THE CHEMISTRY OF WOOD-CELL WALL POLYSACCHARIDES,

WITH SPECIAL REFERENCE TO

THE GALACTAN COMPONENTS.

by

R. M. FAIRWEATHER, B.Sc.

A thesis presented for the degree of Doctor or Philosophy.

University of Edinburgh.

November, 1964.

To KATE AND MY PARENTS.

C O N T E N T S.

page.

page.

EXPERIMENTAL

Bibliography /

ACKNOWLEDGMENTS

I wish to express my sincere debt of gratitude to Professor Sir Edmund Hirst, C.B.E., F.R.S., for his interest in this work and for providing laboratory facilities, and to Dr. G.O. Aspinall for his invaluable advice and encouragement during the course of this research.

I would like to take this opportunity of thanking the many research workers in the department for their helpful discussion, my fiancée for her secretarial help, and Miss J.S. Hunter, Mr. E. Kirkpatrick and Mr. J. Macpherson for the typing and binding of this thesis.

Finally, I would like to thank Messrs. Alex. Cowan and Sons, Ltd., for financial assistance.

INTRODUCTION

The major constituent of plant cell-walls is cellulose. The long threadlike macromolecules of cellulose are arranged in bundles which in certain areas are so well organised, due to hydrogen bonding between free hydroxyl groups in the (1 - 4) - linked glucopyranose residues of adjacent molecules, that crystallinity is observed (1). Other areas are more amorphous in nature and appear to be rich in lignin and non-cellulosic polysaccharides known as 'hemicellulose'. The exact role of these polysaccharides is not known, for while they undoubtedly have a strengthening effect on the cell-wall, many appear to be concerned with metabolic processes.

In spite of much work, the question of whether there is a lignin-hemicellulose linkage is still not settled (2). While many investigators believe that the differences in solubility of these polysaccharides before and after extraction is due to chemical combination with lignin (3 - 9), others believe that the differences can be ascribed to a physical restraint which the individual polymers impose on the solution of each other and especially to the restraint which lignin imposes on the polysaccharides by interpenetration (10 - 12).

Recent evidence in favour of the first theory has been provided by Lindgren who examined electrophoretically a lignin-containing /

lignin-containing polysaccharide from Norway spruce (13). Nelson and Schuerch, however, have shown that the quantity of hemicellulose which can be extracted from birchwood increases as the material is ground up and decreases as the cell-walls are swollen (14).

Extraction

Before the hemicellulose in wood can be extracted, lipids and resins must be removed and this is best done by extraction with an azeotropic mixture of benzene and ethanol (15). Water-soluble hemicelluloses such as larch arabinogalactans and low molecular weight carbohydrate material can then be extracted with hot or cold water. As lignin retards or prevents the complete solution of the alkali-soluble polysaccharides, the material is delignified prior to polysaccharide extraction. This is done by rendering the lignin water-soluble by mild treatment with chlorine (16), sodium chlorite (17) or chlorine dioxide, pyridine and water (18). The material left, known as holocellulose, usually contains about 3% lignin but this does not seem to interfere with the extraction of the hemicelluloses.

The usual extractant is aqueous sodium or potassium hydroxide although sodium carbonate solutions have been used (19,20). The holocellulose is repeatedly extracted with increasing concentrations of extractant (up to 17.5% for sodium hydroxide) at room temperature in an atmosphere of nitrogen to minimise alkaline /

alkaline degradation.

Fractionation

The hemicellulose extract will, in all probability, contain several polysaccharides, and before structural studies can begin the extract must first be fractionated into its component polysaccharides. Heterogeneity may arise from (a) polysaccharides with different structural units (b) polysaccharides with the same structural units but present in different proportions (c) polysaccharides with the same structural units but linked in different ways (d) polysaccharides with the same repeating units but with different molecular weights.

The most common method of fractionation is fractional precipitation, with organic precipitants, of the polysaccharides (21) or their methylated (22) or acetylated (23) derivatives. Another method is graded precipitation from an aqueous solution by the addition of ammonium sulphate (24).

Many polysaccharides form insoluble salts or complexes with copper (25-28) and cetyltrimethylammonium bromide (29,30). The latter was first used to fractionate acidic polysaccharides, but recently the method has been extended to neutral polysaccharides which form complexes with boric acid (31). By using the corresponding base, cetyltrimethylammonium hydroxide, as precipitant on the polysaccharide-boric acid complex, Bouveng and Lindberg have separated /

separated two neutral arabinogalactans from Western larch (32). This procedure has since been used for the fractionation of Japanese larch arabinogalactan (33).

A newer method for polysaccharide fractionations is chromatography on ion-exchange cellulose such as diethyl amino ethyl - (D.E.A.E) - cellulose (34), and ECTEOLA - cellulose (35). These ion-exchange celluloses, in various forms (phosphate, borate etc.) retain acidic polysaccharides but not neutral polysaccharides at neutral pH values, and by using suitable eluants at differing pH values and electrolytic concentrations, many polysaccharide mixtures have been fractionated (36). Recently, separations of acidic mucopolysaccharides have been effected by ion-exchange chromatography on D.E.A.E. Sephadex (37). This process is now being extensively used in the protein field and it should prove to be a valuable technique for effecting and assessing polysaccharide homogeneity.

The fractions obtained by one of the above methods are then examined for homogeneity by chromatography, electrophoresis or ultracentrifugation. Preece and Hobkirk have fractionated, on an analytical scale, several cereal water-soluble polysaccharide mixtures by electrophoresis on filter paper in borate and acetate buffers (38). Better results are obtained when glass-fibre paper (39) is employed as the inert support, as filter paper tends to complex with the polysaccharides and detection of the polysaccharides is difficult, due to the cellulose in the paper. Using this modification, Smith has shown that many polysaccharides which were /

were assumed to be homogeneous appear to be heterogeneous (40).

A method less frequently employed is ultracentrifugation (41). It is, however, particularly useful in determining the homogeneity of fractions from a mixture of polysaccharides of different molecular weights and was used by Lindberg and Bouveng in establishing the homogeneity of two arabinogalactans from western larch (32).

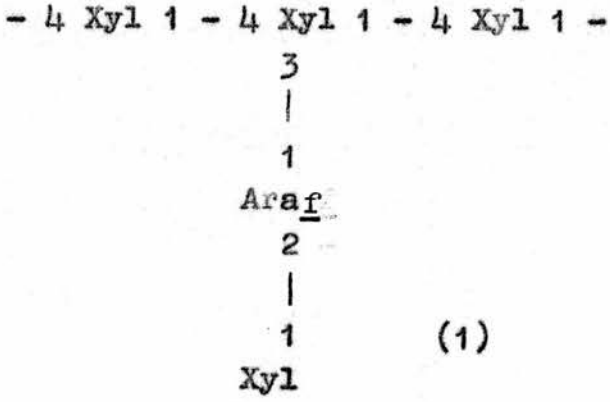
The most common polysaccharides found in hemicellular extracts are xylans, glucomannans and arabinogalactans. On hydrolysis these give rise to D - xylose, D - glucose, D - mannose, D - galactose, L - arabinose, L - rhamnose, D - glucuronic acid and 4-O-methyl - D - glucuronic acid.

Xylans

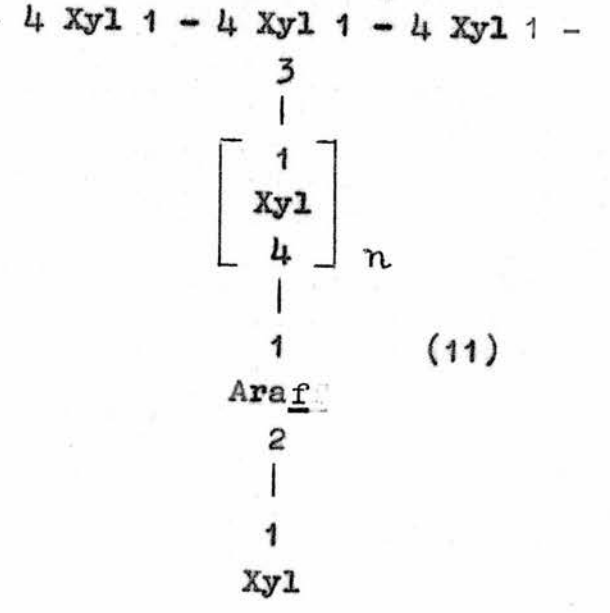
This group of polysaccharides is frequently found in cereals, grasses and woods. They all have a similar backbone composed of (1 - 4) - linked β - D - xylopyranose residues.

The xylans from cereals and grasses are characterised by the presence of L - arabinofuranose residues, linked to the backbone as single-unit side-chains, usually to position C 3 of D - xylose. In many cases, however, D - glucuronic acid or 4 - O - methyl - D - glucuronic acid or both are also present. These acid groups are usually attached to the xylan backbone by a (1 - 2) - linkage, although in one sample of wheat straw xylan (42) the

acidic residues are linked to the D - xylose units by a (1 - 3)- linkage and, in addition to the normally occurring (1 - 2)- linked aldobiouronic acids, an aldobiouronic acid containing a (1 - 4)- linkage has been isolated from corn cob xylan (43). A few cereal xylans contain non-terminal in addition to terminal L - arabino- furanose residues (44 - 51). In most cases there is yet insufficient evidence to show whether the acid-labile residues are attached directly to the main chain (1) or through one or more xylose residues (11).



(1)



(11)

Where /

Where it has been possible to differentiate between the two structures, structure (1) is favoured.

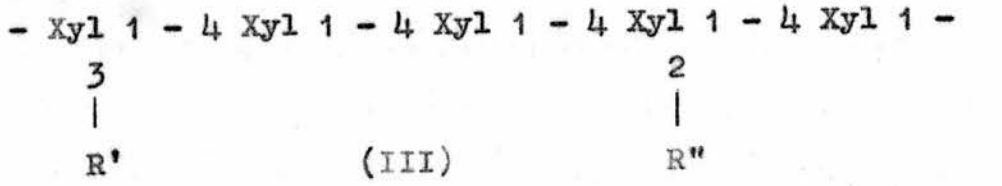
Xylans from hardwoods are characterised by the presence of 4 - O - methyl - D - glucuronic acid residues. These acid groups are attached as single-unit side-chains to approximately every tenth xylose residue in the main chain by a (1 - 2)- linkage. A xylan from trembling aspen was at one time thought to contain L - arabinofuranose end-groups (52) but a later publication has shown that an arabinose - containing polymer was present as a contaminant (53) and it is now thought that the hardwood xylans are devoid of arabinose.

The xylans from softwoods are characterised by the presence of both 4 - O - methyl - D - glucuronic acid and L - arabinose. As in the hardwood xylans, the acidic residues are present as single-unit side-chains and are attached to position C 2 of the xylose residues. The proportion of acidic residues is higher than in the hardwood xylans, there being approximately six xylose units present per acidic unit. The L - arabinofuranose residues could be attached either directly to the main chain or through one or more xylose residues. Where it has been possible to distinguish between these two possibilities, the evidence has always favoured direct attachment with the arabinose units linked, as in the cereal xylans, to position C 3 of the xylose residues.

In a few cases (54 - 58) there have been reports that not all the D - glucuronic acid units in the wood xylans are substituted at position C 4 with O - methyl groups. However a recent /

recent publication (59) has shown that the 4 - O - methyl - D - glucuronic acid residues are slightly demethylated under the conditions normally used for hydrolysis of these polysaccharides.

The general structural features of both the cereal and wood xylans are shown in structure (III).



