

THE MOLECULAR STRUCTURE
OF
MANNOSE-CONTAINING POLYSACCHARIDES

by

Robert Begbie, B.Sc.

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University of Edinburgh



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Introduction

While D-mannose occurs as a minor structural component of a number of polysaccharides the term "mannan" may be employed to describe only those polysaccharides whose principal structural unit is D-mannose; where other monosaccharide residues are components of the polysaccharide, the mannans may be distinguished by the use of appropriate prefixes indicative of these residues e.g. those polysaccharides which yield D-galactose and D-mannose only on hydrolysis are termed galactomannans, those yielding D-glucose and D-mannose glucomannans, etc. A number of polysaccharides, other than mannans as defined here, but which nevertheless are known to contain appreciable amounts of D-mannose are to be found among the gum exudates, notably those elaborated by certain members of the genus Prunus(1), and among polysaccharides associated with many bacteria; although the nature and mode of linkage of residues in these polysaccharides is somewhat uncertain as yet, it is apparent that D-mannose does not represent a major structural unit and consequently their classification as mannans cannot be justified.

Mannans are widely distributed in nature, but may be primarily classified according to source into the two general groups, (a) Bacterial and Fungal polysaccharides and (b) Phytoglycans or plant polysaccharides. In view of basic differences in the structural type between the two groups it

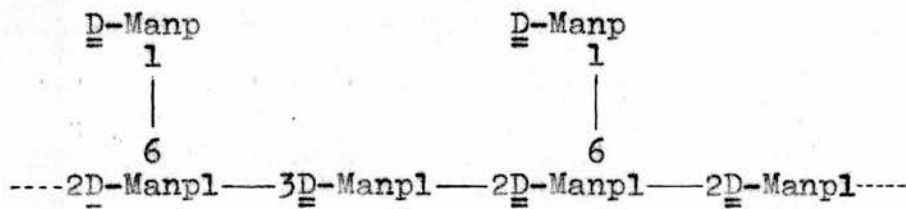
seems desirable to consider them separately and to discuss briefly the characteristics of each group.

(a) Mannose-containing polysaccharides of Bacteria, Fungi and Yeasts.

The production of mannose-containing polysaccharides by many micro-organisms including representatives of the bacterial and fungal groups and by most yeasts has been recognised by many workers although few studies have extended to structural investigation of these materials. Their function is doubtful in some cases but it is accepted that a few may be components of complex antigenic systems capable of eliciting a specific response (production of antibodies) when injected into the animal body while others are merely metabolic by-products of the organism. Many others appear to act as haptens (such as the several polysaccharides isolated from Mycobacterium tuberculosis cultures) which react with immune sera although unable to function as antigens.

Detailed evidence regarding the structure of bacterial mannans is scanty. Adams(2) has examined the mixture of polysaccharides formed during fermentation of glucose, sucrose or lactose by Bacillus polymyxa and has identified one of these as a mannan (associated with 13% protein) of molecular weight of ca. 3000. Hydrolysis of the methylated polysaccharide gave 2,3,4,6 tetra-O-methyl-D-mannose, 2,4,6- and 3,4,6-tri-O-methyl-D-mannoses and 3,4-di-O-methyl-D-mannose in the molar

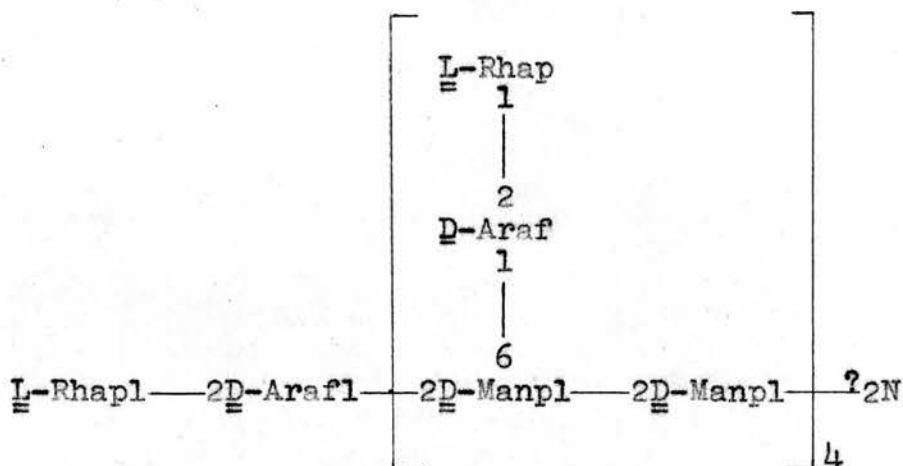
ratio 2:1:1:2. Extensive overoxidation encountered during periodate oxidation(3) indicated that the predominant linkage was not 1,6. On the basis of these results the repeating unit I is proposed.



I

In contrast hydrolysis of a methylated B. anthracis mannan(4) yielded only di-O-methyl mannose and 2,3,6 tri-O-methyl-D-mannose indicating a predominance of 1→4 linkages.

Mannose-containing polysaccharides have been isolated by several workers from different sources in M. tuberculosis culturesnamely from the somatic (intracellular) portion of the cell, from the cell lipids and from the culture medium. Little is known of the molecular structure of these polysaccharides but methylation and subsequent hydrolysis of the somatic polysaccharide (D-arabinose 22%, D-mannose 47%, L-rhamnose25% and amino-sugar 6%) have provided evidence for a constitutional formula similar to II (5).



N = unidentified amino-sugar.

II

While many other bacteria (including species of Salmonella(6) Aerobacter(7) and Clostridium(8) yield polysaccharides which contain D-mannose as a major component, these are generally characterised by a high degree of structural heterogeneity and little approach has been made to the problem of structural elucidation.

Knowledge regarding the structural nature of the mannose-containing polysaccharides of yeast and fungal origin is scarcely more complete; investigations to date have been confined to polysaccharides containing D-mannose only although the occurrence of di- and tri-heteromannans has been claimed (9,10,11).

The mannans isolated from the cell-walls of several species of yeast(12a,12b,13,14) have been shown to be highly branched molecules differing mainly in the length of the branch chains; the relatively high positive optical rotations indicate a predominance of α -links. A comparison of the relative amounts of methylated monosaccharides derived from hydrolysis of the methylated mannans from various yeast species is made in Table 1.

Three possible partial structures for S. cerevisiae mannan have been suggested on the basis of the methylation data in Table 1.(12a,12b.)

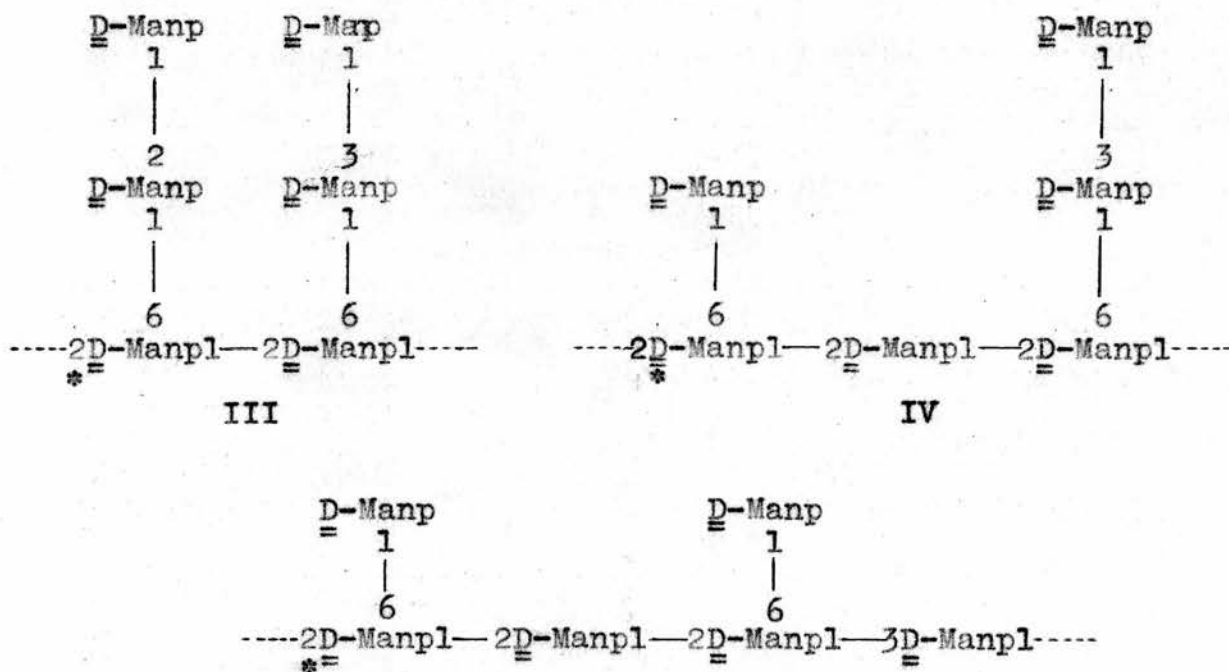


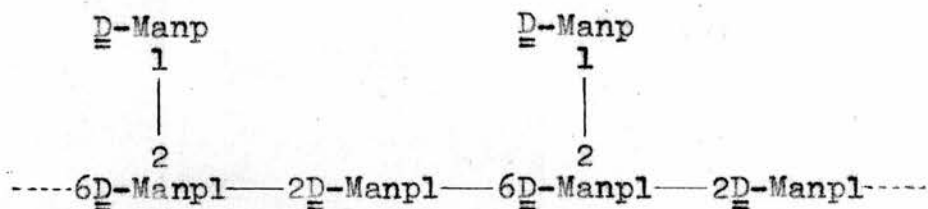
Table 1.

Source	$[\alpha]_D$	2,3,4,6-tetra-O-methyl-D-mannose	3,4,6-tri-O-methyl-D-mannose	2,4,6-tri-O-methyl-D-mannose	3,4 di-O-methyl-D-mannose	Other Methyl ethers of D-mannose	Ref.
<u>S. cerevisiae</u> (baker's yeast)	+ 88°	2	1	1	2	2,3,4-tri-O-methyl-D-mannose	12a,b
<u>S. rouxii</u>	+ 58°	1	1	-	1	3-O-methyl-D-mannose	13
<u>C. albicans</u>	+ 78°	1.65	1.00	-	1.90	2,3,6 tri-O-methyl-D-mannose	14

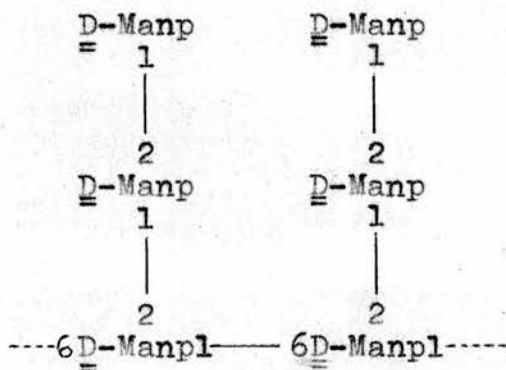
The presence of the small amount of 2,3,4 tri-O-methyl-D-mannose is explained by a small proportion of residues linked through C₁ and C₆ only e.g. at * where no residue is linked through C₂ of the mannose unit. Results obtained from periodate oxidation, hydrolysis and tritylation are in agreement with such structures(15).

A series of oligosaccharides containing 1,6 linked α -D-mannose units has recently been isolated from a partial acid hydrolysate of S. cerevisiae mannan(12c). Although the disaccharide member has been isolated by the same workers and by Jones and Nicholson(12d) as an acid reversion product of D-mannose treated under the conditions used in the partial hydrolysis of the mannan, no higher oligosaccharides were observed and the yield of 6-O- α -D-mannopyranosyl-D-mannose from reversion was considered insufficient to account for the entire yield of the disaccharide from the mannan hydrolysate. It was concluded that yeast mannan contained sequences of α -1,6 bonds, possibly in a molecular backbone with side chains containing other links radiating from the main chain.

By similar interpretation of the methylation data cited in Table 1, Gorin and Perlin(13) have advanced two possible repeating units for the mannan extracted from S. rouxii (VI and VII).



VI



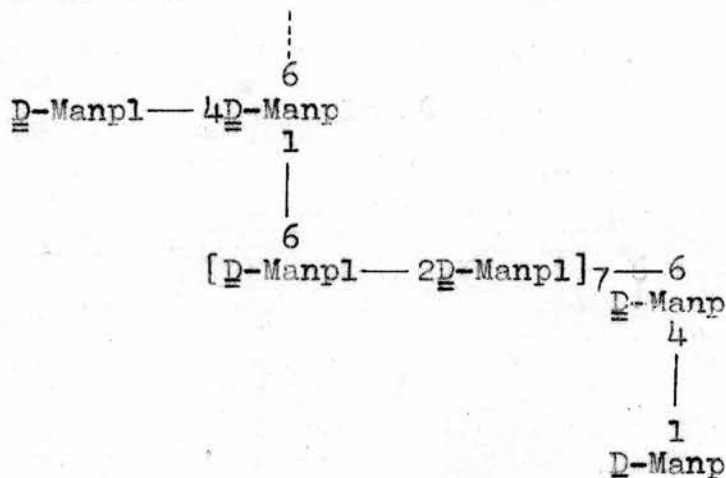
VII

The isolation and identification of 2- $\underline{\underline{O}}\text{-}\alpha\text{-}\underline{\underline{D}}$ -mannopyranosyl- $\underline{\underline{D}}$ -mannopyranose and $\underline{\underline{O}}\text{-}\alpha\text{-}\underline{\underline{D}}$ -mannopyranosyl (1 \rightarrow 2)- $\underline{\underline{O}}\text{-}\alpha\text{-}\underline{\underline{D}}$ -mannopyranosyl (1 \rightarrow 2)- $\underline{\underline{D}}$ -mannopyranose from a partial hydrolysate has confirmed the existence of the (1 \rightarrow 2) linkage in S. rouxii mannan(13).

The C. albicans mannan(14) is probably of very similar structure where short (1 \rightarrow 2) linked chains of varying length are joined by (1 \rightarrow 6) linkages.

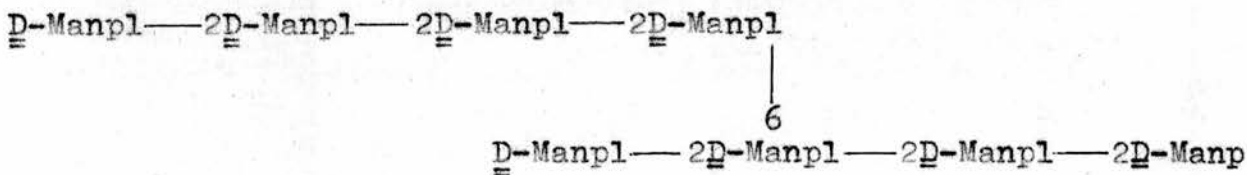
The structures of mannans associated with several fungal species have been investigated. One of these is a branched mannan, mannocarlose, which is a component of the polysaccharide

mixture produced by Penicillium charlesii G. Smith when grown on glucose media(16). Hydrolysis of the methylated polysaccharide(17) yielded 2,3,4,6 tetra-O-methyl, 3,4,6- and 2,3,4-tri-O-methyl and 2,3 di-O-methyl-D-mannoses in the molar ratio of 2:7:7:2; on this evidence the partial structure VIII has been proposed.



VIII

Hough and Perry(3) employing their periodate over-oxidation technique, have derived results which indicate that four (1→2) links lie on either side of a (1→6) link in the manner



IX

Smith(18) has shown that the alkali-soluble mannan from the urediospores of the fungus responsible for the disease Wheat

Stem Rust has a very different structure; on the basis of evidence from methylation and subsequent hydrolysis of the polysaccharide, a repeating unit has been suggested, which consists of six terminal non-reducing residues (three of D-mannose and three of D-glucose), 104 non-terminal D-mannose residues (52 linked through C₁ and C₄ and 52 through C₁ and C₃) and 4 branching residues consisting of D-glucose units linked through C₁, C₂ and C₆; the majority of linkages appear to be of the β - configuration ($[\alpha]_D, -80^\circ$) in contrast to mannan where the high positive rotation ($+66^\circ$) suggest a predominance of α -linkages.

Preliminary studies on one of the extracellular polysaccharides elaborated by the fungus Cryptococcus laurentii have indicated a D-mannose-containing backbone with D-xylose and D-glucuronic acid end-groups(9). Analysis show that the mannan has a mannose : xylose ratio of 5:2 with 11.8% glucuronic anhydride content. 66% of the D-xylose is removed by mild acid treatment with cation exchange resins.

(b) Mannose-containing polysaccharides of plant origin

Polysaccharides of the mannan group have been isolated from various types of plant tissue where they apparently function in the main either as plant food reserves or in association with cellulose and lignin, as skeletal cell-wall components. Differentiation between reserve and structural functions is difficult to demonstrate and indeed it is possible that some mannans especially those occurring in some tuberous cells, may be bifunctional in the sense that they act both as food reserves and as structural material.

Galactomannans occurring as reserve polysaccharides are commonly found in the seeds of the Leguminosae, certain species of palms and in some clovers; the polysaccharides are located in the endosperm and disappear on germination of the seeds. Polysaccharides with similar reserve functions in the plant have been isolated from various other sources notably mannans from vegetable ivory (a term applied to the endosperm of the tagua palm) and several glucomannans from the tubers of various species of Amorphophallus, Iris and Liliaceae. Glucomannans of closely similar structure occur as hemicellulosic components of both coniferous (Gymnospermae) and deciduous (Angiospermae) woods.

Regardless of biological function and source, all plant mannans bear a striking similarity in structural features, sharing in common a linear chain of (1→4) linked β -D-

mannopyranose units (in which (1→4) linked β -D-glucopyranose units may sometimes be incorporated as in the glucomannans). Differences then are confined to the identity of monosaccharide residues attached to the linear backbone, to the mode of attachment of such and to the distribution of residues other than D-mannose where they may be present in the main chain.

Such structures are in direct contrast to the structural arrangement exhibited by the mannose-containing polysaccharides of the bacteria, fungi and yeasts where (1→2), (1→3) and (1→6) links may occur within the same molecule; furthermore the mannans of the latter group are characterised by a predominance of α -linkages as witnessed by the relatively high positive optical rotations.

Mannans

True mannans (which may be defined as polysaccharides yielding more than 95% D-mannose on hydrolysis) have been isolated from two sources only among the spermatophyta (higher land plants).

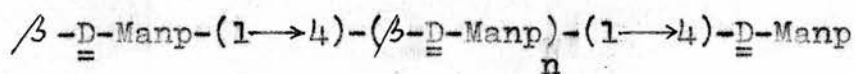
Two mannans have been isolated from the ivory nut, Phytalephas macrocarpa (19a, 19b, 19c). Mannan A which is globular and gives a distinct X-ray diffraction photograph typical of crystalline structures (20) may be extracted with alkali. Mannan B however is separable from cellulose only by precipitation from cuprammonium solution, and in the plant has a microfibrillar structure analogous to cellulose itself.

Methylation of both Mannans A and B with subsequent hydrolysis gave rise to the same series of methyl ethers. (see Table 2) (19b).

Table 2

	<u>Mannan A</u>	<u>Mannan B</u>
2,3,4,6-tetra- <u>O</u> -methyl- <u>D</u> -mannose	4.3	1
2,3,4,6-tetra- <u>O</u> -methyl- <u>D</u> -galactose	1	1
2,3,6-tri- <u>O</u> -methyl- <u>D</u> -mannose	49	63
2,3,4-tri- <u>O</u> -methyl- <u>D</u> -mannose	4	11
2,3-di- <u>O</u> -methyl- <u>D</u> -mannose	0.7	1

The isolation from the products of graded acetolysis of mannans of a homologous series of oligosaccharides containing (1→4) linked β -D-mannopyranose units (X)(19c) is in agreement with the results of methylation studies.

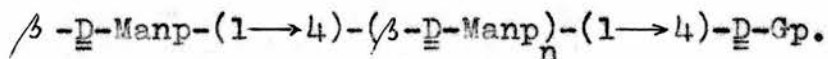


where n=0,1,2,3.

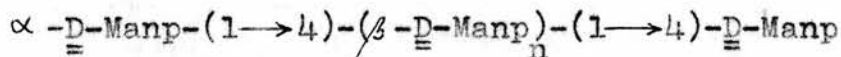
X

It has not yet been determined whether or not representatives of two other oligosaccharide series, XI and XII, are structurally significant., (In the light of recent results from graded acetolysis of mannotriose under similar conditions (21)

it seems probable that the α -linkages arise either from reversion or from acid-catalysed anomerisation of the normal β -glycosidic bond).



XI



XII

Osmotic pressure measurements on the nitrates of mannan A and mannan B gave values for the number average D.P. of 17-21 and 80 respectively(20). These figures would agree with average chain lengths calculated from methylation studies of 12-16 and 75-80 where it is assumed that $\underline{\underline{D}}$ -galactose residues are present only as single-unit side chains; however since structural heterogeneity is also suggested by the methylation data it is as yet impossible to arrive at any simple representation of the molecular structures involved.

A mannan, $[\alpha]_{\text{D}} = -22^{\circ}$, yielding 95% $\underline{\underline{D}}$ -mannose and 2% $\underline{\underline{D}}$ -galactose on hydrolysis has been isolated from the green coffee bean by extraction of the holocellulose with strong alkali(22). Preliminary methylation studies have indicated that the major linkage between $\underline{\underline{D}}$ -mannopyranose units is β (1 \rightarrow 4).

