THE CONSTITUTION OF THREE HEMICELLULOSES FROM THE WOOD OF AMABILIS FIR (ABIES AMABILIS)

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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July 1962

ACKNOWLEDGEMENT

I express my sincere gratitude to

Dr. T.E. Timell

for his generous assistance and encouragement throughout the course of this work and in the preparation of this thesis.

Grateful acknowledgement is also made to Dr. C.B. Purves for his kind interest and encouragement in my work,
to the American Viscose Corporation for a fellowship during the academic years 1959-60 and 1960-61,
to the National Research Council of Canada for financial assistance during the academic year 1961-62,
and to the Pulp and Paper Research Institute of Canada for a summer stipend.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

LIST OF TABLES

LIST OF FIGURES

Ph.D. Chemistry

Eckhard C.A. Schwarz

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ABSTRACT

Pines, spruces, and firs constitute the presently most important conifers. Firs, members of the genus Abies have so far not been studied with respect to their hemicellulosic constituents. The present investigation is concerned with the isolation and detailed structure of the three main hemicelluloses in amabilis fir (Abies amabilis), namely an arabino-glucurono-xylan (7.0 % of the wood), a galactoglucomannan (4.1 %), and a glucomannan (8.1). These polysaccharides were obtained by a series of fractional extractions and precipitaions with alkali from chlorite holocellulose. The techniques used for establishing their structure included the classical methylation method, partial acid hydrolysis and identification of oligosaccharide fragments, and oxidation with periodate. Molecular weights were determined osmometrically.

The xylan consisted of a framework of at least 100 (1→4)-linked β-D-xylose residues to which were directly

attached terminal residues of (1→2)-linked 4-O-methyl-α-D-glucuronic acid and (1→3)-linked α-L-arabinofuranose residues, with one acid for every fifth and one arabinose for every seventh xylose residue. The galactoglucomannan contained galactose, glucose, and mannose residues in a ratio of 1:1:3. It consisted of a slightly branched backbone of at least 76 β-(1→4)-linked D-glucose and D-mannose units, some of which carried α-(1→6)-linked D-galactopyranosyl side chains. The glucomannan was composed of galactose, glucose, and mannose residues in a ratio of 0.1:1:3. It contained a main chain of at least 95 β-(1→4)-linked D-glucose and D-mannose residues, a few of which carried α-(1→6)-linked D-galactopyranosyl side chains. In both hexosans single glucose units were probably interposed between two to six contiguous mannose residues.

GENERAL INTRODUCTION.

Hemicelluloses constitute a large group of polysaccharides of almost universal occurrance in the plant kingdom. Next to cellulose they are the most abundant naturally occurring organic material. In association with cellulose and lignin, hemicelluloses constitute part of the building material of the plant cell walls and may therefore be termed as "non-cellulosic cell wall polysaccharides". The term "hemicellulose" was introduced by Schulze (1) in 1891. He defined hemicelluloses as the alkali soluble fraction of the plant cell wall, readily yielding simple sugars on hydrolysis with hot mineral acids.

The function of the hemicelluloses appears to be that of a cementing material, which binds together the cellulose fibres, thus imparting to the parent tissue strength and resistance to shear. It is interesting to note, that in the case of land plants a relatively small group of sugars serve as building units. They are D-xylose, D-mannose and D-glucose, and to a lesser extent, L-arabinose, D-galactose, L-rhamnose, L-fucose and 4-O-methyl-D-glucuronic acid.

In most land plants these sugars are connected by β-(1→4)-glycosidic bonds to form linear or slightly branched macromolecules of varying lengths.

The chemical structure and quantitative distribution of hemicelluloses in arborescent plants differ somewhat from one species to another. In physical properties and molecular size the members of this group also exhibit quite varying properties.

The difference in average molecular weight between cellulose and hemicelluloses is very large. Native wood cellulose seems to have a weight-average degree of polymerization of approximately 10,000 , whereas the corresponding value for hemicelluloses lies between 100 and 500. It must be borne in mind, however, that isolation and fractionation procedures often degrade the polysaccharide, and that information obtained on degree of polymerization does not always correspond to the true value of a polysaccharide in its native state.

The present study is concerned with the isolation and properties of the main hemicelluloses of amabilis fir (Abies amabilis). Amabilis fir is the most abundant of the firs in the pacific northwest and forms extensive pure forests in many localities. It attains its largest proportions in the Olympic Mountains in western Washington,

where mature trees vary from 140 to 160 ft. in heighth and 2 to 4 ft. in diameter. In mixed stands this species is commonly associated with Sitka spruce, Douglas fir, grand fir, western hemlock and western red cedar. Amabilis fir has been a source of valuable softwood lumber and was once exported in quantities to the Orient. In more recent years most of it has been used by the pulp industry.

The hemicelluloses investigated here include an arabino-4-O-methylglucuronoxylan, an alkali-soluble glucomannan and a water-soluble galactoglucomannan. Similar polysaccharides from other gymnosperms have already been studied and will be dealt with in the historical part of this thesis; however, this has been the first time, that hemicelluloses from the genus Abies have been under investigation, and an attempt has been made to isolate and characterize the three main hemicelluloses in detail.

HISTORICAL INTRODUCTION.

Hemicellulose chemistry underwent an enormous development upon the discovery of new techniques in organic chemistry, especially the various chromatographic methods, which made it possible to elucidate the fine structure of a hemicellulose in a way that would not have been possible before. The classical methylation techniques of Purdie (2) and Haworth (3) still constitute the chief methods in determining the nature of the glycosidic bond; more recently oxidation with periodate (4) and lead tetraacetate (5) and graded hydrolysis have been successfully applied, all of which require the use of chromatography. Careful fractionation and purification of hemicellulose mixtures have to be carried out, to obtain homogeneous products and avoid misleading interpretation of results. Recently these various methods of fractionation and identification have been summarized by Bouveng and Lindberg (6).

Xylans:

Xylans are the most abundantly occurring hemicelluloses. They can be found in wood, cereal straws,

seed hairs, seaweeds etc. Xylans studied so far can be classified into four groups : the true xylans, the arabino-xylans, which have both been isolated, for example, from esparto grass (7,8) and wheat flour (9), glucuronoxylans, chiefly occurring in arborescent angiosperms (hardwoods), and arabino-glucuronoxylans, mainly found in arborescent gymnosperms (softwoods), but also to some extent in cereals and grasses. An extensive review of xylans and other hemicelluloses has recently been given by Aspinall (10), where further classifications as to origin and characteristics have been made.

The basic framework of β-(1→4)-linked D-xylopyranose residues is typical for all land plant xylans. Every wood xylan so far investigated has been found to contain single terminal sidechains of 4-O-methyl-D-glucuronic acid attached glycosidically to the 2-position of a xylopyranose unit of the xylan backbone (11), with the notable exception of Monterey pine (12), where a few of the 4-O-methyl-D-glucuronic acid residues have been found to be (1→3)-linked. In hardwoods the ratio of xylose to uronic acid lies between 7 and 10 to 1, in softwoods between 4 and 8 to 1. Thus softwood xylans are generally more acidic than hardwood xylans. The nature of the linkage of the 4-O-methyl-D-glucuronic acid was first established by the unequivocal identification of an aldobiouronic acid obtained in high

yield on partial hydrolysis of a xylan from trembling aspen by Jones and Wise (13) as 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose. A direct proof of the α-type of the glucosidic linkage has been furnished by Gorin and Perlin (14).

Since then this aldobiouronic acid has been obtained from many woods, including the following softwoods : western hemlock (15), loblolly pine (16), black spruce (17), maritime pine (18), scots pine (19), Norway spruce (20), Sitka spruce (21), Monterey pine (12), european larch (22), eastern white pine (23), tamarack (24), Engelmann spruce (25), southern pine and redwood (26), all indicating the α-(1→ 2)-glycosidic linkage of the 4-O-methyl-D-glucuronic acid side chain.

Other aldobiouronic acids have been obtained on partial hydrolysis of maritime pine (27), namely : 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose, 6-O-(β-D-glucopyranosyluronic acid)-D-galactopyranose and 4-O-(α-D-galactopyranosyluronic acid)-D-xylopyranose. The first two acids have recently been obtained from eastern white pine (23), but their possible connection with the xylan is uncertain.

Softwood xylans contain fairly large amounts of terminal L-arabinofuranose residues. Values of the ratio

arabinose : xylose reported in the literature are ranging from 1:7 to 1:42 (Table I). Xylans isolated from Norway spruce (Picea abies)(20) and loblolly pine (Pinus taeda)(16) are reported to contain no arabinose groups, but in another study (28) of the former species a xylan was isolated containing one arabinose side chain per six xylose residues.

The reason for the conflicting results may be, that some or all of the arabinose groups had been removed during the isolation of the hemicellulose. The possibility, however, that some softwood xylans are really devoid of arabinose residues or that some coniferous woods contain both an arabino-glucuronoxylan and a glucuronoxylan cannot be excluded.

Trembling aspen (Populus tremuloides) was believed earlier to contain also an arabino-glucuronoxylan (29), as the single exception of the hardwood xylans, but a more recent investigation has shown (30), that the arabinose was not an integral part of the xylan. Repeated precipitation of the xylan with Fehling's solution yielded a glucurono-xylan devoid of arabinose groups. The fact that only one non-reducing end group was found per average molecule indicated that the xylan framework was linear.

The first softwood xylan to be thoroughly investigated was that of western hemlock (Tsuga heterophylla) by

Dutton and Smith (15). The mode of linkage of the arabinofuranose side chain, however, was not unequivocally established. Methylation data suggested that the arabinofuranose residues were glycosidically attached to the 3-position of the xylose. Due to the lability of furanosides to acid hydrolysis, disaccharides containing arabinofuranose and xylose have not been obtained by graded acid hydrolysis. However, the use of enzymatic hydrolysis (31) has furnished such fragments thus proving directly that arabinofuranose was attached (1→3) to the xylan backbone.

Recently, Aspinall et al. attacked the problem of the arabinose linkage in a novel way. The primary alcohol groups of the arabinofuranose residues in a cereal arabinoxylan were catalytically oxidized to carboxyl groups, which stabilized the otherwise labile arabinofuranose-xylose linkages. Partial hydrolysis gave in low yield an aldobiouronic acid, which was identified as 3-O-(L-arabinofuranosyluronic acid)-D-xylose. However, the presence of such linkages in any of the softwood xylans has not yet been established in this way.

An arabino-glucuronoxylan from Scots pine (Pinus silvestris) has been studied by Garegg and Lindberg (19). The arabinofuranose (1 per 7 xylose units) was reported to be (1->3)-linked, since equimolar amounts of 2,3,5-tri-O-methyl-L-arabinofuranose and 2-O-methyl-D-xylose were obtained on hydrolysis of the methylated polysaccharide.

Table I.

Number of Xylose Residues per Arabinofuranose in Softwood Xylans.

Wood	Xylose/Arabinose	Reference
Western Hemlock	12	15
Norway Spruce	- [6]	20 [28]
European Larch	few	22
Loblolly Pine	-	16
Maritime Pine	10	18
Eastern White Pine	9	23
Engelmann Spruce	7	25
Scots Pine	7	19
Tamarack	17	24
Sitka Spruce	42	21

The 4-O-methyl-D-glucuronic acid (17 % of the xylan) was attached to position 2 .

Adams (24) isolated a similar xylan from tamarack (Larix laricina), containing arabinose, xylose and uronic acid residues in a ratio of 1:17:3 . The low ratio of arabinose to xylose and the low degree of polymerization (DP=19-22) indicated possibly severe degradation during isolation. A similar low molecular weight xylan from Sitka spruce (DP=16-17) has been characterized by Dutton and Hunt (21). It consisted of arabinose, xylose and uronic acid units in a ratio of 1:42:8.5 .

The manner in which L-arabinofuranose branches are arranged along the xylan chain of wheat flour has been examined by Ewald and Perlin (9). The approach was based on the fact that D-xylose units carrying side chains are resistant to oxidation by periodate, whereas other sugar residues are readily attacked. After selective degradation and removal of the oxidized portions xylose and xylobiose were obtained in equal amounts and also a small quantity of xylotriose. The results indicate that L-arabinofuranose branches occur sometimes on isolated D-xylose units, sometimes on two adjacent D-xylose units, but less frequently on three consecutive D-xylose units along the chain.

This procedure has not been applied to softwood xylans as yet, but it is believed that the side chains are distributed at random along the main xylan backbone.

The majority of the arabino-glucuronoxylans from softwoods have been found to consist of an unbranched framework of (1->4)-β-linked D-xylopyranose residues. Branches are believed to occur in the xylan backbone of loblolly pine (Pinus taeda)(16), since it has been found to contain more than one non-reducing end group per average molecule. However, oligosaccharides, which could be obtained from such branching points upon partial hydrolysis and thus would offer a definite evidence, have not yet been identified. It is interesting to note, that recently an arabino-glucuronoxylan has been isolated from the bark of amabilis fir (33), similar, if not identical to other softwood xylans.

Glucomannans and Galactoglucomannans.

The presence of mannose residues in the wood of gymnosperms was early established (34), but their origin long remained obscure. It was believed that mannose was chemically combined with cellulose, since even repeated alkaline extractions could not produce a mannose-free cellulose. Leech, in 1952 (35), obtained a disaccharide from slash pine (Pinus elliottii)- alpha-cellulose containing D-glucose and D-mannose. Anthis (36) later

proved this compound to be a 4-O-β-D-glucopyranosyl-D-mannose. This indicated that some of the mannose residues were chemically bound to glucose, but the question remained open, whether these sugar units could be ascribed to occluded hemicelluloses rather than to the cellulose framework itself.

In 1956, Jones, Wise and Jappe (37) observed that alkali containing 3 - 4 % metaborate extracted a hemicellulose from slash pine alpha-cellulose, which was enriched in mannan. It was believed that the borate complex, formed with the vicinal cis-hydroxyl groups of the mannose units, might be more acidic than the hemicellulose itself, and would thus be more soluble in alkali.

Hamilton, Kircher and Thompson (38) reported in 1956 the isolation of a true diheteropolymer glucomannan. The polysaccharide, extracted from western hemlock (Tsuga heterophylla), contained glucose and mannose in a ratio of 1:3, linked together by β-(1→4)-glycosidic bonds. Various oligosaccharides containing both glucose and mannose units were isolated and identified, thus proving unequivocally that glucose and mannose were chemically combined in the polysaccharide. A glucomannan of similar composition, accounting for more than 80 % of the mannose residues in white spruce (Picea glauca) was isolated by Timell and Tyminski (39).

During the last five years many similar glucomannans have been isolated from the wood of various gymnosperms, including the genera Ginkgo, Larix, Picea, Pinus, Thuja and Tsuga. They have been found to contain a linear or slightly branched framework of β-(1→4)-linked D-mannose and D-glucose residues, usually in a ratio of 3:1 to 4:1. Their number-average degrees of polymerization fall within the range of 70 to 130, determined after treatment with chlorous acid.

An efficient way to separate glucomannans and galactoglucomannans from accompanying xylans was discovered by Meier (40). He found that mannose-containing polysaccharides, possessing free vicinal cis-hydroxyl groups, were precipitated from aqueous solutions by barium hydroxide.