R' = Araf1 - or Xyl1-2 Araf1

R" = 4 - Me - GA 1- or GA 1 -

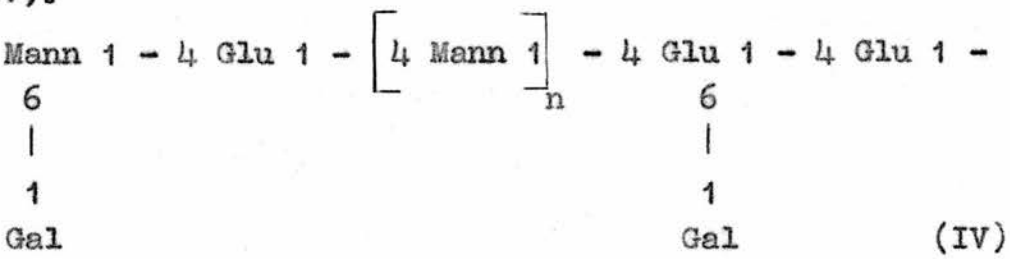
Glucomannans

Polysaccharides containing D-mannose and D- glucose as their main structural units occur in the seeds of various land plants and in woods, particularly the coniferous woods. The polysaccharides are essentially linear with blocks of (1 - 4)- linked β - D- mannopyranose residues separated by one or more (1 - 4)- linked β - D- glucopyranose residues. There are indications however, that two Norwegian spruce glucomannans (60, 61) and a glucomannan from spruce pulp (62) may be branched.

A characteristic feature of the softwood glucomannans is the presence of non-reducing D - galactopyranose end-groups. In many of these glucomannans the galactose residues constitute approximately 20 molar % pf the polysaccharides, while in others only trace amounts are present. In the galactose-rich glucomannans (galactoglucomannans) /

(galactoglucomannans) the D- galactose residues are attached as single-unit side-chains by a (1 - 6) - linkage to mannose, and probably glucose units, in the main chain. It is not yet known whether the galactose residues in the glucomannans which contain only trace amounts of galactose are attached directly to the main chain or through one or more mannose and/or glucose residues.

The general structural features of the glucomannans are shown below (IV).



(IV)

Arabinogalactans

Polysaccharides containing D - galactose and L - arabinose occur in many coniferous woods and are present in largest proportion in larch woods. These water-soluble arabinogalactans are all highly branched with (1 - 3)- and (1 - 6)- linked β - D - galactopyranose residues. The arabinose units are always situated in the periphery of the molecule. Although the proportion of arabinose varies from wood to wood, no galactans devoid of arabinose residues have been isolated.

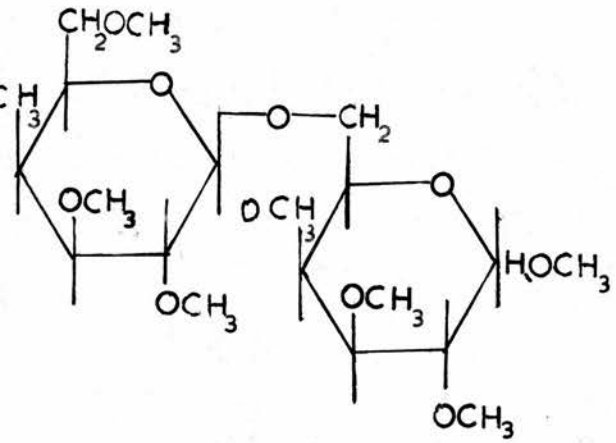
The most extensively studied arabinogalactans, or ζ - galactans, have been those from various larches, particularly Western larch (Larix occidentalis), European larch (Larix decidua) and /

and to a lesser extent Japanese larch (Larix leptolepis).

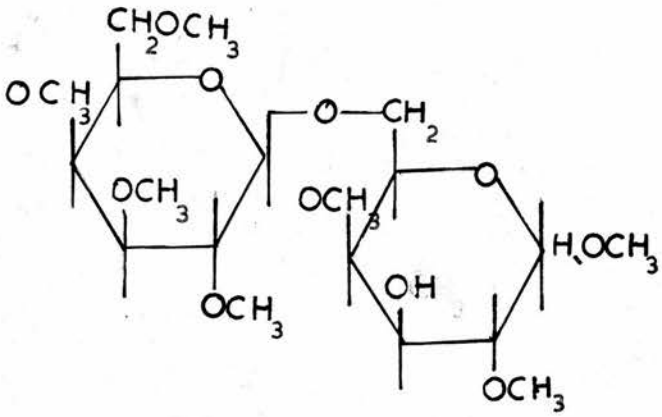
Western larch

Western larch ζ - galactan was first studied in detail by White (63-66). The polysaccharide was fully methylated and methanolysis followed by fractional distillation gave the methyl glycosides of 2, 3, 4, 6 - tetra-O - methyl - D-galactose (2 moles), 2, 3, 4 - tri - O - methyl - D - galactose (1 mole), 2, 4 - di - O - methyl - D - galactose (3 moles), and 2, 3, 5 - tri - O - methyl - L - arabinose (1 mole), indicating that the polysaccharide was highly branched with a galactan framework composed of (1 - 3)- and (1 - 6)- linked D - galactose residues (63). Quantitative analysis (63) gave the molar ratio of D - galactose to L - arabinose as six to one, and since only one species of methylated arabinose was found, White suggested that all the arabinose units were present as non-reducing furanose end-groups.

Partial methanolysis of the methylated ζ - galactan yielded in addition to the methylated methyl glycosides already mentioned, the methyl glycosides of
6 - O - (2,3,4,6 - tetra - O - methyl - β - D - galactopyranosyl) - 2,3,4 - tri - O - methyl - D - galactopyranose (V) and
6 - O - (2,3,4,6 - tetra - O - methyl - β - D - galactopyranosyl) - 2,4 - di - O - methyl - D - galactopyranose (VI), indicating the presence of fragments (VII) and (VIII) in the original polysaccharide (64)/



(V)



(VI)

Gal 1 - 6 Gal 1 -

(VII)

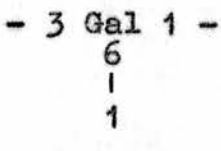
Gal 1 - 6 Gal 1 -
3
1

(VIII)

The degraded methylated polysaccharide gave on methanolysis the methyl glycosides of 2,4 - di - O - methyl - D - galactose (6 moles) and 2,3,4,6,- tetra - O - methyl - D - galactose (1 mole). The degraded material was remethylated and after methanolysis, the methyl glycosides of the following sugars were isolated :- 2, 3, 4, 6, - tetra - O - methyl - D - galactose (41.5 moles %), 2,3,4 - tri - O - methyl - D - galactose (18.7 moles %), 2, 4, 6 - tri - O - methyl - D - galactose (7.8 moles %) and 2, 4 - di - O - methyl - D - galactose (31.8 moles %). The large amount of 2,3,4 - tri - O - methyl - D - galactose compared with the 2,4,6 - isomer suggested that most of the 2,4 - di - O - methyl - D - galactose residues in the undegraded methylated material, were linked to each other by (1 - 6)- linkages /

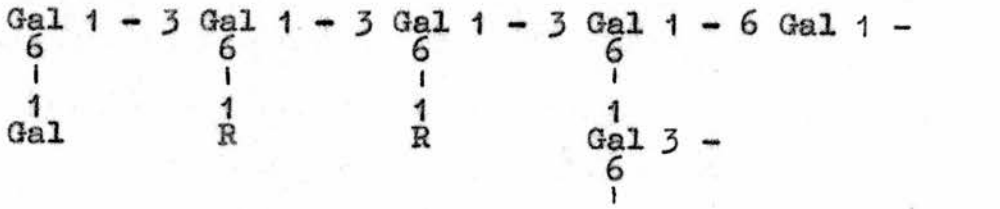
linkages (64,66).

Arabinogalactan was partially hydrolysed under very mild acid conditions in order to remove most of the arabinofuranose residues with relatively little modification to the galactan framework (65). The resulting degraded polysaccharide was methylated and on methanolysis the methyl glycosides of 2,3,4,6 - tetra - O - methyl - D galactose (2 moles), 2,3,4 - tri - O - methyl - D - galactose (1 mole), 2,4,6 - tri - O - methyl - D - galactose (2 moles) were isolated. The appearance of 2,4,6 - tri - O - methyl - D - galactose together with the decrease in amount of 2,4 - di - O - methyl - D - galactose indicated that the L - arabinofuranose residues were attached to position C6 of (1 - 3)-linked β - D - galactose residues (IX).



Araf (IX)

On the basis of these results White proposed the following structure :-



R = Gal or Araf

The /

The arabinogalactan from Western larch (32,67-70) has recently been studied by Bouveng and Lindberg. They showed that under mild conditions of hydrolysis two disaccharides, 3 - O - β - L - arabinopyranosyl - L - arabinose (X) and 6 - O - β - D - galactopyranosyl - D - galactose (XI) were released (67).

Ara p 1 - 3 Ara (X) Gal 1 - 6 Gal (XI)

The isolation of the arabinobiose conflicted with White's methylation results which had shown no indication of either non-terminal arabinose units or terminal arabinopyranose residues. The ease of removal of the galactobiose suggested that some galactofuranose residues were present. This, however, has not been supported by methylation evidence (32,69) and it is now thought that a portion of the (1 - 6) - galactopyranose linkages are weaker than others due to steric factors (68).

In a later publication it was shown that ξ - galactan from the heartwood of Western larch could be separated into two arabinogalactans (A and B) by fractional precipitation of the polysaccharide - boric acid complex with cetyltrimethylammonium hydroxide (32). The two fractions differed in sedimentation velocity and electrophoretic mobility but had approximately the same composition. The molecular weights were of the order of 100,000 for fraction A and 16,000 for fraction B. It is of interest to note that in ultracentrifugal studies on European larch ξ - galactan, Mosimann and Svedberg found two components with /

with similar molecular weights (21). When arabinogalacton A was subjected to prolonged mild acid hydrolysis to remove the arabinofuranose residues, two polymeric galactans were released (68). The two fractions (A' and A") had different electrophoretic mobilities and could easily be separated by fractional precipitation with cetyltrimethylammonium hydroxide on the galactan-boric acid complex. Methylation studies on both subfractions showed that they contained the same types of linkage but in different proportions. This suggested that the two galactans had originated from two polysaccharides. It is possible however, that both fractions corresponded to two fragments of the same molecule which were joined together by a weak galactopyranose linkage of the type already mentioned. Arabinogalactan B was degraded in the same manner to yield a single component with the same electrophoretic mobility as the original polysaccharide.

The major (A) fraction and the minor (B) fraction were both methylated (32,69) and a comparison of the methyl ethers isolated from each fraction (Table I) showed that both polysaccharides had similar galactan frameworks. The arrangement of L - arabinose units however was rather different. In fraction A, two thirds of the L arabinose was present as terminal non-reducing L - arabinofuranose residues and one third as 3 - O - β - L - arabinopyranosyl - L - arabinofuranose residues. The amount of 3 - O - β - L - arabinopyranosyl - L - arabinofuranose was higher in fraction B and since there was an excess of 2,5 - di - O - methyl - L - arabinose over 2,3,4 - tri - O - methyl - L - arabinose, Bouveng suggested /

suggested that some of the arabinose could be accommodated as 3 - O - β - L - arabinofuranosyl - L - arabinofuranose residues. The isolation of 2 - O - methyl - D - galactose and 2,6 - di - O - methyl - D - galactose strongly indicated the existence of (1 - 4)-linkages, although their presence could be due to incomplete methylation or demethylation during hydrolysis.

On treatment with sodium periodate followed by a phenylhydrazine and acetic acid degradation as described by Barry et al. (71), arabinogalactan B gave glyoxal bis phenylhydrazone, glyceroosazone, L - arabinoosazone and a polysaccharide fraction from which one third of the non-oxidisable arabinose residues had been removed. Partial hydrolysis of the degraded polysaccharide afforded 3 - O - β - D - galactopyranosyl - D - galactose with smaller amounts of the (1 - 6) - linked isomer. It is unlikely that the presence of arabinoosazone was due to direct hydrolysis of the arabinofuranose residues during the degradation as no arabinoosazone was found among the products formed on degradation of periodate-oxidised arabinogalactan A. The results show rather that one third of the non-oxidisable arabinose residues were attached to oxidisable galactose residues (XII), and that direct hydrolysis and subsequent osazone formation of the arabinofuranosyl-glyceroosazone, produced on degradation of the oxidised polysaccharide, gave rise to arabinoosazone and glyceroosazone. The remaining two thirds of the non-oxidisable arabinose residues were linked as in (XIII).