Extraction of the dried tubers of Orchis and Eupholia with water yields a mucilaginous polysaccharide (Salep mannan) claimed to consist of D-mannopyranose residues only(23); hydrolysis of the methylated polysaccharide afforded 2,3,6-tri-O-methyl-D-mannose and 2,3,4,6 tetra-O-methyl-D-mannose(24). Re-examination, however, of some polysaccharides originally claimed to be true mannans, employing more sensitive techniques, has shown them to be heteropolymers and this may also be true of salep mannan.

The presence of polymers of D-mannose has been noted in certain seaweeds. Jones(25) has isolated a mannan $[\alpha]_D = -44^\circ$ from the red seaweed Porphyra umbilicalis by extraction with strong alkali and purification through the copper complex. Evidence derived from methylation studies and from periodate oxidation results suggest a branched structure in which one branch-point occurs per twelve (1 \rightarrow 4) linked β -D-mannopyranose residues.

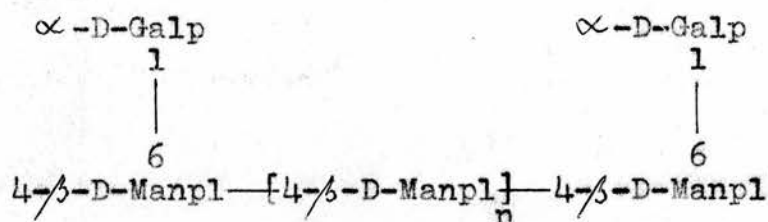
A mannan is also claimed to be present in the brown seaweed Ascophyllum nodosum(26) while mannose-containing oligosaccharides have recently been isolated from the products of partial hydrolysis of Clodium fragile(27).

Galactomannans.

Galactomannans have been isolated from a large number of leguminous endosperms(28) by extraction with water, forming thick, highly viscous solutions which gel in the presence of inorganic salts such as borax. Anderson has shown that variations in the relative proportions of D-galactose and D-mannose occur from species to species but the differences in the ratio of sugars in various samples of the same species reported by some workers may be due merely to differences in analytical procedure or may reflect varietal or geographic types.

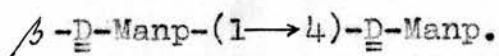
Galactomannans have also been found in other plants including the seeds of clovers(29), coconuts(30), lucerne (alfalfa) (29,31) and in soy bean hulls(32).

Structural studies have largely been confined to examination of the hydrolysates of the methylated polysaccharides and identification of the methyl ethers produced. In the majority of cases (Table 3) the data derived indicate a (1→4) linked backbone of β -D-mannopyranose units to some^{of} which are attached through (1→6) linkages α -D-galactopyranose units, the proportion varying with species. Where periodate oxidation data are available, the suggested general structure XIII has been confirmed.

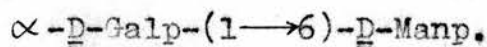


XIII

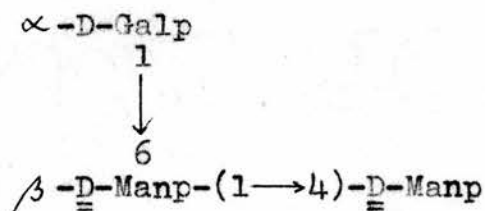
The structure XIII finds further support from the identification from partial hydrolysates of guar(33) and green palmyra palm nut(34) of 4-Q-β-D-mannopyranosyl-D-mannopyranose (XIV), 6-Q-α-D-galactopyranosyl-D-mannopyranose (XV) and Q-α-D-galactopyranosyl-(1→6)-Q-β-D-mannopyranosyl-(1→4)-D-mannopyranose (XVI); the above disaccharides together with Q-β-D-mannopyranosyl-(1→4)-Q-β-D-mannopyranosyl-(1→4)-D-mannopyranose (XVII) have been isolated from enzymic hydrolysates of guar gum(35) employing an enzymic system from the germinating seed.



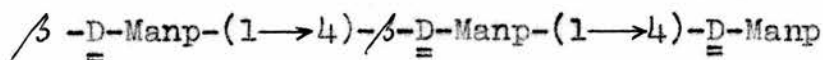
XIV



XV



XVI



XVII

Two exceptions are noteworthy (Table 3); methylation studies have shown that the water-soluble galactomannan from Cocos nucifera (coconut) (30) differs from the common structure XIII in possessing equal numbers of D-mannose and D-galactose terminal units, a proportion of (1→4) linked β -D-galactose units and branch-points on D-galactose units rather than on D-mannose units.

Lucerne yields a second galactomannan on extraction with hot 10% aqueous potassium hydroxide(31) which has a much higher galactose content than the water-soluble galactomannan from the same source. While evidence from methylation data is incomplete it seems likely that all terminal units are supplied by D-galactose, the remainder of the molecule containing D-galactose linked through C₁ and C₃ and D-mannose residues linked through C₁, C₂, and C₃.

Table 3.

Source	$[\alpha]_D$	Terminal Unit	Chain Unit	Branch Unit	Man/ Gal	Ref.
Carob (locust) bean (<u>Ceratonia siliqua L.</u>)	+9°	Galpl- (1)	-4Manpl- (2-4)	-4Manpl- ⁶ (1)	3-5	37
Kentucky coffee bean (<u>Gymnocladus dioica</u>)	+29°	Galpl- (1) Manpl- (+)	-4Manpl- (3)	-4Manpl- ⁶ (1)	4	38
Green Palmyra palm nut (<u>Borassus flabellifer</u>)	+85°	Galpl- (1)	-4Manpl- (1.4)	-4Manpl- ⁶ (0.95)	2.4	34
Guar gum (<u>Cyamopsis tetragonolobus</u>)	+60°	Galpl- (1)	-4Manpl- (1)	-4Manpl- ⁶ (1)	2	36
Soy bean hulls	+65°	Galpl- (2)	-4Manpl- (1)	-4Manpl- ⁶ (2)	1.5	32
Clover (<u>Trifolium pratense</u>)	+78°	Galpl- (7)	-4Manpl- (2)	-4Manpl- ⁶ (7)	1.3	29
Fenugreek (<u>Trigonella foenum graecum</u>)	+70°	Galpl- (5)	-4Manpl- (1)	-4Manpl- ⁶ (5)	1.0-1.2	39
Lucerne (<u>Medicago sativa L.</u>)	+118°	Galpl- (4)	-4Manpl- (1)	-4Manpl- ⁶ (4)	1.25	29
Coconut (<u>Cocos nucifera</u>)	-85°	Manpl- (1) Galpl- (1)	-4Manpl- (11) -4Galpl- (3)	-4Galpl- ⁶ (?) (2)	2	30
Lucerne (alkali extract)	+89°	Galpl-	-3Galpl- +unident- fied tri- -O-methyl hexose	⁶ Manpl- 2 ¹ +unident- fied di-O- methyl hexose	0.5	31

Glucomannans.

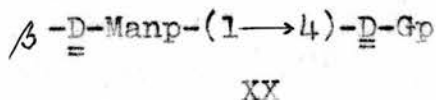
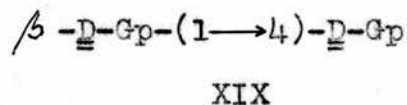
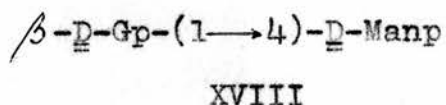
Less intense study has been devoted to plant glucomannans (excluding those from wood tissues) than to the galactomannans, although their distribution in nature is equally wide. The problem of structural elucidation in the group is generally complicated by difficulties in purification and in some cases by loss of solubility of the isolated glucomannans.

Mucilaginous glucomannans are found in the tubers of various species of Amorphophallus, in the seeds of some Iris species, in orchid and lily bulbs and in the leaves of Aloe vera but structural studies have been limited to only a few cases.

As with the other plant mannans already considered the general structural feature appears to be the familiar (1→4) linkage between β -D-mannopyranose units; the glucomannans differ however from the groups already discussed in the incorporation of β (1→4) linked D-glucopyranose residues into the linear backbone of the molecule. In some cases the presence of a small percentage of D-galactose in the polysaccharide has been noted(40).

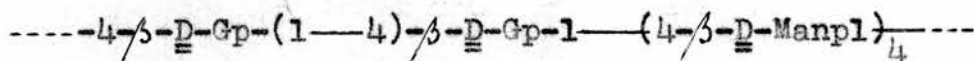
Smith and co-workers(41) have isolated a mixture of glucomannan and glucan from Iles mannan meal (the crude product from the macerated, dried tubers of Amorphophallus oncophyllus and A. variabilis) by extraction with 50% aqueous sodium

xylenesulphonate and 30% aqueous potassium hydroxide and have resolved the mixture by fractionation of the copper complexes and by fractionatal precipitation of the methylated polysaccharides. Hydrolysis of the methylated glucomannan yielded only the 2,3,6-tri-O-methyl ethers of D-mannose and D-glucose (2:1 respectively) indicating a (1→4) linked linear chain of considerable D.P. The low negative rotation of the free polysaccharide and its methylated derivative ($[\alpha]_D = -21^\circ$ and -41° respectively) suggest most of the glycosidic bonds to be of the β -configuration. Further support for such a structure has been obtained by the identification of oligosaccharides produced on deacetylation of the products of graded acetolysis of the glucomannan(42): these include 4-O- β -D-glucopyranosyl-D-mannose (XVIII), 4-O- β -D-glucopyranosyl-D-glucose (XIX) and 4-O- β -D-mannopyranosyl-D-glucose (XX).



The failure to isolate oligosaccharides containing more than two hexose residues does not provide evidence for the distribution of D-glucose and D-mannose units in the molecule and does not permit the postulation of a detailed structural

pattern but the ratio of mannose to glucose (2:1) demands the existence of contiguous mannose residues as in the formulation



XXI

The structure of Konjak glucomannan present in the bulbs (43) (and in leaves and stalk(44)) of Amorphophallus konjak is basically similar to that of Iles glucomannan but the molecule which contains a rather higher proportion of D-glucose, is claimed to be slightly branched(43). Methylation data and evidence from periodate oxidation studies(43) indicate one possible structure for the repeating unit to consist of a linear chain of about 29hexose units, 12 of D-glucose and 17 of D-mannose joined by (1→4) glycosidic bonds; to C₃ of two of the D-glucose and one of the D-mannose units are attached single unit side chains, two of them constituted by D-glucose and one by D-mannose. Partial acetolysis followed by deacetylation of the products(45) and enzymic hydrolysis(46) have produced various oligosaccharide fragments but in the absence of structural data concerning these, the evidence cannot be utilised in ascertaining the structure of the polysaccharide.

The endosperms of two species of Iris, I. ochroleuca and I. sibirica contain glucomannans, which may be extracted with 10% potassium hydroxide and purified through their copper complexes(40).

(Early workers claim that three other Iris species I. germanica, I. pseudocorus and I. foetidissima contain arabomannans(47) but in the absence of efficient means of identifying the monosaccharides at that time, the validity of the claims is to be doubted).

The two polysaccharides appear to be very similar. Both contain equal amounts of D-glucose and D-mannose units (97%) with small amounts of D-galactose units (3%) also present. The isolation of 2,3,4,6 tetra-O-methyl D-galactose (with traces of the tetra-O-methyl ethers of D-mannose and D-glucose and the tentative identification of the di-O-methyl ethers as 2,3-di-O-methyl D-glucose and 2,3-di-O-methyl D-mannose suggest a structure where the D-galactose residues are attached, together with some D-glucose and D-mannose residues, either as terminal units or as side chains linked to C₆ of D-glucose and D-mannose units in the main chain.

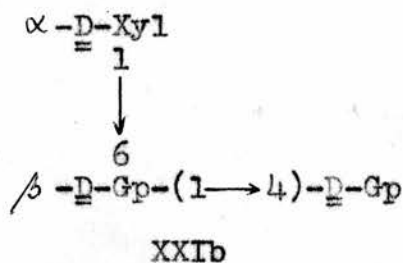
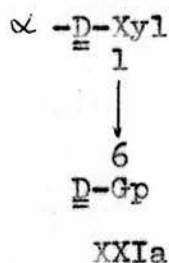
Glucomannans have also been extracted from the bulbs of three Lilium species, L. umbellatum, L. henryii and L. candidum (48). All possess a mannose : glucose ratio of approximately 2:1 and have relatively low specific rotations indicative of β -linked polymers ($-25 \pm 5^\circ$). Structural studies(48) on L. umbellatum and L. henryii glucomannans have shown that both polysaccharides are terminated by D-glucose residues at the non-reducing end of the chain while the former contains a small

proportion of D-glucose units linked through C₁, C₃ and C₆ as indicated by the isolation of 2,4-di-O-methyl D-glucose in the hydrolysates of the methylated polysaccharide. L. henryii glucomannan may also contain a small proportion of branch points.

Glucomannans of Wood.

Glucomannans belonging to a structural group closely similar to that of the mucilaginous glucomannans of seed endosperms have been found in coniferous woods (ca. 10% of the extractive-free wood) and to a lesser extent (up to 2% of the extractive-free wood) in deciduous woods. Purification is often rendered somewhat difficult due to large amounts of accompanying hemicelluloses of the xylan and galactan groups. Contamination from low molecular weight β -glucan (probably degraded cellulose) has also been encountered(49,50) while the persistence of xylose in certain glucomannan preparations has been accounted for in terms of binding of glucomannan and xylan components by incompletely removed lignin(51). (It has recently been shown that the lignin-polysaccharide complex may not be responsible for traces of D-xylose in every case by the isolation of a di- and trisaccharide containing both D-xylose and D-glucose residues (XXIa and XXIb) (62) from an enzymic

hydrolysate of a jack pine glucomannan



The isolation of XXIa and XXIb thus represents the first unequivocal evidence for the existence of polysaccharides containing D-xylose chemically linked to D-glucose units e.g. a xyloglucan or xyloglucomannan).

Despite claims to the contrary(52,53) it is unlikely that a homopolymeric mannan comparable to the type found in ivory nut has ever been isolated from any wood. (The possibility however, of heterogeneous mixture of mannans and glucomannans, or of closely related polymers containing differing proportions of constituent D-glucose and D-mannose units cannot yet be dismissed).

Evidence for the existence of chemically linked D-glucose and D-mannose units in wood polysaccharides was first provided by the isolation of 4-O- β -D-glucopyranosyl-D-mannose (XVIII) and a mannosyl glucose (probably 4-O- β -D-mannopyranosyl-D-glucose (XX)) from a partial hydrolysate of slash pine α -cellulose (54,55). That the linkage is truly integral in a hemicellulosic component of the wood has been subsequently demonstrated by the isolation of XVIII and XX (together with

higher oligosaccharides containing both D-glucose and D-mannose residues after partial hydrolysis (by the agency of both acid and enzymic systems) of hemicellulosic fractions from several wood species(56-62).

Data obtained by the methylation, partial hydrolysis and periodate oxidation techniques on glucomannans from various gymnosperm species including representatives of the genera Ginkgo, Larix, Picea, Pinus, Thuja and Tsuga indicate a general structure of linear (or slightly branched) chains of (1→4) linked β -D-mannose and β -D-glucose units in the ratio 2.5-4.0:1 (with one exception) (63).

The number-average degrees of polymerisation of the isolated glucomannans are relatively low (ca. 70-130) but it is unlikely that these values are representative of the polymers in situ, since in all cases depolymerisation may have occurred during delignification procedures with acid chlorite(64).

The problem of determining the fine structure of the wood glucomannans is complicated by difficulty in ascertaining homogeneity of the polysaccharide material. Although fractionation of glucomannans by various procedures leads to fractions of similar constitution(65-68), Aspinal and co-workers report two cases where glucomannans isolated by alkaline extraction have been accompanied by a β -glucan which is most probably a degraded cellulose(49,50). Traces of such a contaminant would clearly invalidate the interpretation of

cellobiose in partial hydrolysates as indicative of the presence of such adjacent D-glucose units in some glucomannans.

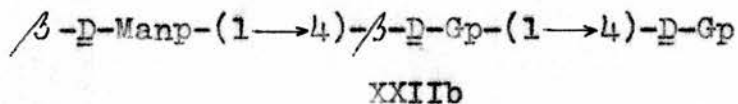
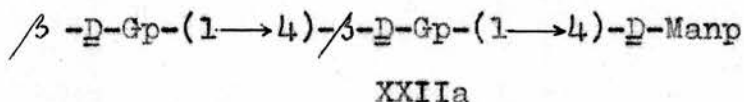
Similarly in this respect, it has yet to be conclusively demonstrated that the D-galactose present in the majority of glucomannan preparations so far examined is derived from a homogeneous tri-heteropolymer (a galactoglucomannan) or from a contaminating galactomannan of a structural group similar to that found in the leguminous endosperms(36).

A comparison of available data suffices to show that the differences in the structural chemistry of the softwood glucomannans may be assigned to four main categories; (a) the distribution of D-glucose and D-mannose in the molecular chain, (b) the identity of the non-reducing terminal unit, (c) the presence or absence of D-galactose in the molecule and (d) the presence or absence of branching. It seems convenient then to examine the structural studies carried out so far in terms of these headings.

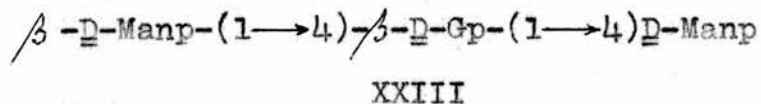
(a) The distribution of D-glucose and D-mannose in wood glucomannans.

To date the sole approach to this problem has been the examination and identification of fragments produced on partial hydrolysis of the polysaccharides. Thus the low yields of cellobiose ($4\text{-O-}\beta\text{-D-glucopyranosyl-D-glucose}$) (XIX) isolated

from partial hydrolysates of unbleached spruce pulp(59), white spruce(66), eastern white pine(61), Sitka spruce(49), western hemlock(58) and western red cedar(57) are considered to indicate the presence of contiguous glucose residues in the molecule; as stated above this interpretation is open to doubt where structural homogeneity has not been ascertained. However the tentative identification of trisaccharides (XXIIa and b containing the cellobiose unit linked directly to a D-mannose residue, from a partial hydrolysate of Norway spruce glucomannan(69) and from an enzymic hydrolysate of jack pine glucomannan(62) indicate unambiguously that blocks of at least two glucose units must occur in these polysaccharides.



That isolated glucose residues occur also in some glucomannan chains is suggested by the failure to isolate cellobiose from the products of partial hydrolysis of loblolly pine glucomannan(56) and by the tentative identification of the trisaccharide (XXIII).



from a similar hydrolysate of Norway spruce glucomannan(69) The relative molar proportions of D-mannose and D-glucose (3-4:1) common in the softwood glucomannans requires the existence of blocks of 3 or 4 adjacent mannose residues and this has been confirmed by the isolation of a mannotriose(56-59,61,62,66) and a mannotetraose(61) belonging to the homologous series represented by structure X.

(b) The non-reducing terminal group.

Evidence regarding the nature of the non-reducing residue terminating the glucomannan chain (other than D-galactose) may be obtained by identification of the 2,3,4,6 tetra-O-methyl ethers of D-glucose and / or D-mannose among the products of hydrolysis of the methylated polysaccharides.

On this basis it has been shown that whereas glucomannans from western hemlock(58), Scots pine(70), eastern white pine(61) contained both D-glucose and D-mannose non-reducing terminal units, jack pine(71), tamarack(72), western red cedar(57) glucomannans possessed only D-glucose terminal units and those from Sitka spruce(49,63), Norway spruce(68), loblolly pine(73), Ginkgo biloba(74) and European larch(50) were terminated at the non-reducing end of the chain by D-mannose units only.

To lay excessive emphasis on such results seems unjustified however, since, in view of (a) the difficulty in chromatographic separation of the tetra-O-methyl ethers of D-mannose and D-

glucose and (b) the possibility of modification of the original molecule by depolymerisation during the extraction procedure.

(c) Significance of D-galactose in the molecule.

The presence of significant amounts of D-galactose (2-4%) has been noted in all softwood glucomannans so far examined (with two possible exceptions(49,65)). Re-examination of a glucomannan from white spruce(66) previously reported devoid of D-galactose, has shown that D-galactose is present to the extent of 3% in the wood. In the majority of reported methylation data, the only D-galactose ether has been the 2,3,4,6 tetra-O-methyl D-galactose(63,56,50) but in two examinations small amounts of di-O-methyl D-galactose have been detected(57,71). The tetra-O-methyl galactose isolated by Dutton and Hunt from a hydrolysate of methylated Sitka spruce glucomannan was not detected after dialysis of a similar methylated fraction and these workers suggest that the ether was derived from a short-chain dialysable polysaccharide(63). Meier has recently isolated a galactosyl mannose (XV), and a galactosyl manno-biose (XVI) from a Norway spruce glucomannan by partial acid hydrolysis(69) and suggests the presence of a mixture of glucomannan and either a galactomannan, belonging to a structural group akin to the mucilage-forming galactomannans, or a galactoglucomannan. Galactoglucomannan

contaminants have been proposed by other workers (56,57) to account for the traces of D-galactose encountered in hydrolysates of glucomannans. Bishop and Cooper(71) have shown however that galactose could not be eliminated from jack pine glucomannan by repeated fractionation and that the polysaccharide was electrophoretically homogeneous, hence that the galactose units were integral in the macromolecule.