Recently Jayme and Kringstad (41) obtained a spruce glucomannan containing galactose by precipitation with a ferric sodium tartaric acid complex. The ratio of galactose, glucose and mannose residues was determined as 1:5:15 . The complex also precipitated straw and poplar xylan, but failed to precipitate a spruce xylan.

Bishop and Cooper studied a glucomannan from Jack pine (Pinus banksiana)(42) containing galactose, glucose and mannose residues in a ratio of 2: 17:49 . The galactose

units were believed to be present as single terminal side chains attached to position 6 of glucose or mannose, thus being an integral part of the polysaccharide. Methylation data indicated the usual (1→4)-linked framework of D-glucose and D-mannose residues. Branching at C-2 or C-3 was not found to occur because no monosaccharides survived periodate oxidation. The degree of polymerization as determined by three different methods was only 18 - 21 .

The first conclusive evidence that galactose and mannose residues are chemically combined in wood, and that the galactose most likely does not originate from a contaminating galactan, was obtained when Meier (43) succeeded in isolating a 6-O-α-D-galactopyranosyl-D-mannose and an O-α-D-galactopyranosyl-(1→6)-O-β-D-mannopyranosyl-D-mannose on partial hydrolysis of a glucomannan from Norway spruce. Since a trihetero-oligosaccharide has so far not been obtained from any galactoglucomannan, the question remains still open, whether this is a true triheteropolymer or a mixture of a glucomannan and a galactomannan, as suggested by Meier (40).

In another investigation of Jack pine wood by Perila and Bishop (44) a crude glucomannan was obtained in 17 % yield. Repeated delignification and copper complexing provided a pure product, which contained galactose,

glucose and mannose residues in a ratio of 1:10:25, but also 3 % xylose. The hemicellulose was subjected to enzymic hydrolysis to give oligosaccharides, which were typical products also of acid hydrolysis of glucomannans. The galactosylmannose, which was reported by Meier (43) and especially looked for, could not be detected, however a 6-O-α-D-xylopyranosyl-D-glucose and a O-α-D-xylopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-D-glucose were obtained. It appeared that these fragments originated from a xyloglucan or xyloglucomannan, the presence of which had not been suspected before.

A glucomannan from tamarack (Larix laricina) has recently been studied by Kooiman and Adams (45). It contained galactose, glucose and mannose units in a ratio of 3:25:70 . Periodate oxidation indicated a linear framework of approximately 35 β-(1→4)-linked D-glucose and D-mannose units. Branched points, if at all present, must have occurred at C-6 of the hexoses in the main chain.

Meier (46) has obtained a glucomannan from Scots pine (Pinus silvestris) under conditions mild enough to leave ester groups intact. By periodate oxidation it was shown that almost all of the acetyl groups were attached to carbon 2 or 3 of the mannose residues. An almost pure arabino-glucuronoxylan isolated from the holocellulose

by extraction with dimethyl sulphoxide contained only traces of acetyl groups. It seems likely that most of the acetyl groups in pine wood are associated with the glucomannan fraction.

The occurrance of galactoglucomannans in various softwoods having a sugar ratio of approximately 1:1:3 has been referred to on several occasions by Hamilton and his co-workers (47,48,49,50). The first wood galactoglucomannan isolated was that of white spruce (Picea glauca), which was found by Adams (51) in the water-soluble fraction of the wood together with an arabinoglucuronoxylan. The two hemicelluloses were separated by precipitating the galactoglucomannan with Fehling's solution. The ratio of the galactose, glucose and mannose residues was determined as 1:1:3.7, and the polysaccharide was obtained in a yield of 0.08 % of the wood.

The question, whether or not the isolated galactoglucomannans constitute true triheteropolymers, has been much discussed. Lindberg and co-workers in connection with their studies of glucomannans from Norway spruce (43) and Scots pine (52) find little evidence for such an assumption. They have shown that residual lignin in mixtures of hemicelluloses has to be removed completely before such mixtures become amenable to resolution. A β-(1$\rightarrow$4)-linked galactan has recently been discovered in spruce compression wood by Bouveng and Lindberg (53).

Hamilton, Partlow and Thompson (54) isolated a galactoglucomannan from a mixture of slash pine (Pinus elliottii) and longleaf pine (Pinus palustris) by the conventional kraft process, containing galactose, glucose and mannose residues in a ratio of 1:1:3 . Methylation data suggested that D-galactose was attached to the framework of β-(1→4)-linked D-glucose and D-mannose residues as single terminal side chains predominantly, if not exclusively, to the 6-position of the mannose units. By graded hydrolysis with 0.05 N oxalic acid it was shown that the galactosidic linkage was more labile than the mannosidic, and the mannosidic more so than the glucosidic linkage. The yield of this polysaccharide was 0.44 % of the wood, however, much higher yields of water-soluble galactoglucomannans have recently been obtained from several softwoods (52).

It has been noticed in the literature that even the socalled true glucomannans contain 1 - 4 % galactose residues, which could not be removed by repeated fractionation. The suggestion has been made (52) that the glucomannans are of the same nature as the galactoglucomannans, only with a lower galactose content. The ratio of the mannose and glucose residues in most of these hemicelluloses was found to be between 3:1 and 4:1, however, quite varying results have been reported with regard to which monosaccharide terminates the chain at the non-reducing end.

Table II.

Composition of Non-reducing End Groups of the Main Chain of Glucomannans and Galactoglucomannans.

Wood	% D-Mannose	% D-Glucose	References
Sitka Spruce	100	-	57
Norway Spruce	100	-	59
Loblolly Pine	100	-	16
Slash Pine	100	-	54
Longleaf Pine	100	-	54
Eastern White Pine	appr. 95	5	55
Tamarack	75	25	45
Scots Pine	appr. 50	50	52, 56
Western Hemlock	50	50	50
Jack Pine	-	100	42

The overall ratio of 3:1 to 4:1 has not always been obtained in the tetra-O-methyl-hexoses on hydrolysis of the methylated polysaccharides. In Table II the composition of the non-reducing end groups of the main chain from various glucomannans and galactoglucomannans has been summarized.

Glucomannans and galactoglucomannans are a group of hemicelluloses, which occur in varying amounts and composition in the wood of gymnosperms; they are less abundant in angiosperms. Some gymnosperms might contain as much as 20 % of a closely related series of galacto-glucomannans, differing mainly in their relative galactose content and perhaps also in their average molecular weight and degree of branching.

RESULTS AND DISCUSSION

Acid Constituents of the Wood

Partial hydrolysis of extractive-free wood meal gave a mixture of sugars, the acid portion of which was resolved on a column of anion exchange resin to yield sugar acids, which were further separated by gradient elution with aqueous ethanol from a charcoal-Celite column. The results of the resolution are shown in Table III.

Table III.

Resolution of Acid Fraction on a Charcoal-Celite Column.

Fraction	Sugar acid	Weight(mg.)
A	D-Galacturonic acid	930
B	4-O-Methyl-D-glucuronic acid	410
C	2-O-(4-O-methyl-α-D-glucuronic acid)-D-xylopyranose	2,550
D	Mixture of aldobiouronic acids, giving rise to galacturonic acid, rhamnose and glucose	45

Fraction A was chromatographically identified as galacturonic acid, probably arising from pectic material known to be present in wood. Fraction B was identified as 4-O-methyl-D-glucuronic acid. Fraction C was an aldobiouronic acid, identified through the tetraacetate of its methyl ester - methyl glycoside as the common 2-O-(4-O-methyl-α-D-glucuronic acid)-D-xylopyranose (23,58,60,61). The 4-O-methyl-D-glucuronic acid residues of Fractions B and C represented 1.0 % of the wood. Quantitative evaluation of the xylan components later revealed that all or almost all of the 4-O-methyl-D-glucuronic acid residues must have originated from the xylan part of the wood (Table V).

Fraction D moved on the paper chromatogram at the rate of an aldobiouronic acid. Hydrolysis yielded galacturonic acid, rhamnose and a trace of glucose. This fraction was probably a mixture of two aldobiouronic acids rather than an aldotriouronic acid. A 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose has earlier been obtained from maritime pine (27) and eastern white pine (23). A galacturonosyl glucose has been isolated from the latter wood (23). It is hard to ascertain whether the galacturonic acid - containing disaccharides are derived from pectin or from the hemicellulosic part of the wood. This fraction was not further resolved or identified.

Isolation of the Hemicelluloses

While most of the hemicelluloses present in hardwoods are easily obtained by direct extraction of the wood with aqueous alkali, softwood hemicelluloses are not amenable to this treatment. This is probably due to the higher lignin content of softwoods, especially in the secondary wall. Lindberg and co-workers have pointed out (43,52) that different hemicelluloses might be bound to one lignin molecule, and they have shown that residual lignin present in mixtures of hemicelluloses has to be removed in order to make the mixtures amenable to resolution. It is well known, however, that yields of hemicelluloses decrease with increasing degree of delignification, which is undoubtedly due to the fact that once the protecting lignin is removed, the underlying carbohydrates are rapidly degraded by the chlorous acid.

For preparation of holocellulose three treatments with acid chlorite were applied according to the method of Wise, Murphy and D'Addieco (73), giving a slightly higher than theoretical yield.(73.1 %). This indicated a residual lignin content of approximately 2 %. Further delignification was not attempted, since in a preliminary experiment it had been shown that a fourth treatment with acid chlorite resulted in severe degradation of the polysaccharides.

Exhaustive extraction of the holocellulose with 24 % aqueous potassium hydroxide removed a mixture of an arabino-glucurono-xylan and a galactoglucomannan. The latter was separated from the xylan by repeated precipitation with barium hydroxide from an alkaline solution (40). After the third precipitation the soluble portion yielded on hydrolysis uronic acids, arabinose and xylose, with only traces of galactose, glucose and mannose. The insoluble portion gave galactose, glucose and mannose in a ratio of 1:1:3 , with only traces of xylose and arabinose.

The residual holocellulose was washed with water until neutral and then extracted with 17 % sodium hydroxide containing 3.5 % boric acid. The extract on hydrolysis gave galactose, glucose and mannose in a ratio of 0.1:1:3 . The glucomannan, $[\alpha]_D$ -38°, was not further purified. The yields of the hemicelluloses given in Table IV are estimated values, taking into account the losses during the purification, especially in the case of the xylan and the galactoglucomannan.

Arabino-glucuronoxylan

The hemicellulose, $[\alpha]_D$ -40°, was easily soluble in water or alkali. Its methoxyl content, uronic anhydride content and equivalent weight suggested the presence of

Table IV

Composition of Amabilis Fir Wood	%
Alpha-cellulose	47.5
Lignin	29.2
Acetyl	1.4
Ash	0.2
Uronic Anhydride	2.6
Arabino-glucuronoxylan	7.0
Galactoglucomannan	4.0
Glucomannan	8.1

5.2 xylose residues per each 4-O-methyl-D-glucuronic acid residue. The ratio between xylose, arabinose and uronic acid was 76:10:14 , corresponding to one arabinose per 7.6 xylose residues.

A portion of the hemicellulose was methylated four times with dimethyl sulphate (3) and twice with methyl iodide in the presence of silveroxide (62) in dimethyl formamide. The product obtained had a methoxyl content of 39.5 %. The infrared diagram indicated complete substitution of the hydroxyl groups. The methylated hemicellulose, $[\alpha]_D$ -42°, was subjected to methanolysis under conditions known to cause no cleavage of the glycosidic linkage of aldobiouronic acids. The mixture of glycosides was

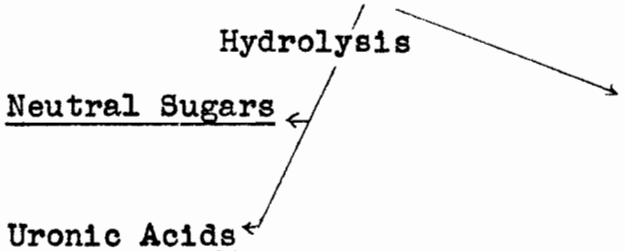
resolved into a neutral and an acidic portion on an anion exchange resin column. Chromatographic examination of the acidic fraction revealed the presence of only one compound, the methyl ester - methyl glycoside of which was reduced with lithium aluminum hydride to give the crystalline methyl 2-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyl)-3-O-methyl-α,β-D-xylopyranoside. The original acid was accordingly 2-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyl-uronic acid)-3-O-methyl-D-xylopyranose.

The weight of this fraction corresponded to 14 % uronic acid in xylan and agreed very well with the amount of uronic anhydride of the original hemicellulose, 15 %. Taking into account the yield of the xylan (7.0 %), this fraction, when calculated as 4-O-methyl-D-glucuronic acid residue, amounted to 0.98 % of the wood, thus indicating that nearly all of the 4-O-methyl-D-glucuronic acid residues present in the wood are associated with the xylan (Table V), attached to the 2-position of xylose units by an α-glucosidic bond.

The neutral glycosides were hydrolyzed to the corresponding mixture of neutral sugars which was resolved on a charcoal-Celite column by gradient elution with aqueous ethanol. The amount of each sugar obtained, in conjunction with the quantity and constitution of the partially methylated aldobiouronic acid, gave the composition of the hydrolysate presented in Table VI.

Table V

4-O-Methyl-D-glucuronic Acid in Amabilis Fir Wood



4-O-Methyl-D-glucuronic acid residues :	1.0 % of Wood	contains 14 % 4-O-Methyl-D-glucuronic acid residues =	0.98 % of Wood

Table VI

Resolution of hydrolyzed methylated Xylan

	moles	
3-O-Methyl-D-xylose	3	$[17]^{x}$
2-O-Methyl-D-xylose	10	
2,3-di-O-Methyl-D-xylose	48	
2,3,4-tri-O-Methyl-D-xylose	1	
2,3,5-tri-O-Methyl-L-arabinofuranose	6	$[10]^{xx}$
2-O-(2,3,4-tri-O-Methyl-α-D-glucuronic acid)-3-O-methyl-D-xylose	14	
Total xylose residues	76	

$[\ ]^{x}$ after addition of the xylose residues present in the aldobiouronic acid

$[\ ]^{xx}$ calculated value from composition of original polysaccharide

The large quantity of 2,3-di-O-methyl-D-xylose together with the high negative rotation of the original and methylated xylan indicated a β-(1→4)-linked framework of D-xylopyranose residues. Other xylans (23) have been reported to have a rotation even more negative, but the relatively large amount of the α-linked 4-O-methyl-D-glucuronic acid residues was expected to render the optical rotation less negative in this case. The fact that the aldobiouronic acid obtained on methanolysis of the methylated xylan was unsubstituted at the 3-position of the xylose moiety, and the absence of 3,4-di-O-methyl-D-xylose, indicated that the acid groups were attached directly to the xylan framework by (1→2) glycosidic bonds. The molar composition of the hydrolyzate from the methylated xylan showed the presence of 76 xylose residues for each non-reducing end group, obtained as 2,3,4-tri-O-methyl-D-xylose.