Arap 1 - 3 Araf 1 - 6 Gal 1 - (XII)

Arap 1 - 3 Araf 1 - 6 Gal 1 - (XIII)
3
1

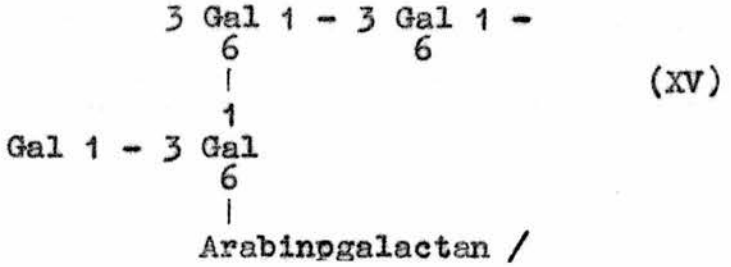
The /

The degraded polysaccharide was subjected to a second periodate oxidation and Barry degradation. Partial hydrolysis of the resulting polysaccharide gave 3 - O - β - D - galactopyranosyl - D - galactose with only trace amounts of the (1 - 6) - linked isomer, indicating that the polysaccharide possessed a periodate-resistant backbone of (1 - 3) - linked - β - D - galactopyranose residues.

The polysaccharide isolated after the first degradation was fully methylated and on hydrolysis gave 2,3,5 - tri - O - methyl - L - arabinose (6.5%), 2,5 - di - O - methyl - L - arabinose (1.7%), 2,3,4,6 - tetra - O - methyl - D - galactose (21.6%), 2,4,6 - tri - O - methyl - D - galactose (38.9%), 2,3,4 - tri - O - methyl - D - galactose (3.3%), 2,6 - di - O - methyl - D - galactose (4.8%) 2,4 - di - O - methyl - D - galactose (18.5%), 4 - O - methyl - D - galactose (1.6%) and 2 - O - methyl - D - galactose (3.2%). The large proportion of 2,4,6 - tri - O - methyl - D - galactose was further evidence in favour of the (1 - 3) - linked backbone. As non-terminal arabinofuranose units were still present, residues containing three arabinose units must have occurred in the original polysaccharide (XIV).

Arap 1 - 3 Araf 1 - 3 Araf 1 - (XIV)

The high proportion of 2,3,4,6 - tetra - O - methyl - D - galactose could only be accounted for if most of the terminal units in the degraded polysaccharide originated from doubly branched D - galactose residues in the original polysaccharide (XV).



(XV)

Arabinogalactan A was oxidised and degraded in a similar manner to give a degraded polysaccharide which on partial hydrolysis gave 3 - O - β - D - galactopyranosyl - D - galactose as the main product with only trace amounts of the (1 - 6)-linked isomer. The absence of arabinoosazone indicated that all the non-oxidisable arabinose units were attached to periodate-resistant residues as in (XIII) and (XIV).

European larch

The first detailed structural studies on European larch arabinogalactan were carried out by Campbell, Hirst and Jones (22), who showed that the polysaccharide contained D - galactose and L - arabinose in the molar ratio of six to one. Fractional precipitation of the methylated ζ - galactan with petroleum ether gave two components. The main fraction gave on hydrolysis equimolecular amounts of 2,3,4,6 - tetra - O - methyl - D - galactose, 2,3,4 - tri - O - methyl - D - galactose and 2,4 - di - O - methyl - D - galactose. The minor fraction was rich in arabinose and on hydrolysis gave 2,3,5 - tri - O - methyl - L - arabinose as the only methylated arabinose derivative.

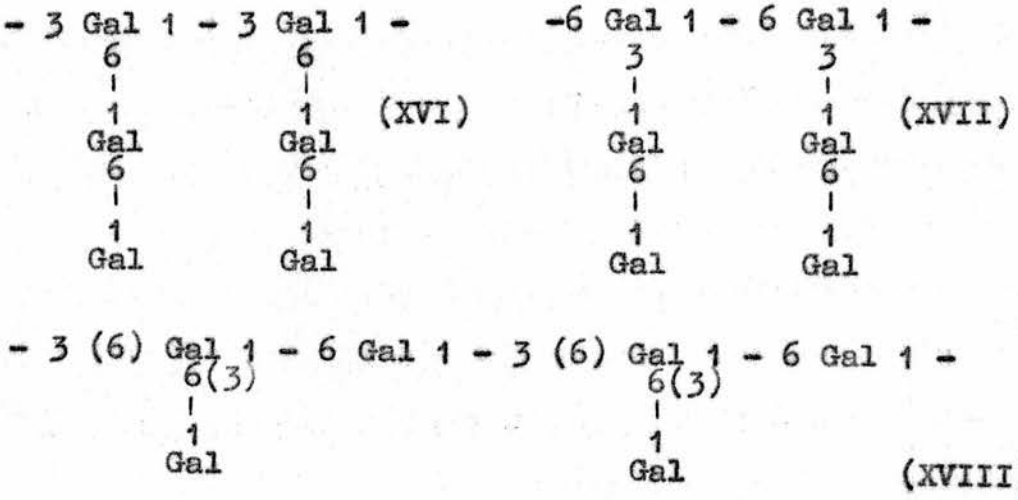
Unfractionated ζ - galactan was partially hydrolysed under mild acid conditions to remove the acid-labile arabinofuranose residues. The degraded material was methylated and on hydrolysis yielded, in addition to the three methylated galactoses mentioned above, 2,4,6 - tri - O - methyl - D - galactose. They concluded that the ζ - galactan was a mixture of polysaccharides, containing /

containing (a) a true galactan and (b) a galactoaraban or an araban and a second galactan differing in structure from the one just mentioned.

More recent investigations by Aspinall et al., however, failed to confirm the above results regarding heterogeneity (72). Both immunological studies (73) and ultracentrifugal measurements (72) indicated that the polysaccharide was homogeneous. Methylation and hydrolysis of ζ - galactan gave equimolecular proportions of 2,3,4,6 - tetra - O - methyl - D - galactose, 2,3,4 - tri - O - methyl - D - galactose and 2,4 - di - O - methyl - D - galactose together with smaller amounts of 2,3,4 - tri - O - methyl - L - arabinose, 2,5 - di - O - methyl - L - arabinose, 2,4,6 - tri - O - methyl - D - galactose and 2 - O - methyl - D - galactose. As only trace amounts of 2,3,5 - tri - O - methyl - L - arabinose were found, it was suggested that most of the arabinose residues were accommodated as 3 - O - β - L - arabinopyranosyl - L - arabinofuranose units. This was in agreement with an earlier finding by Jones who isolated 3 - O - β - L - arabinopyranosyl - L - arabinose as a product from partial hydrolysis of the polysaccharide (74). However, a subsequent examination by gas - liquid chromatography of the methanolysis products from methylated ζ - galactan showed that approximately one third of the arabinose units were present as terminal non-reducing L - arabinofuranose residues (75).

From the above methylation results three possible partial structures for the galactan framework were proposed (XVI, XVII, XVIII)./

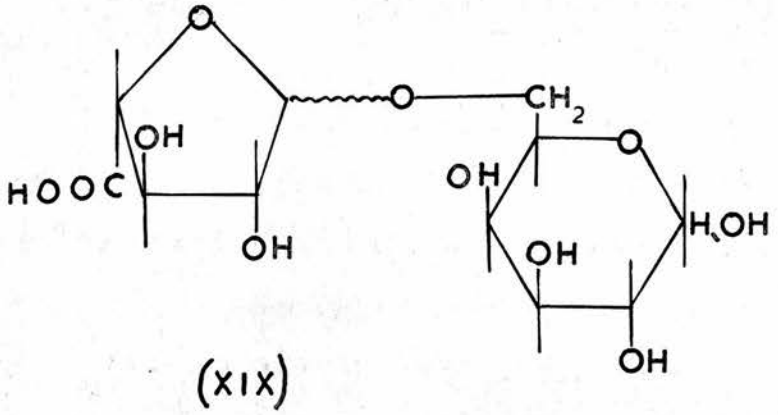
XVIII).



Periodate oxidation followed by a Barry degradation gave a degraded polysaccharide which on partial hydrolysis afforded 3 - O - β - D - galactopyranosyl - D - galactose and a trace of the (1 - 6) - linked isomer. Structure (XVIII) was immediately eliminated as only isolated periodate-resistant residues would be present. Structure (XVII), on partial hydrolysis, would have yielded 6 - O - β - D - galactopyranosyl - D - galactose as the main product. Further evidence in favour of structure (XVI) came from an examination of the partial hydrolysis products from undegraded ζ - galactan, when a galactotriose, probably O - β - D - galactopyranosyl - (1 - 3) - O - β - D - galactopyranosyl - (1 - 3) - D - galactose was isolated. Such a trisaccharide could only have arisen from a polysaccharide with a molecular structure as in (XVI). By selective catalytic oxidation of the primary hydroxyl groups in the arabinofuranose and terminal non-reducing galactose residues to carboxylic acid groups, Aspinall and Nicholson isolated among the products from partial hydrolysis of the oxidised polysaccharide, two aldobiouronic acids,

one /

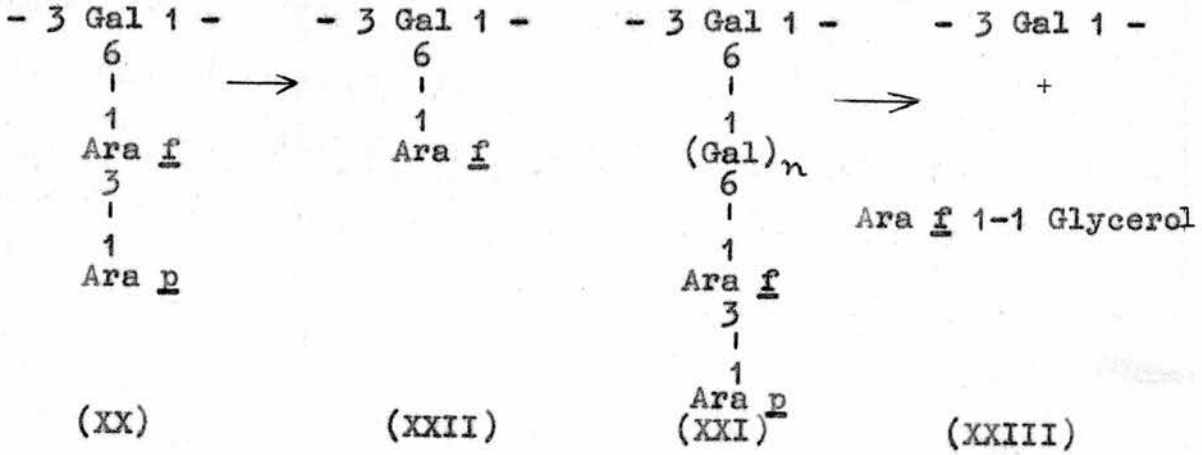
one of which was characterised as 6 - O - (L - arabinofuranosyl-uronic acid) - D - galactose (XIX) (76).



(XIX)

This was the first definite proof that arabinofuranose residues were attached to the D - galactose units by a (1 - 6) - linkage, for although previous investigations had indicated that such a linkage was probable (65,22), the evidence was based on methylation studies on degraded and unde graded ζ - galactan and the presence of large amounts of 2,4,6 - tri - O - methyl - D - galactose in the degraded material could have been due to the rupture of 6 - O - β - D - galactopyranose linkages, particularly since some of them have been shown to be weaker than others (68).

Two possible partial structures were advanced to indicate the mode of attachment of the arabinofuranose residues to the galactan framework (XX,XXI).



The /

The two partial structures could be distinguished by treatment with periodate followed by borohydride reduction and cold hydrolysis as described by Smith et al. (77). Structure (XX) would give among the degradation products, a polysaccharide containing both D - galactose and L - arabinose (XXII) whereas structure (XXI) would furnish a pure galactan and arabinofuranosylglycerol (XXIII) together with other low molecular weight products.

On treatment with sodium periodate followed by a Smith degradation, ζ - galactan gave a degraded polysaccharide which, on hydrolysis gave D - galactose and L - arabinose. A chromatographic examination of the low molecular weight products showed that a very small amount of arabinofuranosylglycerol was present indicating that while most of the arabinofuranose residues were attached directly to the main chain (XX), a small proportion were linked through one or more galactose residues (XXI).