Recent work by Timell(75) describes the isolation of galactoglucomannans from several coniferous woods; these had D-galactose contents of 15-30% and were shown by boundary electrophoresis and ultracentrifugation to be structurally homogeneous. (From one species, eastern hemlock, a glucomannan distinct from the above and with a much lower galactose content(4.8%) was isolated.) Similar galactoglucomannans have been reported as constituents of a mixture of slash pine and longleaf pine by Hamilton, Partlow and Thompson(76).

In the light of these findings it has been suggested that those "glucomannans" so far isolated are indeed true triheteropolymers of galactose, glucose and mannose and are members of a closely related series of galactoglucomannans differing only in relative galactose content and possibly in average molecular weight and degree of branching(75).

(d) Branching in glucomannans from wood.

The majority of softwood glucomannans are reported to be essentially linear molecules although evidence of branching has been suggested in two Norway spruce glucomannans(67-69) and in the glucomannan from Scots pine(51,70) on the basis of a comparison of number average molecular weights (as determined by osmotic pressure measurements on nitrates) with average chain lengths calculated from methylation data.

The resistance to periodate oxidation of a small percentage of glucose units (with traces of mannose) in glucomannans from western hemlock(65), Norway spruce(68), Scots pine(70) and unbleached Spruce pulp(59) has been interpreted as indicative of branching through C₂ or C₃ of the glucose units. Cooper and Bishop(71) have pointed out that certain glucose residues may be relatively resistant to attack by the periodate ion if held in the trans position in a rigid conformation such as might obtain in the glucomannan chain. This would account for the presence of the predominance of intact glucose units in periodate-oxidised glucomannans.

The small amounts of di-O-methyl hexoses almost invariably found in hydrolysates of methylated glucomannans may be structurally significant but the possibility that they originate from under- or de-methylation cannot be ignored.

Several trisaccharides claimed to contain possible branch points were isolated from the products of partial acid hydrolysis

of a glucomannan from unbleached spruce pulp(59) but incomplete identification obviates their use in structural elucidation.

Hardwood Glucomannans.

Timell(77) has recently shown that glucomannans, similar in general structure to those from gymnosperms, may also be extracted from several typical angiosperms (hardwoods) including maple, birch, beech, elm and aspen. The average yield from the extractive-free wood is lower (of the order of 2%) and the ratio of mannose to glucose in the polysaccharides is also somewhat lower (ca. 2:1).

Partial hydrolysis of a glucomannan extracted from red maple(78) has produced a series of oligosaccharides similar to that obtained from softwood species while methylation of the polysaccharide and subsequent hydrolysis gives di-O-methyl hexoses, 2,3,6 tri-O-methyl ethers of D-glucose and D-mannose and 2,3,4,6 tetra-O-methyl D-glucose.

A glucomannan has also been isolated from aspenwood in 2% yield(79). 2,3,6 Tri-O-methyl D-mannose and 2,3,6 tri-O-methyl D-glucose (2:1) have been identified among the hydrolysis products of the methylated polysaccharide.

Although few woods have been examined as yet, it is of interest to note the general absence of D-galactose in hydrolysates of the hardwood glucomannans.

Recently Timell has examined the polysaccharides from a representative of the third member of the Pteropsida, Filicineae (ferns) and has isolated a glucomannan (6% of the extractive-free wood) similar to those from the hardwoods(80). Since the xylan content is correspondingly lower than in the case of the hardwoods, it would appear that the distribution of the hemicelluloses in the phylogenetically much older Filicineae is more or less "intermediate" between that of the Gymnospermae and Angiospermae.

European larch (Larix decidua) glucomannan.

In common with the woods of other coniferous species European larch wood contains xylan, glucomannan(50) and arabinogalactan ("ε-galactan") (81) as the major hemicellulosic components.

Glucomannan-rich hemicellulose fractions have been obtained previously (50a) by extraction of the chlorite holocellulose with 10% aqueous sodium hydroxide under nitrogen and a virtually xylan-free glucomannan isolated as the acetate by fractional precipitation of the acetylated polysaccharides from chloroform solution(50b). Hydrolysis of the glucomannan obtained on saponification of the acetate gave D-mannose, D-glucose and D-galactose in the molar proportions 61:31:7.5. Hydrolysis of the methylated polysaccharide prepared by simultaneous deacetylation and methylation of the acetate afforded 2,3,4,6

tetra-O-methyl D-mannose, 2,3,4,6 tetra-O-methyl D-galactose,
2,3,6-tri-O-methyl D-mannose, 2,3,6-tri-O-methyl D-glucose
and a mixture of di-O-methyl D-mannoses (tentatively identified
as the 2,6- and 2,3-di-O-methyl ethers) in the approximate
molar proportions of 1:5:66:30:8.

On this evidence a structure may be suggested for the
glucomannan in which β -D-glucose and β -D-mannose units are
linked through C₁ and C₄ in the ratio (2-2.5 : 1); D-galactose
appears to be present only as non-reducing terminal units and
as such can only arise from a galactomannan or
galactoglucomannan. Branching is confined to mannose residues
(assuming the valid structural significance of the di-O-methyl
hexoses) and the linkage must be through C₃ or C₆ of these units.

Tamarack (Larix laricina) glucomannan.

Comparison of the above data may be made with that for a
glucomannan from a second Larix species recently isolated by
Kociman and Adams (72). Homogeneity of the polysaccharide
was here ascertained under conditions of free boundary
electrophoresis and sedimentation in the ultracentrifuge.
Analysis showed a composition of D-mannose, D-glucose and
D-galactose in the molar proportions of 70:25:3. Hydrolysis
of the methylated polysaccharide gave 2,3,4,6 tetra-O-methyl
D-glucose, 2,3,4,6 tetra-O-methyl-D-galactose, 2,3,6 tri-O-
methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose and 2,3 di-

O-methyl D-glucose and 2,3,di-O-methyl-D-mannose, (1.2:2.2:68.8:24.1:1.7:1.6). Periodate oxidation and estimations of the non-reducing and reducing end-groups indicated an average chain length of approximately 35 units.

The authors prefer to interpret their results on the basis of a mixture of molecular species; thus two-thirds of the linear chains of (1→4) linked β -D-mannose and β -D-glucose units are terminated at the non-reducing ends by D-galactose and the remainder by D-glucose. The di-O-methyl hexoses are considered to be products of demethylation or of incomplete methylation, rather than representing branch-points at C₆ of the hexose units in the chain.

Thus the main difference in structural features regardless of the interpretation of the experimental data is the presence of Larix decidua of non-reducing terminal units of D-mannose as opposed to those of D-glucose occupying the same position in Larix laricina. While the D-galactose content is somewhat lower in the case of tamarack, the mannose : glucose proportions are very similar for both polysaccharides.

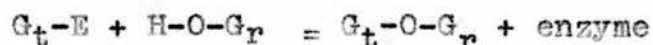
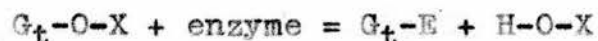
In general both polysaccharides appear to compare with glucomannans already isolated from other softwood species.

The Biosynthesis of Mannans.

The biosynthetic route to the mannans is still relatively obscure; the nucleotide guanosine diphosphate mannose has been found in baker's yeast and Cabib and Leloir(82) have postulated that this may be present as a component of the "glycoside pool" described by Neish(83), acting as a glycosyl donor for the synthesis of the mannan found in the cell-wall of yeast. Neish(84) has suggested that the hemicellulose mannan precursor may also be guanosine diphosphate mannose, with the biosynthetic pathway composed of a series of transglycosylation reactions similar to those occurring in the synthesis of polysaccharides of the starch and dextran classes(85).



or more completely



where G_t-O-X a sugar residue (G_t-O-) substituted at the anomeric centre by the aglycon X and G_r-O-H is a carbohydrate receptor molecule, the products being a higher saccharide (G_t-O-G_r) and a hydroxy compound ($X-O-H$).

The biosynthesis of mannose-containing polysaccharides by Torula utilis yeast has been studied by Sowden et al.(11,86). The yeast, grown on D-glucose-1-¹⁴C as the sole source of carbon, yielded three distinct polysaccharides; a water-soluble mannan devoid of radioactivity, a second mannan, soluble in 6% aqueous sodium hydroxide and composed of polymerised D-mannose-1-¹⁴C

and a third polysaccharide (soluble in 30% potassium hydroxide) which contained both radioactive D-glucose and D-mannose, the relative ratios of which were unchanged by fractional precipitation. The D-mannose from the second mannan and the D-mannose and D-glucose from the glucomannan possessed identical specific radioactivities; further all radioactivity was located in C₁ of the hexose residues.

Thus it appears that the mannans of T. utilis (which, it should be noted, differ in their mode of retention in the cell-wall) may be synthesised by several routes. While the pathway to the non-radioactive mannan must involve a drastic rearrangement of the carbon-chain of the D-glucose substrate, the radioactive mannan and glucomannan probably arise from condensation of D-mannose residues produced by the enzymically induced isomerisation of D-glucose with the concurrent incorporation of D-glucose-1-¹⁴C in the case of the glucomannan.

Measurement of the distribution of radioactivity in D-mannose, D-glucuronic acid and D-xylose residues produced on hydrolysis of the mannan from Cryptococcus laurentii grown on C¹⁴ sugar media has shown that D-mannose and D-glucuronic acid are formed from the hexose substrates without appreciable breakdown of the hexose skeleton and that D-xylose is formed mainly by a process involving loss of C₆.(104)

Although the identification of the methylated monosaccharides obtained on hydrolysis of methylated glucomannans, affords valuable information regarding the mode of linkage of monosaccharide residues in the polysaccharides and the location of non-reducing terminal and branching units, no evidence may be derived from such studies to assess the order of linkage of the residues. An approach to this problem has been made by the isolation and characterisation of oligosaccharide fragments produced on graded acetolysis of larch glucomannan acetate described in Section 1.

In a partial depolymerisation such as acetolysis however, the hydrolytic attack on glycosidic links is seldom purely random and variations in the sensitivity to acid hydrolysis of such links is normally encountered (D-mannosidic links are claimed to be cleaved more readily than the corresponding anomeric D-glucosidic links, for example(105)); it may be anticipated also that some oligosaccharide fragments may suffer further hydrolysis after formation in the hydrolytic medium. Thus no quantitative assessment of the distribution of D-glucose and D-mannose residues in glucomannans is possible and only dominant features may be revealed. Hence it is desirable to develop methods whereby one or other of the residues (D-glucose or D-mannose) may be selectively removed while leaving the other residues and linkages intact. Various approaches have been made to this problem and the

results of two such procedures investigated are reported in Sections II and III.

GENERAL METHODS OF INVESTIGATION.

General Methods of Investigation

Paper chromatography was carried out on Whatman No.1 filter paper using the following solvent systems (v/v):-

- (A) Ethyl acetate : pyridine : water (10:4:3)
- (B) Ethyl acetate : acetic acid : water (9:2:2)
- (C) Ethyl acetate : acetic acid : formic acid : water (18:3:1:4)
- (D) Butan-1-ol : ethanol : water (1:1:1)
- (E) Butan-1-ol : ethanol : water (4:1:5, upper layer)
- (F) Butan-2-one : acetic acid : water saturated with boric acid (9:1:2)

Except where otherwise stated chromatograms were run with standard sugars for comparison, air-dried and sprayed with the appropriate reagent.

Chromatographic spray reagents

- (a) Aniline hydrogen phthalate(87) (reducing sugars)

Unless otherwise stated chromatograms were sprayed with a solution of aniline (0.93g.) and phthalic acid (1.66g.) in water-saturated butan-1-ol (100ml.) and developed at 120° for 2-3 minutes.

- (b) Silver nitrate reagent(88) (non-reducing sugars)

This reagent was employed to detect glycitols and sugar glycosides. The air-dried chromatograms were dipped in the

silver nitrate reagent (saturated aqueous silver nitrate (1ml.) in acetone (20ml.)), dried (7-10mins.) and sprayed with ethanolic sodium hydroxide (5g. sodium hydroxide in 15ml. water diluted to 250ml. with ethanol.) Reducing-sugars gave brown or black spots appearing rapidly (1-2mins.), non-reducing-sugars brown or black spots which appeared more slowly (2-3mins.). The chromatograms were preserved by washing with 10% aqueous sodium thiosulphate, washing with water and drying at 120°.

Urea oxalate(89) (hexuloses)

Fructose and fructose-containing oligosaccharides were revealed by spraying the chromatograms with a saturated solution of urea oxalate and developing at 120-140° for 2-3 minutes. Free or combined fructose gave dark, blue-grey spots.

Triphenyltetrazolium chloride reagent(88) (2-O-substituted reducing sugars).

Chromatograms were sprayed with a 0.5% solution of the reagent in chloroform, dried and then sprayed with ethanolic sodium hydroxide solution and heated for a few seconds at 80°. Carmine spots developed slowly at room temperature where reducing sugars were present except where there was a substituent on C₂ adjacent to the free reducing group. Excess reagent was removed by washing with water.

Periodate/permanganate reagent(90)

Air-dried chromatograms were sprayed with a mixture of 4 parts 2% aqueous sodium metaperiodate with 1 part potassium permanganate in 2% sodium carbonate. On standing at room temperature for ca. 15 minutes, yellow spots on a pink background were obtained for both reducing and non-reducing sugars.

The following abbreviations have been used to describe the mobility of sugars on paper chromatograms:-

$$R_x = \frac{\text{Distance travelled by sugar}}{\text{Distance travelled by sugar X}}$$

$$R_f = \frac{\text{Distance travelled by sugar}}{\text{Distance travelled by solvent front.}}$$

The abbreviation M_G , quoted in ionophoretic examinations, refers to the ratio

$$\frac{\text{Distance travelled by sugar}}{\text{Distance travelled by D-glucose}}$$

correction being made for electro-endosmotic flow by incorporating a standard of 2,3,4,6-tetra-O-methyl-D-glucopyranose.

Paper ionophoresis(91,92) was carried out on Whatman NO.1 paper in borate buffer at pH 10. A potential of 750 volts was applied over 4-5 hours and the dried papers were sprayed either with saturated aniline oxalate solution containing 5% acetic acid or with the periodate-permanganate reagent.

Whatman 3MM paper sheets (a thick paper with medium flow rate used for chromatographic fractionation of sugar mixtures) were first extracted with methanol in a Soxhlet extractor.

Cellulose columns(93,94) were packed dry and washed with water and butan-1-ol before applying the solvent to be used for the separation of the sugars.

Sugar mixtures were absorbed on powdered cellulose from a solution in the irrigant to be used and the material packed as a thin layer at the top of the column. A small volume (ca.20-30ml.) of the eluant was allowed to soak in till the level of the cellulose was reached and finally a constant head reservoir containing the irrigant was inverted on the top of the column.

The eluate was collected on an automatic fraction collector and a sample from every fourth or^r fifth tube examined chromatographically. Those fractions containing the same sugars or mixtures of sugars were combined and evaporated to dryness. The weights of fractions were recorded after purification of the residues by dissolution in ethanol water, filtration and evaporation to dryness. The fractions were finally dried in vacuo over phosphorus pentoxide.

Absorption chromatography on charcoal-Celite mixtures (1:1) was employed to fractionate mixtures of monosaccharides and oligosaccharides (95,96). B.D.H. activated charcoal (1 part) was mixed with Celite 545 (1 part) and the mixture washed with

concentrated hydrochloric acid, followed by a large volume of water until the washings were chloride free. The mixture was packed as a water slurry into columns and allowed to settle under gravity before being washed thoroughly with water. The sugar mixtures (as 10% solutions in water) were applied to the top of the column and allowed to soak into the absorbent before step-wise or gradient elution with water and water : ethanol mixtures. Fractions were collected automatically in small-scale fractionations and were examined by the procedures used in cellulose column chromatography above.

Evaporations were carried out under reduced pressure at 40°.

Demethylations were carried out by heating the sugar (5mg.) with hydriodic acid (1ml, 96%) in a sealed tube at 100° for 5-10 minutes. The solution was cooled, diluted with water (10ml.) and neutralised with silver carbonate. Silver ions were removed by treatment with Amberlite IR 120(H) resin, and the filtrates concentrated and examined chromatographically for sugars.

Estimation of methoxyl content was made by a semi-micro modification of the Zeisel procedure(97).

Acetyl content was determined by the Weissenberger method(98)

Optical rotations were observed at 18° ± 2°.

Melting-points were usually determined using a Kofler hot-stage microscope and are uncorrected.

Small-scale hydrolyses were carried out by the following methods:-

- (a) N Sulphuric acid hydrolyses: samples (5-10mg. for polysaccharides, 1-3mg. for oligosaccharides) were heated with N acid (1-2ml.) at 100° for an appropriate period. (6 hours for polysaccharides, 1-3 hours for oligosaccharides). The solutions were neutralised with barium carbonate, filtered, the barium ions removed with Amberlite IR 120(H) resin and the solutions concentrated. In partial hydrolyses of oligosaccharides with 0.1 N sulphuric acid the barium carbonate neutralisation was omitted and hydrolysates deionised directly with Amberlite IR 45(OH) resin or IR 4B(OH) resins.
- (b) Formic acid hydrolyses: samples (10-20mg.) were heated with 95% formic acid (2ml.) for 6 hours at 100°; the formic acid was removed by repeated distillation under reduced pressure and the resulting formyl esters hydrolysed with N sulphuric acid (2ml.) at 100° for 1-2 hours.
- (c) Hydrochloric acid hydrolyses: samples (10-20mg.) were heated with acid (2ml.) at 100° for 6 hours; the hydrolysates were neutralised with silver carbonate, filtered, treated with Amberlite IR 120(H) resin to remove silver ions and evaporated to dryness. The residue was then extracted with

methanol, filtered and concentrated.

- (d) 72% sulphuric acid hydrolyses: samples (10-20mg.) were allowed to stand for 48 hours in acid (2ml.) at room temperature; the solutions were then diluted to 50ml. and heated for 4 hours at 100°. The hydrolysates were then treated as in (a) above.

Estimations of sugars were carried out by determination of the formic acid released on periodate oxidation of sugars.(99).

Consumption of periodate ion in oxidation of sugars with sodium metaperiodate followed spectrophotometrically at 222.5 m μ (100).

Acetylation of Polysaccharides

Freeze-dried polysaccharides (10g.) were dispersed in formamide (250ml.) at room temperature for 10 days with vigorous agitation in the presence of glass beads. Purified pyridine (250ml.) was added slowly over 2 hours with stirring followed by acetic anhydride (200ml.) over a further period of 1 hour. The reaction was allowed to proceed for 5-7 days with continuous agitation. The reaction mixture was poured into iced water (5l.) and the flocculent brown precipitate was centrifuged off and drained on sintered glass. The solid was extracted with chloroform and the solution dried over anhydrous sodium sulphate before being concentrated to a small volume (100ml.). The acetates were

precipitated by pouring dropwise into light petroleum (b.p. 60-80°, 5 volumes.) with vigorous agitation. The buff product was finally dried over phosphorus pentoxide and paraffin wax in vacuo.

Tritylation of Polysaccharides

The polysaccharide was initially activated by treatment with pyridine-water azeotrope (pyridine : water = 3:2 w/w) so that the solid : liquid ratio was approximately 1:15 (w/v). The mixture was mechanically stirred and heated so that the azeotrope (b.p. 93°) distilled. As the azeotrope was removed, the volume of the mixture was maintained constant by addition of purified pyridine (b.p. 115°). Loss of water by distillation was indicated by a gradual rise in the temperature of the boiling-point of the distillate. The last traces of water were removed by addition of anhydrous pyridine when the boiling-point reached 114-115°.

Stirring was continued and the temperature of the mixture allowed to fall to 100° when triphenylmethyl chloride (10 moles) was added. Stirring at 100° was maintained for a further 7 hours when the mixture was allowed to cool to room temperature. The tritylated product was precipitated by pouring dropwise in 4 volumes of stirred methanol. The suspension was stirred for 2-3 hours when the product was separated at the centrifuge, washed several times with methanol to remove traces of triphenylcarbinol and finally with ether to remove methanol.

Ether was removed in air at room temperature followed by drying in vacuo at 50-60°. [In some cases it was found necessary to remove small amounts of occluded triphenylcarbinol or triphenylmethyl chloride by dissolution of the product in chloroform and reprecipitation in methanol.]

Estimation of Triphenylmethoxyl Content(101)

Tritylated polysaccharide (50-100mg.) was treated with concentrated sulphuric acid (4-5ml.) and gently agitated until dissolved. Water (40-50ml.) was added slowly with swirling and the precipitated triphenylcarbinol was filtered into a preweighed porosity 4 sintered glass crucible, for drying and reweighing. Due to the presence of some foreign material in most products it was found advisable to treat the weighed precipitate with methanol and re-weigh in order to estimate the methanol-soluble material (triphenylcarbinol).

Detritylation of Tritylated Polysaccharides.

The trityl ether (1.0g.) was dissolved in anhydrous chloroform (ethanol-free, 30ml.) and chloroform saturated with dry hydrogen chloride (60ml.) was added at once with vigorous shaking. The detritylated polysaccharide was precipitated almost immediately and after standing for 15 minutes was separated from the supernatant by centrifugation. [Addition of a small amount of anhydrous acetone (20ml.) rendered the polysaccharide less flocculent and improved sedimentation]. The residue was washed

with chloroform/acetone, acetone (except where otherwise stated) ether and finally air-dried.