When the number-average molecular weight of the methylated xylan was determined by osmometry (63), a value of 21,400 was obtained, corresponding to 95 xylose units per average molecule. On the basis of these data, 0.25 branch points per molecule would be present.

The isolation of 2,3,5-tri-O-methyl-L-arabinofuranose showed that the xylan contained terminal residues of L-arabinofuranose. The amount obtained (6.0 moles) was lower than the value expected from the composition of the

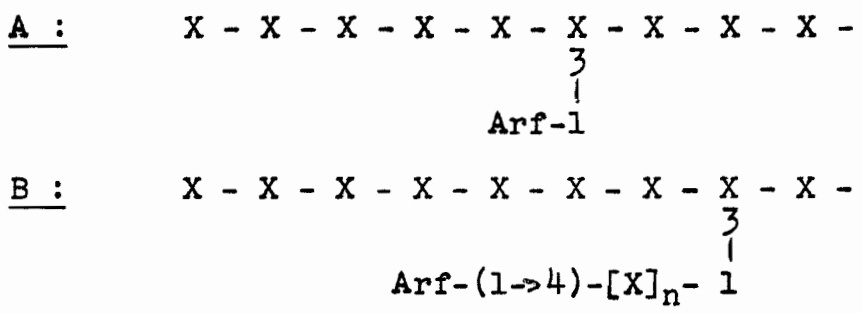
original xylan, which should have been 10.0 moles. The presence of 10.0 moles of 2-O-methyl-D-xylose suggested that the arabinofuranose groups were attached to the 3-position of the xylose residues. The lower value of the tri-O-methylarabinose was probably due to losses during evaporations, a phenomenon which has been observed before (23,64).

From the 17 moles of 3-O-methyl-D-xylose 14 were linked (1→2) to 2,3,4-tri-O-methyl-D-glucuronic acid. The excess of 3.0 moles was probably due to branching or demethylation during the methanolysis of the methylated hemicellulose (65). The possibility that some of the L-arabinose residues were linked (1→2) to the xylan backbone cannot be excluded.

From the above evidence the mode of attachment of the L-arabinose groups is not entirely established. The methylation data suggest that they are linked (1→3), either directly (Figure I,A) or through interposed xylose residues (Figure I,B).

Two methods have so far given proof of a direct (1→3)-linkage (31,32) of the arabinose side chains, but neither of them accounted for all of the arabinose residues involved. Another method consists of the selective removal of the acid-labile arabinofuranose groups (8), followed by estimation of the amount of non-reducing xylose end groups as compared to the original polysaccharide. This method has

Figure I



- X - = 4-β-D-Xylopyranosyl-1-

Arf = L-Arabinofuranose

---

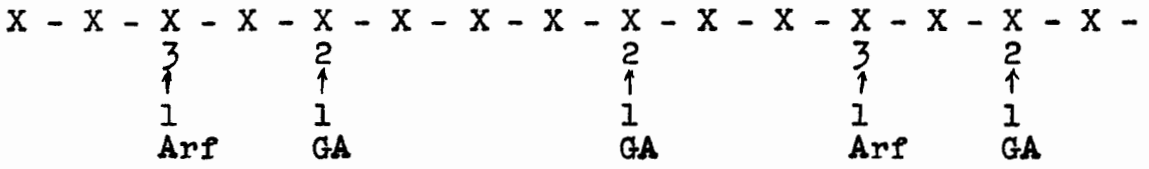
recently successfully been applied to an arabino-glucurono-xylan from eastern white pine (23), and also suggested a direct attachment (Figure I,A) of the arabinose groups. Whether the (1→3)-glycosidic bonds are of the α- or β- type cannot be decided on the basis of the present evidence. The occurrence of a β-linkage has been suggested for an arabino-glucuronoxylan from southern pine (26). It has since been found (99), however, that removal of the arabinose groups from softwood xylans gives polysaccharides with a less negative rotation. This evidence suggests that the arabinose residues are linked to the xylan framework by α-L (=β-D) glycosidic bonds. The arabinose units in the arabinoxylans occurring in the gum fractions of flour from barley, rye and wheat are evidently also present in the α-L-modification (99).

From the above evidence it is now possible to suggest a tentative structure for the arabino-4-O-methylglucuronoxylan present in amabilis fir wood. This polysaccharide contains an average number of 104 β-D-xylopyranose residues linked by (1→4)-glycosidic bonds to a framework containing approximately 0.25 branch points per average molecule. A single terminal side chain of 4-O-methyl-D-glucuronic acid is attached by a (1→2)-glycosidic bond to every fifth xylose residue on the average. An arabinofuranose residue is attached directly by a (1→3)-glycosidic bond to every seventh xylose residue (Figure II)..

The present hemicellulose is similar to the acidic xylans previously isolated from other coniferous woods. Whether or not it contains O-acetyl groups in its native state cannot be ascertained on the basis of the present evidence. Recently all O-acetyl groups present in Scots pine (46) have been found to be attached to the glucomannan part; however, this phenomenon cannot yet be generalized for all softwoods.

Figure II

Repeating Unit of Amabilis Fir Xylan :



- X - = 4-O-β-D-Xylopyranosyl-1→

Arf = L-Arabinofuranose

GA = 4-O-Methyl-D-glucuronic Acid

Glucomannan

The glucomannan, $[\alpha]_D$ -38°, obtained from the pre-extracted holocellulose contained very little xylose and arabinose residues and was not further purified. The ratio of galactose, glucose and mannose residues was found to be 0.1 : 1 : 3 . When oxidized with periodate, the polysaccharide consumed 1.06 mole of oxidant per hexose residue. The number-average degree of polymerization of the nitrate derivative as determined by osmometry was 95.

A portion of the glucomannan was methylated to completion and subsequently hydrolized to a mixture of sugars which was resolved on a charcoal-Celite column. The molar ratios of the methylated sugars are given in Table VII.

Table VII

Methylated Hexoses from methylated Glucomannan	Mole
3,6-Di-O-methyl-D-glucose	0.2
2,3-Di-O-methyl-D-mannose	1.5
2,3,6-Tri-O-methyl-D-mannose	33.1
2,3,6-Tri-O-methyl-D-glucose	12.5
2,3,4,6-Tetra-O-methyl-D-galactose	1.3
2,3,4,6-Tetra-O-methyl-D-mannose	1.0

The large quantity of 2,3,6-tri-O-methylhexoses indicates that the polysaccharide is composed of (1→4)-linked hexose residues, and this conclusion is supported by the results of the periodate oxidation. The negative rotation of the glucomannan shows that the linkage is of the β-type. All galactose residues present are attached as single terminal side chains at the 6-position of mannose mainly, as can be concluded from the isolation of approximately equimolar amounts of 2,3,4,6-tetra-O-methyl-D-galactose and 2,3-di-O-methyl-D-mannose, and from the amount of periodate consumed by the original polysaccharide. The number-average degree of polymerization as determined by osmometry was found to be 282. This is considerably higher than that of the original glucomannan. A possible explanation of this phenomenon is that the lower molecular weight fractions of the methylated glucomannan were lost during the recovery. One mole of 2,3,4,6-tetra-O-methyl-D-mannose was present per 56 hexose units (not counting the galactose residues) . This indicates that the non-reducing end groups consist of mannose residues only, and that four branch points are present in the average molecule. It is not certain, however, where these branch points occur; the presence of 3,6-di-O-methyl-D-glucose may originate from demethylation during methanolysis and hydrolysis rather than indicate a branch point at C_2 of a glucose unit. Branching at C_6 in a glucomannan from

tamarack has been considered by Bishop and Cooper (42).

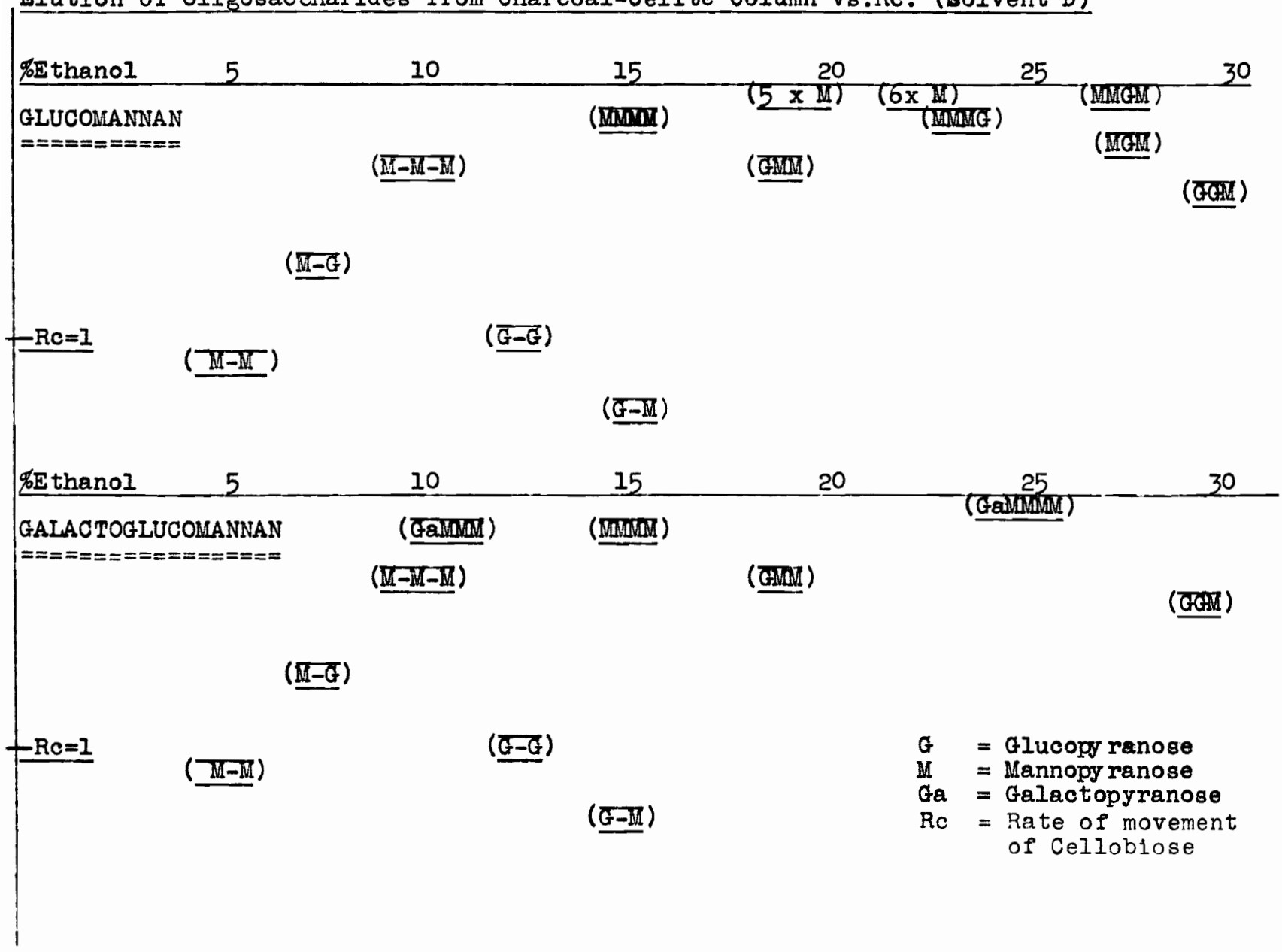
The above conclusions were corroborated by the nature of the oligosaccharides formed on partial hydrolysis of the glucomannan with aqueous formic acid. The mixture of oligosaccharides obtained was resolved by gradient elution with aqueous ethanol from a charcoal-Celite column. The results are presented in Table XII, and the elution pattern is given in Figure III.

Significant for the structure of the glucomannan is the high yield of only mannose-containing oligosaccharides, from mannobiose to mannohexaose, and the low yield of cellobiose and 4-O-β-D-glucopyranosyl-β-(1→4)-D-glucopyranosyl-D-mannose (G→G→M). The lower yield of 4-O-β-D-mannopyranosyl-D-glucose as compared to 4-O-β-D-glucopyranosyl-D-mannose can readily be explained by the relative stabilities of the glucosidic and mannosidic linkages (54). Oligosaccharides indicating branch points in the main chain were not observed, and galactose-containing oligosaccharides, although especially looked for, could not be detected.

From this evidence a tentative structure can now be assigned to the glucomannan present in amabilis fir. The structure of the β-(1→4)-linked main chain, having single terminal side chains of galactose residues and four branch points per average molecule of 95 hexose units, has already been discussed in connection with the methylation data.

Figure III

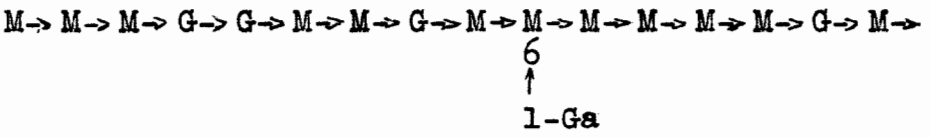
Elution of Oligosaccharides from Charcoal-Celite Column vs.Rc. (Solvent D)



The results of the partial hydrolysis indicate that mannose and glucose residues are probably distributed at random along the chain, that few glucose residues are contiguous, and that mannose residues can occur contiguously at least up to six units. Figure IV gives the theoretical repeating unit of the glucomannan molecule. It is similar to that of other glucomannans isolated from various gymnosperms.

Figure IV

Repeating Unit of Glucomannan



M = D-Mannopyranose

G = D-Glucopyranose

Ga = D-Galactopyranose

→ = β-(1→4)-glycosidic linkage

Galactoglucomannan

The pure galactoglucomannan, $[\alpha]_D$ -8°, containing galactose, glucose and mannose residues in a ratio of 1:1:3, was a white powder, soluble in water and aqueous alkali. When oxidized with periodate, it consumed 1.23 mole of oxidant per hexose unit. The number-average molecular weight of the nitrate derivative as determined by osmometry (63) was 27,000 , corresponding to a degree of polymerization of 76 . Boundary electrophoresis indicated that the polysaccharide was chemically homogeneous.

A portion of the galactoglucomannan was methylated to completion and subsequently hydrolyzed to a mixture of methylated sugars which was resolved on a charcoal-Celite column. The results are presented in Table VIII.

Table VIII

Methylated Hexoses from methylated Galactoglucomannan

	Mole
2,3-Di-O-methyl-D-mannose	5.7
3,6-Di-O-methyl-D-mannose	0.6
2,3-Di-O-methyl-D-glucose	1.1
2,3,6-Tri-O-methyl-D-mannose	15.6
2,3,6-Tri-O-methyl-D-glucose	5.3
2,3,4,6-Tetra-O-methyl-D-galactose	4.5
2,3,4,6-Tetra-O-methyl-D-mannose	1.0

The results suggest that the methylated galactoglucomannan contained galactose, glucose and mannose residues in a ratio of 1 : 1.4 : 5.1 . This indicates that some of the galactose residues must have been lost during the isolation procedure.