Japanese larch

A water-soluble arabinogalactan from Japanese larch has recently been studied by Aspinall and Wood (33). Electrophoretic and ultracentrifugal examinations showed that the ζ - galactan was heterogeneous and contained two polysaccharides. These were separated by fractional precipitation of the polysaccharide-boric acid complex with cetyltrimethylammonium hydroxide. Like Western larch /

larch ζ - galactan, both fractions differed in electrophoretic mobility and sedimentation velocity but had the same D - galactose to L - arabinose ratio, namely, six to one.

Examination of the hydrolysis products from methylated arabinogalactan A by paper chromatography and gas-liquid chromatography showed that the polysaccharide was very similar to other larch ζ - galactans. Approximately equal molecular proportions of 2,3,4,6 - tetra - O - methyl - D - galactose, 2,3,4 - tri - O - methyl - D - galactose and 2,4 - di - O - methyl - D - galactose were found together with smaller amounts of 2,3,5 - tri - O - methyl - L - arabinose, 2,5 - di - O - methyl - L - arabinose and 2,3,4 - tri - O - methyl - L - arabinose. Only trace quantities of 2,6 - di - O - methyl - D - galactose, 2 - O - methyl - D - galactose and/or 2,4,6 - tri - O - methyl - D - galactose were observed.

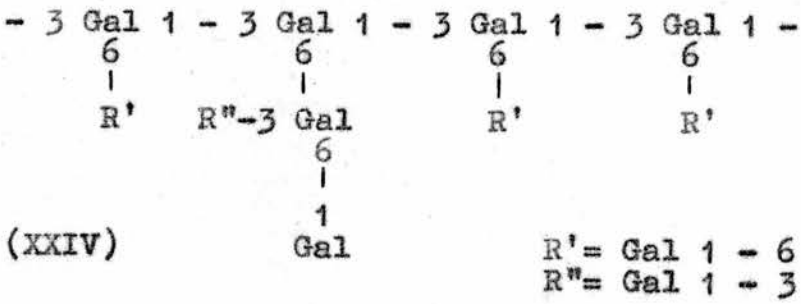
Periodate oxidation of fraction A, followed by a Smith degradation gave a degraded polysaccharide which contained arabinose and galactose in the ratio of one to ten. As no monosaccharides were detected on a chromatographical examination of the reduced, hydrolysed, low molecular weight, degradation products, all the non-oxidisable arabinofuranose residues must have been linked to periodate-resistant galactose residues as in (XIII).

The main oligosaccharide found in the hydrolysate from partially hydrolysed degraded arabinogalactan A was 3 - O - β - D - galactopyranosyl - D - galactose with trace amounts of the (1 - 6) - linked isomer. Hydrolysis of the methylated periodate degraded polymer /

polymer gave 2,4,6 - tri - O - methyl - D - galactose as the main component together with smaller amounts of 2,3,4,6 - tetra - O - methyl - D - galactose, 2,4 - di - O - methyl - D - galactose and 2,3,5 - tri - O - methyl - L - arabinose. Trace amounts of 2,5 - di - O - methyl - L - arabinose were observed suggesting that, like Western larch ζ - galactan (70), side chains containing at least three arabinose units were present (XIV).

A second periodate oxidation and subsequent Smith degradation resulted in the isolation of a polysaccharide which on partial hydrolysis furnished 3 - O - β - D - galactopyranosyl - D - galactose in large amount with only trace amounts of the (1 - 6) - linked isomer. Methylation studies on the twice degraded polysaccharide showed that over 90% of the β - D - galactopyranose residues were (1 - 3) - linked.

The small number of (1 - 6)-linked residues which remained after the second degradation indicated that branching might occur in some of the galactose side-chains (XXIV).



(XXIV)

The water-soluble arabinogalactans from Western larch, European /

European larch and Japanese larch have a similar molecular structure. The backbone is composed almost entirely of (1 - 3) - linked β - D - galactopyranose residues although periodate oxidation results suggest that a small proportion of (1 - 6) - linked β - D - galactopyranose residues may be present. This backbone is highly ramified by substitution at positions C 6 and since only trace amounts of 2,4,6 - tri - O - methyl - D - galactose were found in the hydrolysates from the methylated ζ - galactans, virtually all the galactose units in the main chain are substituted. Each galactan side-chain contains an average of two galactose residues which are linked to each other and to the main chain by (1 - 6) - β - pyranose linkages. There are indications that some residues in the side-chains are substituted at position C 3 as well as position C 6.

Arabinose residues, which constitute approximately 14 molar % of the polysaccharides are always situated in the periphery of the molecule. Two thirds of the residues are accommodated as 3 - O - β - L - arabinopyranosyl - L - arabinofuranose units while the remainder are present as terminal non-reducing arabinofuranose residues. Arabinofuranose residues in European larch ζ - galactan, and probably the ζ - galactans from Western and Japanese larches, are attached to galactose residues by a (1 - 6) - linkage. The majority of arabinofuranose residues are linked directly to the main chain although periodate oxidation studies suggest that in Western larch arabinogalactan B and European larch arabinogalactan, some of these residues may be attached to the galactan backbone through one or more (1 - 6) - linked /

linked β - D - galactopyranose residues.

Uronic acid - containing arabinogalactans

For sometime now, the presence of D - glucuronic acid residues as constituents of coniferous wood arabinogalactans has been suspected as small amounts of the uronic acid have been detected in hydrolysates from several of these polysaccharides (72, 78 - 82). Recent work on the arabinogalactans from tamarack larch (Larix laricina) (82-84), mountain larch (Larix lyallii) (85) and maritime pine (Pinus maritima) (86) has shown that D - glucuronic acid residues are present as constituents of these three polysaccharides and it is now thought that many, if not all, of the coniferous wood arabinogalactans contain a small amount (ca. 2%) of D - glucuronic acid.

The above three polysaccharides have similar structural arrangements but differing proportions of D - galactose, L - arabinose and D - glucuronic acid (table 2).

The main methyl ethers found in the hydrolysates from the methylated polysaccharides were:- 2,3,4,6 - tetra - O - methyl - D - galactose, 2,3,4 - tri - O - methyl - D - galactose, 2,4 - di - O - methyl - D - galactose, 2,3,5 - tri - O - methyl - L - arabinose, 2,3,4 - tri - O - methyl - L - arabinose, 2,5 - di - O - methyl - L - arabinose and 2,3,4 - tri - O - methyl - D - glucuronic acid. In addition 2,3,4 - tri - O - methyl - D - xylose was isolated from methylated maritime pine arabinogalactan.

Periodate oxidations of mountain larch and maritime

pine /

pine arabinogalactans followed by Smith degradations, gave degraded polysaccharides which furnished on hydrolyses, D - galactose and L - arabinose. A small amount of L - arabinosylglycerol was detected amoung the low molecular weight degradation products from oxidised mountain larch arabinogalactan. Partial hydrolyses of the degraded polysaccharides gave large amounts of 3 - O - β - D - galactopyranosyl - D - galactose with only traces of the (1 - 6) - linked isomer.

Partial hydrolyses of the two undegraded polysaccharides yielded 6 - O - β - D - galactopyranosyl - D - galactose, 3 - O - β - D - galactopyranosyl - D - galactose, 3 - O - β - L - arabinopyranosyl - L - arabinose and, from maritime pine arabinogalactan, 3 - O - α - D - xylopyranosyl - L - arabinose. When tamarack larch arabinogalactan was partially hydrolysed, four galactotrioses (XXV) and two galactotetraoses (XXVI) were isolated in addition to the above three homodisaccharides. In no case did more than two (1 - 6)-linkages or two (1 - 3) - linkages occur consecutively.

Gal 1 - 3 Gal 1 - 3 Gal Gal 1 - 6 Gal 1 - 6 Gal

Gal 1 - 3 Gal
6
|
1
Gal

Gal 1 - 3 Gal
6
|
1
Gal

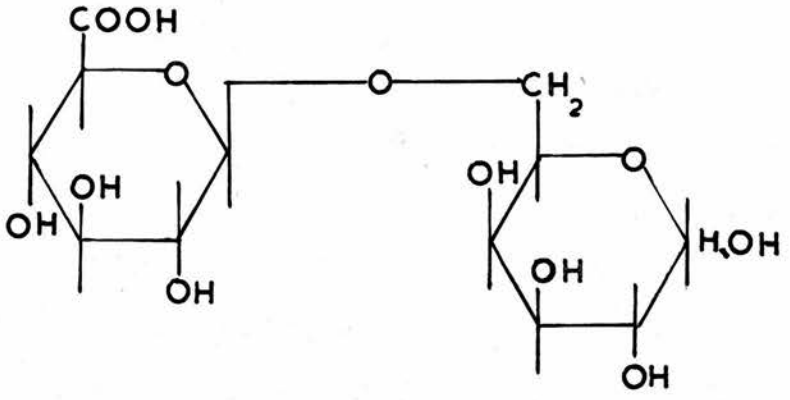
(XXV)

Gal 1 - 6 Gal 1 - 6 Gal 1 - 3 Gal

Gal 1 - 6 Gal 1 - 3 Gal 1 - 3 Gal (XXVI)

Hydrolyses of these acidic polysaccharides gave neutral monomers, /

monomers, D - glucuronic acid and the aldobiouronic acid 6 - O - β - D - (glucopyranosyluronic acid) - D - galactose (XXVII).



(XXVII)

An examination of the partial hydrolysis products from methylated maritime pine arabinogalactan showed that the acidic residues could be attached either directly to the galactan backbone by a (1 - 6) - linkage or through one or two (1 - 6) - linked - D galactopyranose residues.

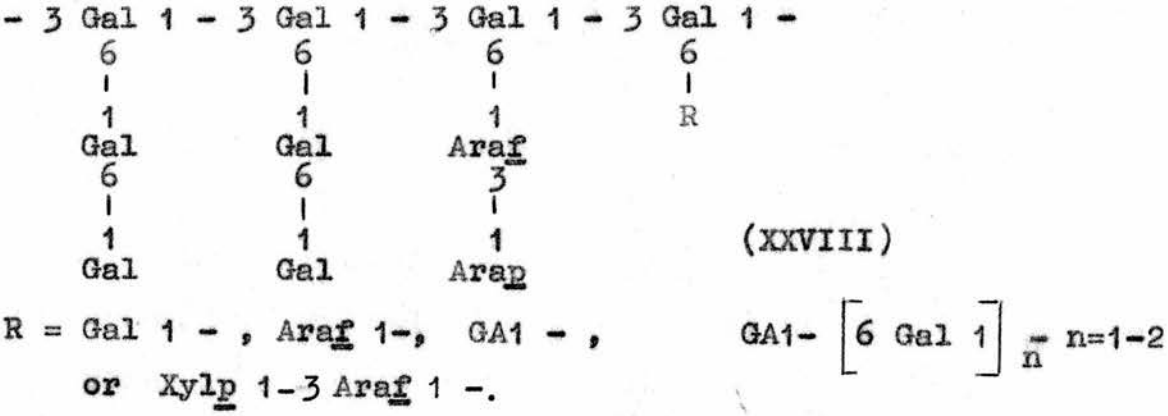
The above results clearly show that the arabinogalactans from maritime pine and mountain larch not only resemble one another in their general structural arrangement, but also the neutral larch arabinogalactans in containing a galactan backbone, composed almost exclusively of (1 - 3) - linked β - D - galactopyranose residues, which is highly branched by substitution at positions C 6.

The arabinose units, which are accommodated as terminal non-reducing L - arabinofuranose residues and 3 - O - β - L - arabinopyranosyl - L - arabinofuranose units, are attached directly to the periodate-resistant backbone although in mountain pine arabinogalactan, /

arabinogalactan, a small proportion of them may be linked through one or more (1 - 6) - linked β - D - galactopyranose residues.

Glucuronic acid residues are always present as non-reducing end-groups and are linked to D - galactose residues by a (1 - 6) - linkage. With the exception of maritime pine arabinogalactan, where the glucuronic acid groups can be linked either directly to the main chain or through one or two galactose residues, there is no evidence to indicate the position of linkage of the aldobiouronic acid residues to the rest of the molecule.