Tosylation of Tritylated Polysaccharides.

Dry tritylated polysaccharide (10g.) was dissolved by warming in dry pyridine (120ml.). p-Toluenesulphonyl chloride (57g., 10 molar excess per hydroxyl group) was then dissolved in dry pyridine (30ml.) and mixed with the above solution. The mixture was shaken vigorously and stored at 35° for 7 days.

The product was precipitated from the reaction mixture by adding the solution dropwise to stirred methanol (1 l.) and the suspension was stirred continuously for 3-4 hours. The precipitate was collected by centrifugation and washed with methanol (3x100ml.) and finally with ether. [A solution of sodium chloride in methanol was added when colloidal solutions were encountered]. The product was dried in air and at 60° in vacuo.

Estimations of sulphur content of sulphonylated materials were carried out by determination of the oxides of sulphur (as sulphuric acid) produced by catalytic combustion of the material.(102)

Purification of organic solvents for column chromatography and for reactions where specified was effected by the methods described in the literature(103).

SECTION I.

Graded Acetolysis of a Glucomannan

from

European Larch (*Larix decidua*).

SECTION I

Experimental

Isolation of the Hemicelluloses.

Extraction of Lipids, Colouring matter, etc.

450g. batches of Larch saw-dust were continuously extracted in a Soxhlet extractor with 3l. of a benzene-ethanol azeotrope (2:1 v/v) over a period of 36 hours. The extractive-free wood was air-dried at room temperature for 15 hours prior to delignification.

Average yield = 420g.

Hydrolysis of the extractive-free wood with 72% sulphuric acid gave glucose (++++), xylose (+++), mannose (++) , galactose (+), arabinose (+) and aldobiuronic acids (tr.).

Delignification of the Extractive-free Wood.

Delignification was effected by the acid chlorite method of Wise(106). 50g. portions of the extractive-free sawdust were heated for 1 hour at 60-70° with a mixture of 250g. sodium chlorite, 250ml. glacial acetic acid and 2500ml. water and allowed to stand at room temperature for a further 24 hours. The creamy-white product was separated from the delignification liquors by filtration under reduced pressure, washed with cold water, then with acetone and finally air-dried at 40°.

Average yield = 37-38g. (74-76% of the extractive-free wood).

Hydrolysis of a sample of the holocellulose with 72% sulphuric acid gave glucose (++++), xylose (++++), mannose (+++), galactose (+), arabinose (+), aldobiouronic acids (tr.) and traces of monosaccharides with chromatographic mobility (solvent A) greater than that of xylose ($R_{xy1} = 1.39$ - major component).

A sample of the pale yellow chlorite liquor was deionised by dialysis through cellophane sheeting and concentrated to a small volume. The polysaccharides precipitated on addition of 3 vols. of acetone to the solution, were washed with acetone and ether and finally air-dried. Hydrolysis of the white powder, $[\alpha]_D = +10^\circ$, with N sulphuric acid for 12 hours at 100°C gave galactose (++++), arabinose (++) , xylose (+) with small amounts of mannose, glucose, aldobiouronic acids and a component with a chromatographic mobility ($R_{xy1} = 1.50$ in solvent A) corresponding to that of rhamnose.

Alkaline Extraction of the Holocellulose.

The air-dried holocellulose was exhaustively extracted with aqueous solutions of increasing alkaline concentration (6% and 10% sodium hydroxide) and subsequently with aqueous sodium hydroxide containing sodium borate (10% sodium hydroxide to which 4% metaboric acid was added) following the procedure of Jones, Wise and Jappe(107).



100g. batches of holocellulose were extracted by agitation with 1000ml. of the extractant over 15 hours in an atmosphere of nitrogen. The extracts were filtered through several layers of alkali-washed muslin. No precipitation occurred on acidification of the filtrate (pH 4-5) with glacial acetic acid and the polysaccharides were precipitated by addition of an equal volume of acetone. The precipitates were separated by centrifugation, washed with 50% aqueous acetone and dried by solvent exchange through acetone to ether.

The precipitated polysaccharide from the alkaline borate extract was washed with hot 95% ethanol (X2) and with hot methanol till borate free, before drying as above.

All polysaccharides were finally dried over phosphorus pentoxide in vacuo.

Table 4

Extract		Products of Hydrolysis						[α] _D	Average yield from 100g. holocellulose
		Gal	G	Man	Xyl	Ara	$\overline{\text{UAc}}$		
6% NaOH	F	+++	+++	+++	++++	+	tr	-44° ($\pm 4^\circ$)	20g.
10% NaOH	F	+	++	++++	++	tr	tr	-37° ($\pm 2^\circ$)	16g.
Alkaline borate	F	++	++	++++	tr	tr	tr	-	4g.
Residue	S	tr	++++	+	tr	-	tr	-	48g.

$\overline{\text{UAc}}$ = aldobiouronic acid.

F. indicates 95% formic acid as hydrolysing agent.

S. indicates 72% sulphuric acid as hydrolysing agent.

Acetylation of the Hemicelluloses.

Method - see "General Methods" section.

Batch acetylation of the 10% sodium hydroxide extracts was effected employing various dispersion and reaction conditions. The most efficient procedure was that described (p. 46). From 30g. of the hemicellulose extract 21g. of chloroform-soluble acetates were obtained

$$\text{OAc}\% = 39.4\%.$$

$$[\alpha]_D = -38.1^\circ (\pm 1.5^\circ) \text{ (c. 1.365 in chloroform).}$$

Hydrolysis of a sample with 95% formic acid gave mannose (++++), xylose (+++), glucose (++) , galactose (+), arabinose (tr) and aldobiouronic acids (tr).

Fractionation of the Acetylated Hemicelluloses.

Trial experiments indicated that a virtually pure glucomannan acetate could be isolated by fractional precipitation from a 0.8% w/v chloroform solution of the mixed hemicellulose acetates employing light petroleum (b.p. 60-80°) as the precipitant.

The hemicellulose acetates (20g.) were dissolved in chloroform (2500ml.) and light petroleum (b.p. 60-80°) added in 100ml. aliquots with stirring. Precipitated material was isolated by decantation and reprecipitated from a minimum volume of chloroform by addition of 5 volumes of petroleum ether

with vigorous stirring. (This procedure gave clean fibrous material). The following fractions were obtained after washing with petroleum ether and drying in vacuo over paraffin wax. The remaining supernatant solution was concentrated under reduced pressure to a dark brown syrup which appeared to be mainly organic impurity.

Table 5

Fraction	Petrol in supernatant	Products of Hydrolysis					Weight of Fraction	OAc%	[α] _D	
		Gal	G	Man	Xyl	Ara				UAc
1	39.5%	tr	+	+++	+++	tr	tr	10.8g.	34.0%	-38.4°
2	53.3%	tr	+	+++	tr	-	-	6.5g.	41.8%	-30.4°
3	56.6%	tr	+	+++	+	tr	-	0.1g.	-	-21.8°
Residue	-	tr	tr	tr	tr	tr	-	0.5g.	-	-

A sample of fraction 2 (60mg.) was deacetylated using sodium methoxide in methanol to give the free glucomannan (30mg.)

$$[\alpha]_D = -33.6^\circ (\pm 1.4^\circ) \text{ (C. 0.873 in 2N NaOH).}$$

Hydrolysis of the polysaccharide and estimation of the sugars released indicated the composition - D-galactose (4.5%), D-glucose (20.6%), D-mannose (70.2%) and D-xylose (4.7%).

Acetolysis of Glucomannan Acetate.

Glucomannan acetate (fraction 2) was subjected to graded acetolysis and the saponified products fractionated on charcoal-Celite (1:1). Several oligosaccharides have been characterised.

Trial acetolysis

Glucomannan acetate (fraction 2, 1.0g.) was added in small portions with shaking to an ice-cooled acetolysis mixture (acetic anhydride (6.0ml.), glacial acetic acid (6.0ml.) and concentrated sulphuric acid (0.6ml.)). The dark-brown solution was allowed to stand at room temperature and samples (2ml.) were withdrawn at 24 hour intervals. The samples were pipetted into water (20ml.) and the suspension extracted with chloroform (2x15ml.). The extracts were washed with saturated aqueous sodium hydrogen carbonate (15ml.) and water (15ml.) and dried over anhydrous sodium sulphate before concentrating to syrups under reduced pressure. The syrupy acetates were dissolved in a mixture of dry methanol (1ml.) and chloroform (0.5ml.). Sufficient of a normal barium methoxide solution (methanol) was added to render the solutions alkaline to phenolphthalein and the mixtures were allowed to stand at 0°C for 18 hours, before being diluted with water. The aqueous solutions were treated successively with Amberlite IR 120 (H) resin and Amberlite 45 -OH (OH) resin. The deionised solutions were

concentrated and the residues examined on paper chromatograms in solvents A. and B. The proportion of D-galactose increased with duration of acetolysis. Traces of oligosaccharides of the xylose series (pink spots with aniline hydrogen phthalate) disappeared after 48 hours.

Disaccharide and trisaccharide (higher oligosaccharide) fractions were separated from the saponified products after 72 hours acetolysis. Hydrolysis of the disaccharide fraction gave mannose (++++), glucose (++) and galactose (tr.) while hydrolysis of the tri- and higher oligosaccharide fraction gave the same products with only a very slight trace of galactose.

Large Scale Acetolysis of Acetylated Glucomannan.

Acetylated Glucomannan (fraction 2, 8.7g.) was added gradually with continuous agitation to an ice-cooled mixture of acetic anhydride (52ml.), glacial acetic acid (52ml.) and concentrated sulphuric acid (5.2ml.). The viscous amber solution was allowed to reach room temperature (20°C) and maintained at this temperature for 71 hours. The mixture was poured into water (300ml.) to give an amber-coloured suspension. Addition of sodium hydrogen carbonate (pH 4-5) caused aggregation of the precipitated sugar acetates. These were separated by decantation, washed with water and dissolved in chloroform (150ml.). The aqueous solution was extracted with

chloroform (4x150ml.). The combined chloroform solutions were washed with saturated sodium hydrogen carbonate solution (250ml.), water (300ml.), and finally dried over anhydrous sodium sulphate. Concentration under reduced pressure gave a yellow syrup (ca. 10g.). Barium methoxide (1.8g.) in methanol (9ml.) was added to a solution of the oligosaccharide acetates in chloroform (25ml.) and methanol (50ml.) and the mixture kept at 0°C for 20 hours. The resultant yellow paste was poured into water (350ml.), barium ions were removed as barium sulphate by neutralisation with 0.5 N sulphuric acid to pH 7 and the filtrate concentrated to a syrup (5.0g.).

Chromatography of the syrup in solvent B showed the presence of mannose (++++), glucose (++) , galactose (+), xylose (tr) and arabinose (tr) with oligosaccharides having R cellobiose values of 1.55, 1.22, 1.00, 0.83, 0.31 and 0.22.

Fractionation of Oligosaccharides on Charcoal-Celite.

The mixture of sugars (5.0g.) in water (130ml.) was absorbed on a column (495mm.x30mm.) of charcoal-Celite (1:1. 150g.) The sugars were eluted successively with water and water containing increasing proportions of ethanol (0-30% ethanol). The concentration of ethanol in the eluant was changed in 2.5% increments with every 1000-1500ml. elute. Samples (4ml.)

of each fraction (350-400ml. eluate) were evaporated to dryness in an air-blast at 60°C and examined chromatographically in solvent B (for mono- and disaccharides) or solvent D (for tri- and higher oligosaccharides). Identical fractions were combined and where necessary further fractionation was carried out by chromatography on Whatman filter sheets using an appropriate solvent system (Solvents A,B or D). Elution with water yielded a mixture of monosaccharides (1.14g. mannose, glucose, galactose with traces of xylose) which were not examined further.

Table 6

Fraction	Eluant	Weight	Component Sugars.
1	water	20mg.	Mainly inorganic salts with traces of monosaccharides.
2-3	water	1141mg.	Mannose (++++), glucose (++), galactose (+), arabinose (tr.), xylose (tr.) O ₁ (tr.)
4	2.5% ethanol	405mg.	O ₁
5	"	333mg.	O ₁ , O ₂ .
6	"	37mg.	O ₁ (tr.), O ₂ , O ₅ (tr.).
7	"	17mg.	O ₁ (tr.), O ₂ , O ₅ .
8	5.0% ethanol	89mg.	O ₂ (tr.), O ₆ , O ₇ .
9	"	129mg.	O ₄ , O ₇ , O ₈ .
10	"	51mg.	O ₄ , O ₈ , O ₉ .
11	"	81mg.	O ₃ , O ₄ .
12	7.5% ethanol	73mg.	O ₃ , O ₄ (tr.).
13	"	28mg.	O ₃ , trs. of slower-moving sugars.
14	"	30mg.	O ₁₀ , trs. of slower-moving sugars.
15	10.0% ethanol	32mg.	O ₁₃ , trs. of faster- and slower-moving sugars.
16	"	43mg.	O ₁₁ , tr. of faster-moving sugar.
17	"	20mg.	Mixture of oligosaccharides.
18-20	12.5% ethanol	22mg.	O ₁₂ , trs. of faster-moving sugars.
21	"	35mg.	O ₁₃ , trs. of faster-moving sugars.
22-23	15.0% ethanol	132mg.	O ₁₃ .
24-25	"	72mg.	O ₁₃ , O ₁₄ , trs. of slower-moving sugars.
26	17.5% ethanol	28mg.	O ₁₄ , trs. of slower-moving sugars.
27-28	"	48mg.	Complex mixture of oligosaccharides
29	20.0% ethanol	73mg.	O ₁₅ , higher oligosaccharide (pentasaccharide?).
	20-30.0% ethanol	313mg.	Higher oligosaccharides.

Identification and Characterisation of Oligosaccharides.

Oligosaccharide 1. (O₁) 405mg.

R_G = 0.50 in solvent A.

M_G = 0.66

The sugar gave only mannose on hydrolysis and was indistinguishable from authentic 4-O-β-D-mannopyranosyl -D-mannose on paper chromatograms in solvents A and B. The disaccharide crystallised from moist ethanol with some difficulty; a solution of the sugar in this solvent was cooled to 0°, allowed to reach room temperature and seeded with a small crystal of authentic 4-O-β-D-mannopyranosyl-D-mannose. The acicular crystals had m.p. and mixed m.p. 203-204° and $[\alpha]_D = -5.3^\circ (\pm 0.7^\circ)$ (equil.) (c 5.8 in water.)

An X-ray powder photograph was identical with that obtained from authentic sample of 4-O-β-D-mannopyranosyl-D-mannopyranose.

Oligosaccharide 2. (O₂) 120mg.

R_G = 0.40 in solvent A.

M_G = 0.34

The disaccharide gave only mannose and glucose (1:1) on hydrolysis and was chromatographically and electrophoretically indistinguishable from authentic 4-O-β-D-mannopyranosyl-D-glucose.

The syrupy material crystallised on long standing at 35°C and was re-crystallised from aqueous ethanol (fine needles, 44mg.)

m.p. and mixed m.p. 199-201°

$[\alpha]_D + 33.7^\circ \quad +19.7^\circ$ (equil. 2 hours)

(C. 2.7 in water)

X-ray powder photographs of the crystalline disaccharide and of an authentic specimen of 4-0-β-D-mannopyranosyl-D-glucose were identical.

Oligosaccharide 3. (O₃) 75mg.

R_G = 0.85 in solvent A.

M_G = 0.60

The sugar gave only glucose and mannose on hydrolysis and showed a chromatographic mobility (solvents A and B) and electrophoretic mobility identical with that of authentic 4-0-β-D-glucopyranosyl-D-mannose. The disaccharide which crystallised from aqueous ethanol after several weeks at 35°C. showed only a small mutarotation $[\alpha]_D + 5.7^\circ \longrightarrow +6.6^\circ$ (equil.) (c. 1.2 in water) and the crystals probably consisted of a mixture of α - and β -anomeric forms. The sugar had m.p. and mixed m.p. (authentic 4-0-β-D-glucopyranosyl-D-mannose monohydrate) 169-172° with sintering in the region 135-140°. (Kofler hot-stage microscope). Melting-point determinations in sealed tubes on the disaccharide and on the authentic monohydrate

showed m.p. 128-130° and it is probable that the higher figure represents the melting-point of the anhydrous crystalline form. Similar anomalies in melting behaviour have been noted in the case of mannotriose trihydrate (19c).

An X-ray powder photograph of the disaccharide was identical with that given by 4-O- β -D-glucopyranosyl-D-mannose monohydrate. The sugar was further characterised by conversion into 4-O- β -D-glucopyranosyl - α -D-mannose octa-acetate (following the method of Merler and Wise (59)). After two re-crystallisations from aqueous ethanol the needle-shaped crystals had m.p. and mixed m.p. 200-202° and $[\alpha]_D + 34.9^\circ$ (c 0.36 in chloroform). An X-ray powder photograph of the acetate was identical with that of octa-acetate prepared from authentic 4-O- β -D-glucopyranosyl-D-mannose.

Oligosaccharide 4. (O₄) 31mg.

R_G = 0.53 in solvent A.

M_G = 0.25.

The sugar which gave only glucose on hydrolysis and had chromatographic and electrophoretic mobilities identical with those of 4-O- β -D-glucopyranosyl-D-glucose (cellobiose), crystallised readily from aqueous solution and was recrystallised from aqueous ethenol to give crystals with m.p. and mixed m.p. 229-231°,

$[\alpha]_D + 15.2^\circ \longrightarrow +36.7^\circ$ (equil., 5 hours) (c. 1.2 in water).

X-ray powder photographs of the disaccharide and of authentic cellobiose were indistinguishable.

Oligosaccharide 5. (O₅) 8mg.

R_G = 0.66 in solvent A.

M_G = 0.58.

The sugar, $[\alpha]_D + 54 \pm 5^\circ$ (c. 0.4 in water), gave only mannose on hydrolysis and was chromatographically and electrophoretically indistinguishable from 4-O- α -D-mannopyranosyl-D-mannose.

Oligosaccharide 6. (O₆) 4mg.

R_G = 0.40 in solvent A.

M_G = 0.49

The sugar, $[\alpha]_D + 59^\circ \pm 5^\circ$ (c. 0.2 in water) afforded only galactose on hydrolysis and was indistinguishable on paper chromatograms and electrophoretograms from 4-O- β -D-galactopyranosyl-D-galactose although distinguishable from 3-O- β -D-galactopyranosyl-D-galactose and 6-O- β -D-galactopyranosyl-D-galactose. A portion of the syrup (2mg.) was oxidised with lead tetra-acetate (5mg.) in glacial acetic acid (0.5ml.) (108). The excess lead tetra-acetate was destroyed after 2 hours by addition of oxalic acid, the solution was diluted with water

2ml. and heated on a boiling water-bath in the presence of Amberlite 120 (H) resin. Galactose and a tetrose $R_{xylose} = 1.84$, (presumably threose) were detected whereas similar treatment of 3-O- β -D-galactopyranosyl-D-galactose gave galactose and lyxose.

Oligosaccharide 7. (O₇) 40mg.

$R_G = 0.17$ in solvent A.

$M_G = 0.55$

The sugar yielded only mannose/^{on}hydrolysis and had chromatographic and electrophoretic mobilities identical with those of O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose. Crystallisation occurred readily from aqueous ethanol; the crystals exhibited m.p. and mixed m.p. 162-166° (slow heating on a Kofler hot-stage microscope) and m.p. 131-132.5° (sealed tube), $[\alpha]_D -13.5^\circ \rightarrow -20.3^\circ$ (equil. 24 hours) (c . 0.59 in water) and X-ray powder photographs were identical with those of O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose monohydrate.

Oligosaccharides 8-15.

Oligosaccharides 8-15 were obtained in the amorphous state, and have been characterised as far as possible by chromatographic comparison of the oligosaccharides formed on partial acid hydrolysis of the various oligosaccharides and of the derived

glycitols (from reduction of the reducing oligosaccharide with potassium borohydride) with authentic samples. To avoid repetition complete details of the oligosaccharides and of the products of partial acid hydrolysis of oligosaccharides and derived glycitols have been recorded in Tables 7 and 8.

Table 7.

Oligosaccharide. ¹	Wt.(mg.)	R _G ²	M _G	Hydrolysis of Products.
8. $\beta M \rightarrow \beta M \rightarrow G$	25	0.10	0.40	mannose, glucose
9. $\alpha M \rightarrow \beta M \rightarrow M^3$	2	0.21	0.78	mannose
10. $\alpha M \rightarrow \beta M \rightarrow G$	10	0.15	0.49	mannose, glucose
11. $\beta G \rightarrow \beta M \rightarrow M$	11	0.24	0.49	mannose, glucose
12. $\beta M \rightarrow \beta G \rightarrow G$	44	0.14	0.31	mannose, glucose
13. $\beta M \rightarrow \beta G \rightarrow M$	100	0.21	0.57	mannose, glucose
14. $\beta G \rightarrow \beta G \rightarrow M$	20	0.29	0.49	mannose, glucose
15. $\beta M \rightarrow \beta G \rightarrow \beta G \rightarrow M$	22	0.07	0.40	mannose, glucose

1. M = D-mannopyranose, G = D-glucopyranose; in all cases the residues are linked through C₁ and C₄.
2. R_{glucose} values recorded in solvent A.
3. Trisaccharide was compared with authentic samples from ivory nut mannans.