The large quantity of 2,3,6-tri-O-methylhexoses shows that the polysaccharide is composed of (1→4)-linked D-mannose and D-glucose residues; the isolation of 2,3,4,6-tetra-O-methyl-D-galactose (4.5 moles) and the absence of any other galactose ethers indicates that all galactose units are present as single terminal side chains. The presence of 2,3-di-O-methyl-D-mannose (5.7 moles) and 2,3-di-O-methyl-D-glucose (1.1 mole) suggests that the galactose residues are attached (1→6), predominantly to mannose. Mannose only terminates the chain at the non-reducing end, as can be derived from the isolation of 2,3,4,6-tetra-O-methyl-D-mannose, one such end group being present per 28 mannose and glucose residues. The number-average degree of polymerization of the methylated galactoglucomannan as determined by osmometry (63) was 91, which indicates 2.2 branch points per average molecule. Since the amount of 2,3-di-O-methylhexoses exceeded that of 2,3,4,6-tetra-O-methyl-D-galactose by 2.3 moles, branching of the main chain is believed to occur at carbon 6 of glucose and mannose. The small quantity of 3,6-di-O-methyl-

D-mannose might have originated from demethylation. However, some branching through carbon 2 cannot be excluded.

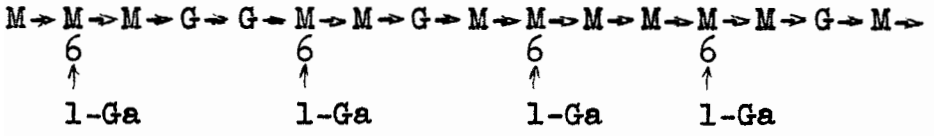
Partial hydrolysis of the galactoglucomannan corroborated the above conclusions. When resolving the mixture of oligosaccharides by gradient elution with aqueous ethanol from a charcoal-Celite column, the elution pattern (Figure III) and relative yields of the oligosaccharides (Table XIV) were almost identical to that of the glucomannan, with two notable exceptions. One tetra- and one pentasaccharide were obtained, containing galactose and mannose residues in a ratio of 1:3 and 1:4, respectively. On partial hydrolysis of these oligosaccharides a disaccharide was obtained in both cases which was chromatographically identical with an authentic sample of 6-O-α-D-galactopyranosyl-D-mannose. Other products of the partial hydrolysis were mannose, mannobiose and mannotriose. This evidence suggests that galactose is linked α-(1→6)-glycosidically to mannose. The α-type linkage of the galactose can also be derived from the less negative optical rotation of the polysaccharide in comparison with the glucomannan. The relatively higher amount of oxidant consumed on periodate oxidation (1.23 mole) in comparison with glucomannans (1.0 mole) also indicates that branching of the galactose residues must occur at carbon 6 of the hexose units of the main chain. This conclusion is corroborated by the fact that no hexoses survived excessive

periodate oxidation, which would have indicated branching through carbon atoms 2 or 3.

From the above evidence a tentative structure can now be drawn as presented in Figure V. The average molecule consists of at least 75 units of D-mannose and D-glucose residues linked by β-(1→4)-glycosidic bonds, having 2.2 branch points per molecule probably at carbon atom 6, and mannose only at the non-reducing ends. D-Galactose residues are linked as single terminal side chains by α-(1→6)-galactosidic bonds, predominantly, if not exclusively, to mannose residues. The results of the partial hydrolysis suggest that few glucose residues are linked contiguously, but that mannose residues are occasionally together at least up to six units.

Figure V

Repeating Unit of Galactoglucomannan



M = D-Mannopyranose

G = D-Glucopyranose

Ga = D-Galactopyranose

→ = β-(1→4)-glycosidic linkage

The results of the Tiselius boundary electrophoresis show that the present galactoglucomannan is a true triheteropolymer. The assumption has been made (43,59,72) that galactoglucomannans really represent a mixture of a galactomannan and a glucomannan. This possibility, however, can be excluded in this case, since a highly branched galactomannan would have solubility characteristics very different from those of a straight chain glucomannan. It is interesting to note in this connection that true mannans occurring in vegetable ivory are even less soluble in aqueous alkali than the wood glucomannans (70).

It has been suggested in the literature (48,49,54) that the alkali-soluble "glucomannans" in softwood are actually also true galactoglucomannans, since these polysaccharides have almost always been found to contain 3-4 % galactose, which could not be removed by repeated fractionation. In methylation studies these galactose residues are usually obtained as tetra-O-methylgalactose. The different degrees of solubility of the glucomannans and galactoglucomannans can be explained by the relative frequency of the galactose side chains. The situation here is similar to that existing in softwood xylans with respect to the arabinofuranose side chains (56,60,68). Only in two cases (38,57) have galactose residues been reported to be absent, but the possibility that they were originally present and were lost during isolation (sulphite cooking)

is very likely (71). It has long been known that galactose appears in the cooking liquor during the early stages of a sulphite cook, and that the galactosidic linkage is considerably more labile than the glucosidic and mannosidic bonds (54). It appears therefore more correct to consider the glucomannan and galactoglucomannan portion of amabilis fir and other softwoods as constituting a closely related series of hemicelluloses, differing mainly in their relative galactose content and average molecular weight. Other differences, such as degree of branching and distribution of sugar residues might also exist.

EXPERIMENTAL

REAGENTS AND METHODS

Paper Chromatography

The following solvent systems were used :

System	Solvents	Ratio (v/v)
A	Ethyl acetate - acetic acid - water	9 : 2 : 2
B	Ethyl acetate - acetic acid - water	9 : 3.5 : 4
C	n-Butanol - pyridine - water	10 : 3 : 3
D	Ethyl acetate - pyridine - water	8 : 2 : 1
E	2-Butanone, saturated with water at 4°C, containing 2 % ammonia.	

Separations were carried out by the descending technique on Whatman No. 1 filter paper for analytical and No. 3MM for preparative purposes. For separation of methylated sugars No. 3MM was used with a strip of Whatman No. 50 filter paper attached to the top end. This slowed down the rate of movement of the solvent and yielded better separation with solvent system E.

The spray reagent consisted of :
o-Aminodiphenyl (3 g.) dissolved in 100 ml. acetic acid, to which 1.3 ml. of 85 % reagent grade orthophosphoric acid was added. After spraying, the papers were heated for 2 - 3 min. at 105°C. Hexoses appeared then as brown spots, pentoses as red, mono-uronic acids as orange changing to violet on further

heating, and aldobiouronic acids as orange spots changing to red on further heating.

Electrophoresis

Electrophoretic separations were carried out on Whatman No. 3MM filter paper in a 0.05 M borate buffer at pH 10 at 750 volts for 3 - 6 hours. The same detection method was used as in the paper chromatographic separations. The borate buffer contained 4.0 g. of sodium hydroxide and 7.5 g. of boric acid per litre (75).

Methanolic Hydrogen Chloride

Anhydrous methanol was prepared as described by Vogel (76). Dry hydrogen chloride was passed through the methanol for 2 - 3 min. and the concentration of acid was determined by titration with 0.1 N sodium hydroxide, using phenolphthalein as indicator. The required strength was obtained by diluting the dry methanolic hydrogen chloride with anhydrous methanol.

Tetrahydrofuran

Reagent grade tetrahydrofuran was refluxed over sodium hydroxide for 3 hours and then distilled. Before use it was freshly distilled over lithium aluminum hydride.

Dimethyl Formamide

Before use the reagent grade product was refluxed over barium hydroxide and then distilled.

Methoxyl Analysis

The procedure as described by Vieböck and Schwappach (77), modified by Timell and Purves (78) was used.

Molish Test

1 - 2 drops of 1 % α-naphthol in ethanol was added to 1 ml. of sugar solution. Concentrated sulphuric acid was added carefully to form a layer beneath the sugar solution. A purple ring between the two layers indicated the presence of neutral sugars, a greenish-purple ring the presence of uronic acids.

Silver Oxide

Silver oxide was prepared according to the procedure of Helferich and Klein (79). The substance was dried *in vacuo* in the dark prior to its use.

Charcoal-Celite Column

Darco G-60 charcoal and Celite (1:1 by volume) was mixed and treated with concentrated hydrogen chloride for a few hours. The slurry was diluted with water and washed with aqueous ethanol until neutral. At the bottom of a column (3.5 x 55 cm.) a small round wire net was placed, which was covered with cotton pad, followed by a bed of cellulose powder and a similar bed of Celite. The charcoal-Celite slurry was slowly poured into the column and never allowed to run dry. The top of the column was connected

with the solvent supply line for gradient elution, and the bottom end with an automatic fraction collector.

Determination of relative Ratios of Sugar Mixtures

Quantitative estimation of ratios of reducing sugars was carried out by the spectrophotometric method of Timell, Glaudemans and Currie (86). The reagent was o-aminodiphenyl, and the relative densities were determined by means of a Beckman Model DU spectrophotometer.

Demethylation of methylated Sugars

Boron trichloride was used as demethylating agent according to the procedure of Bonner, Bourne and McNally (81). The methylated sugar (1-5 mg.) was dissolved in 2 ml. of methylene chloride and cooled to -77°C in an ethanol - dry ice mixture. Liquid boron trichloride (2 ml., cooled to -77°C) was added and the reaction mixture kept at this temperature for 30 min., after which it was left at room temperature overnight. Any remaining boron trichloride was decomposed by addition of methanol, and boric acid was removed by evaporation from methanol.

Partial Hydrolysis of Oligosaccharides

Samples (1-2 mg.) were refluxed in 5 ml. of 0.225 N sulphuric acid for 30 min.. The acid was neutralized with Amberlite IR-45 anion exchange resin. The resin was removed by filtration, the filtrate evaporated to 0.5 ml. and examined by paper chromatography (Solvents A,C and D).

Total Hydrolysis of Oligosaccharides

Samples (1-2 mg.) were heated in a pressure vessel at 105°C for 1 1/2 hours in N-sulphuric acid. The acid was neutralized with barium carbonate and filtered through Celite. The filtrate was treated with Amberlite IR-120 cation exchange resin and evaporated to 0.5 ml.. Paper chromatographic examination was carried out using Solvents A, C and D.

Reduction of Oligosaccharides

Samples (ca. 5 mg.) were dissolved in 1 ml. of water and 5 ml. of a freshly prepared solution of 5 % sodium borohydride in water was slowly added. The reaction mixture was kept at room temperature overnight, and Amberlite IR-120 cation exchange resin was added until gas evolution ceased. The resin was filtered off, the filtrate was evaporated to dryness, and the residue was evaporated to dryness three times from methanol.

Determination of Degree of Polymerization of Oligosaccharides

The degree of polymerization of reducing oligosaccharides was determined according to the method of Peat, Whelan and Roberts (88), modified by Timell (89). A Beckman Model DU spectrophotometer was used for estimating the sugar ratios of the hydrolysates before and after reduction of the oligosaccharides.

Wood Analyses

Determination of alpha-cellulose, lignin, pentosan, ash and moisture was carried out according to TAPPI standard methods. Uronic anhydride was determined according to the procedure of Browning (80).

Evaporations

Solutions were evaporated in a rotary evaporator at 15-20 mm. mercury pressure below 50°C.

Infrared Spectra

A Perkin Elmer No.21 double beam spectrophotometer was used. Crystalline substances were mixed with potassium bromide and pressed into disks. Syrups were smeared on sodium chloride plates.

Melting Points

A Fisher-Johns apparatus was used for melting point determination. All values reported are corrected.

Optical Rotations

All specific rotations are equilibrium values and were determined at 20°C.

Examination of the Acidic Constituents of Amabilis Fir Wood

Wood meal (200 g., 40-60 mesh) was suspended in 300 ml. of 72 % sulphuric acid, cooled to 0°C, and left standing for one hour, after which the thick paste had thinned. The mixture was then diluted to 4 litres and boiled under reflux for 6 hours. After cooling to 10°C, saturated barium hydroxide solution was added slowly under vigorous stirring until the hydrolysate reached a pH of 7.0 . Most of the barium sulphate was removed by centrifuging and the remainder filtered through Celite. The filtrate was treated with Amberlite IR-120 cation exchange resin and then passed through a column containing Dowex 1-X4 anion exchange resin (acetate form, 100 mesh), followed by distilled water, until a Molish test was negative. The acidic components were recovered by eluting with 30 % acetic acid to a negative Molish test. The acidic eluate was evaporated to yield a syrup (5.05 g.). Examination by paper chromatography indicated the presence of four main uronic acids.

The mixture was added to the top of a charcoal-Celite column (6 x 64 cm.) and the sugar acids were removed by gradient elution with aqueous ethanol in the following order : 2 - 15 % ethanol (8 litres), 15 - 25 % ethanol (8 litres), 25 - 40 % ethanol (8 litres). The eluate was collected in 20-ml. fractions at intervals

of 12 to 35 min.. The results of the fractionation are presented in Table IX.

Table IX

Fraction	% Ethanol	Tube No.	Weights of Sugar Acids
A	4 - 7	96 - 112	0.930 g.
B	7 - 10	113 - 132	0.410 g.
C	10 - 21	133 - 260	2.550 g.
D	21 - 27	261 - 350	0.045 g.
			3.935 g.

Identification of Fractions :

Fractions A and B

These fractions corresponded in rate of movement on a paper chromatogram (Solvents A, B and D) to galacturonic acid and 4-O-methylglucuronic acid, respectively.

Fraction C

This sugar acid, $[\alpha]_D^{20}$ +86°(c 1.0, in water) formed a white powder on evaporation from methanol and had an infrared diagram identical with that of an authentic specimen of 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose (60).

% OMe calcd. for $C_{12}H_{20}O_{11}$: 9.1 %

found : 9.2 %

A portion of this aldobiouronic acid was converted to the methyl ester methyl glycoside of the tetraacetate (59) as described earlier (61). Chrystallization from chloroform and recrystallization from ethanol gave crystals, m.p. and mixed m.p. 202°C, $[\alpha]_D^{20}$ +101°(c 0.5 in chloroform). The infrared diagram was identical with that of an authentic sample of methyl 2-O-(methyl 2,3-di-O-acetyl-4-O-methyl-α-D-glucopyranosyl uronate)-3,4-di-O-acetyl-D-xylopyranoside (74).

Fraction D

This fraction moved on a paper chromatogram (Solvent A) at the rate of an aldobiouronic acid and gave on hydrolysis galacturonic acid, rhamnose and a trace of glucose.

Isolation and Purification of Hemicelluloses from Amabilis Fir Wood Meal

Preparation of Holocellulose :

Holocellulose (I) was prepared by the method of Wise, Murphy and D'Addieco (73) from extractive-free wood meal (40-60 mesh). The wood meal was suspended in 16 litres of water (1200 g. wood meal, oven dry) and was delignified by successive treatments with sodium

chlorite (360 g.) and acetic acid (120 ml.). The reaction was allowed to proceed at a temperature of 78°C, and the intervals between each treatment were kept at one hour. After three treatments the holocellulose was recovered by filtration, washed until neutral with water, followed by 5 litres of ethanol, and then dried in air overnight. Yield 73.1 % (oven dry basis).

A second batch of holocellulose was prepared by the same method (1400 g. wood meal). This was later used for preparation of the galactoglucomannan.
Yield of holocellulose (II) : 76.0 %.