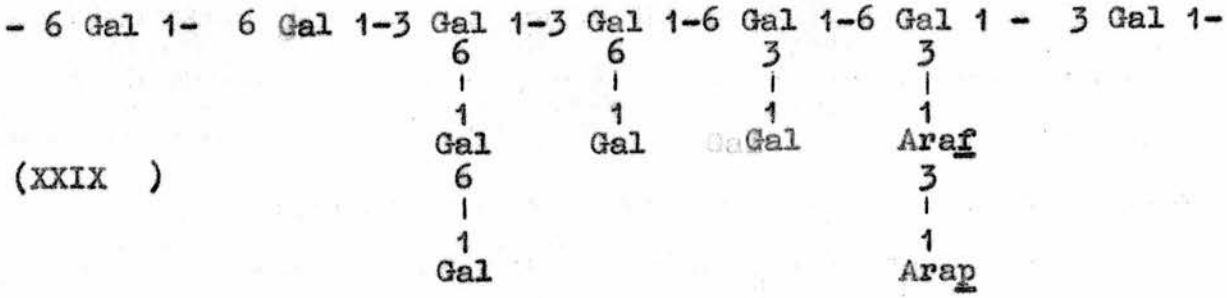
The following simplified, and by no means complete, partial structure embodies most of the structural features known to exist in both the neutral and acidic arabinogalactans (XXVIII).



(XXVIII)

On the basis of methylation and partial hydrolysis results, Adams et al. proposed the following structure for the repeating unit in tamarack larch arabinogalactan (XXIX).

- 6 Gal 1 - /



This structure differs from structure (XXVIII) in containing both (1 - 3)- and (1 - 6)- linkages in the main chain, and side-chains of (a) terminal galactose residues and (b) 3 - O - β - L - arabinopyranosyl - L - arabinofuranose units attached to the main chain by (1 - 3)- linkages. It should be pointed out that while the experimental findings are in agreement with the proposed structure, structure (XXVIII) is equally applicable as there is no evidence for the presence of either (1 - 6)- linkages in the main chain or side-chains linked to the main chain by a (1 - 3)- linkage being necessary. The two structures can easily be differentiated by periodate oxidation followed by a Smith degradation. Structure (XXVIII) would give a linear periodate-resistant (1- 3)- linked galactan, whereas structure (XXIX) would fragment to give low molecular weight products and a hexasaccharide containing both (1 - 3)- and (1 - 6)- linkages (XXX). A second periodate oxidation and Smith degradation would result in the complete breakdown of this oligosaccharide as only two of the six residues are periodate-resistant.

Gal 1 - 3 /

Gal 1 - 3 Gal 1 - 6 Gal 1 - 6 Gal 1 - 3 Gal
3
1
1
Araf

(XXX)

It is extremely unlikely that the glucuronic acid residues arise from contaminating material and are not constituents of the above three arabinogalactans. The homogeneity of each arabinogalactan extract was checked by ultracentrifugation and/or electrophoresis. Where heterogeneity was found the material was fractionated and the homogeneity of arabinogalactan fractions verified as above. Furthermore, the only other uronic acid-containing polysaccharides known to occur in woods are xylans and pectins. In the former, D - glucuronic acid units are always linked to D - xylose residues, and the latter furnishes D - galacturonic acid on hydrolysis.

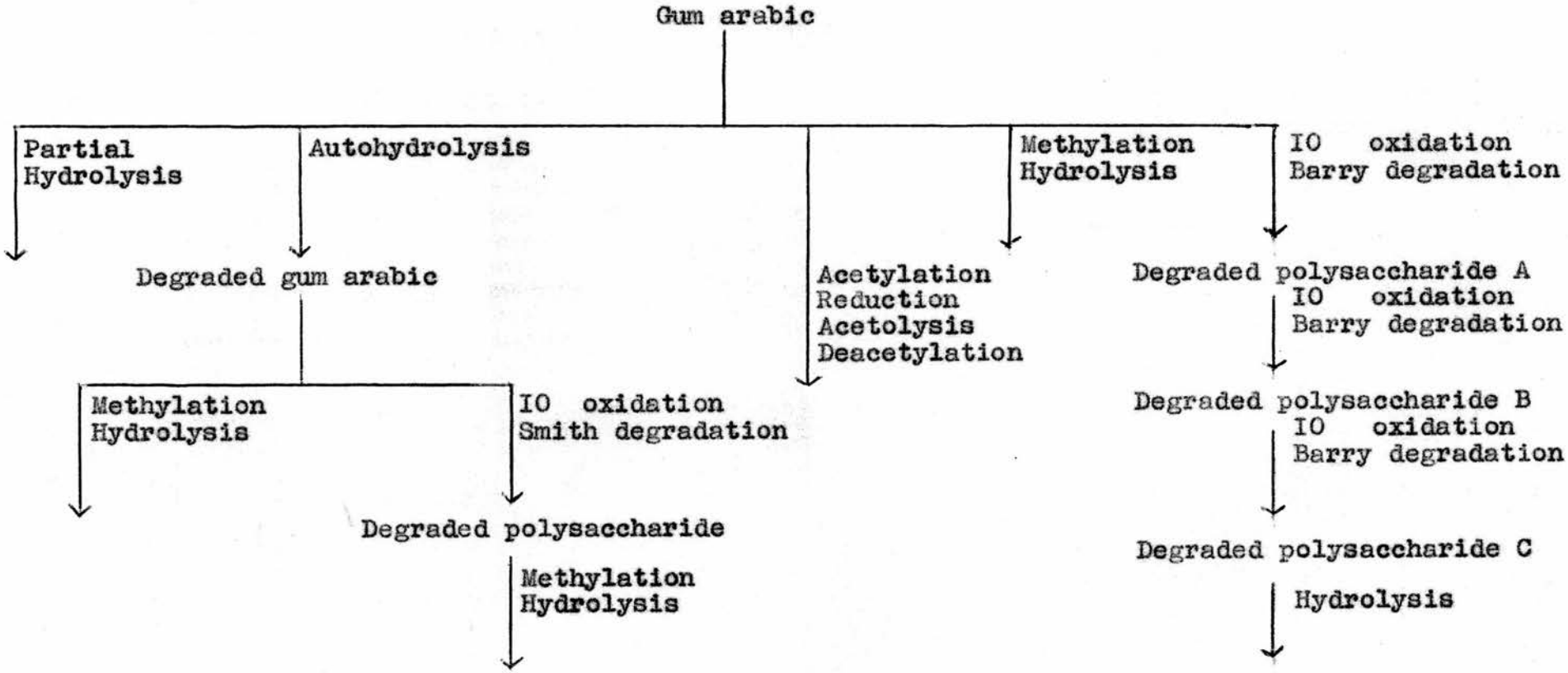
The isolation of 2,3,4 - tri - O - methyl - D - xylose as the only methylated xylose derivative from hydrolysed methylated maritime pine arabinogalactan shows that the xylose residues in the original polysaccharide are present as non-reducing end-groups and are not derived from an accompanying xylan.

Polysaccharides containing D - galactose and L - arabinose as their main structural units are found in several plant gum exudates, notably those from the genus Acacia. Polysaccharides from Acacia senegal gum (36,87-89), Acacia pycnantha gum (90-92), Khaya senegalensis gum (B fraction) (36) and Asafoetida /

Asafoetida gum (93) possess, like the neutral and acidic arabinogalactans, galactan frameworks consisting of (1 - 3)- and (1 - 6)- linkages. In addition to D - galactose and L - arabinose, these polysaccharides contain varying amounts of L - rhamnose and D - glucuronic acid, often present in part as its 4 - O - methyl ether (table 2).

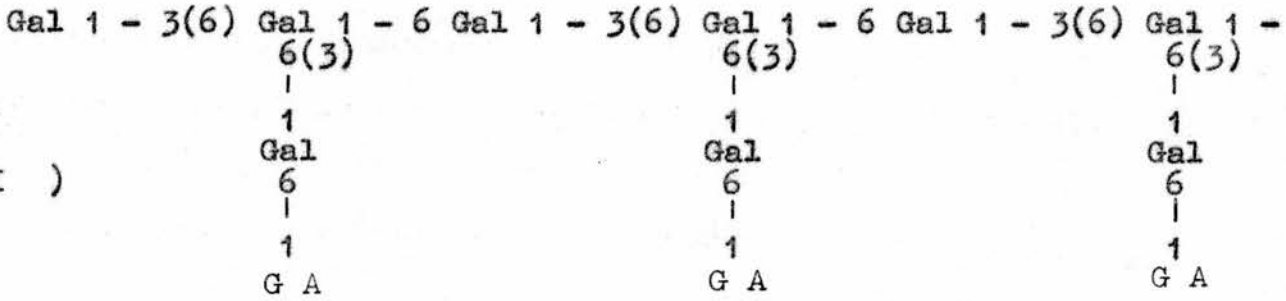
A summary of the structural studies carried out on the polysaccharide from Acacia senegal (gum arabic) is shown on page 32. Autohydrolysis of the polysaccharide gave L - arabinose, L - rhamnose and 3 - O - α - D - galactopyranosyl - L - arabinose together with a degraded polysaccharide. Hydrolysis under more drastic conditions liberated 3 - O - β - D - galactopyranosyl - D - galactose and the aldobiouronic acid 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose. The polysaccharide recovered after autohydrolysis (degraded gum arabic) contained nine parts of galactose to three parts of glucuronic acid. On methylation and hydrolysis it yielded 2,3,4,6 - tetra - O - methyl - D - galactose (1 mole), 2,3,4 - tri - O - methyl - D - galactose (5 moles), 2,4 - di - O - methyl - D - galactose (3 moles) and 2,3,4, - tri - O - methyl - D - glucuronic acid (3 moles), indicating that degraded gum arabic contained both (1 - 3) - and (1 - 6) - linkages.

Gum /

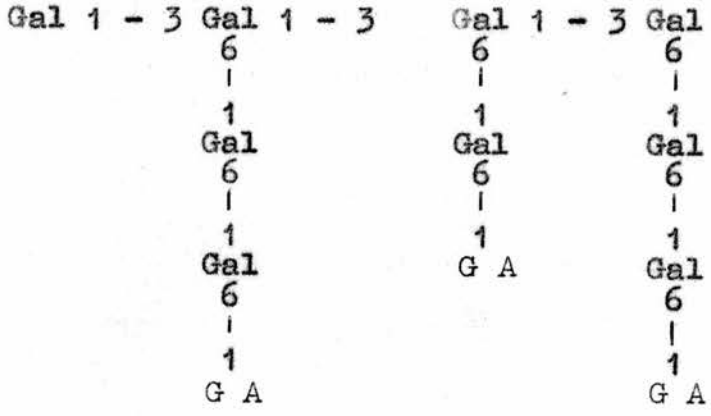

Gum arabic
Partial Hydrolysis
Autohydrolysis
Methylation Hydrolysis
IO oxidation Barry degradation
Degraded gum arabic
Acetylation Reduction Acetolysis Deacetylation
Degraded polysaccharide A
IO oxidation Barry degradation
Methylation Hydrolysis
IO oxidation Smith degradation
Degraded polysaccharide B
IO oxidation Barry degradation
Degraded polysaccharide
Degraded polysaccharide C
Methylation Hydrolysis
Hydrolysis

and that the glucuronic acid residues occurred exclusively as non-reducing end-groups. Partial hydrolysis of methylated degraded gum arabic led to the isolation of the hexamethyl ether of 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose showing that glucuronic acid groups were attached to the galactan backbone through at least one galactose residue. On the basis of the above results, several possible partial structures can be put forward to represent the repeating unit in degraded gum arabic (XXXI - XXXIII).