Table 8

$[\alpha]_D$ in H_2O	Oligosaccharides on partial acid hydrolysis	Oligosaccharides on partial hydrolysis of derived glycitols
8. $-12^\circ \pm 3^\circ$	$\beta M \rightarrow M, \beta M \rightarrow G$	$\beta M \rightarrow M$
9.	$\beta M \rightarrow M, \alpha M \rightarrow M$	$\alpha M \rightarrow M$
10.	$\alpha M \rightarrow M, \beta M \rightarrow G$	$\alpha M \rightarrow M$
11.	$\beta G \rightarrow M, \beta M \rightarrow M$	$\beta G \rightarrow M$
12. $+15.0^\circ \pm 0.8^\circ$	$\beta M \rightarrow G, \beta G \rightarrow G$	$\beta M \rightarrow G$
13. $-6.1^\circ \pm 0.2^\circ$	$\beta M \rightarrow G, \beta G \rightarrow M$	$\beta M \rightarrow G$
14. $-5.5^\circ \pm 0.8^\circ$	$\beta G \rightarrow M, \beta G \rightarrow G$	$\beta G \rightarrow G$
15. $-5.0^\circ \pm 0.5^\circ$	$\beta G \rightarrow M, \beta M \rightarrow G,$ $\beta M \rightarrow \beta G \rightarrow G, \beta G \rightarrow \beta G \rightarrow M$	$\beta M \rightarrow G, \beta G \rightarrow G$ (tr.) $\beta M \rightarrow \beta G \rightarrow G$

Acetolysis of Mannotriose.

α -D-Mannopyranosyl-(1→4)- α -D-mannopyranosyl-(1→4)-
D-mannopyranose trihydrate (mannotriose) (1.0g.) was treated with
the acetolysis mixture (acetic anhydride, acetic acid and
concentrated sulphuric acid, 10:10:1 (v/v)) at 0° under the
conditions employed for the acetolysis of the glucomannan and
the resulting mixture of sugar acetates de-acetylated as described
previously. The oligosaccharides thus obtained (0.88g.) were
fractionated on charcoal-Celite by gradient elution with water
containing 0.0→10.0% ethanol and the fractions were examined
chromatographically in solvent A. The main fractions contained
mannose, mannobiose and mannotriose but minor fractions contained
sugars chromatographically and ionophoretically indistinguishable
from 4-O- α -D-mannopyranosyl-D-mannose and O- α -D-mannopyranosyl-
(1→4)- α -D-mannopyranosyl-(1→4)-D-mannose.

Discussion

Extractive-free Larch sawdust was partially delignified by the acid chlorite procedure⁽¹⁰⁶⁾ prior to alkaline extraction. Hemicellulose fractions rich in glucomannan were isolated by extraction of the holocellulose (previously extracted with 6% aqueous sodium hydroxide) with 10% aqueous sodium hydroxide and also by subsequent extraction of the residue with a 10% sodium hydroxide solution to which 4% boric acid had been added. (107). The alkaline borate was shown to contain a smaller proportion of xylan than the mixture of polysaccharides extracted by 10% caustic soda; it is of interest that the proportion of galactose-containing polymers extracted increased however. The nature of the latter (galactan, galactomannan or galactoglucomannan) has not been determined.

Acetylation of the glucomannan-rich extract by a modification of the method of Carson and Maclay(109) (acetic anhydride and pyridine in a formamide medium) proved difficult. Investigation of the optimum conditions indicated that efficient dispersion was of prime importance and improved yields were obtained by the use of freeze-dried hemicelluloses with prolonged dispersion periods.

The acetylated hemicelluloses were partially separated by fractional precipitation from chloroform solution with light petroleum. The curious fractionation pattern, in which xylan

acetates were present in the earlier and later fractions while almost absent from intermediate fractions may have resulted from variations in the degree of substitution of the acetylated xylans.

An acetylated glucomannan relatively free from contaminating xylan acetate was isolated by this procedure, giving on hydrolysis D-mannose (70%), D-glucose (21%), D-galactose (4.5%) and D-xylose (4.5%) and having specific rotation -30.4° and OAc, 41.8%. This material was subjected to graded acetolysis with acetic anhydride, acetic acid and concentrated sulphuric acid (10:10:1 v/v) and resulting sugar acetates were saponified by treatment with barium methoxide. The mixture of sugars was fractionated by chromatography on charcoal-Celite followed where necessary by partition chromatography on cellulose filter sheets. Five oligosaccharides were obtained crystalline and were characterised by comparison with authentic specimens (m.p., specific rotation, X-ray powder photographs). These were:- 4-O- β -D-mannopyranosyl-D-mannose, 4-O- β -D-mannopyranosyl-D-glucose, 4-O- β -D-glucopyranosyl-D-mannose, 4-O- β -D-glucopyranosyl-D-glucose and O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose. Five of the six possible trisaccharides containing both 1,4-linked β -D-mannose and β -D-glucose residues were also isolated and though not obtained in the crystalline state were characterised by chromatographic examinations of the partial hydrolysis products of the oligosaccharides and of the derived

glycitols from potassium borohydride reduction of the reducing oligosaccharides. These trisaccharides were as follows:-
 $\underline{O}-\beta-\underline{D}$ -mannopyranosyl-(1→4)- $\underline{O}-\beta-\underline{D}$ -mannopyranosyl-(1→4)- \underline{D} -glucose, $\underline{O}-\beta-\underline{D}$ -glucopyranosyl-(1→4)- $\underline{O}-\beta-\underline{D}$ -mannopyranosyl-(1→4)- \underline{D} -mannose, $\underline{O}-\beta-\underline{D}$ -mannopyranosyl-(1→4)- $\underline{O}-\beta-\underline{D}$ -glucopyranosyl-(1→4)- \underline{D} -mannose, $\underline{O}-\beta-\underline{D}$ -mannopyranosyl-(1→4)- $\underline{O}-\beta-\underline{D}$ -glucopyranosyl-(1→4)- \underline{D} -glucose and $\underline{O}-\beta-\underline{D}$ -glucopyranosyl-(1→4)- $\underline{O}-\beta-\underline{D}$ -glucopyranosyl-(1→4)- \underline{D} -mannose. Four of these trisaccharides have been identified among the products of partial acid hydrolysis of a Norway spruce glucomannan (69) and the chromatographic and ionopheretic mobilities were similar to those of the above trisaccharides. In addition a tetrasaccharide $\underline{O}-\beta-\underline{D}$ -mannopyranosyl-(1→4)- $\underline{O}-\beta-\underline{D}$ -glucopyranosyl-(1→4)- $\underline{O}-\beta-\underline{D}$ -glucopyranosyl-(1→4)- \underline{D} -mannose has been isolated and characterised by the procedures employed in the identification of the trisaccharides.

It is unlikely that small quantities of other oligosaccharides encountered represent fragments of the main glucomannan. Thus 4- $\underline{O}-\beta-\underline{D}$ -galactopyranosyl- \underline{D} -galactose probably originate from a small proportion of a 1,4/ β -linked galactan of the structural type recently found in the chlorite delignification liquors of spruce compression wood(110). The disaccharide has also been isolated from the products of partial hydrolysis of Norway spruce glucomannan and of White birch α -cellulose. The apparent differences in extractability exhibited by these galactans may be

related to differences in chain length or to positions in the cell wall, a situation comparable to that of the glucomannans of sprucewood(68).

Three oligosaccharides containing 1,4-linked α -D-mannopyranose residues have been tentatively identified. Although these sugars could have arisen from a proportion of anomalous linkages in a predominantly β -linked polymer, it is considered more probable that they result from acid-catalysed anomerisation of β -D-mannopyranosyl linkages(111). $4\text{-O-}\alpha\text{-}\underline{\text{D}}\text{-mannopyranosyl-}\underline{\text{D}}\text{-mannose}$ and $\text{O-}\alpha\text{-}\underline{\text{D}}\text{-mannopyranosyl-(1}\rightarrow\text{4)-O-}\beta\text{-}\underline{\text{D}}\text{-mannopyranosyl-(1}\rightarrow\text{4)-}\underline{\text{D}}\text{-mannose}$ have been isolated from the products of graded acetolysis of ivory nut mannans under similar conditions(19c) and the oligosaccharides were not detected on paper chromatograms when crystalline mannobiose and mannotriose were subjected to acetolysis. This control experiment has been repeated with $\text{O-}\beta\text{-}\underline{\text{D}}\text{-mannopyranosyl-(1}\rightarrow\text{4)-O-}\beta\text{-}\underline{\text{D}}\text{-mannopyranosyl-(1}\rightarrow\text{4)-}\underline{\text{D}}\text{-mannose trihydrate}$ and the saponified products fractionated by gradient elution from charcoal-Celite with water containing ethanol. Small amounts of di- and tri-saccharides containing 1 4-linked α -D-mannopyranose residues were formed and were shown to be chromatographically and ionophoretically identical with the α -linked oligosaccharides from partial acid hydrolysis of the larch glucomannan and ivory nut mannans. The third oligosaccharide containing an α -D-mannopyranose residue, $\text{O-}\alpha\text{-}\underline{\text{D}}\text{-mannopyranosyl-(1}\rightarrow\text{4)-O-}\beta\text{-}\underline{\text{D}}\text{-mannopyranosyl-(1}\rightarrow\text{4)-}\underline{\text{D}}\text{-glucose}$, would be derived

by a similar anomerisation of the β -glycoside bond in \underline{O} - β - \underline{D} -mannopyranosyl-(1 \rightarrow 4)- \underline{O} - β - \underline{D} -mannopyranosyl-(1 \rightarrow 4)- \underline{D} -glucose which is a component of the acetolysis products from the glucomannan.

Earlier methylation and hydrolysis of a glucomannan isolated by a similar procedure(50b) afforded 2,3,4,6-tetra- \underline{O} -methyl- \underline{D} -mannose, 2,3,4,6-tetra- \underline{O} -methyl- \underline{D} -galactose, 2,3,6-tri- \underline{O} -methyl- \underline{D} -mannose, 2,3,6-tri- \underline{O} -methyl- \underline{D} -glucose and a mixture of di- \underline{O} -methyl- \underline{D} -mannoses in the approximate molar proportions of 1:5:66:30:8. Chromatographic mobility and the products of demethylation and periodate oxidation indicated the presence of 2,3 - and 2,6-di- \underline{O} -methyl- \underline{D} -mannoses. It was considered possible that this fraction also contained a small proportion of tri- \underline{O} -methyl galactose.

The methylation data thus indicate that the polysaccharide contained chains of 1,4-linked β - \underline{D} -mannopyranose and β - \underline{D} -glucose residues in the proportion 2.5:1. The mannose : glucose ratio is somewhat lower than that estimated for the glucomannan employed in acetolysis studies here (3.3:1). It seems unlikely that the polysaccharide used in the methylation studies was contaminated by cellulose and the difference in the proportion of mannose to glucose may reflect the isolation of two distinct glucomannans belonging to the same structural group.

While methylation studies indicated that the majority of \underline{D} -galactose residues were present as non-reducing end-groups

(probably arising from a galactomannan or galactoglucomannan) no oligosaccharides were detected comparable to the galactosyl mannose (XV) and galactosyl mannobiose (XVI) isolated by Meier (69) from partial hydrolysis of Norway spruce glucomannan. As already discussed the isolation of 4-O- β -D-galactopyranosyl-D-galactose points to the presence of small amounts of a 1,4-linked galactan; the absence of significant amounts of 2,3,6-tri-O-methyl-D-galactose in the hydrolysis products of the methylated glucomannan preparation can only be explained by postulating selective removal of the galactan contaminant during methylation.

From evidence derived from methylation studies therefore, and from the acetolysis data presented here some insight into the structure of European larch glucomannan has been gained. The polysaccharide possesses a main chain of 1,4-linked β -D-glucopyranose and β -D-mannopyranose residues in the molar proportions of 1:2.5-3.3. The D-galactose residues are present largely as terminal non-reducing groups and are probably attached as side-chains to the D-mannose residues in the main chain by 1,6 and/or 1,3-linkages (assuming the valid structural significance of the di-O-methyl mannose). In the absence of evidence regarding the structural homogeneity of the polysaccharide, these results could be interpreted on the basis of a single molecular species, a galactoglucomannan, or a mixture of linear glucomannan and a galactomannan of the type found in the

endosperms of leguminous plants. The isolation of 4-O- β -D-mannopyranosyl-D-glucose and 4-O- β -D-glucopyranosyl-D-mannose together with higher oligosaccharide containing both monosaccharide units provides evidence for a heteropolymer. The presence of 4-O- β -D-glucopyranosyl-D-glucose, O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-glucose, O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-mannose, and O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-mannose among the hydrolysis products, indicates that contiguous glucose residues occur in the glucomannan. It is at the same time noteworthy that the trisaccharide found in largest amount was O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-mannose, reflecting the presence of a large number of isolated glucose units in the chain.

The glucomannan component of European larch exhibits the same structural features as glucomannans isolated from other coniferous woods, e.g. spruces(49,63,66-69), pines(53,61,62,70,71,73,76), western red cedar(57), western hemlock(58,65) and Ginkgo biloba(74). The structural similarity is most marked in the case of Norway spruce(67-69), loblolly pine(56,73) and Ginkgo biloba(74) where, as with the larch glucomannan, the chains are terminated at the non-reducing end by D-mannose residues rather than by D-glucose residues.

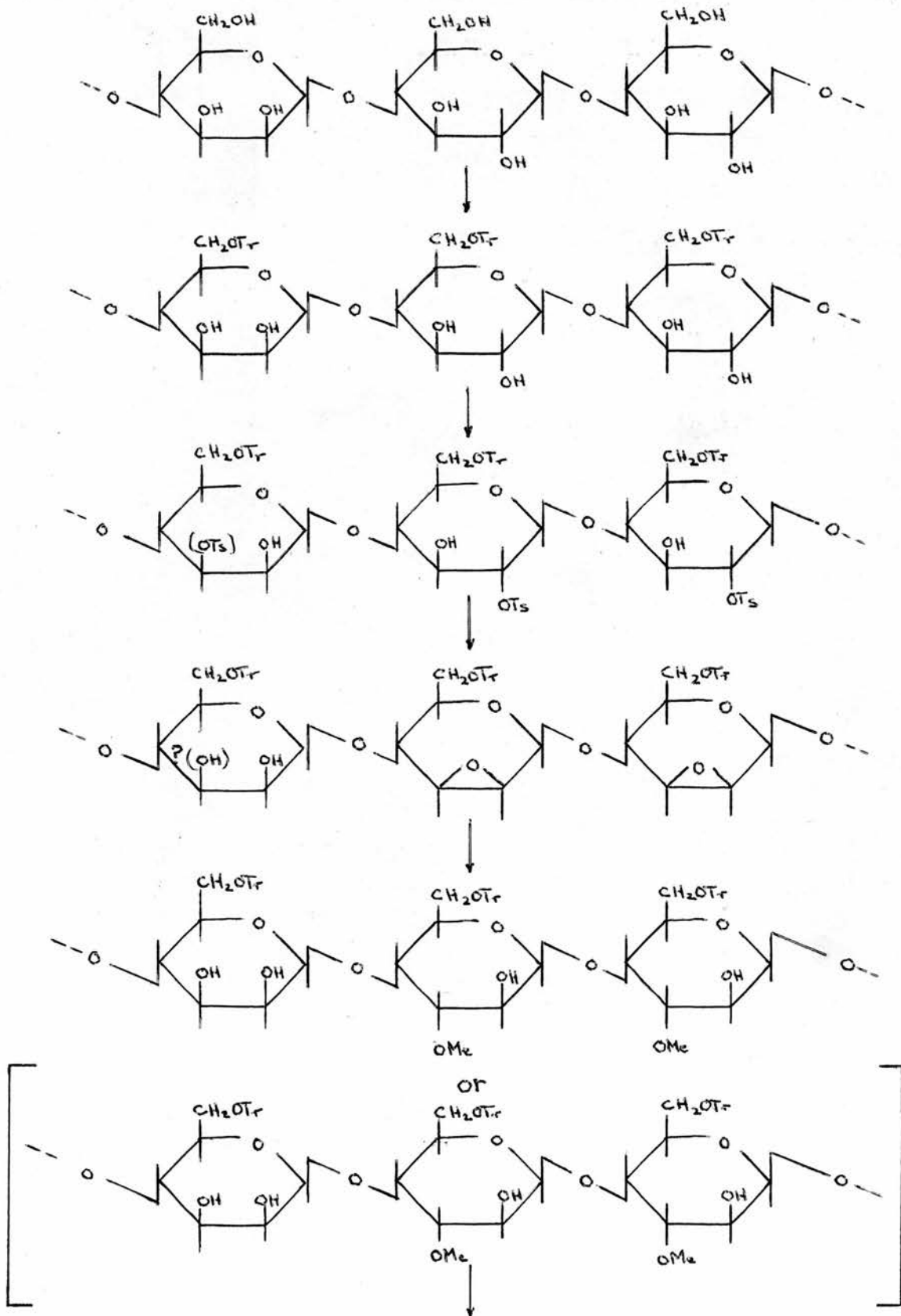
SECTION II

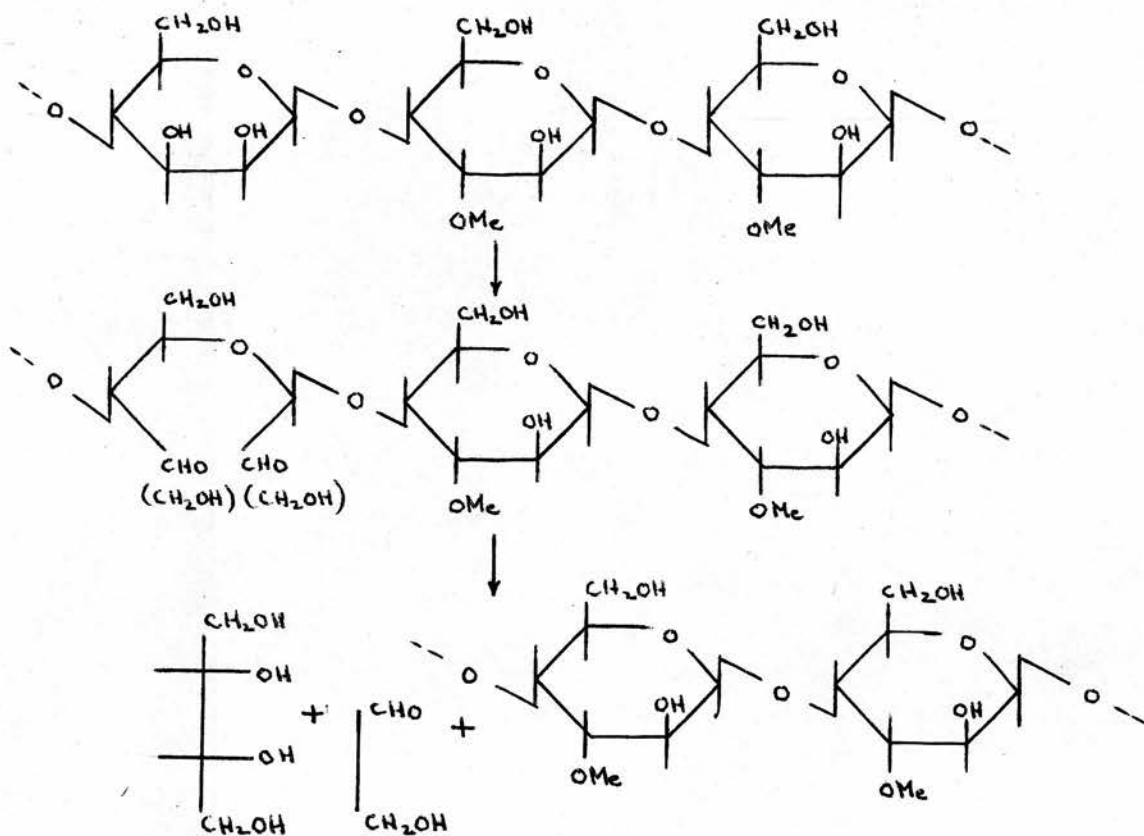
Chemical Modification of Amylose.

To obtain an accurate assessment of the distribution of various hexose residues in a polysaccharide (e.g. D-glucose and D-mannose residues in glucomannans) it is desirable to develop methods whereby the glycosidic bonds joining like residues in the polysaccharide are preserved intact with simultaneous destruction of all other glycosidic linkages. Thus, with respect to glucomannans, the end-products in such selective degradations will consist of oligosaccharide series derived from D-mannose or D-glucose units, some or all of which may be modified.

Section II describes results obtained from the chemical modification of amylose. The proposed scheme for the selective degradation of glucomannans is represented below and was subject to the following assumptions based on the behaviour of monosaccharide analogues; (a) that tosylated D-glucose residues possessing the requisite trans configuration of reactive substituents at C₂ and C₃ would undergo epoxide formation and subsequent ring-opening (with Walden inversion) to give mono-O-methyl hexoses thus conferring resistance to oxidation by the periodate ion; (b) that tosylated D-mannose residues with a cis arrangement of substituents at C₂ and C₃ would not undergo the ring closure (but possibly be subject to O-sulphonyl fission without inversion) and hence would be cleaved by periodate attack after removal of blocking substituents.