Preparation of Hemicelluloses :

Holocellulose (I) was suspended in a 10-litre solution of 24 % potassium hydroxide and shaken overnight in a nitrogen atmosphere at room temperature. After filtration on a sintered glass funnel the residue was washed with 3 litres of 24 % potassium hydroxide. The filtrate and washings were poured into four times their volume of ethanol containing an excess of acetic acid, cooled to 0°C. The precipitate formed was recovered by filtration and washed in succession with 70 % ethanol, ethanol and petroleum ether (b.p. 30-60°C). Drying in vacuo gave a white powder (106 g., 11.1 % of wood). Hydrolysis yielded uronic acids, xylose and arabinose, some glucose, galactose and mannose.

The crude xylan was dissolved in 10 % sodium hydroxide (2 litres) and the solution clarified by centrifuging. An equal amount of saturated barium hydroxide solution was then added dropwise under vigorous stirring. The soluble portion was recovered on the centrifuge, cooled to 0°C, and neutralized with ice-cold acetic acid. After centrifuging again the soluble portion was poured into four times its volume of ethanol. The precipitate formed was recovered by filtration, washed successively with 70 % ethanol, ethanol and petroleum ether, and dried in vacuo. Since the hemicellulose still yielded some glucose, galactose and mannose on hydrolysis, the procedure was repeated twice. After the third precipitation with barium hydroxide the hemicellulose gave upon hydrolysis uronic acid ; xylose and arabinose in a ratio of 14:76:10, and was almost devoid of galactose, glucose and mannose residues. Yield of purified xylan : 29 g. Ash : 9.1 %.

The residual holocellulose was washed with water until neutral and suspended in 10 litres of 17 % sodium hydroxide (w/w) containing 3.5 % of boric acid. After shaking for 16 hours at room temperature the soluble portion was recovered by filtration and washed with 2-3 litres of 17 % sodium hydroxide. The filtrate and washings were poured into four times their volume of ethanol at 0°C, containing an excess of acetic acid. The precipitate

formed was recovered by filtration on a large Büchner funnel and successively with 70 % ethanol, ethanol and petroleum ether (b.p. 30-60°C). After drying in vacuo the white powder yielded on hydrolysis glucose and mannose in the ratio of 1:3, and also approximately 3 % of galactose. Xylose and arabinose were present in trace amounts. Yield of glucomannan : 88 g., 8.1 % of the wood. $[\alpha]_D^{20}$ -38° (c 2.4, in 10 % KOH). Ash : 2.2 %.

Holocellulose (II) (1200 g.) was suspended in 10 litres of 24 % potassium hydroxide (w/w) and shaken at room temperature for 16 hours. The solute was recovered in the above described manner by filtration and precipitation in acidic ethanol. The hemicellulose obtained (120 g.) was dissolved in 2 litres of 10 % potassium hydroxide and a saturated solution of barium hydroxide (2 litres) was added over a period of one hour. The precipitated barium complex was recovered by centrifuging; the centrifugate, containing still substantial amounts of galactose, glucose and mannose besides xylose and arabinose, was recovered by precipitation into acidic ethanol and filtration, and again dissolved in potassium hydroxide (1 litre) for a second barium hydroxide precipitation. The barium complex from the second precipitation was recovered by centrifuging and combined with the first one. The complex was dissolved in 50 % acetic acid, and the acidic solution was poured into four times its volume

ethanol. The precipitate was recovered in the above described way and yielded on hydrolysis galactose, glucose and mannose and still approximately 15 % xylose and arabinose. A third barium hydroxide precipitation was carried out to yield a galactoglucomannan (18 g.) containing galactose, glucose and mannose residues in a ratio of 21.4 : 20.2 : 58.4 with only traces of xylose and arabinose. $[\alpha]_D^{20}$ -8° (c. 3.0, in water), ash 1.1 %.

Another portion of galactoglucomannan (7.0 g.) was obtained by barium hydroxide precipitation of a hemicellulose mixture recovered from the combined centrifugate of the previous precipitations. This portion yielded on hydrolysis galactose, glucose and mannose in a ratio of 15 : 25 : 60 , but was less pure with respect to the amounts of xylose and arabinose present.

Preparation of ash-free Xylan

Xylan (1 g.) was shaken with ice-cold N-hydrochloric acid (25 ml.) for 15 min. and the mixture poured into 120 ml. of ethanol with vigorous stirring. After filtering, the xylan was washed free from acid with 70 % ethanol, ethanol and petroleum ether in succession and dried in vacuo. Yield : 0.91 g., $[\alpha]_D^{20}$-40° (c. 1.5, in water).

Anal.: Methoxyl : 3.05 %

Uronic anhydride : 15.0 %

Xylose : arabinose : uronic acid = 76:10:14.

Equivalent Weight of repeating Unit in Xylan

The average repeating unit with regard to uronic acid side chain was determined in the following way : Ash-free xylan (25-30 mg.) was suspended in excess of 0.01 N sodium hydroxide. After shaking for one hour, excess sodium hydroxide was backtitrated with 0.01 N hydrochloric acid, using phenolphthalein as indicator. Equivalent weight of repeating unit of xylan was calculated from :

$$\text{Eq.wt} = \frac{\text{mg. of xylan}}{\text{N of base} \times \text{ml. of base consumed}}$$

Found : 990, meaning 6.1 anhydropentose units per one 4-O-methylglucuronic acid unit.

Periodate Oxidation of Xylan

Xylan (1 g.) was dissolved in water (300 ml.) and neutralized with 0.1 N sodium hydroxide. Sodium meta-periodate was added to yield a 0.05 molar solution. Aliquots of 40 ml. were taken out every 24 hours and the periodate consumed was measured by the excess arsenite method (92). The molar consumption of periodate per 76 xylose units was 81.

Methylation of Xylan

The hemicellulose (15 g.) was dissolved in 100 ml. of 10 % sodium hydroxide; water and solid sodium hydroxide were added to give a solution of 250 ml. of 40 % (w/w) sodium hydroxide. After stirring the mixture for 12 hours in an atmosphere of nitrogen, dimethyl sulphate was added dropwise to the reaction mixture at the rate of one drop every 10 seconds. After the dimethyl sulphate was consumed, three more additions of 100 g. sodium hydroxide and 200 ml. dimethyl sulphate were made. The rate of addition of the dimethyl sulphate was increased to one drop per every 4-6 seconds. The reaction mixture was neutralized with sulphuric acid; when heated to boiling, a portion of the partly methylated xylan separated out and could be collected by filtration. After briefly washing with water and drying in vacuo, this portion yielded 6.5 g.. The remainder of the partly methylated xylan was recovered by dialyzing the filtrate against running tap water, which removed the sodium sulphate. After evaporating and drying the dialyzed portion, another 4.0 g. of partly methylated xylan was obtained.

The combined partly methylated xylan (11.5 g.) was dissolved in dry dimethyl formamide (250 ml.); silver oxide (25 g.) and methyl iodide (25 ml.) were added and the mixture shaken for 24 hours. This treatment was repeated twice, after which 1000 ml. of chloroform were added and the insoluble

salts removed by filtration. The filtrate was washed twice with 200 ml. of 5 % potassium cyanide and twice with water (200 ml.). The chloroform solution was dried over sodium sulphate and concentrated to 100 ml.. Slow addition to 1000 ml. of petroleum ether (b.p. 30-60°C) caused the polysaccharide to precipitate. The fully methylated xylan was washed with petroleum ether and dried *in vacuo* to yield 8.5 g. of a white fluffy powder. The infrared diagram showed a negligible hydroxyl peak.

Anal.: Calcd. methoxyl content for fully methylated and esterified xylan : 39.6 %

found : 39.5 %

$[\alpha]_D^{20}$ - 42° (c 1.5, in chloroform).

Methanolysis of methylated Xylan

The methylated xylan (4.1 g.) was refluxed with 2 % anhydrous methanolic hydrogen chloride (100 ml.) for seven hours. After neutralization with silver carbonate the syrup was saponified with 30 ml. of 5 % barium hydroxide at 60°C for two hours. Solid carbon dioxide was added and the barium carbonate formed was removed by filtration. The filtrate was treated with Amberlite IR-120 cation exchange resin and evaporated to 5 ml..

Separation of the Acidic and Neutral Components

The acidic solution was passed through a column (3.5 x 18 cm.) containing Dowex 1-X4 exchange resin (acetate form, 100 mesh). Neutral sugars were eluted with water to a negative Molish test, the acidic portion was recovered by eluting with 30 % acetic acid. The fractions were evaporated to a clear syrup and dried *in vacuo* for four days at room temperature. Examination by paper chromatography (Solvent B) indicated, that the acidic fraction consisted of only one component.

Yields : Neutral glycosides : 2.9 g.
Acids : 1.0 g.

Hydrolysis of Neutral Glycosides

Neutral glycosides (2.9 g.) obtained after methanolysis and separation from the acidic fraction were hydrolysed with N-sulphuric acid (50 ml.) for 6 hours to yield free sugars. The solution was neutralized with barium carbonate, deionized with Amberlite IR-120 and IR-45 exchange resins and further purified with Darco G-60 charcoal. The solution was filtered and the filtrate evaporated and dried *in vacuo* to yield a syrup containing a mixture of methylated sugars (2.3 g.).

Separation and Identification of the neutral methylated Sugars from methylated Xylan

The neutral methylated sugars (2.3 g.) were added to the top of a charcoal-Celite column (3.5 x 55 cm.) and resolution was effected by gradient elution with aqueous ethanol in the following order :

2 to 20 % ethanol : 8 litres

20 to 40 % ethanol : 8 litres.

Fractions, 25 ml., were collected at intervals ranging from 10 min. initially to 35 min. at the later stages of the separation. Every third fraction obtained was examined by paper chromatography (Solvent E). The results of the fractionation are summarized in Table X.

Table X

Tube No.	Fraction	Methylated Sugars	Weight (g.)
30 - 34	A	3-O-methyl-D-xylose	0.034
35 - 39	B	mixture	0.158
40 - 49	C	2-O-methyl-D-xylose	0.080
50 - 53	D	mixture	0.011
54 -112	E	2,3-di-O-methyl-D-xylose	1.052
113-220	F	mixture	0.088
221-282	G	2,3,5-tri-O-methyl-L-arabinofuranose	0.039
283-390	H	2,3,4-tri-O-methyl-D-xylose	0.025

Identification of 3-O-Methyl-D-xylose (fraction A)

The compound was distinguished from its 2-O-methyl-isomer by electrophoresis in borate buffer, and moved at the same rate as an authentic specimen.

Anal.: $[\alpha]_D^{20}$ +20.3° (c,1.8, in methanol)

Calcd. for $C_6H_{12}O_5$: OMe , 18.9 %

found : OMe , 18.7 %

The aniline derivative was prepared by heating a portion (17 mg.) in ethanol (3 ml.) containing 0.03 ml. of aniline for 2 hours. After standing overnight the aniline was removed in vacuo, and the 3-O-methyl-N-phenyl-D-xylopyranosylamine crystallized from ethyl acetate; m.p. and mixed m.p. 136°C.

Identification of 2-O-Methyl-D-xylose (Fraction C)

On electrophoresis this compound moved at the same rate as an authentic specimen. A portion (2 mg.) was demethylated with boron trichloride (81), and the demethylated sugar was identified as xylose by paper chromatography (Solvents A and D). The syrup crystallized after a few days and was recrystallized from ethyl acetate. m.p. and mixed m.p. 133-134° C.

Anal.: $[\alpha]_D^{20}$ +35.5 ° (c, 1.5 in methanol)

Calcd. for $C_6H_{12}O_5$: OMe, 18.9 %

found : OMe, 18.4 %

Identification of 2,3-di-O-Methyl-D-xylose (Fraction E)

The syrup, $[\alpha]_D^{20}$ +21.5° (c, 1.5 in water), crystallized spontaneously from ethyl acetate. After one recrystallization from ethyl acetate - petroleum ether it had m.p. and mixed m.p. 92°C.

Anal.: Calcd. for $C_7H_{14}O_5$: OMe, 34.8 %
found : OMe, 34.6 %

The aniline derivative (2,3-di-O-methyl-N-phenyl-D-xylopyranosylamine) had after two crystallizations from ethyl acetate m.p. 130°C and $[\alpha]_D^{20}$ +194° (c, 1.3 in ethyl acetate).

Identification of 2,3,5-tri-O-Methyl-L-arabinofuranose (Fraction G)

This fraction (39 mg.) had $[\alpha]_D^{20}$ -38° (c, 1.0 in methanol) and moved on a paper chromatogram (Solvent E) at the same rate as an authentic specimen. On demethylation it yielded arabinose.

Final characterization was achieved by preparing the 2,3,5-tri-O-methyl-L-arabonamide (23). The tri-O-methyl sugar was dissolved in water (4 ml.) to which five drops of bromine were added. Oxidation was allowed to proceed for 48 hours in the dark. Excess bromine was removed by aeration and the solution neutralized with silver carbonate. The filtrate was deionized with Amberlite IR-120 cation exchange resin and condensed to a syrup, which was distilled

in vacuo at a bath temperature of 130°C on a fingertip condenser. The lactone was dissolved in methanol, the solution saturated with dry ammonia gas at 0°C and kept at 4°C for 48 hours. The solvent was removed at 50°C, and the syrup crystallized from isopropyl ether, m.p. 130-132°C, $[\alpha]_D^{20}$ -17.5° (c, 1.0 in water) (24).

Identification of 2,3,4-tri-O-Methyl-D-xylose (Fraction H)

This fraction (25 mg.) had $[\alpha]_D^{20}$ +19.8° (c, 2.5 in ethanol) and could not be induced to crystallize. On a paper chromatogram (Solvent E) it moved at the same rate as an authentic sample. Demethylation (81) gave xylose only.

Anal.: Calcd for $C_8H_{16}O_5$: OMe, 48.4 %
found : OMe, 47.6 %

Composition of Mixtures in Fractions B, D and F

Fraction B

The syrup (158 mg.) had $[\alpha]_D^{20}$ +23.2 and the amounts of the two compounds were accordingly estimated as :

3-O-methyl-D-xylose : 30 mg.
2-O-methyl-D-xylose : 128 mg.

The ratio as obtained by electrophoresis was approximately 1:4 .

Fraction D

The ratio between 2-O-methyl- and 2,3-di-O-methyl-D-xylose was determined by paper chromatography (Solvent E) as follows :

2-O-methyl-D-xylose : 5 mg.
2,3-di-O-methyl-D-xylose : 6 mg.

Fraction F

The mixture (88 mg.) had a rotation of $[\alpha]_D^{20}$ -1.0° (c, 3.0 in ethanol). The amounts of the two compounds were calculated as :

2,3-di-O-methyl-D-xylose : 52 mg.
2,3,5-tri-O-methyl-L-arabinose : 36 mg.