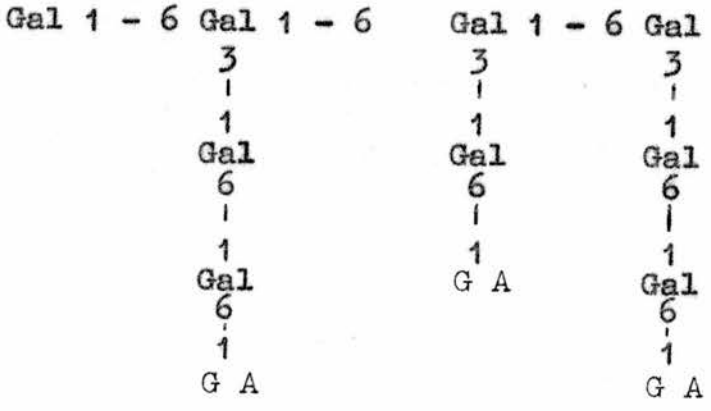
(XXXI)



(XXXII)



(XXXIII)

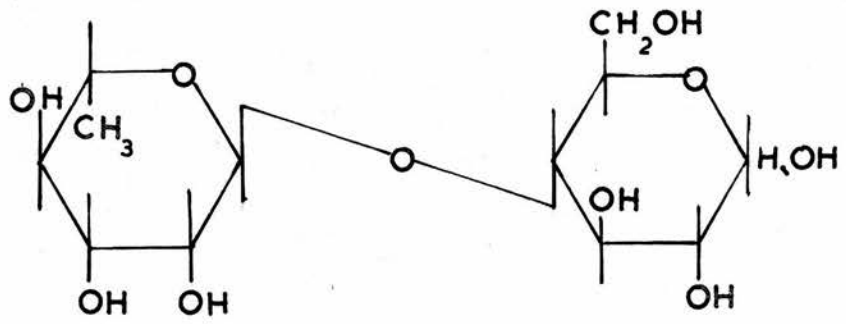


Further information about the structure of the backbone was obtained by treating the undegraded gum with sodium periodate and subjecting the oxidised polysaccharide to a Barry degradation, when a degraded polysaccharide was isolated. The recovered polymer was further twice degraded in the same manner to give a polysaccharide which was resistant to periodate oxidation and which gave galactose only on hydrolysis. This indicated that the main chain in degraded gum arabic was composed of (1 - 3) - linked β - D - galactopyranose residues as in (XXXII). The same conclusion was reached when degraded gum arabic was periodate-oxidised and Smith-degraded to give a polysaccharide fraction which was shown by methylation studies to contain only (1 - 3) - linked β - D - galactopyranose residues.

Undegraded gum arabic on methylation and hydrolysis gave 2,3,4 - tri - O - methyl - L - rhamnose, 2,3,5 - tri - O - methyl - L - arabinose, 2,5 - di - O - methyl - L - arabinose, 2,3,4,6 - tetra - O - methyl - D - galactose, 2,4 - di - O - methyl - D - galactose, 2,3,4 - tri - O - methyl - D - glucuronic acid and 2,3 - di - O - methyl - D - glucuronic acid. In addition to the above sugars, a gas-liquid chromatographic examination of the cleavage products from methanolysed methylated gum arabic identified trace amounts of the methyl glycosides of 2,3,4 - tri - O - methyl - L - arabinose and 2,4,6 - tri - O - methyl - D - galactose (36). The detection of the former methyl glycoside was in agreement with an earlier finding by Andrews and Jones (89) who isolated the disaccharide 3 - O - β - L - arabinopyranosyl - L - arabinose as a product from partial hydrolysis of some samples of gum arabic. A comparison of /

of the methyl ethers isolated on hydrolysis of methylated degraded gum and methylated undegraded gum showed that acid-labile groupings were attached to position C 3 of the galactose residues in the side-chains and to position C 4 of most of the glucuronic acid units.

Since rhamnose and glucuronic acid are present in approximately equimolecular proportions, it has been suggested that rhamnose residues might be linked to glucuronic acid units. Acetolysis of acetylated carboxyl-reduced gum arabic followed by deacetylation (88), led to the isolation of a neutral disaccharide which was characterised as 4 - O - α - L - rhamnopyranosyl - D - glucose (XXXIV).



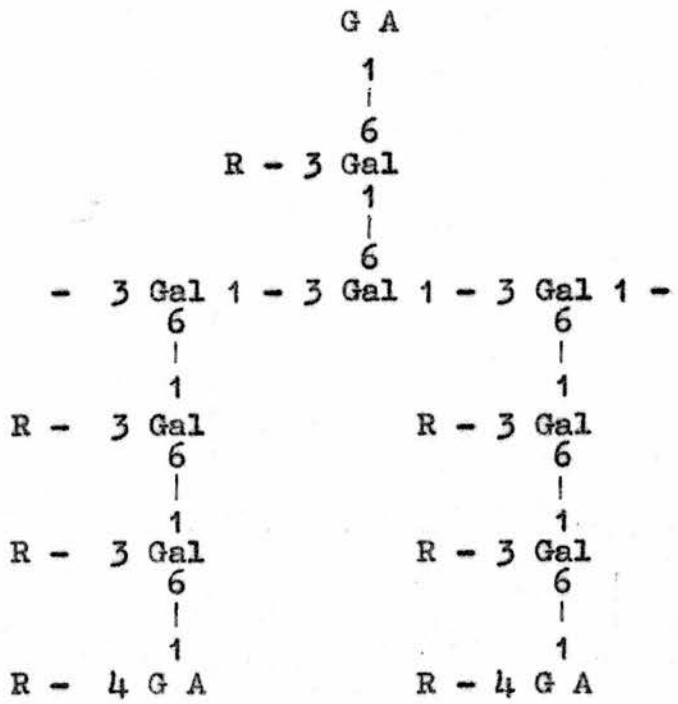
(XXXIV)

This provided clear proof that in the original gum, some of the L - rhamnopyranose residues were linked to position C 4 of D - glucuronic acid units.

Since partial hydrolysis of methylated degraded gum gave the hexamethyl ether of 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose and since no 2,3,4 - tri - O - methyl - D - galactose was detected among the hydrolysis products from methylated /

methylated undegraded gum, it may be concluded that 4 - O - α - L - rhamnopyranosyl - D - glucopyranosyluronic acid units terminate galactose-containing side-chains.

On the basis of the above results from degraded and undegraded gum arabic, structure (XXXV) can be put forward as an approximation for the main repeating unit in the polysaccharide.



R = Araf 1 - , Rhap 1 - ,
Gal 1 - 3 Araf 1 - ,
or in some cases
Arap 1 - 3 Araf 1 -

(XXXV)

The molecular structures of the polysaccharides from Acacia pycnantha gum, Khaya senegalensis gum (B fraction) and Asafoetida gum were determined by similar methods. Mild acid hydrolysis or autohydrolysis of the gums liberated acid-labile residues the nature of which is shown in table 3.

Methylation and partial hydrolysis studies showed that all three gums had a highly branched galactan framework of (1 -3)- and /

and (1 - 6) - linkages. The isolation of 3 - O - β - D - galactopyranosyl - D - galactose as the major product from periodate-oxidised Smith degraded gums indicated that, as in gum arabic, the polysaccharides contained main chains of (1 - 3) - linked β - D - galactopyranose residues.

In addition to neutral oligosaccharides, partial hydrolyses afforded 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose. A second aldobiouronic acid, 6 - O - (4 - O - methyl - β - D - glucopyranosyluronic acid) - D galactose was also isolated from Asafoetida gum and Khaya senegalensis gum (B fraction). The acidic units in Acacia pycnantha gum and Asafoetida gum, and four out of five of the acidic units in Khaya senegalensis gum (B fraction) were present as non-reducing end-groups.

In all three gums, acid-labile residues were attached, by a (1 - 6) - linkage, to (1 - 3) - linked galactose units in the main chain. Acid-labile residues were also linked to (a) (1 - 6) - linked galactose units in the side-chains in Acacia pycnantha gum, by a (1 - 6) - linkage and (b) 3,6 - di - O - substituted galactose residues in Asafoetida gum, by a (1 - 4) - linkage.

It is evident from what has already been said that the above gum polysaccharides are structurally similar to the coniferous wood arabinogalactans.

It would appear that there is a family of polysaccharides which have a main chain of (1 - 3) - linked β - D - galactopyranose residues to which are attached, through positions C 6, side-chains of (1 - 6) - linked β - D - galactopyranose residues. Acid-labile pentose /

pentose units, and acidic hexose residues when present, are always attached to, or present as, side-chains and are never incorporated in the main chain. The nature of these peripheral residues is tabulated in table 3.

Table I

Methyl ethers isolated from methylated Western larch Arabinogalactans A and B.

Methyl ethers	Molar %	Molar %
2,3,5 - trimethylarabinose	32.2	7.6
2,3,4,6 - tetramethylgalactose		24.1
2,5 - dimethylarabinose	3.2	8.1
2,3,4 - trimethylarabinose	2.6	5.1
2,4,6 - trimethylgalactose	4.2	4.0
2,3,4 - trimethylgalactose	13.7	11.8
2,6 - dimethylgalactose	2.9	3.9
2,4 - dimethylgalactose	37.5	31.0
2 - monomethylgalactose	3.7	2.3
2,3 - dimethylarabinose	-	0.5
2, - monomethylarabinose	-	0.6
4 - monomethylgalactose	-	0.3
unmethylated monomers	-	0.1
unknown methylgalactose	-	0.7

Table 2.

Source	Galactose	Arabinose	Xylose	Rhamnose	Glucuronic acid	4 - O - methyl glucuronic acid
Mountain larch	78.6	14.6	-	-	6.8	-
Tamarack larch	77.2	20.3	-	-	2.5	-
Maritime pine	85.9	9.7	1.5	trace	2.9	-
Asafoetida	55.6	33.3	-	trace	11.1	
K. senegalensis	55	25	-	-	20	
A. senegal	36.8	30.3	-	11.4	13.8	-
A. pycnantha	65	27	-	2	5	-

Table 3.

Source of polysaccharide	Acid-labile groups	Acid units
European, Western and Japanese larches	Araf 1 - Arap 1 - 3 Araf 1	
Tamarack and Mountain larches	Araf 1 - Arap 1 - 3 Araf 1	GA 1 - 6 Gal
Maritime pine	Araf 1 - Arap 1 - 3 Araf 1 Xylp 1 - 3 Araf 1	GA 1 - 6 Gal
Asafoetida gum	Araf 1	GA 1 - 6 Gal 4 - Me - GA 1 - 6 Gal
Khaya senegalensis gum (B fraction)	Araf 1 -	GA 1 - 6 Gal 4 - Me - GA 1 - 6 Gal
Acacia pycnantha gum	Araf 1 - Araf 1 - 3 Araf 1 - Rhap 1 -	GA 1 - 6 Gal
Acacia senegal gum	Araf 1 Arap 1 - 3 Araf 1 Galp 1 - 3 Araf 1 Rhap 1 -	Rhap 1 - 4 GA 1 - 6 Gal

SECTION I

ARABINOGALACTAN A FROM

JAPANESE LARCH (*Larix leptolepis*)

DISCUSSION.

Japanese larch arabinogalactan A has recently been studied by Aspinall and Wood (33). Hydrolysis of the polysaccharide afforded galactose and arabinose in the approximate ratio of 6 to 1. Examination of the cleavage products from the fully methylated polysaccharide by gas-liquid chromatography of the methyl glycosides and paper chromatography of the sugars showed approximately equal amounts of 2,3,4,6 - tetra - O - methylgalactose, 2,3,4 - tri - O - methylgalactose and 2,4 - di - O - methylgalactose with smaller amounts of 2,3,5 - tri - O - methylarabinose, 2,3,4 - tri - O - methylarabinose and 2,5 - di - O - methylarabinose. Only trace quantities of 2,6 - di - O - methylgalactose, 2 - O - methylgalactose and 2,4,6 - tri - O - methylgalactose were observed.

Periodate oxidation and Smith degradation of the polysaccharide furnished a degraded polysaccharide (ADI) which contained galactose and arabinose in the approximate ratio of ten to one. A second periodate oxidation and Smith degradation gave a further degraded polysaccharide (ADII) which contained only traces of arabinose. Both degraded polysaccharides were examined by partial hydrolysis, methylation, and periodate oxidation.

The results of these experiments are shown below.