Proposed Scheme for Degradation of Glucomannan





Thus the occurrence of contiguous D-glucose units in the glucomannan would be reflected by the isolation of members of an oligosaccharide series containing mono-O-substituted D-altrose units and/or mono-O-substituted D-glucose units (where substituents would be $-OCH_3$ as here, or for example $-H$ if fission of the epoxide ring were effected by an H^- nucleophile.

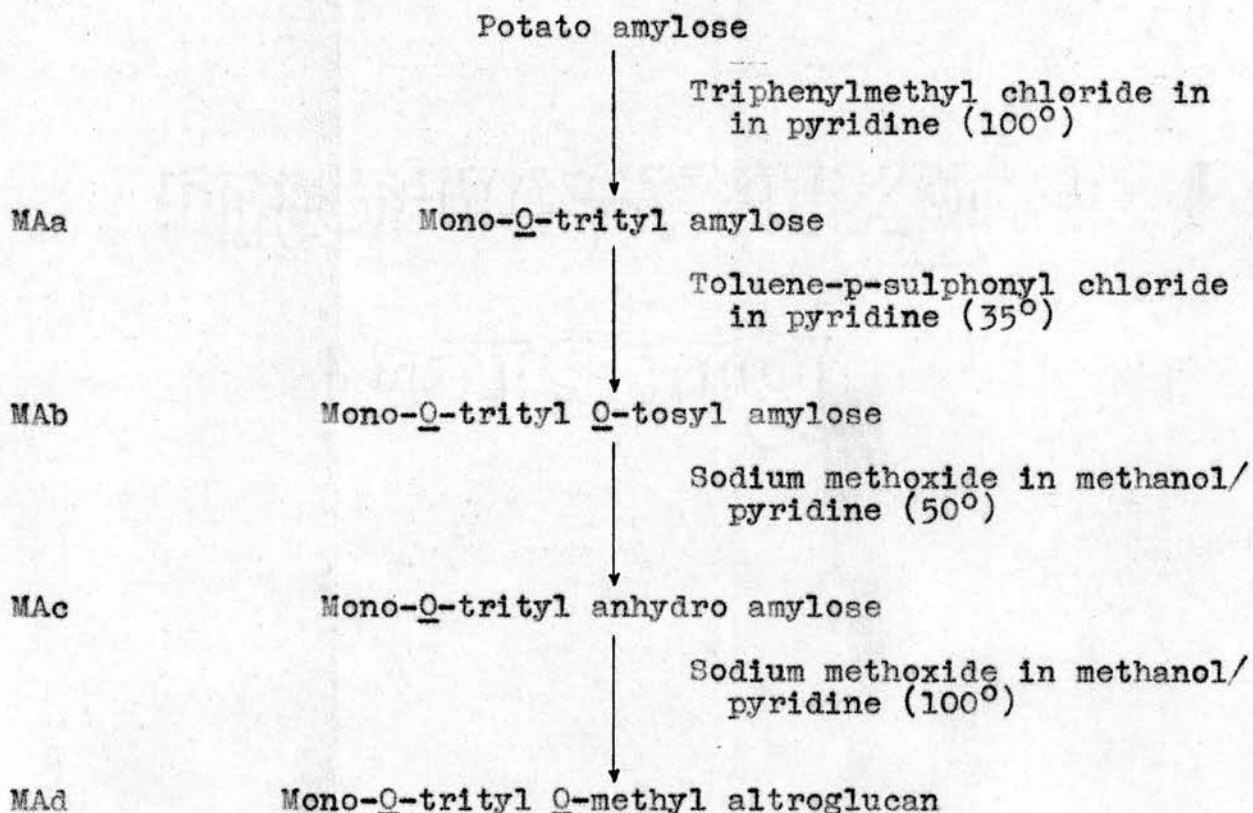
In order to test the validity of these assumptions and to investigate optimum reaction conditions etc., potato amylose was selected as a model polysaccharide containing 1,4-linked D-glucose residues and was subjected to the procedures proposed for the modification of the glucomannan. The reaction conditions and results obtained are presented in this section and the occurrence of unanticipated "anomalies" is discussed.

SECTION II

Experimental

Scheme for the Modification of D-glucose residues in Amylose.

To avoid needless repetition and confusion in terminology, the substituted and modified amyloses isolated at the several stages in the modification procedure represented below will be referred to in this section and in later discussion by the abbreviations shown.



Experimental

Extraction and Purification of Amylose (112)

Amylose was extracted in batches and purified as described.

A slurry of potato starch (30 g.) in water was added with stirring to boiling water (3l.) and the temperature maintained at 100° for a further 1 hour. The solution was allowed to cool to 60° when thymol (3g./l.) was added with vigorous agitation and the mixture set aside for 3 days. The thymol-amylose complex was isolated by centrifugation at 25000 revs./minute and added to boiling water (2l.). Boiling was continued for 15 minutes; butan-1-ol (ca. 300ml.) was added with shaking and the mixture was allowed to stand for 18 hours. The butan-1-ol-amylose complex was collected by centrifugation and the butanol complexing procedure was repeated. The final butanol-amylose complex was stored under butan-1-ol; when amylose was required for use, the complex was drained of supernatant, dialysed against running water and the free amylose finally freeze-dried from aqueous solution.

Average yield = 6.0g., $[\alpha]_D = +155.4^\circ (\pm 3.6^\circ)$ (c. 2.53
in N sodium hydroxide)

Tritylation of Amylose.

In a small-scale run, tritylation of air-dried amylose (5g.) under the conditions already described (p.47) gave a cream product (10.4g.).

$[\alpha]_D$, +95.7° ($\pm 2.4^\circ$) (C. 0.86 in dioxan)

OTr, 61.5% (Calc. for $[C_6H_9O_4(OC_{19}H_{15})]_n$ OTr, 64.2% i.e. one triphenylmethoxyl group per D-glucose residue)

Tritylation of freeze-dried amylose (10g.) under the same conditions gave rise to a buff product (25g.) - MAa.

$[\alpha]_D$, +105.5° ($\pm 3.5^\circ$) (C. 1.175 in chloroform)

OTr, 64.6%

This analysis corresponds to a degree of substitution of 1.00

A determination of periodate uptake in 50% aqueous dioxan at 35° showed a fairly rapid consumption of 0.97 moles (20 hours) (Fig.1.)

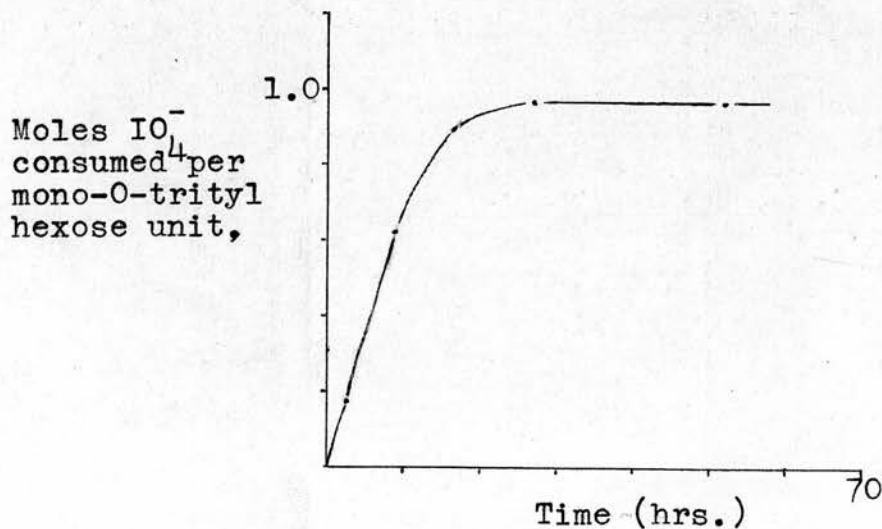


Fig.1.

Tosylation of Tritylated Amylose.

In a trial experiment, tosylation of trityl amylose (7.0g.) under the conditions described (p.49) gave a buff product (8.9g.)

$[\alpha]_D, +57.7^\circ (\pm 2.5^\circ)$ (C. 0.80 in dioxane)

OTr, 46.4% (Calc. for $[C_6H_8O_3(OC_{19}H_{15})(OSO_2C_7H_7)]_n$, i.e. one triphenylmethoxyl group and one toluene-p-sulphonyl group per D-glucose residue, 46.4%)

S, 5.11% (Calc. for $[C_6H_8O_3(OC_{19}H_{15})(OSO_2C_7H_7)]_n$, 5.73%)

(Early analysis indicated a triphenylmethoxyl content much higher than expected (ca. 55%). Prolonged treatment (24 hours) with concentrated sulphuric acid at 30-35° gave the results recorded above. The high results may have been due to the estimation (as triphenylcarbinol) of water-insoluble tosylated oligosaccharides arising from the increased stability of the glycosidic links towards acid-hydrolysis where glycoside residues are substituted in the 2-position by a sulphonyl group).

Repetition of the above procedure on a larger scale (Trityl amylose 20g.) gave a product (27.5g.), MAb, having

$[\alpha]_D, +41.4^\circ (\pm 1.1^\circ)$ (C. 1.84 in chloroform)

OTr, 46.8%

S, 5.44%

The analytical figures correspond to a degree of substitution of ca. 1.95.

oxidation

The course of periodate/oxidation of a sample of the Q-tosyl amylose (detritylated MAb) was followed in a medium of acetone containing 20% water; after 24 hours, the consumption was 0.19 moles of periodate per substituted residue, i.e. $[C_6H_8O_3(OC_{19}H_{15})(OSO_2C_7H_7)]$, and no further uptake was observed.

Infra-red analysis of the trityl tosyl amylose (MAb) (Nujol mull) indicated a sharp peak (1180 cm.^{-1}) in the characteristic absorption band for Q-sulphonates, ($1150-1200\text{ cm.}^{-1}$)(113), absent in the absorption spectrum of the parent trityl amylose.

Assessment of Optimum Conditions for Desulphonylation of Trityl Tosyl Amylose.

Small-scale experiment.

Trityl tosyl amylose (MAb 5g., 0.009 mole) was dissolved in chloroform (65ml.) (dried over anhydrous sodium sulphate and distilled) and the solution cooled to 0° . Sodium methoxide (2.3g.) in methanol (15ml.) (i.e. 2.8N with respect to sodium methoxide) was cooled to 0° and added to the above solution, the flask shaken vigorously and transferred to the refrigerator (0°).

The reaction was followed by observation of the change in optical rotation of 0.5ml. samples diluted to 5ml. with a mixture of chloroform and methanol (4:1 v/v).

Time(hrs)	0.5	3.2	7.5	33	70	96	100	117
Optical Rotation	+0.19°	+0.22°	+0.22°	+0.23°	+0.24°	+0.26°	+0.26°	+0.26°

When the observed optical rotation reached a constant value (117 hours) the solution was diluted with water (100ml.) and the emulsified chloroform layer was separated and washed with water (2x100ml.). The chloroform emulsion was dried over anhydrous sodium sulphate and the clear yellow solution concentrated to a small volume (ca. 150ml.). The product was precipitated by dropwise addition of the chloroform solution to stirred methanol (750ml.) and the precipitate washed on sintered glass with methanol (3x100ml.) and ether (3x100ml.) before air-drying at room temperature (3.5g.); $[\alpha]_D$, +84.9($\pm 2.0^\circ$) (C. 0.966 in dioxan)

S, 3.58%

Further treatment of the material under similar conditions resulted in only a small increase in the degree of desulphonylation (S, 3.17%). (Determination of sulphated ash, 8.9%, indicated considerable contamination of the product by either sodium toluene -p-sulphonate or sodium sulphate, and later products were deionised by treatment of their chloroform solutions with Amberlite IR 120(H) and IR 45(OH) resins.).

The partially desulphonylated material (S, 3.17%, 1.9g.)
was dissolved in dry chloroform (25ml.). Sodium methoxide in/^{methanol}
(3.3 N, 8ml.) was added with stirring and the solution was
heated under reflux (55-60°) for 3 hours. The product was
isolated and dried as described previously. Yield (1.0g.)

[α]_D, +104.9° (+3.0°) (0.67 in dioxane).

S, 2.56%

The gradual formation of a white, granular precipitate in
the desulphonylation reactions described above was investigated.
The material was assumed to be sodium toluene-p-sulphonate but
analysis indicated the true identity to be sodium chloride.
Examination of the literature(114) showed that alkyl orthoformates
are formed with great facility by the action of sodium methoxide
and chloroform, sodium chloride being produced simultaneously



Examination of alternative solvent systems for the
desulphonylation reaction showed that anhydrous pyridine and
methanol offered the best possibilities.

The partially desulphonylated product (S, 2.56%, 0.7g.) was
dissolved in anhydrous pyridine (20ml.). Sodium methoxide in
methanol (3.8 N, 12ml.) was added dropwise with vigorous agitation
and the mixture was heated under reflux at 55-60° for 18 hours.

The product was precipitated by pouring the pale amber reaction solution dropwise into stirred methanol. The resulting precipitate was redissolved in chloroform (50ml.), the solution washed with water (2x50ml.) and treated with Amberlite IR120(H) and Amberlite IR 45(OH) resins. The deionised solution was dried over anhydrous sodium sulphate and concentrated to a small volume before precipitating the product by dropwise addition of the solution to stirred methanol. The product was washed and dried as before (0.3g.)

S, 0.34%

Desulphonylation of Trityl Tosyl Amylose. (MAB)

Trityl tosyl amylose (MAB, 13.0g.) were dissolved in anhydrous pyridine (750ml.). Sodium methoxide (108g.) in methanol (450ml.) was added dropwise with vigorous agitation and the mixture was heated under reflux at 60° for 24 hours.

The desulphonylated product was isolated as before by dropwise addition of the reaction mixture to stirred methanol; a chloroform solution of the precipitate was treated with Amberlite IR 120(H) and IR 45(OH) resins, dried over anhydrous sodium sulphate, concentrated to a small volume and the product reprecipitated in stirred methanol. (3.9g.)

S, 0.085%

OMe, 1.05%

A second batch (13.0g.) of trityl tosyl amylose gave a desulphonylated product (5.3g.) MAc

S, 0.066%

OMe, 0.32%

The combined yields from batches I and II (9.2g.) had

$[\alpha]_D, +99.6^\circ (+0.8^\circ)$ (C. 2.59 in chloroform)

and

OTr, 66.5% (Calc. for $[C_6H_7O_3(OC_{19}H_{15})]_n$ i.e. one trityl group per 2,3-anhydro-D-glucose residue, 69.6%)

A sample was detritylated (see "General Methods") and the consumption of periodate ion by the "anhydro" amylose was measured spectrophotometrically in an aqueous medium. 0.40 Moles/2,3-anhydro D-glucose unit were consumed after 50 hours; no further uptake was observed on prolonged oxidation (ca. 100 hours) (Fig.2.)

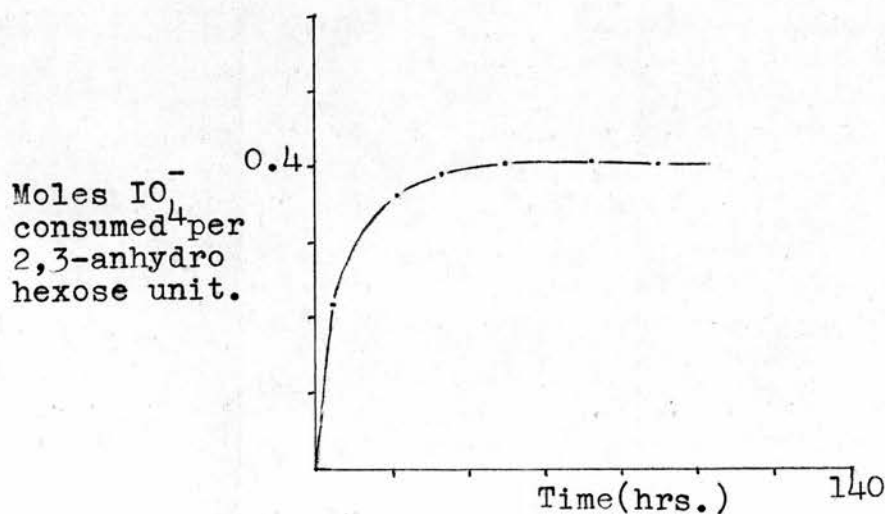


Fig.2.

Examination of the infrared absorption spectrum of the trityl 2,3-anhydro amylose showed the complete absence of peaks in the range 1150-1200 cm^{-1} .

Fission of the Epoxide Ring in Trityl 2,3-anhydro-Amylose.

Trityl "anhydro" amylose (0.47g.) was dissolved in anhydrous pyridine (30ml.) and the solution treated with sodium methoxide (4.05g.) in methanol(15ml.) at 100° for 48 hours. The reaction mixture was then poured dropwise into stirred methanol (250ml.). The flocculent white precipitate was isolated on sintered glass and redissolved in anhydrous chloroform. (A small amount of chloroform insoluble material (sodium methoxide) was removed by centrifugation. The product was finally obtained by precipitation in stirred methanol and was washed and dried as previously. (0.23g.)

$[\alpha]_D^{25}$, +54.5° ($\pm 3.8^\circ$) (C. 0.91 in dioxan)

OTr, 60.4% (Calc. for $[\text{C}_6\text{H}_8\text{O}_4(\text{OCH}_3)(\text{OC}_{19}\text{H}_{15})]_n$, i.e. 62.0%)

Poor solubility of the tritylated polysaccharide in the reaction mixture employed in methoxyl estimation gave very low results. A detritylated sample however had OMe, 7.4% (Calc. for $[\text{C}_6\text{H}_9\text{O}_4(\text{OCH}_3)]_n$, 17.6%).

The detritylated product was easily soluble in water and gave on hydrolysis with N sulphuric acid reducing sugars with the following chromatographic mobilities (solvent A):-

R_{3A} , 0.42, 0.51, 0.78, 1.04 and 1.17.

(R_{3A} here and henceforth = R_{3-O} -methyl-D-altrose.

The bulk of the trityl anhydro amylose (MAc, 5g.) was treated with sodium methoxide under the conditions employed in the small-scale experiment to effect ring fission and a product MAd (3.0g.)

$[\alpha]_D$, +59.1° (+1.0°) (C. 2.00 in chloroform) was obtained.

OMe (detritylated material), 9.8% (Calc. for $[C_6H_9O_4(OCH_3)]_n$, 17.6%)

The detritylated material consumed 0.48 moles of periodate per monomethyl hexose residue over a period of 30 hours after which time no further uptake was observed .(Fig.3.)

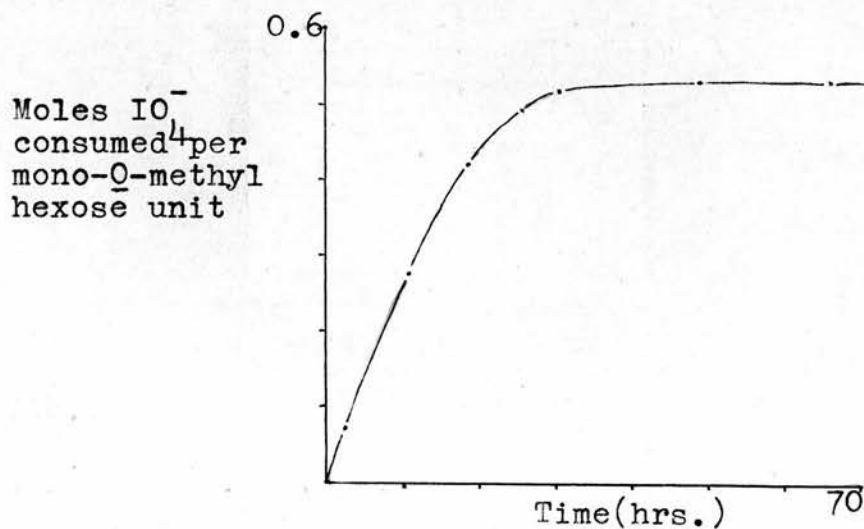


Fig.3.

The remainder of the trityl polysaccharide obtained by fission of the epoxide ring of trityl 2,3-anhydro amylose (2.0g.) was detritylated as described in "General Methods" (p.48); the material so obtained (0.7g.) showed low solubility in methanol but dissolved very readily in water.

Hydrolysis of Detritylated MAd.(O-methyl altroglucan).

Detritylated MAd (0.7g.) was dissolved in N sulphuric acid (10ml.) in a sealed tube and heated on a boiling water bath for 4 hours. The hydrolysate was neutralised with barium carbonate and the precipitated barium sulphate removed by centrifugation. Ba⁺⁺ ions were removed by successive treatments of the solution with Amberlite IR 120(H) and IR 4 B(OH) resins before concentration to a syrup (0.56g.).

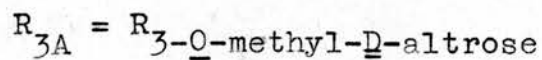
Chromatography in solvents A indicated D-glucose to be the major component of the hydrolysate accompanied by smaller amounts of other reducing sugars corresponding in chromatographic mobility and colour reaction with aniline oxalate to mono-O-methyl hexoses.

Fractionation of Detritylated MAd Hydrolysis Products.

The syrup (0.5g.) was fractionated on a cellulose column (410x16mm.) by stepwise elution with benzene/ethanol systems. (Benzene : ethanol 4:1, 3:1, 2:1, 1:1 (v/v) all containing 1% water). The following fractions were obtained by combination of tubes containing the same sugars as indicated on paper chromatograms (Solvent E).

Table 9.