Formula for calculating the weights of fractions in a mixture by means of optical rotation :

$$\frac{[\alpha]_D \text{ of compound A} - [\alpha]_D \text{ of mixture}}{[\alpha]_D \text{ of mixture} - [\alpha]_D \text{ of compound B}} = \frac{\text{weight of B}}{\text{weight of A}}$$

Identification of Acidic Fraction from methylated Xylan

The acid glycoside (1.0 g.) was converted to its methyl ester by refluxing with 2 % methanolic hydrogen chloride (50 ml.) for 8 hours. The methyl ester - methyl glycoside was recovered after neutralizing with silver carbonate, filtering through Celite and treating with Amberlite IR-120 cation exchange resin. The resin was removed by filtration, the filtrate evaporated to dryness and further dried in vacuo over anhydrous calcium chloride. The ester (1.0 g.) was reduced with lithium aluminum hydride (3 g.) in dry tetrahydrofuran (50 ml.). After the addition of the lithium aluminum hydride, the mixture was refluxed for one hour and left standing overnight at room temperature. Excess reducing agent was destroyed by addition of ethyl acetate and water, and the aluminum hydroxide was removed on the centrifuge. The aqueous solution was deionized with Amberlite IR-120 and evaporated to a syrup (700 mg.) which crystallized from ethyl acetate. Recrystallization from ethyl acetate gave crystals, m.p. 164-165° C, which has previously been reported for methyl 2-(2,3,4-tri-O-methyl-α-D-glucopyranosyl)-3-O-methyl-β-D-xylopyranoside. The infrared spectrum was found to be identical with that of an authentic specimen.

Anal.: $[\alpha]_D^{20}$ +84° (c, 1.5 in water) (23)(21)

Calcd. for $C_{16}H_{30}O_{10}$: OMe , 40.6 %

found : OMe , 40.4 %

Preparation of fully acetylated Xylan

Xylan (1.0 g.) was dissolved in 50 ml. of dry formamide and freshly distilled pyridine (100ml.) was added followed by 25 ml. of acetic anhydride. The reaction mixture was shaken by hand and cooled until no more heat was evolved, and then placed on a shaker for 24 hours. Two more additions of acetic anhydride (25 ml.) at intervals of 24 hours were made, and the mixture was poured into 1,500 ml. of ice-water, containing 2 % hydrochloric acid. The precipitate was recovered by filtration on a Büchner funnel and washed with cold water until neutral. The acetate was then washed successively with ethanol and petroleum ether and dried *in vacuo* over potassium hydroxide. Yield : 1.25 g.

Methylation of the Glucomannan

Glucomannan (20 g.) was dissolved in 200 ml. of 10 % sodium hydroxide. After complete dissolution water and solid sodium hydroxide were added to yield 300 ml. of 40 % (w/w) sodium hydroxide solution. 200 ml. of dimethyl sulphate were added at 0°C over a period of 10 hours. After this 30 g. of solid sodium hydroxide was allowed to dissolve in the reaction mixture and 200 ml. of dimethyl sulphate were again added at the same rate. This treatment was repeated three times. During the whole experiment the reaction vessel was kept under an atmosphere of nitrogen. The reaction mixture was neutralized with 50 % sulphuric acid and heated to boiling. A sticky material separated out, which could be collected by filtration and dissolved in chloroform. The chloroform solution was dried over sodium sulphate, evaporated and further dried *in vacuo*.

This partially methylated material (12.5 g.) was further methylated according to the method of Kuhn, Trischmann and Löw (62). The product was dissolved in dry dimethyl formamide, silver oxide (40 g.) and methyl iodide (40 ml.) were added to the solution and the mixture was shaken at room temperature in the dark for 24 hours. The same amounts of silver oxide and methyl iodide were added a second time and the mixture was again shaken for 24 hours. Chloroform (1000 ml.) was added and insoluble salts were

removed by filtering through Celite. The filtrate was washed twice with 200 ml. of 5 % potassium cyanide and twice with water (200 ml.). The chloroform solution was dried over sodium sulphate, evaporated to a syrup and further dried *in vacuo* for four days. This product (OMe = 43.5 %) was then methylated according to Purdie (82) with methyl iodide (200 ml.) and silver oxide (80 g.). The mixture was shaken for 48 hours, filtered through Celite and the insoluble part thoroughly washed with chloroform. The combined washings and filtrate were dried over sodium sulphate and concentrated to 100 ml.. Slow addition to 800 ml. of petroleum ether (b.p. 30-60°C) caused the methylated glucomannan to precipitate, which was recovered by filtration and dried *in vacuo*. Yield 8.5 g.

Anal.: Calcd. for fully methylated
glucomannan : OMe, 45.6 %
found : OMe, 45.1 %
$[\alpha]_D^{20}$ -22.5° (c, 2.0 in chloroform)

The infrared spectrum showed a negligible hydroxyl peak.

Hydrolysis of the fully methylated Glucomannan

The fully methylated glucomannan (6.4 g.) was dissolved in 125 ml. of 2 % methanolic hydrogen chloride and boiled under reflux for seven hours. The methanol was evaporated, N- aqueous hydrogen chloride (150 ml.) was added and the reaction mixture again refluxed for seven hours, after which it was neutralized with silver carbonate

and filtered through Celite. The aqueous solution was treated with hydrogen sulphide and filtered through Celite again. Paper chromatography (Solvent E) showed the presence of di-, tri- and tetra-O-methylhexoses. Yield 5.4 g.

Separation of methylated Hexoses from methylated Glucomannan

The mixture of the methylated sugars (2.37 g.) was added to the top of a charcoal-Celite column (3.5 x 55 cm.) and was resolved by gradient elution with aqueous ethanol in the following sequence : 3 to 18 % ethanol (6 litres), 18 to 35 % ethanol (8 litres). The eluate was collected in 25 ml. fractions and every third fraction was examined by paper chromatography (Solvent E). The results are presented in Table XI.

Table XI

Resolution of methylated Hexoses from methylated Glucomannan

Tube No.	Fraction	Compound	Weight (g.)
49 - 53	A	mainly 3,6-di-O-Methyl-D-glucose	0.009
54 - 58	B	Mixture	0.006
59 - 67	C	2,3-di-O-Methyl-D-mannose	0.025
68 - 75	D	Mixture	0.170
76 -150	E	2,3,6-tri-O-Methyl-D-mannose	0.876
151-166	F	Mixture	0.068
167-216	G	2,3,6-tri-O-Methyl-D-glucose	0.388
217-225	H	Mixture	0.015
226-243	I	2,3,4,6-tetra-O-Methyl-D-galactose	.030
244-350	J	2,3,4,6-tetra-O-Methyl-D-mannose	0.035

Characterization of di-O-Methylhexoses

Fraction A

On electrophoresis in borate buffer this fraction had the same rate of movement as an authentic sample of 3,6-di-O-methyl-D-glucose. It yielded glucose on demethylation with boron trichloride (81).

Fraction B

Electrophoresis indicated the presence of predominantly 2,6-di-O-methyl-D-glucose with traces of 3,6-di- and 2,3-di-O-methylhexoses. Demethylation yielded glucose and mannose in an approximate ratio of 5:1.

Fraction C

This fraction was electrophoretically identical with that of an authentic sample of 2,3-di-O-methyl-D-mannose. Demethylation yielded mannose with a trace of glucose.

Fraction D

The mixture (170 mg.) was chromatographically estimated as consisting of 10 mg. of Fraction C and 160 mg. of Fraction E.

Identification of 2,3,6-tri-O-Methyl-D-mannose (Fraction E)

The syrup (876 mg.) was homogeneous on paper chromatographic examination (Solvent E) and had $[\alpha]_D^{20}$ -11.8° (c, 3.5 in water)(74). A portion (150 mg.) was dissolved in 9 ml. of dry pyridine and 600 mg. of p-nitro-benzoyl chloride was added. The reaction mixture was kept at 70°C for 30 min. and then at room temperature overnight. Saturated sodium carbonate solution was slowly added until no further effervescence occurred. Water (150 ml.) was added and the solution extracted with chloroform. The chloroform extract was dried over sodium sulphate and condensed to 3-4 ml.. Addition of petroleum ether (b.p. 30-60°C) induced crystallization of the 1,4-di-p-nitro-benzoate derivative (110 mg.). Recrystallization from ethyl acetate gave needles, m.p. and mixed m.p. 189°C, $[\alpha]_D^{20}$ + 33° (c, 0.6 in chloroform).

Refluxing another portion of the syrup with aniline in ethanol for 6 hours yielded the 2,3,6-tri-O-methyl-N-phenyl-D-mannopyranosylamine, which after recrystallization from ethyl ether had m.p. 125°C, $[\alpha]_D^{20}$ -149° (c, 0.9 in methanol)(43).

Identification of 2,3,6-tri-O-Methyl-D-glucose (Fraction G)

The syrup (388 mg.) crystallized on standing for a few weeks and was recrystallized from ethyl ether,

m.p. and mixed m.p.120-121°C (55,84), $[\alpha]_D^{20}$ + 69° (c, 3.1 in water). Demethylation (81) yielded glucose only.

Identification of 2,3,4,6-tetra-O-Methyl-D-galactose (Fraction H)

This fraction (30 mg.) was not chromatographically pure (Solvent E). The preponderant part moved at the same rate as an authentic sample of 2,3,4,6-tetra-O-methyl-D-galactose. Demethylation yielded galactose, glucose and mannose in the ratio of 4:1:1 . $[\alpha]_D^{20}$ +101°(c, 1.0 in water).

Identification of 2,3,4,6-tetra-O-Methyl-D-mannose (Fraction J)

The syrup (35 mg.) had $[\alpha]_D^{20}$ + 5.3° (c, 3.3 in water)(55) and yielded on demethylation mannose, with a slight trace of glucose. The rate of movement on paper chromatography (Solvent E) was the same as that of an authentic sample.

Fraction F

On paper chromatographic examination (Solvent E) this fraction (68 mg.) was found to consist of approximately 50 mg. of Fraction E and 18 mg. of Fraction G.

Partial Hydrolysis of the Glucomannan

Glucomannan (10 g.) was dissolved in 100 ml. of 90 % formic acid and diluted with water to 200 ml. to give a 45 % solution, which was heated to 98°C for 3 1/2 hours. The cooled solution was condensed to a syrup and boiled with 200 ml. of 0.5 N sulphuric acid for 10 min. to hydrolyse any formic esters formed. The solution was then neutralized with barium carbonate, filtered through Celite, and the filtrate treated with Amberlite IR-120 cation exchange resin. Chromatographic examination (Solvent D) indicated the presence of approximately 50 % monosaccharides, 30 % oligosaccharides ranging in degree of polymerization from 2 to 7, and 20 % higher oligomers.

Separation of Oligosaccharides

8 g. of the hydrolysate obtained on partial hydrlysis was added to the top of a charcoal-Celite column (5 x 56 cm.). The mixture was resolved by gradient elution with aqueous ethanol, and the eluant was collected in 25 ml. fractions at intervals ranging from 12 to 60 min.. Gradient elution was carried out in the following way :

2 to 10 %	aqueous	ethanol	:	8 litres
10 %	"	"	:	2 "
10 to 20 %	"	"	:	8 "
20 %	"	"	:	2 "
20 to 35 %	"	"	:	8 "

The fractions were combined to give pure oligosaccharide solutions. Mixtures of overlapping fractions were further separated by paper chromatography (Solvent D).

Table XII

Fractionation of Oligosaccharides from Glucomannan

Tube No.	Fraction	Oligosaccharide	Weight (mg.)
1 -146	A	Monosaccharides	
147-210	B	4-O-β-D-Mannopyranosyl-D-mannose	310
222-237	C	4-O-β-D-Mannopyranosyl-D-glucose	30
273-294	D	Ma→Ma→Ma	238
339-360	E	Glu→Glu	22
366-438	F	Ma→Ma→Ma→Ma	94
366-438	G	Glu→Ma	185
489-528	H	Ma→Ma→Ma→Ma→Ma	40
489-528	I	Glu→Ma→Ma	87
579-591	J	Ma→Ma→Ma→Ma→Ma→Ma	78
618-639	K	Ma→Ma→Ma→Ma→Glu	48
660-687	L	Ma→Ma→Glu→Ma	48
660-687	M	Ma→Glu→Ma	65
768-792	N	Glu→Glu→Ma	62

Ma = Mannopyranose

Glu = Glucopyranose

→ = β-(1→4)-glycosidic linkage

Identification of Oligosaccharides from Glucomannan

Fraction B; 4-O-β-D-Mannopyranosyl-D-mannose (Mannobiose)

The syrup (310 mg.), $[\alpha]_D^{20}$ -8.3° (c, 3.0 in water), crystallized from methanol, m.p. and mixed m.p 213°C (86). Hydrolysis yielded mannose only.

Fraction C: 4-O-β-D-Mannopyranosyl-D-glucose

This compound (30 mg.) could not be induced to crystallize. It was chromatographically identical with an authentic sample in solvents A and D. Hydrolysis yielded equal amounts of mannose and glucose, and after reduction of the disaccharide with sodium borohydride and subsequent hydrolysis mannose only. $[\alpha]_D^{20}$ +14° (c, 3.0 in water), reported values range from +5° to +29° (44,55).

Fraction D: Ma→Ma→Ma (Mannotriose)

The trisaccharide (238 mg.) was chromatographically identical with an authentic sample (Solvent A and D) and yielded mannose only on hydrolysis. The syrup was dissolved in methanol and crystallized, when a few drops of n-butanol were added. Recrystallization from methanol gave crystals, m.p. and mixed m.p. 166-168°C, $[\alpha]_D^{20}$ -24° (c, 2.4 in water) (55,87).

Ma = Mannopyranose

Glu = Glucopyranose

→ = β-(1→4)-glycosidic linkage

Fraction E: 4-O-β-D-Glucopyranosyl-D-Glucose (Cellobiose)

This fraction (22 mg.) crystallized from methanol after addition of n-butanol, m.p. and mixed m.p 231-233°C. The compound was chromatographically identical with an authentic sample and yielded glucose on hydrolysis. $[\alpha]_D^{20}$ +35° (c, 2.2 in water)(90).

Fraction F: Ma→Ma→Ma→Ma (Mannotetraose)

Hydrolysis of this tetrasaccharide (94 mg.) gave only mannose. Partial hydrolysis yielded mannose, mannobiose and mannotriose. The degree of polymerization was determined according to the method of Peat, Whelan and Roberts (88) as 3.7. $[\alpha]_D^{20}$ -28° (c, 2.0 in water)(91).

Fraction G: 4-O-β-D-Glucopyranosyl-D-mannose

This compound (185 mg.) crystallized from methanol and had after recrystallization from methanol m.p. and mixed m.p. 137-138°C, $[\alpha]_D^{20}$ +7° (c, 2.0 in water). It was chromatographically identical with an authentic sample (Solvent A and D) and gave equal amounts of mannose and glucose on hydrolysis. Reduction of the disaccharide prior to hydrolysis gave glucose only.

Fraction H: Ma→Ma→Ma→Ma→Ma (Mannopentaose)

The product (40 mg.) was obtained pure by separation on paper from higher oligosaccharides. Partial hydrolysis yielded mannose, mannobiose and mannotriose (Solvent D). The degree of polymerization was determined as 5.15 (88,89). $[\alpha]_D^{20}$ -35° (c, 4.0 in water).