Partial hydrolysis /

Partial hydrolysis

Disaccharides	Arabinogalactan		
	A	ADI	ADII
(1-3) - galactobiose	small	large	large
(1-6) - galactobiose	large	trace	trace
(1-3) - arabinobiose	small	none	none

Periodate oxidation

Polysaccharide	Reagent consumed Moles/sugar unit	Formic acid liberated Moles/sugar unit
Arabinogalactan A	1.22	0.57
Arabinogalactan ADI	0.26	0.08
Arabinogalactan ADII	0.08	-

Methylation

Methyl ether	Arabinogalactan		
	A	ADI	ADII
2,3,5 - Trimethylarabinose	+	+	trace
2,3,4 - Trimethylarabinose	+	-	-
2,5 - Dimethylarabinose	+	trace	-
2,3,4,6 - Tetramethylgalactose	++++	++	+
2,3,4 - Trimethylgalactose	++++	-	-
2,4,6 - Trimethylgalactose	trace	++++	++++
2,4 - Dimethylgalactose	+++++	++	+
2,6 - Dimethylgalactose	trace	-	-
2 - Monomethylgalactose	trace	-	-

The /

The above results of Aspinall and Wood show that Japanese larch arabinogalactan A possesses a highly branched framework consisting of a (1-3)- linked galactose main chain to which are attached, through positions C6, (1-6)- linked galactose side-chains. Arabinose residues, which constitute approximately 14 molar % of the polysaccharide are accommodated as non-reducing end-groups in both the furanose and pyranose form, and as non-terminal furanose residues.

The object of the present investigations on Japanese larch arabinogalactan A was:-

(1) To isolate and characterise the methyl ethers formed on hydrolysis of the fully methylated polysaccharide.

(2) To isolate and characterise the arabinose-containing disaccharide formed on partial hydrolysis of the polysaccharide.

(3) To ascertain the mode(s) of linkage of arabinofuranose residues to galactose units.

Methylation studies

Fully methylated arabinogalactan A was hydrolysed with hydrochloric acid and the resulting mixture of methyl ethers was separated by partition chromatography on a cellulose column. The following methylated sugars were characterised by the formation of crystalline derivatives.

Table 4 /

Table 4

Methyl ether	Molar %
2,3,5 - Trimethyl - L - arabinose	7
2,3,4 - Trimethyl - L - arbinose	4
2,5 - Dimethyl - L - arabinose	3
2,3,4,6 - Tetramethyl - D - galactose	28
2,4,6 - Trimethyl - D - galactose	2
2,3,4 - Trimethyl - D - galactose	16
2,6 - Dimethyl - D - galactose	1
2,4 - Dimethyl - D - galactose	38
2 - Monomethyl - D - galactose	1

In addition trace amounts of 2 - O - methylarabinose, 2,3,6 - tri - O - methylgalactose, 3 - O - methylgalactose and 2,3,4 - tri - O - methylglucuronic acid were identified chromatographically.

Partial hydrolysis studies

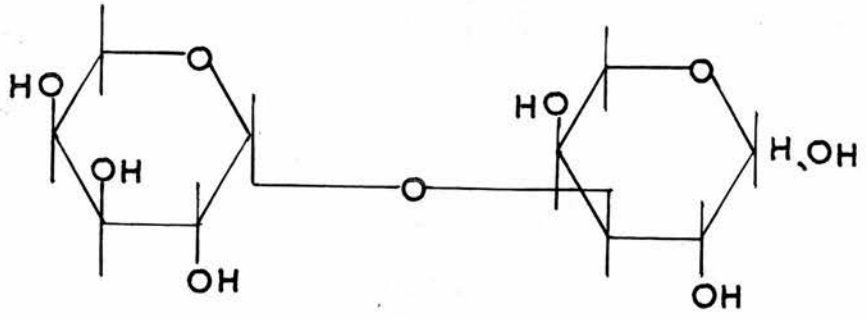
Arabinogalactan A was heated with hydrochloric acid (0.01N) at 100° for 2.5 hours and the resulting degraded polysaccharide was precipitated with ethanol and removed at the centrifuge. The centrifugate and ethanol washings were combined and concentrated to give low molecular weight sugars. The mixture of mono-and oligosaccharides was separated by adsorption chromatography on a charcoal-celite column using water and aqueous ethanol as eluting agents.

Fraction 3, /

Fraction 3, $[\alpha]_D$, + 157° contained only one sugar which was chromatographically identical to 3 - O - β - L - arabinopyranosyl - L - arabinose. Hydrolysis of the fraction gave arabinose only. The fraction gave a phenylosazone which was identical (mixed m.p. and x-ray powder photograph) to that of an authentic sample of 3 - O - β - L - arabinopyranosyl - L - arabinose. Periodate oxidation of the phenylosazone gave formaldehyde but no mesoxalaldehyde 1,2 - bisphenylhydrazone. This result is indicative of a 3 - O - substituted pentose phenylosazone since neither the (1-4)- or (1-5)- linked isomer will yield formaldehyde, and only the (1-5)- linked isomer will furnish mesoxalaldehyde 1,2 - bisphenylhydrazone as a product of periodate oxidation.

Methylation of fraction 3 followed by a chromatographic examination of the cleavage products by gas-liquid chromatography of the methyl glycosides and paper chromatography of the sugars, gave approximately equal amounts of 2,3,4 - tri - O - methylarabinose and 2,4 - di - O - methylarabinose with a trace of 2,5 - di - O - methylarabinose.

On the above evidence the sugar is assigned the following structure:-

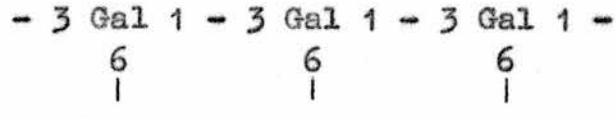


Fractions /

Fractions 5 and 6 both contained a sugar with the same chromatographic mobility as 6 - O - β - D - galactopyranosyl - D - galactose. The two fractions were combined and fractionated by paper chromatography. The major component gave a phenylosazone which had an identical X-ray powder photography to that of an authentic sample of 6 - O - β - D - galactopyranosyl - D - galactose phenylosazone.

Twelve other fractions were collected and examined chromatographically when galactose, arabinose, and trace amounts of a disaccharide with the chromatographic mobility of 3 - O - β - D - galactopyranosyl - D - galactose and several unidentified oligosaccharides, were observed.

The above methylation and partial hydrolysis results, which confirm the chromatographic results of Aspinall and Wood, clearly show that the galactan framework of Japanese larch arabinogalactan A contains (1-3)- and (1-6)- linked β - D - galactopyranose residues. Since the Smith degradation studies showed that the polysaccharide contains a (1-3)- linked galactan backbone, and since only small amounts of 2,4,6 - tri - O - methyl - D - galactose were found among the hydrolysis products from the methylated polysaccharide, the vast majority of galactose residues in the main chain must be substituted at position C6. (I).



(I)

Approximately half of the arabinose residues are present

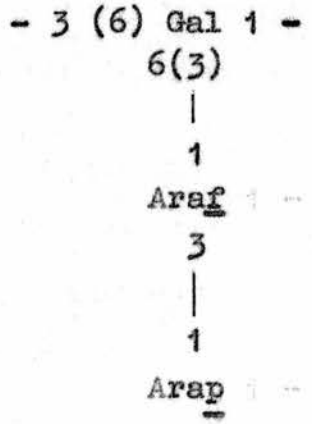
as /

as non-reducing L - arabinofuranose end-groups (II) with the remainder being accommodated as 3 - O - β - L - arabinopyranosyl - L - arabinofuranose units. Since L - arabinopyranose residues were only found as non-reducing end-groups, the arabinobiose units must be present as is shown below (III).

Araf 1 - (II)

Arap 1 - 3 Araf 1 - (III)

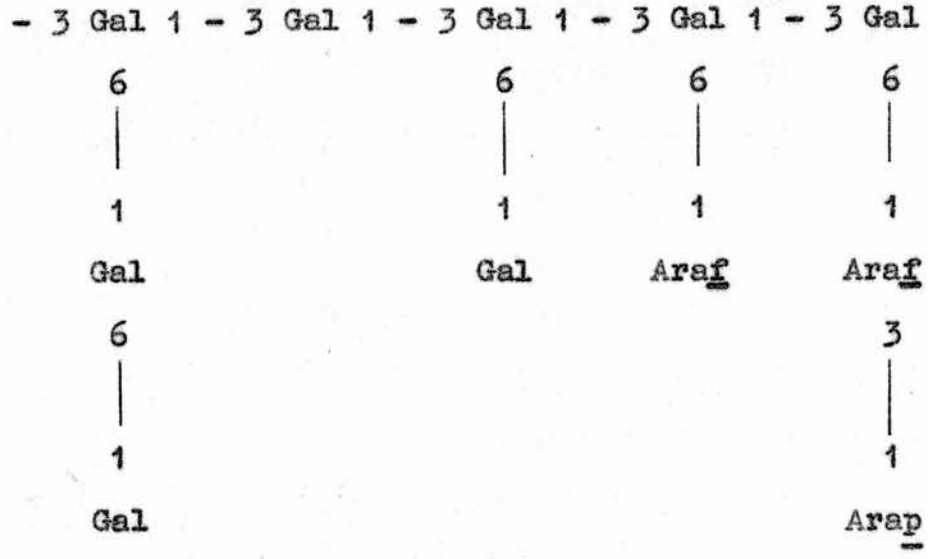
The isolation of a degraded polysaccharide which contained 10% arabinose from the first Smith degradation on Japanese larch arabinogalactan A shows that non-terminal arabinofuranose residues are attached to periodate resistant galactose residues (IV). This point will be discussed in greater detail in connection with the oxidation studies on the polysaccharide.



(IV)

On the basis of the above results and those of Aspinall and Wood, the following structure for Japanese larch arabinogalactan A is proposed /

proposed (V). The point of attachment of arabinofuranose residues is only one of several alternatives.



(V)

Oxidation studies

With the exception of European larch ζ - galactan, direct evidence for the mode of attachment of arabinose to galactose units in coniferous wood arabinogalactans has been lacking as arabinofuranose residues are readily cleaved by acid and it has been impossible to isolate, as products of partial hydrolysis, oligosaccharides containing both arabinose and galactose residues. Aspinall and Nicolson catalytically oxidised the primary hydroxyl groups of arabinofuranose and terminal non-reducing galactose residues in European larch arabinogalactan to carboxylic acid groups (76).

Both the galactopyranosyluronic acid and arabinofuranosyluronic acid linkages in the oxidised polysaccharide thus formed resisted acid hydrolysis. Partial hydrolysis of the oxidised polysaccharide afforded