Tube No.	Fraction	Wt.(mg.)	$[\alpha]_D$	R_{3A}	Wt. % of hydrolysate
0-20	1	4.8	-	1.83 (yellow) 1.49 (pink)	1.7
21-40	2	35.4	-202°	1.49, 1.68 (faint)	12.8
41-70	3	23.0	-31.0°	1.07 (brown)	8.4
71-115	4	34.3	+19.1°	1.26, 0.98 (brown)	12.4
116-190	5	39.5	+45.7°	0.83 (brown)	14.3
191-220	6	10.9	-	0.91, 0.77 (brown)	3.9
220-300	7	11.2	-	0.76, 0.67, 0.57, 0.41	4.0
300-420	8	118.3	+51.1°	0.45 (brown)	43.2



Examination of the Fractions.

Fraction 1.

The small amount of material isolated from this first fraction appeared to be mainly non-carbohydrate in nature. The identity of traces of substituted reducing sugars has not been determined.

Fraction 2. (35mg.) $[\alpha]_D^{20}$, -202° (1.23 in water)

The fraction contained only non-reducing sugars (negative Fehling's test). No spots were observed on chromatograms sprayed with neutral aniline oxalate; on spraying with aniline hydrogen phthalate containing 5% acetic acid and developing at 150° , faint trace spots appeared slowly with R_{3-O} -methyl altrose 1.49 and 1.68 (solvent E). The fraction (1mg.) was treated with N sulphuric acid (0.5ml.) for 4 hours on a boiling water bath and the solution was neutralised and deionised as described in "General Methods". The syrup obtained on concentration of the solution gave two spots for reducing sugars having R_{3-O} -methyl-D-altrose 0.96 and 0.75 (solvent A) (authentic 2-O-methyl-D-altrose had R_{3-O} -methyl-D-altrose 1.00 in solvent A). Demethylation of the fraction gave reducing sugars with mobilities corresponding to altrose (major component) and glucose (tr.) (solvent A). Periodate oxidation and chromatography of the

(138)
oxidation products/(solvent E) gave a single intense yellow spot with R_f 0.83.

Fraction 3. (23mg.) $[\alpha]_D, -31^\circ$ (C. 0.91 in water)

Chromatography in solvent E showed the presence of a single reducing sugar R_{3-O} -methyl-D-altrose=1.07 (hexose) (the fraction probably contained a small amount of non-reducing material from fraction 2).

The sugar gave only a very faint reaction with the triphenyltetrazolium salt reagent on paper chromatograms.

Demethylation gave altrose and glucose (ca. 1:1, visual ratio)

Periodate oxidation of the fraction and chromatography of the oxidation products gave^a pink spot, R_f 0.62, a yellow spot (faint), R_f 0.15 and a more intense yellow spot, R_f 0.83.

Fraction 4. (34mg.) $[\alpha]_D, +19.1^\circ$ (C. 0.79 in water)

Chromatography in solvent E indicated an equimolecular mixture (visual estimation) of two components R_{3-O} -methyl-D-altrose 0.98 and 1.26 (hexoses).

Fractionation on 3MM paper in solvent E gave two sub-fractions.

Sub-fraction (a) (12mg.) R_{3O} -methyl-D-altrose = 1.00 (solvent E)

$[\alpha]_D, +25.3^\circ$ (C. 1.0 in water)

The sugar gave a strong positive reaction with triphenyl-tetrazolium salt reagent.

Periodate oxidation and chromatographic examination of the oxidation products showed only a pink spot, R_f 0.59.

Demethylation gave altrose, glucose (tr.) and a pentose (tr.).

Sub-fraction (b) (9mg.) R_3 -O-methyl-D-altrose 1.25 (solvent E)

$[\alpha]_D$, +20° (C. 0.7 in water)

The sugar gave a negative reaction with the triphenyl-tetrazolium salt reagent.

Periodate oxidation of the sugar, followed by chromatography of the oxidation products, gave a yellow spot with R_f 0.15-0.20 (methoxymalondialdehyde) and a second yellow spot with R_f 0.73.

Demethylation gave altrose only.

Fraction 5. (39.5mg.) $[\alpha]_D$, +45.7° (C./in water)^{0.75}

Chromatography in solvent E showed the presence of a single component with R_{SA} 0.83.

The sugar gave a strong positive reaction on a chromatogram with the triphenyltetrazolium salt reagent.

Periodate oxidation and chromatography of the oxidation products gave only a pink spot (pentose) with R_f 0.60.

Demethylation gave glucose only.

Fractions 6 and 7. (22mg.) contained a mixture of sugars (cf. Table 9, p.91) and were not further examined.

Fraction 8. (118.3mg.) $[\alpha]_D +51.1^\circ$ (2.36 in water)

Chromatography indicated the presence of glucose only (with a trace of a hexose with slightly faster mobility in solvent E).

The identity of the sugar was confirmed by the preparation of the di-O-isopropylidene derivative(115); m.p. and mixed m.p. with authentic 1,2;5,6 di-O-isopropylidene D-glucose, 110° .

Discussion

Amylose was obtained by fractionation of potato starch via the thymol and butan-1-ol complexes as described in the literature (112). Since maximal solubility of the polysaccharide seemed desirable the normal precautions taken to minimise depolymerisation (e.g. exclusion of oxygen) were ignored.

The amylose, $[\alpha]_D^{+155^\circ}$ in N sodium hydroxide, was subjected to tritylation by the agency of triphenylmethyl chloride in a pyridine medium. The trityl content of the resulting product (MAa $[\alpha]_D^{+105^\circ}$) was close to that required for a mono-O-trityl ether (OTr 64.6%; calc. 64.2%), while the consumption of 0.97 moles of periodate per mono-O-trityl residue indicated that the 6-O-trityl ether was formed. (This is to be expected for the bulky triphenylmethoxyl group and on account of the relative availability of the primary and secondary hydroxyls). Whelan et al. (116a and 116b) whose procedure was followed here, have recently reported the isolation of a trityl amylose with the same composition.

Tosylation in pyridine yielded a mono-O-trityl O-tosyl amylose (MAb, $[\alpha]_D^{+41^\circ}$) for which the sulphur analysis was only slightly lower than that required for a polysaccharide containing one toluene-p-sulphonyl group per glucose residue (S.5.11%;

calc. 5.73%). That di-O-tosylation probably occurred was evidenced by a low consumption of periodate (0.19 moles per mono-O-tosyl glucose residue). The substituted polysaccharide exhibited a strong absorption maximum (1180 cm.^{-1}) in the range characteristic for the O-S stretching frequencies of O-sulphonates ($1150-1200 \text{ cm.}^{-1}$ (113)).

Optimum desulphonylation conditions were found to be rather more drastic than might be anticipated from the general facility of epoxide formations encountered among O-tosyl- α -D-glucosides under alkaline conditions (117). Thus treatment of the mono-O-trityl mono-O-tosyl amylose with sodium methoxide methanol/pyridine solution at $50-60^\circ$ gave a mono-O-trityl 'anhydro' amylose (MAc $[\alpha]_D, +99.6^\circ$). Examination of the infra-red spectrum showed the absence of peaks in the O-S stretching range $1150-1200 \text{ cm.}^{-1}$. The low methoxyl value (OCH_3 , 0.32%) indicated that the extent of fission of newly-formed epoxide rings was negligible. The anhydro amylose (de-tritylated MAc) consumed 0.40 moles of periodate per 2,3-anhydro glucose unit; assuming that the 2,3-epoxide is stable under the conditions of periodate oxidation, this uptake can only be interpreted as indicative of the presence of unsubstituted glucose units in the polymer. The

uptake is much higher than in the case of the O-tosyl amylose, hence there must occur an increase of D-glucose residues during desulphonylation. Although O-S cleavage with retention of configuration is known to occur in secondary sulphonates where other reactive sites are blocked (118) and must occur as the first stage in the formation of epoxides from di-O-sulphonates, it is unexpected where the carbon atom adjacent to the sulphonylated C-atom carries the reactive substituent (-OH or -OSO₂ C₆ H₄ CH₃). Although knowledge is far from complete regarding the conformation of hexose units in polysaccharide chains and the restrictions of flexibility of the pyranose ring which might ensue, it seems possible that a degree of rigidity might be conferred on the ring structure of the glucoses units of the amylose chain, which would impede the adoption of the diaxial position of the reactive groups required/for the intramolecular S_N2 reaction, and hence prevent epoxide formation. It is of interest that the mono-O-trityl hexosan derived by Whelan et al. (116b) from treatment of mono-O-trityl, mono-O-tosyl amylose (with analysis very similar to that described here) with sodium hydroxide in dioxan gave on detritylation^{and}/hydrolysis D-glucose (50%), D-altrose (20%) and 1,6 anhydro-D-altrose (30%). It seems likely in the light of the current

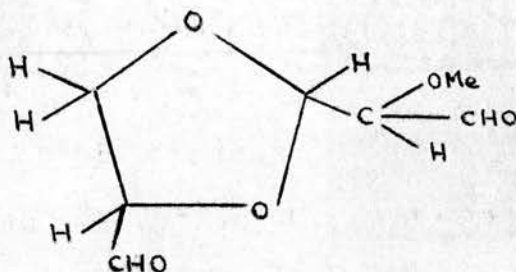
evidence that a proportion of the D-glucose produced arose from O-S cleavage without Walden inversion as distinct from that derived from epoxide ring fission.

Further treatment of the mono-O-trityl anhydro amylose with sodium methoxide in methand/pyridine at 100° resulted in fission of the epoxide ring. Analysis of the detritylated product indicated a methoxyl content of 9.8% (calc. for a mono-O-methyl hexosan, 17.6%). 0.48 Moles of periodate were consumed per residue (calculated as a monomethyl hexose unit). The slight discrepancy in the consumption of periodate compared with that of the parent anhydro amylose cannot be explained other than by experimental error (or presence of impurities in one or other of the determinations).

Hydrolysis of the O-methyl altroglucan (detritylated MAd) gave rise to a mixture of D-glucose (43.2%), mono-O-methyl hexoses and a small amount of unidentified sugars. The mixture was fractionated on cellulose employing mixtures of benzene and ethanol as the irrigant. Where necessary further fractionation was effected by chromatography on filter sheets in solvent E.

Fraction 1 consisted of traces of reducing sugars with chromatographic mobilities greater than the mono-O-methyl hexose range and was not further examined.

Fraction 2 contained only non-reducing sugars. The high negative rotation of the fraction ($[\alpha]_D = -202^\circ$) is characteristic of 1,6-anhydro D-altrose and its derivatives where the glycosidic C-O bond is held rigidly in the β - configuration. Demethylation gave altrose with a trace of glucose while treatment with N acid at 100° produced two reducing sugars one of which possesses a mobility on paper chromatograms identical with that of 2 - or 3 - O -methyl D-altrose. Periodate oxidation gives a product moving close to the solvent front on paper chromatograms: this reaction might be consistent with the production of a 1, 3-dioxolane structure such as



from e.g. 1,6-anhydro-2-O-methyl D-altrose (The 1,6-anhydro 3-O-methyl-D-altrose would resist periodate oxidation).

Fraction 3 gave a single spot on paper chromatograms but was probably contaminated by a small amount of material from fraction 2 (as witnessed by the presence of a yellow spot among the periodate oxidation products with the same mobility as that observed in fraction 2). Demethylation gave equimolecular amounts

of glucose and altrose. (The latter may be derived from a 1,6 anhydro 0-methyl altrose as in fraction 2). The weak positive reaction obtained with the triphenyltetrazolium salt reagent is suggestive of a 2-0-methyl hexose. (The chromatographic mobility does not correspond to a 2-0-methyl glucose however).

Fraction 4a appears to be identical with 3-0-methyl D-altrose while the demethylation product of fraction 4b is also altrose. Periodate oxidation of the latter fraction gives methoxymalondialdehyde indicative of a 2-0-methyl hexose while the negative triphenyltetrazolium reaction is also consistent with the presence of a 2-0-substituted sugar. The experimental data is most consistent with a 2-0-methyl altrose; the chromatographic mobility $R_{3-0\text{-methyl altrose}}$, 1.26 differs from that of authentic 2-0-methyl D-altrose however ($R_{3-0\text{-methyl } \underline{D}\text{-altrose}}$ 1.00) (solvent E.)

Fraction 5 gave glucose only on demethylation and the observation on paper chromatograms of a pink spot (mono-0-methyl pentose) as the sole product of periodate oxidation suggests a 3-0-methyl hexose (The sugar also shows a strong positive triphenyltetrazolium reaction. The optical rotation $[\alpha]_D$, +45.7° is lower than that expected for 3-0-methyl D-glucose, $[\alpha]_D$ + 55.1°⁽¹³⁹⁾ but the chromatographic mobility is identical with that

of 3-O-methyl D-glucose in solvents A and E.

Fraction 6 and 7 contained mixtures of reducing sugars with mobilities intermediate between those of glucose and the mono-O-methyl hexoses already discussed. The fractions were not further examined.

Fraction 8 contained glucose plus a trace of a sugar with a slightly faster chromatographic mobility (not altrose). The optical rotation, $[\alpha]_D, +51^\circ$ is also consistent with the identification of the sugar as D-glucose. The identity of the sugar was confirmed by preparation of 1,2: 5,6 di-O-isopropylidene D-glucose (115).

Clearly the picture regarding the reactions concerned in the modification of amylose by the procedures adopted is rather more complex than might be predicted from the behaviour of analogous monosaccharide models. In so far as identification may be considered valid it is clear that the extent of ^{the}tosylation reaction is not limited to production of a polysaccharide containing a single tosyl group per glucose residue. The mono-O-methyl hexose isolated in largest amount appears to be 3-O-methyl D-glucose. This can only be derived from a 2,3-anhydro D-alloside configuration which in turn must result from a 2,3-di- or 3-mono-O-tosyl D-glucose residue. Thus the extent of

di-O-sulphonylation must be greater than that of mono-O-sulphonylation or alternatively the sulphonyl group enters preferentially at C_3 (in contradistinction to the situation encountered among α -methyl D-glucoside analogues where C_2 is preferentially substituted (119,120) 3-O-methyl D-altrose is derived from the 2,3-anhydro D-mannosidic structure; the latter would be the expected product from ring closure across $C_2 - C_3$ in 2-O-tosyl D-glucose residues. Although the identification of 1,6 anhydro 2-O-methyl D-altrose is only very tentative such a product would be expected to occur in small amounts as the alternative product of ring fission a 2,3-anhydro-D-alloside structure. The possible occurrence of 4-O-methyl hexoses cannot be overlooked; these might result from epoxide formation at C_3, C_4 on non-reducing terminal D-glucose residues (by epoxide migration (121,122)). The yield would necessarily be small but might be enhanced by virtue of the flexibility of the singly-linked pyranose ring as distinct from the rigidly held residues in the chain. Overend and Ricketts have postulated such an epoxide migration to account for the production of D-mannose units in polysaccharide derived from alkaline hydrolysis of dextran sulphates (123). The simultaneous occurrence of D-gulose is explained by formation via a 3,4-anhydro-D allose or 3,4-anhydro-D-galactose which would result from a D-glucose 2- or 3-O-sulphate ester in the dextran. Such a situation might also hold for tosylation of non-reducing end

groups in amylose.

Although of stereochemical interest the results obtained from such a modification of amylose are not encouraging when the original aim (i.e. the application to the degradation of glucomannans) is considered; consequently the scheme was abandoned in favour of the approach outlined in section III.

SECTION III

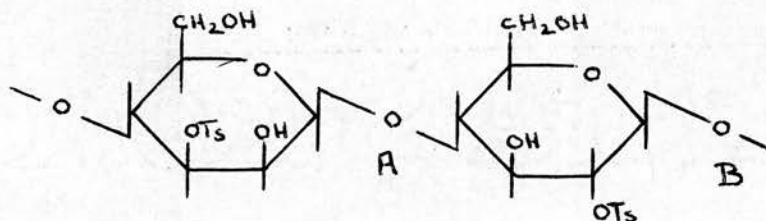
Attempted Selective Hydrolysis

of

Mannosidic Links in a Glucomannan

A second approach to the problem of selective degradation of glucomannans has been made by the attempted utilisation of the resistance towards acid hydrolysis exhibited by 2-O-sulphonylated glycosides(124).

By analogy with monosaccharide models e.g. methyl 4,6-O-benzylidene- α -D-glucoside(119,120), it was anticipated that the D-glucose residues of a 1,4-linked glucomannan would be preferentially sulphonylated at C₂; further that, by similar analogy with methyl 4,6-O-benzylidene- α -D-mannoside, D-mannose residues would be substituted at C₃ preferentially(125) although possibly with less ease than in the corresponding D-glucoside.

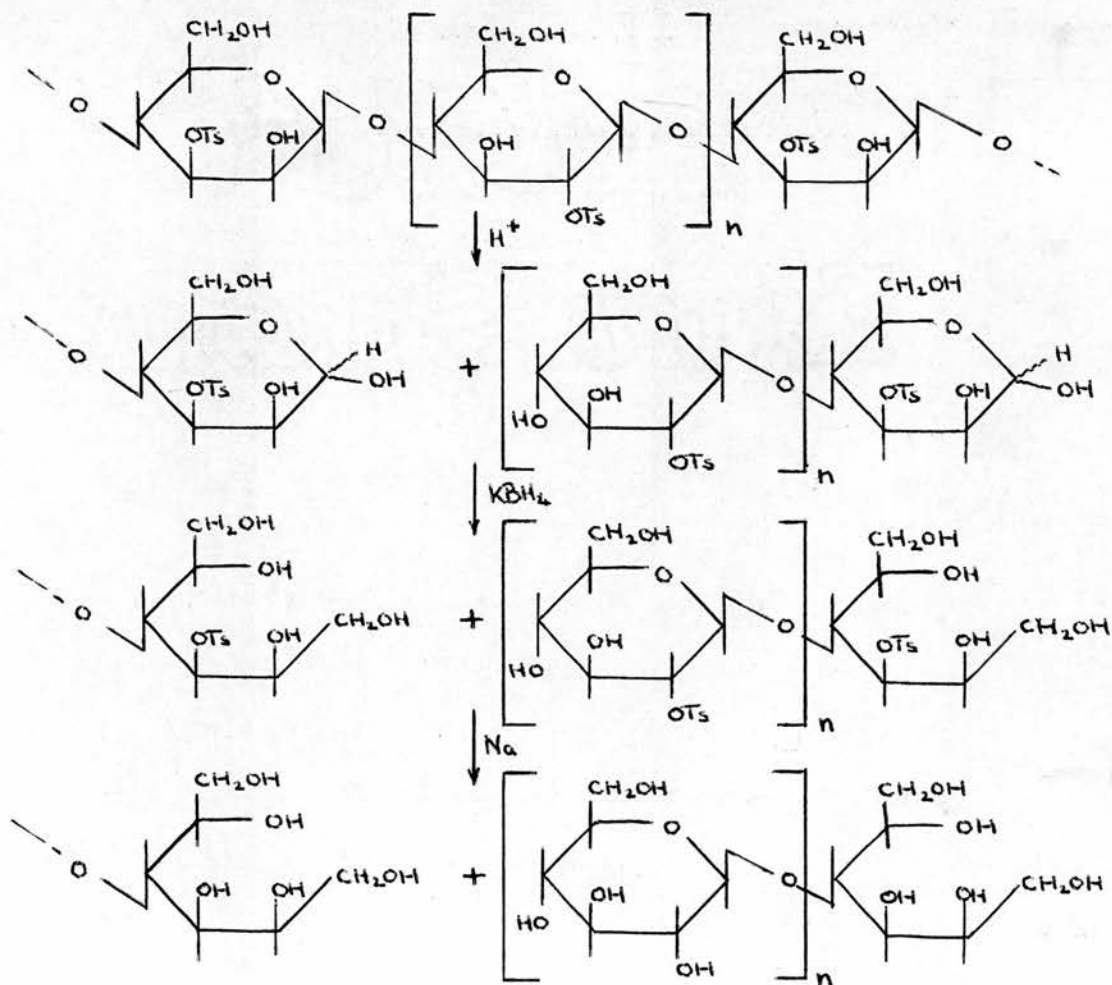


Thus it might be expected that the D-glucosidic bond at B would be somewhat stabilised towards acid hydrolysis by virtue of the "protecting" effect of the adjacent sulphonyl group while bond A would remain relatively labile to acid. The end-products of hydrolysis of a mono-O-tosyl glucomannan would then consist of 3-O-tosyl/^{D-mannose} and a series of oligomers of 2-O-tosyl

D-glucose where each member is terminated at the reducing end by a 3-O-tosyl D-mannose residue; identification of the oligosaccharide glycitols obtained on subsequent reduction and detosylation would thus give a more quantitative picture of the relationship of D-glucose units in the polymer.

The results obtained by treatment of model compounds under the conditions for the proposed scheme are described and discussed in this section.

Proposed Scheme for the Selective Hydrolysis of Glucomannans.



SECTION III

Experimental

Acid hydrolysis of methyl 3-O-tosyl- α -D-mannoside.

Methyl 3-O-tosyl- α -D-mannoside (m.p. 90-100°) (450mg.) (kindly supplied by Dr. J.C.P.Schwarz) was dissolved as far as possible in cold 0.5 N sulphuric acid (10ml.). The waxy, insoluble material was removed by filtration and the clear, colourless filtrate was transferred to a 10ml. flask. The solution was heated on a boiling water bath and samples were withdrawn at intervals, cooled and the optical rotation measured.

