. Timell, Can. J. Chem., 38, 1957 (1960)

87. R. Lebel, private communication.

88. T.E. Timell and E.C. Jahn, Svensk Paperstidn., 54, 831 (1951)

89. G.G.S. Dutton and K. Hunt, J. Am. Chem. Soc., 80, 4420 (1958)

90. G.O. Aspinall, E.L. Hirst and R.S. Mohamed, J. Chem. Soc., 1734 (1954)

91. J. Saarnio, K. Wathen and C. Gustafsson, Acta Chem. Scand., 8, 825 (1954)

92. T.E. Timell, Chem. and Ind., 999 (1959)

93. idem, Can. J. Chem., 37, 893, (1959)

94. G.O. Aspinall, M.E. Carter and J. Los, J. Chem. Soc., 4807 (1956)

95. R.L. Whistler and D.F. Durso, J. Am. Chem. Soc., 72, 677 (1950)

96. T.E. Timell, Svensk Paperstidn., 63, 668 (1960)

97. B.H. Zimm and I. Myerson, J. Am. Chem. Soc., 68, 911 (1946)

98. J.V. Stabin and E.H. Immergut, J. polymer Sci., 14, 209 (1954)

99. T.J. Painter and C.B. Purves, Tappi, 43, 729 (1960)

100. T.E. Timell, Svensk Paperstidn., 64, 744 (1961)

101. M. Abdel-Akher, J.K. Hamilton, R. Montgomery and F. Smith, J. Chem. Soc., 74, 4970 (1952)

102. G.A. Adams, Can. J. Chem., 38, 2402 (1960)

103. T.E. Timell, C.P.J. Glaudemans and A.L. Currie, Anal. Chem., 28, 1916 (1956)

104. B.L. Browning, Tappi, 32, 119 (1949)

105. T.E. Timell and C.B. Purves, Svensk Paperstidn., 54, 303 (1951)

106. T.E. Timell, J. Am. Chem. Soc., 81, 4989 (1959)

107. R.A. Laidlaw and E.G.V. Percival, J. Chem. Soc., 528 (1950)

108. I. Ehrenthal, M.C. Rafique and F. Smith, J. Am. Chem. Soc., 74, 1341 (1952)

109. S. Allen, T.G. Bonner, E.J. Bourne and N.M. Saville, Chem. and Ind., 630 (1958)

110. A.E. Carruthers and E.L. Hirst, J. Chem. Soc., 2299 (1922)

111. G.O. Aspinall and R.J. Ferrier, ibid, 638 (1958)

112. M. Abdel-Akher and F. Smith, Nature, 166, 1037 (1950)

113. T. Purdie and J.C. Irvine, J. Chem. Soc., 85, 1049 (1904)

114. P.A. Rebers and F. Smith, J. Am. Chem. Soc., 76, 6097 (1954)

115. W.N. Haworth, E.L. Hirst and H.R.L. Streight, J. Chem. Soc., 1349 (1931)

116. J.C. Irvine and E.L. Hirst, ibid, 121, 1213 (1922)

117. R.L. Whistler and J.Z. Stein, J. Am. Chem. Soc., 73, 4169 (1951)

118. R.L. Whistler and C.G. Smith, ibid, 74, 3795 (1952)

119. Z.H. Skraup and J. König, Monatsh., 22, 1011 (1901)

120. M. Bergman and H. Schotte, Ber., 54, 1564 (1921)

121. A. Tyminski and T.E. Timell, J. Am. Chem. Soc., 82, 2823 (1960)

122. J.K. Hamilton, H.W. Kirchner and N.S. Thompson, ibid, 78, 2508 (1956)

123. G.O. Aspinall, R.A. Laidlaw and R.B. Rashbrook, J. Chem. Soc., 444 (1957)

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. The extractive free wood of Engelmann spruce (Picea engelmannii (Parry) Engelm.) was delignified with acid chlorite to give a holocellulose in 73% yield. Extraction of the hemicellulose with potassium hydroxide solution gave a crude extract (14.5%) which was resolved into two fractions by treatment with barium hydroxide solution. The portion remaining in solution, was an arabino-glucurono-xylan (8%), while the insoluble portion was a galactoglucomannan. Further extraction of the holocellulose with sodium hydroxide solution containing borate gave a crude extract (10%) which was purified by precipitation of its barium complex to give the glucomannan fraction (8%).