Fraction I: Glu→Ma→Ma

The syrup could not be induced to crystallize. The hydrolysate of the trisaccharide (87 mg.) contained glucose and mannose in a ratio of 1:2 , reduction prior to hydrolysis yielded glucose and mannose in a ratio of 1:1. Partial hydrolysis gave glucose, mannose, glucosyl mannose and mannobiose. $[\alpha]_D^{20}$ -9° (c, 4.0 in water).

Fraction J: Ma→Ma→Ma→Ma→Ma→Ma (Mannohexaose)

Separation by paper chromatography (Solvent D) from higher oligosaccharides present in the same fraction gave a pure compound (78 mg.). Hydrolysis yielded mannose only. The degree of polymerization was determined as 5.7 (89). $[\alpha]_D^{20}$ -41° (c, 7.8 in water).

Fraction K: Ma→Ma→Ma→Ma→Glu

Hydrolysis of this product (48 mg.) gave mannose and glucose in a ratio of 4:1. Reduction prior to hydrolysis yielded mannose only. The degree of polymerization was determined as 5.0 (89). $[\alpha]_D^{20}$ -23° (c, 4.8 in water).

Fraction L: Ma→Ma→Glu→Ma

Solvent A was used for paper chromatographic separation of this compound (48 mg.) from Fraction M. Hydrolysis yielded mannose and glucose in a ratio of 3:1, reduction prior to hydrolysis in a ratio of 2:1. Partial hydrolysis of the reduced tetrasaccharide gave mannose, glucose, mannosyl glucose and mannobiose. $[\alpha]_D^{20}$ - 17° (c, 4.8 in water).

Fraction M: Ma→Glu→Ma

This fraction (65 mg.) yielded on hydrolysis mannose and glucose in a ratio of 2:1 . Hydrolysis of the reduced trisaccharide gave mannose and glucose in a ratio of 1:1 . The products obtained on partial hydrolysis were mannose, glucose and glucosyl mannose. The DP was determined as 2.8 . $[\alpha]_D^{20}$ +5.5° (c, 6.5 in water).

Fraction N: Glu→Glu→Ma

The compound (62 mg.) gave on hydrolysis mannose and glucose in a ratio of 1:2 . Reduction prior to hydrolysis yielded glucose only, and the products of partial hydrolysis were glucose, mannose, cellobiose and glucosyl mannose. $[\alpha]_D^{20}$ +9° (c, 6.2 in water).

Periodate Oxidation of the Glucomannan

Samples of glucomannan (150-200 mg.) were oxidized for various lengths of time with 50 ml. of 0.05 M sodium metaperiodate solution. Reaction was allowed to proceed in the dark according to a standard procedure (83). The consumption of periodate was determined by the excess arsenite method (92). The molar consumption of periodate per hexose unit was 0.918 (24 hours), 1.01 (36 hours), 1.06 (48 hours), 1.07 (72 hours), remaining constant after 72 hours.

Preparation of the Nitrate Derivative of the Glucomannan

Nitric acid (100 ml.), 85 %, reagent grade, was cooled to -20°C and 60 g. of phosphorus pentoxide slowly added. The mixture was kept at 0°C overnight and undissolved phosphorus pentoxide was filtered off on a sintered glass funnel. Glucomannan (2.0 g.) was added to the mixture and kept for 1 1/2 hours at 17°C. The reaction mixture was then poured into 50 % aqueous acetic acid. The nitrate was recovered by filtration and washed with ice-water until neutral. Yield : 1.6 g.

Examination of Sugar Residues unoxidized by Periodate

Glucomannan and galactoglucomannan (1 g. each) were oxidized by 0.4 M sodium metaperiodate (50 ml.) at room temperature in the dark for four weeks. Iodate and excess periodate were precipitated by addition of 0.5 M barium acetate and the precipitate was filtered. The filtrate was condensed to a syrup and refluxed in N-sulphuric acid for six hours (25 ml.). The acid was neutralized with barium carbonate and filtered through Celite. The filtrate was treated with Amberlite IR-120 cation exchange resin and the resin filtered off. The solution was condensed to 0.5 ml. and examined by paper chromatography (Solvent A and D). No sugars had survived the oxidation.

Methylation of Galactoglucomannan

The galactoglucomannan (10 g.) was methylated in the same way as previously described for the glucomannan. Methylation with dimethyl sulphate was carried out in three steps (instead of four steps as in the case of the glucomannan) , followed by two steps according to Kuhn (62) and one step according to Purdie (82). The infrared spectrum of this product showed a negligible hydroxyl peak. Yield 4.3 g.

Anal.: Calcd. for fully methylated
galactoglucomannan : OMe, 45.6 %,
found : OMe, 45.0 %
$[\alpha]_D^{20}$ -7° (c, 2.0 in chloroform)

Hydrolysis of methylated Galactoglucomannan

The fully methylated galactoglucomannan (3.0 g.) was refluxed in 2 % methanolic hydrogen chloride for seven hours; the methanol was evaporated and the product again refluxed for seven hours in N-hydrochloric acid. The mixture was recovered as described before for the glucomannan, and showed on paper chromatographic examination (Solvent E) the presence of di-, tri- and tetra-O-methylhexoses. Yield 2.5 g.

Separation of methylated Hexoses from Galactoglucomannan

The mixture of methylated sugars (2.0 g.) was added to the top of a charcoal-Celite column (3.5 x 56 cm.) and was resolved by gradient elution with aqueous ethanol in the following sequence : 3 to 18 % ethanol (6 litres), 18 to 35 % ethanol (8 litres).

The eluate was collected in 25 ml. fractions and every third fraction was examined by paper chromatography (Solvent E). The results are presented in Table XIII. Where necessary, further paper chromatographic resolution and purification of the fractions was applied.

Table XIII

Resolution of methylated Sugars from Galactoglucomannan

Tube No.	Fraction	Sugar	Weight (mg.)
1 - 89	A	Mono-O-methylhexoses	
90 -135	B	di-O-Methyl-D-mannoses	220
110-135	C	2,3-di-O-Methyl-D-glucose	40
136-202	D	2,3,6-tri-O-Methyl-D-mannose	590
203-225	E	2,3,6-tri-O-Methyl-D-glucose	200
226-286	F	2,3,4,6-tetra-O-Methyl-D-galactose	180
287-450	G	2,3,4,6-tetra-O-Methyl-D-mannose	40

Identification of methylated Sugars from Galactoglucomannan Fraction B

Paper chromatography was used (Solvent E) to separate this fraction (220 mg.) from the overlapping Fraction C. Demethylation yielded mannose. Paper eletrophoresis in borate buffer indicated the presence of 2,3-di- and 3,6-di-O-methylhexoses. The mixture was tentatively

identified as consisting of 2,3-di-O-methyl-D-mannose (200 mg.) and 3,6-di-O-methyl-D-mannose (20 mg.).

Fraction C

This fraction (40 mg.) was electrophoretically identical with an authentic sample of 2,3-di-O-methyl-D-glucose. Demethylation yielded glucose only.

Fraction D 2,3,6-tri-O-Methyl-D-mannose

The syrup (590 mg.) had $[\alpha]_D^{20}$ -12.2° (c, 3.5 in water)(55). The 1,4-di-p-nitrobenzoate derivative was prepared and gave m.p. and mixed m.p. 188-189°C, $[\alpha]_D^{20}$ +33° (c, 1.0 in chloroform).

Treatment with aniline yielded the 2,3,6-tri-O-methyl-N-phenyl-D-mannopyranosylamine, which after recrystallization from ethyl ether had m.p. and mixed m.p. 125°C, $[\alpha]_D^{20}$ -152° (c, 1.0 in methanol)(83).

Fraction E 2,3,6-tri-O-Methyl-D-glucose

The syrup (200 mg.), dried in vacuo crystallized on standing and was recrystallized from ethyl ether, m.p. and mixed m.p. 120-121°C, $[\alpha]_D^{20}$ +70.5° (c, 2.0 in water).

The 1,4-di-p-nitrobenzoate derivative (93) on recrystallization from methanol had m.p. and mixed m.p. 189-190°C, $[\alpha]_D^{20}$ -33.5° (c, 1.0 in chloroform).

Fraction F 2,3,4,6-tetra-O-Methyl-D-galactose

The syrup (180 mg.) had $[\alpha]_D^{20}$ -109° (c, 1.0 in water) and yielded galactose on demethylation. Treatment with aniline

gave crystalline 2,3,4,6-tetra-O-methyl-N-phenyl-D-galacto-pyranosylamine, m.p. and mixed m.p. 192°C, $[\alpha]_D^{20}$ +120° (c, 1.0 in water) (95).

Fraction G: 2,3,4,6-tetra-O-Methyl-D-mannose

This fraction was chromatographically identical with an authentic sample (Solvent E). The compound (40 mg.) yielded on demethylation mannose, with a trace of glucose, indicating the presence of a small amount of 2,3,4,6-tetra-O-methyl-D-glucose. $[\alpha]_D^{20}$ +5.5° (c, 4.0 in water) (55).

Partial Hydrolysis of Galactoglucomannan and Separation of Oligosaccharides

Partial hydrolysis was carried out in the same manner as for the glucomannan. A portion (6 g.) of the hydrolysate was added to the top of a charcoal-Celite column (5 x 56 cm.). Fractionation was carried out by gradient elution with aqueous ethanol in the following way :

2 to 10 %	aqueous	ethanol	: 8 litres
10 %	"	"	: 2 "
10 to 20 %	"	"	: 8 "
20 %	"	"	: 2 "
20 to 35 %	"	"	: 8 "

The eluant was collected in 25 ml. fractions and every third fraction was examined by paper chromatography; mixtures of overlapping fractions were further resolved by paper chromatography (Solvent A and D). The results of the fractionation are presented in Table XIV.

Table XIV

Fractionation of Oligosaccharides from Galactoglucomannan

Tube No.	Fraction	Oligosaccharide	Weight (mg.)
1 -151	A	Monosaccharides	
152-217	B	Ma→Ma	240
218-240	C	Ma→Glu	14
271-300	D	Ma→Ma→Ma	126
336-366	E	Glu→Glu	18
282-366	F	Ga(1→6)Ma→Ma→Ma	35
370-450	G	Ma→Ma→Ma→Ma	40
370-450	H	Glu→Ma	90
480-520	I	Glu→Ma→Ma	42
635-660	J	Ga(1→6)Ma→Ma→Ma→Ma	28
752-800	K	Glu→Glu→Ma	32

Ga = Galactopyranose

Glu = Glucopyranose

Ma = Mannopyranose

→ = β-(1→4)-glycosidic linkage

Identification of Oligosaccharides from Galactoglucomannan

Fraction B: 4-O-β-D-Mannopyranosyl-D-mannose (Mannobiose)

The compound (240 mg.) was obtained crystalline from methanol-n-butanol and had after recrystallization from methanol m.p. and mixed m.p. 209-210°C, $[\alpha]_D^{20}$-8° (c, 4.0 in water)(86). Hydrolysis yielded mannose only.

Fraction C: 4-O-β-D-Mannopyranosyl-D-glucose

The syrup (14 mg.), $[\alpha]_D^{20}$ +29° (c, 1.4 in water), could not be induced to crystallize. Hydrolysis yielded equal amounts of mannose and glucose. Hydrolysis of the reduced disaccharide gave mannose only.

Fraction D: Ma→Ma→Ma (Mannotriose)

This trisaccharide (126 mg.) gave on partial hydrolysis mannose and mannobiose. The compound crystallized from ethanol and had after recrystallization from ethanol m.p. and mixed m.p. 167-168°C, $[\alpha]_D^{20}$ -25° (c, 2.0 in water) (87).

Fraction E: 4-O-β-D-Glucopyranosyl-D-glucose (Cellobiose)

The disaccharide (18 mg.), $[\alpha]_D^{20}$ +33.5° (c, 1.8 in water), could not be induced to crystallize. Hydrolysis yielded glucose only. The octaacetate derivative was prepared by treatment with acetic anhydride (2 ml.) and anhydrous sodium acetate at 98°C for six hours. The reaction mixture was neutralized with sodium bicarbonate solution, extracted with chloroform (20 ml.), and the chloroform solution dried over sodium sulphate. The solution was evaporated to dryness,

and the octaacetate crystallized from methanol (yield 8 mg.), having m.p. and mixed m.p. 220-221°C (96).

Fraction F: Ga(1→6)Ma→Ma→Ma

This tetrasaccharide (35 mg.) yielded on hydrolysis galactose and mannose in a ratio of 1:3 . The degree of polymerization was determined as 3.7 (89). The products obtained on partial hydrolysis were chromatographically (Solvent A and D) identical with authentic samples of 6-O-α-D-galactopyranosyl-D-mannose, mannobiose and mannotriose. Reduction prior to hydrolysis yielded galactose and mannose in a ratio of 1:2 . The product was tentatively identified as a tetrasaccharide consisting of three contiguous units of D-mannose with D-galactose attached to one of the three mannose residues at the 6-position. $[\alpha]_D^{20}$ -7° (c, 3.5 in water).

Fraction G: Ma→Ma→Ma→Ma

Hydrolysis of this fraction (40 mg.) yielded mannose only. The product was chromatographically identical with Fraction F of the glucomannan. $[\alpha]_D^{20}$ -28° (c, 2.0 in water).

Fraction H: 4-O-β-D-Glucopyranosyl-D-mannose

Hydrolysis of this disaccharide (90 mg.) gave equal amounts of glucose and mannose. Reduction prior to hydrolysis yielded glucose only. The compound crystallized from ethanol, m.p. and mixed m.p. 135-137°C, $[\alpha]_D^{20}$ +10° (c, 3.0 in water)(44).

Fraction I: Glu→Ma→Ma

This compound (42 mg.) had $[\alpha]_D^{20}$ -9° (c, 4.2 in water) and was identical to Fraction I of the glucomannan part.

Fraction J: Ga(1→6)Ma→Ma→Ma→Ma

This pentasaccharide (28 mg.) yielded on hydrolysis galactose and mannose in a ratio of 1:4 . The degree of polymerization was determined as 4.6 (89). Partial hydrolysis was carried out and the products obtained were chromatographically (Solvent A and D) identical with authentic samples of 6-O-α-D-galactopyranosyl-D-mannose, mannobiose and mannotriose. The compound was thus tentatively identified as consisting of four contiguous units of β-(1→4)-linked D-mannose, with D-galactose attached to the 6-position of a mannose residue. $[\alpha]_D^{20}$ -11° (c, 2.5 in water).

Fraction K: Glu→Glu→Ma

This compound (32 mg.) was found to be identical with Fraction N of the glucomannan part. $[\alpha]_D^{20}$ +9.5° (c, 3.2 in water).(44).

Periodate Oxidation of the Galactoglucomannan

Samples of galactoglucomannan (150-200 mg.) were oxidized for various lengths of time with 50 ml. of 0.05 M sodium metaperiodate solution in the dark. The consumption of periodate was determined by the excess arsenite method (53). Molar consumption of periodate per hexose unit was 1.15 (36 hours), 1.20 (48 hours), 1.23 (72 hours), 1.25 (96 hours), remaining constant after 96 hours.