Time	0	25	65	110	230	300	335	420
Optical Rotation	+1.12°	+0.98°	+0.78°	+0.56°	+0.15°	0.00°	-0.05°	-0.06°

After 2-3 hours the solution became yellow and eventually precipitation of a dark brown material occurred. This was removed by centrifugation before recording the optical rotation.

After 6.5 hours the insoluble material was removed at the centrifuge; the centrifugate was neutralised by addition of barium carbonate and decolourised with a small amount of charcoal. The colourless aqueous solution was concentrated to a syrup (200mg.). Attempts to induce crystallisation failed but the syrup reduced Fehling's solution and had an absorption maximum at 1180 cm^{-1} ; the sugar moved as a single spot on paper chromatograms, R_f 0.69, (with a slight trace of material with faster mobility).

Treatment of methyl 2-O-tosyl- α -D-glucopyranoside under conditions of acid hydrolysis.

Methyl 2-O-tosyl- α -D-glucopyranoside was prepared by debenzylidenation(126) of methyl 4,6 benzylidene 2-O-tosyl- α -D-glucopyranoside as prepared by the method of Robertson and Griffith(127).

m.p. 136-137° $[\alpha]_D, +93^\circ(\pm 3.4^\circ)$ (C. 1.0 in chloroform)

Methyl 2-O-tosyl- α -D-glucoside (100mg.) was heated with sulphuric acid (3ml., 1.5 N) at 100°C. The optical rotation and reducing power were unchanged after 6 hours under these conditions. The solution was neutralised with barium carbonate deionised by passage through Amberlite IR 120(H) and IR 45(OH) resins and finally concentrated to a syrup which crystallised readily on standing at room temperature (95mg.). The crude material was recrystallised from methanol

m.p. and mixed m.p. 135-136° ; $[\alpha]_D, +91^\circ$

The infra-red spectrum was identical with that of the starting material.

Reduction of 3-O-tosyl-D-mannose with potassium borohydride.

3-O-Tosyl-D-mannose (89mg.) was dissolved in water (5ml.). Potassium borohydride in water (15mg. in 2ml.) was added to the solution. After 1.5 hours a drop of the reaction mixture, acidified with acetic acid, no longer reduced Fehling's solution. The solution was acidified (pH 4-5) with acetic acid and passed through Amberlite IR 120(H) and IR 4B(OH) resins before evaporation to dryness under reduced pressure. The syrup failed to crystallise but moved as a single spot on paper chromatograms (R_f , 0.72 in solvent E) and showed an absorption maximum at 1182 cm.^{-1} indicating the presence of an O-sulphonate substituent.

Treatment of methyl 2-O-tosyl- α -D-glucoside under the conditions of borohydride reduction.

Methyl 2-O-tosyl- α -D-glucoside was treated with potassium borohydride under the conditions employed for the reduction of 3-O-tosyl-D-mannose. Methyl 2-O-tosyl- α -D-glucoside (120mg.) was dissolved in a mixture of water and methanol (ca. 3:1 v/v, 2ml.) and the solution added to a solution of potassium borohydride in water (20mg. in 2ml.). The mixture, diluted

to 11ml., was allowed to stand at room temperature and the optical rotation observed at intervals. No change in rotation was noted after 4 hours and excess borohydride was destroyed with acetic acid. The product was isolated as described for 3-O-tosyl-D-mannitol. The syrup obtained crystallised readily on standing and was recrystallised from methanol (105mg.)

m.p. and mixed m.p. with starting material 135-136° ;

$[\alpha]_D, +90^\circ$

The infra-red spectrum was identical with that of the starting material.

Reductive Detosylation of Methyl 2-O-tosyl- α -D-glucopyranoside.

Methyl 2-O-tosyl- α -D-glucopyranoside (250mg.) was dissolved in 80% aqueous methanol (30ml.) in a 3-necked flask fitted with a stirrer. Sodium amalgam (3g.) was added portionwise with efficient stirring at 0-5°C. Sulphuric acid (6 N) was added simultaneously so that the pH was maintained in the range 3-4. The suspension was stirred overnight at ca. pH 4 (room temperature). After adjustment of the pH to 7 by dropwise addition of 0.5 N sodium hydroxide, the suspension was evaporated to dryness under reduced pressure at 40° and dried

in vacuo over phosphorus pentoxide. The remaining solid material was exhaustively extracted with dry methanol; removal of the solvent under reduced pressure gave a syrup which crystallised on standing at room temperature. Recrystallisation from methanol gave colourless crystals m.p. and mixed m.p. 163-165° ; $[\alpha]_D$, +152°(±4.5°) (C. 1.2 in water).

The sugar had a chromatographic mobility identical with that of authentic methyl- α -D-glucoside in solvents A and E. The infra-red spectrum was also identical with that of methyl- α -D-glucoside.

Reductive Detosylation of 3-O-tosyl D-mannitol.

Treatment of 3-O-tosyl D-mannitol (30mg.) with sodium amalgam under the conditions described above gave rise to a syrup which ran as a single spot on chromatography in solvents A and E, having a mobility identical to that of D-mannitol. After desorption from charcoal the syrup crystallised from methanol in the form of colourless needles m.p. and mixed m.p., 164-165°.

Extraction of Glucomannan from *Lilium henryii* bulbs.

L.henryii bulbs were selected as a convenient source of glucomannan; polysaccharide was extracted by a modification of the procedure followed by Andrews, Hough and Jones(48).

L. henryii bulbs were shredded, washed with methanol and macerated (ball-mill) for 3 days with this solvent. The buff, insoluble material was obtained by filtration and air-dried at room temperature.

Glucomannans were extracted from the bulb powder with cold 1% aqueous mercuric chloride (31. per 100g. of powder) for 18 hours. The extracts were clarified on the centrifuge and the polysaccharides precipitated by the addition of 3 volumes of ethanol with stirring. The precipitated polysaccharide gave a strong blue coloration with iodine indicating the presence of starch. The starch contaminant was removed by redissolution of the precipitated polysaccharide in water (11.) and fractional precipitation of the polysaccharides by additions of ethanol. The polysaccharide remaining after addition of 1 volume of ethanol gave no positive iodine test for amylose and after hydrolysis, gave D-mannose and D-glucose in the molar ratio 1:1.99.

$[\alpha]_D, -23.5^{\circ}(\pm 2.4^{\circ})$ (C. 0.85 in water)

Yield, 18g.

Tritylation of L. henryii Glucomannan.

Glucomannan (9g.) was treated under the tritylation conditions previously described. After reprecipitation of the crude product from chloroform solution, the tritylated glucomannan was collected on sintered glass, washed with methanol and ether and air-dried (18.7g.)

$[\alpha]_D, +14.7(\pm 2.4^\circ)$ (C. 0.82 in chloroform)

OTr, 64.4% (Calc. for $[C_6H_9O_4(OC_{19}H_{15})]_n$, 64.2% i.e. one triphenylmethoxyl group per D-glucose residue)

Tosylation of Mono-O-trityl Glucomannan.

Tosylation of the mono-O-trityl glucomannan (9g.) under the conditions described gave a buff product (12.2g.) having

$[\alpha]_D, -9.9^\circ(\pm 3.4^\circ)$ (C. 0.61 in chloroform)

OTr, 52.6% (Calc. for $[C_6H_8O_3(OC_{19}H_{15})(OSO_2C_7H_7)]_n$, 46.4%)

S, 3.59% (Calc. for $[C_6H_8O_3(OC_{19}H_{15})(OSO_2C_7H_7)]_n$, 5.73%)

The reaction time was extended to 11 days without significant increase in the tosyl content.

Quantitative examination of the mono-O-trityl O-tosyl glucomannan for chlorine (Lassaigne sodium test(128)) gave a completely negative result indicating the virtual absence of chlorinated residues.

Examination of the infra-red spectrum of the mono-O-trityl O-tosyl glucomannan showed the characteristic absorption maximum at 1180 cm^{-1} in the O-S stretching range for O-sulphonates.

Detritylation of Mono-O-trityl O-tosyl Glucomannan.

O-Tosyl glucomannan (5.2g.) was obtained by the usual detritylation procedure (p.48) on mono-O-trityl O-tosyl glucomannan (10.2g.). The O-tosyl glucomannan (insoluble in water, soluble in aqueous dioxan) showed an absorption maximum in the infra-red at 1180 cm^{-1} indicating the presence of the O-sulphonate grouping.

S, 6.51% (Calc. for $\text{C}_6\text{H}_9\text{O}_4(\text{OSO}_2\text{C}_7\text{H}_7)$ S, 10.13%)

Acid Hydrolysis of O-tosyl Glucomannan.

O-Tosyl glucomannan (5.0g.) was dissolved in 15% aqueous dioxan and the solution rendered approximately N with respect to sulphuric acid by addition of concentrated sulphuric acid (4ml.) in 15% aqueous dioxan (66ml.). The mixture was heated on a

water bath under reflux; samples (1ml.) of the dark brown solution were withdrawn at intervals and the optical rotation measured after 10-fold dilution.

Time(mins.)	10	110	280	360	600	1380
Optical Rotation	-0.04°	0.00°	+0.04°	+0.05°	+0.06°	+0.06°

100°

85-90°

Hydrolysis was allowed to proceed for 10 hours at 100° when the rotation became constant and heating (at 85-90°) continued for a further 13 hours without further change in rotation. The dark-brown solution was cooled, baryta water added with vigorous stirring/^{till} pH 3-4 was attained and the pH finally adjusted to 7 by addition of solid barium carbonate. The resulting suspension was centrifuged at 1700r.p.m. giving a brown residue. The centrifugate ^{was} / evaporated to dryness and extracted with hot methanol (3x150ml.). The colloidal extract was centrifuged at 5000r.p.m. and the amber supernatant concentrated (5.0g.). Extraction of the resulting amber glass with chloroform (3x150ml.) gave fractions I and III.

Fraction I (chloroform insoluble) was obtained as an amber glass on drying in vacuo over phosphorus pentoxide (2.9g.).

Fraction III (chloroform soluble) was obtained by evaporation of the chloroform extract (1.6g.).

Fraction II was obtained by extraction of the barium sulphate/barium carbonate residue (from neutralisation) with a mixture of chloroform and methanol(1:1 v/v) and evaporation of the extract to dryness (0.5g.).

Fraction IV resulted from extraction of the methanol insoluble residue with pyridine. Filtration of the pyridine extract through a filter aid and evaporation of the filtrate gave a brown powder which failed to redissolve in any common solvent (1.2g.).

Preliminary Examination of the Fractions.

Fraction I (2.9g.). Chromatographic examination of the syrup (solvent E) indicated the presence of mannose and glucose with unidentified spots at R_f , 0.33-0.56 (elongated) (probably oligosaccharide tosylates) and R_f , 0.65-0.82 (probably monosaccharide tosylates).

Fraction II (0.5g.). The fraction appeared to contain the same components as fraction I; distinct spots at R_f , 0.78 and 0.85 were observed however. The material was not further examined.

Fraction III (1.6g.). The syrup partially crystallised on standing. Recrystallisation of the crude crystals from chloroform/methanol gave colourless prisms (0.8g.) m.p. and mixed m.p. (with triphenylcarbinol) 160-163°. The infra-red spectrum was also identical with that of an authentic sample of triphenylcarbinol. While the crystals were Molisch negative and did not react with aniline hydrogen phalate on paper chromatograms, the residual syrup was Molisch positive and when run on chromatograms in solvent E two distinct spots were observed travelling close to the solvent front $R_f = 0.81$ and $R_f = 0.85$. The material was not further examined.

Fraction IV (1.2g.). The identity of the material was not confirmed. The Molisch test for carbohydrate proved negative and it is possible that the material is derived from a polymerisation of decomposition products of dioxan. The fraction was not further examined.

Reduction and Detosylation of Fraction I.

Fraction I (2.3g.) was reduced with potassium borohydride and detosylated with sodium amalgam under the conditions employed for the same reactions on the monosaccharide model compounds. Yield of mixed oligosaccharide glycitols (0.7g.).

Preliminary Fractionation of Oligosaccharide Glycitols on Ion-exchange Resin.

Fractionation of the mixture of oligosaccharide glycitols (0.7g.) on a column of Dowex 50Wx8 resin (Ba⁺⁺ form, 200-400 mesh, 97x2.6cm.) (129) gave a preliminary although incomplete separation of the components. 1.5ml. fractions were collected on an automatic fraction cutter; every third tube was evaporated to dryness and the syrup chromatographed. Fractions containing the same components were combined and further separations effected on Whatman 3MM paper sheets. In this manner the main oligosaccharide glycitols were obtained as below. The chromatographically pure syrups were subjected to complete and partial acid hydrolyses and the products identified by chromatography in solvents C and F.

Table 10.

	Yield	R _m	Products of complete acid hydrolyses	Products of partial acid hydrolyses	Possible identity
OG ₁	16mg.	0.65	G, Mannitol	-	G-Mol
OG ₂	26mg.	0.52	M, Mannitol	-	M-Mol
OG ₃	20mg.	0.42	M, Glucitol	-	M-Gol
OG ₄	5mg.	0.29	G, Glucitol	G-G	G-G-Gol
OG ₅	4mg.	0.24	M, G, Mannitol	G-M	G-M-Mol
OG ₆	8mg.	0.14	M, G(2:1), Glucitol	M-M, M-G	M-M-G-Gol
OG ₇	9mg.	0.10	M, G(2:1), Glucitol	G-M, M-M	G-M-M-Gol

G=D-glucose, M=D-mannose, Gol=D-glucitol, Mol=D-mannitol.

Discussion

In order to assess the applicability of the various stages in the proposed degradation of the glucomannan, the behaviour of a few model compounds was examined.

Although methyl 2-O-tosyl- α -D-glucoside remained resistant to acid hydrolysis by the agency of 1.5N sulphuric acid, methyl 3-O-tosyl- α -D-mannoside (kindly supplied by Dr. J.C.P. Schwarz) was rapidly hydrolysed to the corresponding free aldose without concomitant removal of the tosyl group.

The 3-O-tosyl-D-mannose so obtained was reduced to the glycitol, 3-O-tosyl-D-mannitol, by potassium borohydride (130); the tosyl group again remained unreactive under the reaction conditions as indicated by the appearance of only a single spot on paper chromatograms and by the detection of the characteristic absorption maximum (1180 cm.^{-1}) for O-sulphonates. Methyl 2-O-tosyl- α -D-glucoside treated under similar conditions was unattacked and the starting material was recovered in 90% yield.

In view of the facile epoxide formation in 1,2-diol mono-O-sulphonate systems (such as obtain in 2- or 3-O-tosyl-D-glucose residues in the substituted glucomannan) it seemed undesirable to effect detosylation under the usual alkaline conditions associated with the sodium amalgam procedure. Consequently the reductive detosylation was carried out under conditions similar to those applied in sodium amalgam reduction of lactones (131) (pH maintained ca. 3-4).

Treatment of 3-O-tosyl-D-mannitol with sodium amalgam at pH 3-4 afforded only D-mannitol (identified by chromatographic mobility, m.p. and mixed m.p. with authentic D-mannitol); similar treatment of methyl 2-O-tosyl- α -D-glucoside yielded only methyl- α -D-glucoside (identified by comparison of chromatographic mobility, m.p. and mixed m.p. and infra-red spectrum with those of authentic methyl- α -D-glucoside).

Glucomannan was extracted from L. henryii bulbs by a modification of the method of Andrews, Hough and Jones(48), ($[\alpha]_D$, -23.5° mannose : glucose ca. 2:1). Tritylation (under the conditions employed for the tritylation of amylose) yielded a substituted polysaccharide corresponding closely to a mono-O-trityl glucomannan ($[\alpha]_D$, 4.7° OTr, 64.4%, calc. for $[C_6H_9O_4(OC_{19}H_{15})]_n$ OTr, 64.2%). Tosylation of the mono-O-trityl glucomannan was effected by the method described for tosylation of trityl amylose. The product had $[\alpha]_D$, -9.9° : OTr, 52.6%; S, 3.59%; calc. for $C_6H_8O_3(OC_{19}H_{15})(OSO_2C_7H_7)$ OTr, 46.4%; S, 5.73%. Extension of the reaction time produced no significant increase in the extent of tosyl substitution; hence it may be assumed that at least some of the hexose residues/

are held in conformations unfavourable for the entry of a sulphonyl group at C₂ or C₃. (The possibility that the low sulphur value had resulted from chlorination via the well-known replacement side-reaction of sulphonates on prolonged treatment with sulphonyl chlorides in pyridine media (132)



was dismissed on the grounds of the failure to detect halogen in qualitative tests).

The mono-O-trityl O-tosyl glucomannan was detritylated as already described. Hydrolysis of the resulting O-tosyl glucomannan in 15% aqueous dioxan (N with respect to sulphuric acid) was effected on a boiling water-bath until the optical rotation became constant (10hrs.) and heating was continued at 85° for a further 13 hours without change in rotation.

Three carbohydrate-containing fractions were isolated from the products of hydrolysis as described in the flow-sheet (p. 116). Fraction II appeared to have approximately the same composition as Fraction I, but was not further examined. Fraction III was obtained as a crystalline solid and the infra-red spectrum was identical with that of authentic triphenylcarbinol; (this probably derives from incomplete detritylation of the mono-O-trityl O-glucomannan). Fraction IV after isolation resisted solubility in all solvents and was not further examined. (Molisch

test was negative).

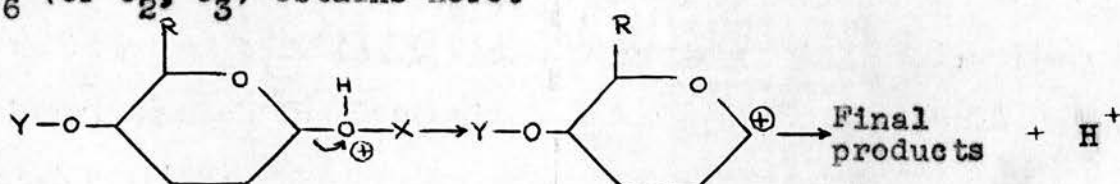
Fraction I. The syrup contained mannose and glucose together with faster moving sugars presumably tosylated oligosaccharides (R_f 0.3 - 0.6) and tosylated monosaccharides (R_f 0.78 and 0.85). This mixture was reduced with potassium borohydride by the procedure already adopted and the resulting glycitols detosylated as described for the detosylation of 3-O-tosyl-D-mannitol and methyl 2-O-tosyl- α -D-glucoside. Preliminary separation of the glycitol mixture was effected on ion exchange resin (Dowex 50 W x 8) (129) and sub-fractionation on cellulose filter sheets in solvents A and E gave several chromatographically pure syrups. Preliminary investigation has indicated the presence of a mannosyl glucitol, mannosyl mannitol and a glucosyl mannitol. Cellobiitol was not detected. Although the nature of the linkages between the aldose and glycitol residues has not been experimentally determined, it is probable that the normal 1,4/ β - linkage exists. Chromatographic identification of the products ^{of} complete and partial acid hydrolysis of the higher oligosaccharide glycitols has further confirmed that the hydrolysis is non-specific since intact D-mannosidic links occur; complete separation of these glycitols may not have been achieved however and structural identification has not yet been attempted.

The results show that the anticipated selectivity of hydrolytic attack was not achieved in practice. Thus the isolation of a manno bitol (probably 4-O- β -D-mannopyranosyl-D-mannitol) and a mannosyl glucitol (probably 4-O- β -D-mannopyranosyl-D-glucitol) suggest that the mannosidic links are rendered stable to acid hydrolysis in the same manner as expected for glucosidic bonds. This would necessitate the preferred entry of a tosyl group at C₂ on a mannopyranose unit (as distinct from the C₃ substitution encountered in monosaccharide analogues where the pyranose ring is in the C1 conformation (125)). Although no direct evidence exists, it seems probable by analogy with cellulose (133) and amylose (134) that the residues in the 1,4-linked glucomannan chain also exist preferentially in the C1 conformation.

Recent work (135) has indicated that the reactivity of hydroxyl groups toward sulphonylating agents may depend on the configuration at the anomeric centre; thus while mono-O-tosylation of methyl 4,6-O-benzylidene- α -D-glucoside occurs almost exclusively at C₂ (119, 120) it appears that C₃ is the more favoured position in the corresponding β -anomer. Such a preference for tosylation at C₃ in the β -D-glucose

residues of the glucomannan would fail to cause stabilisation of the glucosidic links.

The mechanism involved in the stabilising effect of 2-O-sulphonyl substituents is uncertain but has been noted on several occasions (124). While steric and inductive effects are both probable, the latter appears to be more important since the size of the group (eg. methanesulphonyl, benzene sulphonyl, toluene-*p*-sulphonyl) has little influence on the effectiveness. Presumably the inductive mechanism suggested by Bunton et al. (136) to account for the stabilisation of glycosidic bonds by electrophilic substituents on C₆ (or C₂, C₃) obtains here.



where R is electrophilic eg. -C=O or -COOH the electron shift is opposed.

It is noteworthy that the electrophilic substituent responsible for the stabilisation of O - X simultaneously activates the bond Y - O by virtue of the assistance offered in effecting the electron shift (137). Thus there must exist ^{the} ~~and~~ possibility ^{of} that a degree of 'reactivation' of the glycosidic bonds in mono-O-tosylated 1 → 4 linked chains such as the tosylated glucomannan examined here.

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