2. Analysis of the xylan indicated the presence of xylose arabinose and 4-O-methyl glucuronic acid in a ratio of 7:1:1.1. The methylated xylan was hydrolysed and the resulting sugars were resolved by chromatography. The following sugars were obtained: 2,3,5-tri-O-methyl-L-arabinose (6 parts), 2,3,4-tri-O-methyl-D-xylose (1), 2,3-di-O-methyl-D-xylose (49), 2-O-methyl-D-xylose (8), 3-O-methyl-D-xylose (2), and 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-3-O-methyl-D-xylose (10).

3. The xylan was subjected to a mild hydrolysis which selectively removed the arabinose. The arabinose-free xylan was methylated and hydrolysed and the resulting sugars were resolved by chromatography. The above methylated sugars were obtained in a molar ratio of 0.1:1:55:2:4:12.

4. On periodate oxidation the xylan consumed 0.775 moles of oxidant per anhydro-pentose unit. Osmotic pressure measurements on the methylated

and the acetylated xylan gave a number-average degree of polymerisation of 130 and similar measurements on the methylated arabinose-free xylan gave a value of 120.

5. It was concluded that the arabino-4-O-methylglucurono-xylan consisted of an essentially linear chain, composed of a minimum of 95 β-(1--4)-linked D-xylose residues to which were directly attached an average of 1.1 α-(1--2)-linked 4-O-methyl-D-glucuronic acid residues and 1 α-(1--3)-linked L-arabinofuranose residue per 7 xylose residues.

6. Analysis of the glucomannan indicated the presence of galactose, glucose and mannose residues in a ratio of 3:20:63. The glucomannan was methylated and hydrolysed and the resulting sugars were resolved by chromatography. The following sugars were obtained: 2,3,4,6-tetra-O-methyl-D-galactose (2), 2,3,4,6-tetra-O-methyl-D-mannose (1), 2,3,6-tri-O-methyl-D-mannose (56), 2,3,6-tri-O-methyl-D-glucose (18), 2,3-di-O-methyl-D-mannose (1.2) and 2,3-di-O-methyl-D-glucose (0.7).

7. The glucomannan was subjected to a partial hydrolysis with formic acid and the resulting sugars were resolved by chromatography. The monosaccharides glucose, mannose and galactose were obtained together with the disaccharides mannobiose (M--M), cellobiose (G--G), mannosyl glucose (M--G), glucosyl mannose (G--M), the trisaccharides mannotriose (M--M--M), (M--G--M) and (G--M--M), and the tetrasaccharide mannotetrose (M--M--M--M).

8. On periodate oxidation the glucomannan consumed 0.988 moles of oxidant per anhydro-hexose unit. Osmometry data indicated that the methylated glucomannan had a $\bar{P}n$ of 111.

9. It was concluded that the glucomannan was a slightly branched triheteropolymer composed of a minimum of 105 glucose and mannose residues in a ratio of 1:3.2, to which are attached about 3.5% galactose residues.

10. Analysis of the galactoglucomannan indicated the presence of galactose, glucose and mannose residues in the ratio of 1:1:3. The galactoglucomannan was methylated and hydrolysed and the sugars were resolved by chromatography. The sugars were similar to those isolated from the hydrolysate of the methylated glucomannan and were in the ratio of 8:1:25:8:4:4, except that the 2,3-di-O-methyl-D-mannose fraction also contained about 30% of other di-O-methyl sugars.

11. The galactoglucomannan was subjected to a partial hydrolysis and mono-, di- and trisaccharides similar to those obtained from the hydrolysate of the glucomannan were obtained together with galactosyl-mannosyl-mannose and a trisaccharide which contained glucose, galactose and mannose. This constitutes the first isolation of a trisaccharide containing all three sugar units and shows that the polysaccharide is a true triheteropolymer.

12. On oxidation with periodate the galactoglucomannan consumed 1.16 moles of oxidant per anhydro-hexose unit. The methylated polysaccharide had a $\bar{P}n$ of 117.

13. It was concluded that the galactoglucomannan was a branched triheteropolymer composed of a minimum of 90 β-(1→4)-linked glucose and mannose residues in a ratio of 1:3 to form a chain which had on the average 1.5 branch points per molecule. To this framework were attached an average of one galactose residue per one glucose and three mannose residues.