Preparation of the Nitrate Derivative of the Galactoglucomannan

Galactoglucomannan (2.0 g.) was nitrated in the same way as described for the glucomannan. Yield : 1.5 g.

Determination of Number-Average Molecular Weights

The osmometers used were of the Zimm and Mayerson type (97) as modified by Stabin and Immergut (98). The osmotic pressure was determined by the static method at different concentrations according to the procedure of Glaudemans and Timell (63). The (h/w) was plotted against the concentration (w) and the value $(h/w)_{c=o}$ was obtained by extrapolating to zero concentration.

The following equation was used for calculating the number-average molecular weight ($\overline{M}_n$) :

$$\overline{M}_n = \frac{RT}{(h/w)_{c=o}}$$

R = 848 g. cm. / degree mole

T = absolute temperature in °K

Table XV

Osmotic Pressure Results and Number-Average Degrees of Polymerization ($\overline{DP}_n$)

Xylan

	Acetate		Methylated	
	h/w	w	h/w	w
	0.806	1.035	1.258	0.702
	0.892	1.345	1.326	1.133
	[0.692	2.002]	1.375	1.944
	0.812	2.404	1.297	2.826
	0.835	2.715		
$(h/w)_{c=o}$:	0.83		1.20	
$\overline{M}_n$:	31,000		21,400	
$\overline{DP}_n$:	104		95	

Glucomannan

	Nitrate		Methylated	
	h/w	w	h/w	w
	0.984	1.135	0.430	0.600
	0.985	1.738	0.446	1.232
	0.873	2.231	0.513	1.307
	0.959	2.765	0.539	1.977
	0.970	4.054		
$(h/w)_{c=o}$:	0.95		0.43	
$\overline{M}_n$:	27,000		59,700	
$\overline{DP}_n$:	95		282	

Table XV (contd.)

Galactoglucomannan

	Nitrate		Methylated	
	h/w	w	h/w	w
	0.964	1.415	1.295	0.919
	0.861	1.450	1.316	1.300
	0.951	2.802	1.332	1.158
	0.927	3.643	1.370	1.935
	0.850	3.951	1.292	2.387
	0.958	3.999		
$(h/w)_{c=o}$:	0.95		1.20	
$\overline{M}_n$:	27,000		21,400	
$\overline{DP}_n$:	76		91	

BIBLIOGRAPHY

1. Schulze, E., Ber. 24, 2277 (1891).
2. Purdie, T. and Irvine, J.C., J. Chem. Soc. 83, 1021 (1929).
3. Haworth, W.N., J. Chem. Soc., 107, 13 (1915).
4. Hirst, E.L. and Jones, J.K.N., J. Chem. Soc. 1659 (1949).
5. Perlin, A.S. and Lansdown, A.R., Can. J. Chem., 34, 451 (1956).
6. Bouveng, H.O. and Lindberg., Adv. Carbohydrade Chem. Vol. 15, 52 (1960).
7. Aspinall, G.O., Hirst, E.L., Moody, R.W. and Percival, E.G.V., J. Chem. Soc., 1631 (1953).
8. Aspinall, G.O. and Ferrier, R.J., J. Chem. Soc. 638 (1958).
9. Ewald, C.M. and Perlin, A.S., Can. J. Chem. 37, 1254 (1959).
10. Aspinall, G.O., Adv. Carbohydrate Chem., 14, 429 (1959).
11. Timell, T.E. and Gillham, J.K., Pulp & Paper Mag. of Canada, October 1958.
12. Barsch, D.J. and Wise, L.E., Tappi 39, 581 (1956).
13. Jones, J.K.N. and Wise, L.E., J.Chem. Soc. 3389 (1952).
14. Gorin, P.A.J. and Perlin, A.S., Can. J. Chem. 36, 999 (1958).
15. Dutton, G.G.S. and Smith, S., J. Am. Chem. Soc., 78, 2505 (1956).
16. Jones, J.K.N. and Painter, T.J., J. Chem. Soc. 669 (1957).
17. Gorrod, A.R.N. and Jones, J.K.N., J. Chem. Soc. 2522(1954).
18. Roudier, A. and Eberhard, L., Compt. rend., 240, 2012 (1955).
19. Garegg, P.J. and Lindberg, B., Acta Chem. Scand., 14, 871 (1960).
20. Aspinall, G.O. and Carter, M.E., J. Chem. Soc. 3744 (1956).
21. Dutton, G.G.S. and Hunt, K.J., J. Am. Chem. Soc. 82, 1682 (1960).

22. Aspinall, G.O. and McKay, J.E., J. Chem. Soc. 1059 (1958).

23. Banerjee, S.K. and Timell, T.E., Tappi 43, 849 (1960).

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

25. Timell, T.E. and Mills, A.R., unpubl.results.

26. Hamilton, J.K., Partlow,E.V. and Thompson, N.S., Tappi 41, 811 (1958).

27. Roudier, A., "Researches on the Hemicelluloses of Maritime Pine"; Paper presented on the 136th meeting of the Am. Chem. Soc., Atlantic City, N.J., Sept. 1959.

28. Saarnio, J., Suomen Kemistilehti, B 29, 35 (1956).

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

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

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

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

33. Timell, T.E., Svensk Papperstidng., 64, 748 (1961).

34. Wise, L.E. and Jahn, E.C., "Wood Chemistry", Reinhold Publ. Corp., New York, N.Y., 1952.

35. Leech, J.K., Tappi 35, 249 (1952).

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

37. Jones, J.K.N., Wise, L.E. and Jappe, J.P., Tappi 39, 139 (1956).

38. Hamilton, J.K., Kircher, H.W. and Thompson, N.S., J. Am. Chem. Soc. 78, 2508 (1956).

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

40. Meier, H., Acta Chem. Scand., 12, 144 (1958).

41. Jayme, G. and Kringstad, K., Das Papier, 15, 500 (1961).

42. Bishop, C.T. and Cooper, F.P., Can. J. Chem. 38, 793 (1960).

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

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

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

46. Meier, H., Acta Chem. Scand. 15, 1381 (1961).

47. Hamilton, J.K. and Thompson, N.S., Pulp and Paper Mag. Canada (10) 59, 253 (1958).

48. Hamilton, J.K., Partlow, E.V. and Thompson, N.S., Tappi 41, 803 (1958).

49. Hamilton, J.K. and Thompson, N.S., J. Am. Chem. Soc. 79, 6464 (1957).

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

51. Adams, G.A., Tappi 40, 721 (1957).

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

53. Bouveng, H.O. and Meier, H., Acta Chem. Scand. 13, 1884 (1959).

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

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

56. Bouveng, H.O., Garegg, P.J. and Lindberg, B., Acta Chem. Scand. 14, 742 (1960).

57. Aspinall, G.O., Laidlaw, R.A. and Rashbrook, R.B., J. Chem. Soc. 4444 (1957).

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

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

60. Jabbar Mian, A. and Timell, T.E., Svensk Papperstidg. 63, 884 (1960).

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

62. Kuhn, R., Trischmann, H. and Low, I., Angew. Chemie 67, 32 (1955).

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

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

65. Adams, G.A., Can. J. Chem. 35, 556 (1957).

66. Timell, T.E., Svensk Papperstidg. 60, 762 (1957).

67. Bouveng, H.O., Garegg, P.J. and Lindberg, B. Chem. and Ind. 1727 (1958).

68. Timell, T.E., J. Am. Chem. Soc. 82, 5211 (1960).

69. Meier, H., Holzforschung 13, 177 (1959).

70. Meier, H., Biochem. et Biophys. Acta 28, 229 (1958).

71. Sundman, J., Paper and Timber (Finland) 32, 267 (1950).

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

73. Wise, L.E., Murphy, M. and D'Accieco, A.A., Paper Trade J. 122 No.2, 35 (1946).

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

75. Bouveng, H.O. and Lindberg, B., Acta Chem. Scand. 8, 1283 (1956).

76. Vogel, A.E., "A Textbook of practical Organic Chemistry", Longmans, Green and Co., London 1956, p. 169.

77. Vieböck, F. and Schwappach, A., Chem. Ber. 61, 2818 (1930).

78. Timell, T.E. and Purves, C.B., Svensk Papperstidg. 303, (1951).

79. Helferich, B. and Klein, W., Annalen der Chemie, 450, 219 (1926).

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

81. Bonner, T.G., Bourne, E.J. and McNally, S., J. Chem. Soc. 2929 (1960).

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

83. Andrews, P., Hough, L. and Jones, J.K.N., J. Chem. Soc. 181 (1956).

84. Carrington, H.C., Haworth, W.N. and Hirst, E.L., J. Chem. Soc. 1084 (1933).

85. Haworth, W.N., Heath, R.L. and Peat, S., J. Chem. Soc. 833 (1941).

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

87. Whistler, R.L. and Smith, C.G., J. Am. Chem. Soc. 74, 3795 (1952).

88. Peat, S., Whelan, W.J. and Roberts, J.G., J. Chem. Soc. 2258 (1956).

89. Timell, T.E., Svensk Papperstidg. 63, 668 (1960).

90. Skraup, Z.K. and König, J. Monatshefte 22, 1011 (1901).

91. Aspinall, G.O., Rashbrook, R.B. and Kessler, G., J. Chem. Soc. 215 (1958).

92. Fleury, P. and Lange, J., J. Pharm. Chim. (8) 17, 107 (1933).

93. Mitchel, R.L., Ind. Eng. Chem. 45, 2526 (1953).

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

95. Haworth, W.N., Loach, J.V. and Long, C.W., J. Chem. Soc. 3146 (1927).

96. Violet, E.G., Smith, F. and Stacey, M., J. Chem. Soc. 622 (1946).

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

98. Stabin, J.V. and Immergut, E.H., J. Polymer Sc. 14, 209 (1954).

99. Marchessault, R.H. and Timell, T.E., unpublished results

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. Three hemicelluloses have been isolated from a chlorite holocellulose of amabilis fir (Abies amabilis). An arabino-glucurono-xylan (yield 7.0 %) and a galacto-glucomannan (yield 4.1 %) were obtained by extracting the holocellulose with 24 % potassium hydroxide. The poly-saccharides were separated by precipitating the latter with barium hydroxide from an aqueous solution. A gluco-mannan (yield 8.1 %) was obtained by extracting the remaining holocellulose with 17 % sodium hydroxide containing 3.5 % borate.

2. Partial hydrolysis of the wood gave galacturonic acid, 4-O-methyl-D-glucuronic acid, 2-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-xylopyranose, a galact-uronosyl glucose and a galacturonosyl rhamnose. The yield of the total 4-O-methyl-D-glucuronic acid residues was 1 % of the wood.

3. The methylated xylan on methanolysis and hydrolysis yielded 2-O-methyl-D-xylose (10 moles), 3-O-methyl-D-xylose (3), 2,3-di-O-methyl-D-xylose (48), 2,3,4-tri-O-methyl-D-xylose (1), 2,3,5-tri-O-methyl-L-arabinose (10) and 2-O-(2,3,4-tri-O-methyl-α-D-glucuronic acid)-3-O-methyl-D-xylose (14 moles). One non-reducing xylose end group was thus present per 76 xylose residues.

4. On oxidation with periodate 1.06 mole of oxidant was consumed per anhydroxylose unit. The number-average degree of polymerization of the methylated and the original xylan was 95 and 104, respectively.

5. On the basis of this evidence it is concluded that the arabino-4-O-methylglucurono-xylan in amabilis fir wood contains a straight or slightly branched framework of at least 100 β-(1→4)-linked D-xylopyranose residues to which are directly attached terminal residues of α-(1→2)-linked 4-O-methyl-D-glucuronic acid and (1→3)-linked L-arabinofuranose, one acid being present every fifth and one arabinose every seventh xylose residue.

6. The galactoglucomannan, containing galactose, glucose and mannose in a ratio of 1:1:3 , yielded on methylation and hydrolysis 2,3-di-O-methyl-D-mannose (5.7 moles), 3,6-di-O-methyl-D-mannose (0.6), 2,3-di-O-methyl-D-glucose (1.1), 2,3,6-tri-O-methyl-D-mannose (15.6), 2,3,6-tri-O-methyl-D-glucose (5.3), 2,3,4,6-tetra-O-methyl-D-galactose (4.5) and 2,3,4,6-tetra-O-methyl-D-mannose (1 mole). One non-reducing mannose end group was thus present per 28 anhydroglucose and mannose residues.

7. Partial hydrolysis of the galactoglucomannan yielded 4-O-β-D-mannopyranosyl-D-mannose (M→M), 4-O-β-D-mannopyranosyl-D-glucose (M→G), 4-O-β-D-glucopyranosyl-D-mannose (G→M), cellobiose (G→G), mannotriose (M→M→M), manno-

tetraose (M→M→M→M), G→M→M, G→G→M, Ga(1→6)M→M→M, (Ga = D-galactopyranosyl) and Ga(1→6)M→M→M→M→M.

8. On periodate oxidation of the galactoglucomannan 1.23 moles of oxidant was consumed per anhydrohexose unit. The number-average degrees of polymerization of the methylated and the original galactoglucomannan were determined 91 and 76, respectively. Tiselius boundary electrophoresis showed that the polysaccharide was homogeneous.

9. It is concluded that the galactoglucomannan is composed of a minimum of 76 glucose and mannose residues linked together by β-(1→4)-glycosidic bonds with an average of two branch points per molecule and with mannose at the non - reducing ends. Branching is believed to occur at C-6 of a glucose or mannose unit. Single glucose unit are probably interposed between two to six contiguous mannose residues. Galactose is an integral part of the molecule and linked α-(1→6)-glycosidically, predominantly to mannose.

10. The glucomannan, which contained galactose, glucose and mannose residues in a ratio of 0.1 : 1 : 3, yielded on methylation and hydrolysis 3,6-di-O-methyl-D-glucose (0.2 moles), 2,3-di-O-methyl-D-mannose (1.0), 2,3,6 -tri-O-methyl-D-mannose (33.0), 2,3,6-tri-O-methyl-D-glucose (12.5), 2,3,4,6-tetra-O-methyl-D-galactose (1.3) and 2,3,4,6-tetra-O-methyl-D-mannose (1.0). One non-reducing end group was thus present per 57 anhydroglucose and mannose residues.

11. Partial hydrolysis of the glucomannan yielded 4-O-β-D-mannopyranosyl-D-glucose (M→G), M→M, G→G, G→M, M→M→M, M→M→M→M, M→M→M→M→M, M→M→M→M→M→M, G→M→M, G→G→M, M→G→M, M→M→G→M, M→M→M→M→G.

12. On periodate oxidation 1.06 moles of oxidant was consumed per anhydrohexose unit. The number-average degrees of polymerization of the methylated and the original polysaccharide were 282 and 95, respectively.

13. On the basis of this evidence it is concluded that the glucomannan consists of at least 95 glucose and mannose residues linked together by β-(1→4)-glycosidic bonds with an average of four branch points per molecule. Branching is believed to occur at C-6 of a mannose unit. The galactose residues are assumed to be an integral part of the molecule attached as single terminal side chains by (1→6)-glycosidic bonds to mannose. The glucose residues are probably interposed between two to six or more contiguous mannose residues; occasionally two glucose residues are linked contiguously.