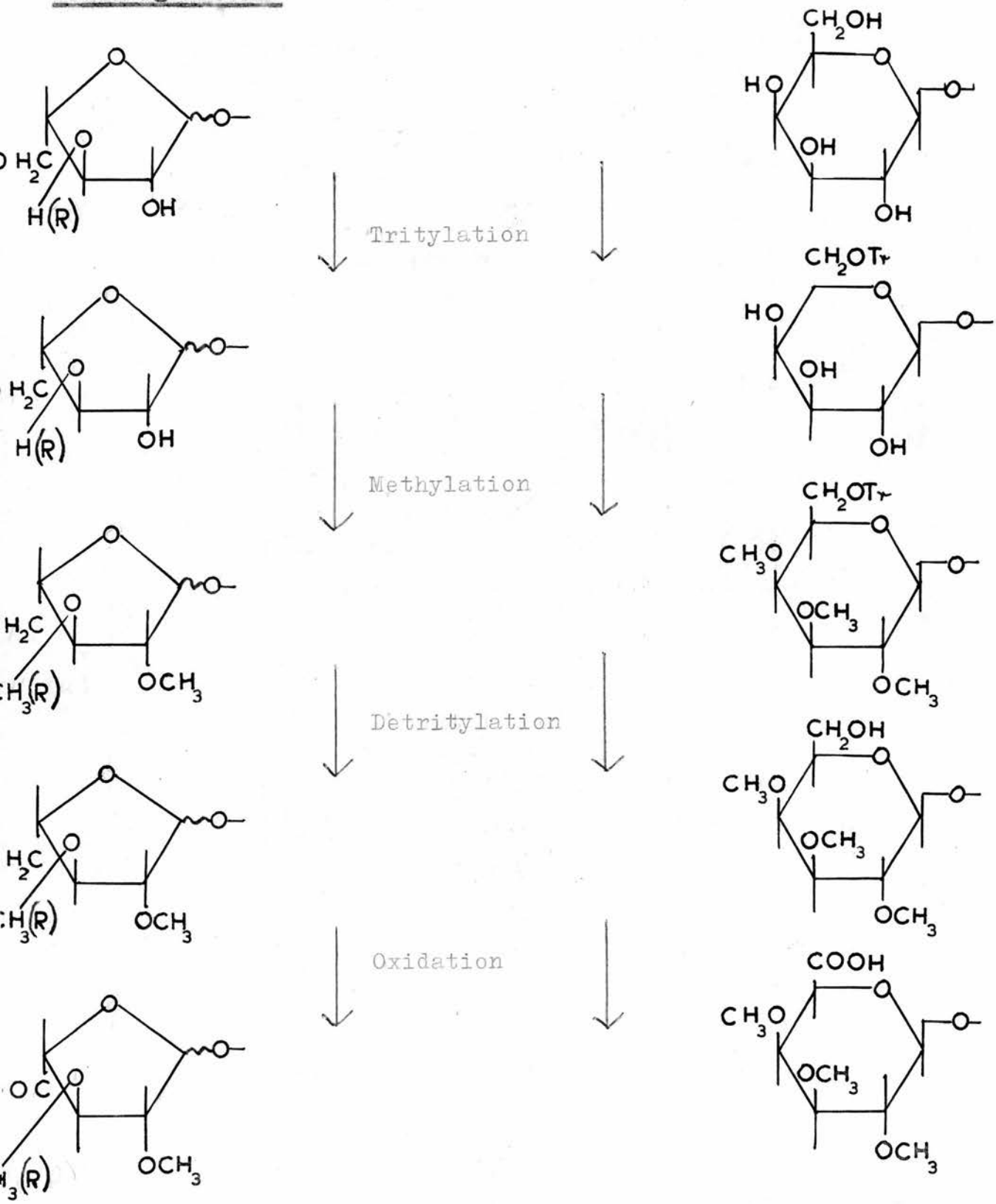
two /

two aldobiouronic acids, 6 - O - (β - D - galactopyranosyluronic acid)- D - galactose and 6 - O - (L - arabinofuranosyluronic acid) - D - galactose. The formation of the second aldobiouronic acid (VI) showed that arabinofuranose residues were attached to galactose units by a (1-6)- linkage.

Araf A 1 - 6 Gal (VI)

In the present investigations the primary hydroxyl groups of arabinofuranose and terminal non-reducing galactose residues in Japanese larch arabinogalactan A were selectively blocked by tritylation. Methylation and detritylation of the tritylated polysaccharide resulted in the isolation of a partially methylated polysaccharide in which only primary hydroxyl groups were available for oxidation. Oxidation of the primary hydroxyl groups to carboxylic acid groups was effected with chromium trioxide.

Scheme leading to the isolation of oxidised methylated arabinogalactan.



Arabinogalactan A was tritylated with trityl chloride in pyridine : dimethylformamide solution. The trityl content of the resulting product, $[\alpha]_D$, + 31°, was 38.3%. Since the ratio of galactose to arabinose is 6 to 1, and assuming that one third of the galactose and two thirds of the arabinose residues have primary hydroxyl groups, the theoretical trityl content is ca. 35%. The slightly higher value obtained suggests that a small proportion of secondary hydroxyl groups had been tritylated.

Methylation of the tritylated polysaccharide with barium oxide and methyl iodide followed by several treatments with silver oxide and methyl iodide afforded a methylated tritylated polysaccharide, $[\alpha]_D$, - 43°, (Tr, 33.7%, OMe, 23.2%. Calc. Tr, 33.6%, OMe, 27.0%). The low value for the methoxyl content was probably due to some steric effect of the bulky trityl group. Examination of the cleavage products from the methylated tritylated polysaccharide by gas-liquid chromatography of the methyl glycoside and paper chromatography of the sugars showed mono - O - methylgalactoses, 2,4 - di - O - methylgalactose, 2,3,4 - tri - O - methylgalactose, 2,4,6 - tri - O - methylgalactose, 2 - O - methylarabinose, 2,3 - di - O - methylarabinose and 2,3,4 - tri - O - methylarabinose. As only traces of 2,3,4,6 - tetra - O - methylgalactose, 2,5 - di - O - methylarabinose and 2,3,5 - tri - O - methylarabinose were observed it is evident that the vast majority of the primary hydroxyl groups in the polysaccharide had been tritylated.

Since arabinofuranose residues are readily cleaved by acid it was felt inadvisable to detritylate the methylated tritylated polysaccharide with acid, the standard method for cleaving trityl ethers.

However /

However all attempts to detritylate the methylated polysaccharide by catalytic hydrogenolysis with platinum catalysts, and reductive hydrogenolysis with lithium aluminium hydride, failed.

The methylated tritylated polysaccharide was detritylated with hydrogen chloride in methanol : chloroform solution. The solution was neutralised with silver carbonate and extracted with an aqueous solution of potassium cyanide to remove colloidal silver salts to give chloroform-soluble detritylated methylated arabinogalactan A. The aqueous extracts were deionised and freeze-dried to give a chloroform-insoluble fraction (fraction C). The chloroform-soluble material was fractionated into 4 fractions (fractions 1-4) by stepwise extraction with boiling light petroleum containing increasing proportions of chloroform. The major fractions were analysed for trityl and methoxyl content and the results are given below.

Fraction	% chloroform in extractant	Tr %	O Me %	$[\alpha]_D$
1	50	-	-	-
2	60	-	-	-
3	80	0	34.1	- 23°
4	100	0	31.8	- 21°
C	-	0	29.1	- 4°

Examination of the cleavage products from methanolyses by gas-liquid chromatography of fractions 3 and C showed that both /

both methanolysates contained methyl glycosides of the following sugars; 2,3,4 - tri - O - methylarabinose, 2,3 - di - O - methylarabinose, 2,3,4 - tri - O - methylgalactose, 2,4 - di - O - methylgalactose and traces of 2,3,5 - tri - O - methylarabinose, 2,3,4,6 - tetra - O - methylgalactose and 2,4,6 - tri - O - methylgalactose. Since the trityl content of the five fractions was nil, it appeared that the different solubility characteristics of the fractions were due to differing methoxyl contents. The five fractions were then combined.

The detritylated methylated polysaccharide in glacial acetic acid was oxidised with chromium trioxide. Water was added and extraction of the aqueous solution with chloroform gave chloroform-soluble oxidised methylated arabinogalactan A, $[\alpha]_D$, - 20°, O Me, 32.6%. The aqueous solution was deionised and freeze-dried to give a chloroform insoluble fraction. A paper chromatographic examination of the hydrolyses products from both fractions showed that three acid sugars were present in both hydrolysates. The two fractions were combined, hydrolysed with N sulphuric acid at 100° for 4 hours and the products were separated into acidic and neutral sugars on a D.E.A.E. Sephadex column, in the formate form.

A gas-liquid chromatographic examination of the derived methyl glycosides from the neutral sugar fraction showed that appreciable quantities of methyl 2 - O - methylarabinoside and methyl 2,3 - di - O - methylarabinoside were present indicating that only a proportion of the available primary hydroxyl groups had been oxidised.

The /

The cleavage products from methanolysis of the acidic sugar fraction were examined by gas-liquid chromatography both before and after reduction with lithium aluminium hydride. A portion of the acidic sugar fraction was methylated with methyl iodide and silver oxide. The cleavage products from methanolysis of the resulting remthylated material were examined by gas-liquid chromatography both before and after reduction with lithium aluminium hydride. The results obtained from the above four analyses are shown in tables 5 and 6. The $\underline{\underline{T}}$ values in parenthesis are common to more than one glycoside.

Table 5

Methyl glycosides of	$\underline{\underline{T}}$ values for Column (b)	
	Acidic sugars	Remethylated acidic sugars
2,3,4 - Trimethylgalactose	2.47, 2.71	2.36, 2.73
2,4 - Dimethylgalactose	3.40, (4.00)	
2,3,4 - Trimethylgalacturonic acid	3.65, (4.00)	3.68, 4.03

In addition to the $\underline{\underline{T}}$ values quoted above, several peaks with low retention times were observed on both chromatograms. Such peaks could have been due to 2 - $\underline{O}$ - methylaraburonic acid and/or 2,3 - di - $\underline{O}$ - methylaraburonic acid the retention times of which are not known.

Table 6 /

Table 6

Methyl glycosides of	$\underline{T}$ values for column (b)	
	Reduced acidic sugars	Reduced remethylated acidic sugars
2,3 - Dimethylarabinose	0.64, 0.82, 0.97	0.63, 0.81, 0.95
2,3,4 - Trimethylgalactose	2.47, 2.72	2.46, 2.73
2,4 - Dimethylgalactose	3.40, 4.02	
Unknown		2.16

Although methyl 2 - O - methylarabinoside was not detected among the cleavage products from methanolysis of the reduced acidic sugar fraction, hydrolysis of this material followed by paper chromatography gave a faint trace of 2 - O - methylarabinose.

The presence of an unrecognised peak ($\underline{T}$ = 2.16) in the reduced remethylated acidic sugar fraction (table 6) and the distortion of the first methyl 2,3,4 - tri - O - methylgalactoside peak ($\underline{T}$ = 2.36 as compared with 2.47) in the unreduced remethylated acidic sugar fraction (table 5) indicated that an unknown methyl glycoside(s) with similar retention times as methyl 2,4,6 - tri - O - methylgalactoside ($\underline{T}$ = 1.97 and 2.25) was present. Several sugar mixtures containing varying proportions of authentic methyl 2,4,6 - tri - O - methylgalactoside and methyl 2,3,4 - tri - O - methylgalactoside were analysed and it was shown that the second methyl 2,4,6 - tri - O - methylgalactoside peak ($\underline{T}$ = 2.25) and the first methyl 2,3,4 - tri - O - methylgalactoside peak ($\underline{T}$ = 2.47) could be recognised even when only traces of methyl

2,4,6 - /

2,4,6 - tri - O - methylgalactoside were present. Thus any methyl 2,4,6 - tri - O - methylgalactoside present in the above four samples must be present in very small amount.

The relative proportions of the methylated sugars found among the cleavage products from the above four samples are shown in table 7.

Table 7

	Relative proportions in samples			
Methylated sugars	1	2	3	4
2,3 - Dimethylarabironic acid	?	-	?	-
2 - Monomethylaraburonic acid	?	-	-	-
2,3,4 - Trimethylgalacturonic acid	++++	-	++++	-
2,3 - Dimethylarabinose	-	+	-	+
2 - Monomethylarabinose	-	trace	-	-
2,3,4 - Trimethylgalactose	+++	+++++	++++	+++++
2,4 - Dimethylgalactose	++	++	-	-

Sample 1 : Acidic sugar fraction

Sample 2 : Reduced acidic sugar fraction

Sample 3 : Remethylated acidic sugar fraction

Sample 4 : Reduced remethylated acidic sugar fraction.

It can be seen from the results shown in table 7 that the proportion of 2,4 - di - O - methylgalactose found among the cleavage products /

products from the reduced acidic sugar fraction was greater than the proportion of 2,3 - di - O - methylarabinose, and that 2,3,4 - tri - O - methylgalactose was the predominant neutral sugar found among the cleavage products from the unreduced acidic sugar fraction. Since no 2,4,6 - tri - O - methylgalactose was observed among the cleavage products from either the reduced or unreduced remethylated acidic sugar fractions, it follows that terminal non-reducing galactose residues in Japanese larch arabinogalactan A are attached to position C6 of galactose residues, some of which are substituted at position C3 (VII, VIII). As the majority of 3,6 - di - O - substituted galactose residues are known to be present in the main chain, the terminal non-reducing galactose residues in (VIII) are probably attached directly to the galactan backbone (IX).

Gal 1 - 6 Gal 1 - (VII)

Gal 1 - 6 Gal 1 - (VIII)

3
|

- 3 Gal 1 - 3 Gal 1 - 3 Gal 1

6
|

1

Gal (IX)

Although no methylated araburonic acid derivatives were positively identified among the cleavage products from the acidic sugar fraction, the presence of 2,3 - di - O - methylarabinose among the cleavage products from the reduced acidic sugar fractions could only be due to reduction of 2,3 - di - O - methylaraburonic acid.

It /

It is clear then from the results shown in table 7 that two possible modes of attachment of non-reducing arabinofuranose end-groups to galactose residues in the polysaccharide can be proposed (X, XI). Again it is probable that the 3,6 - di - O - substituted galactose residue in structure (XI) is accommodated in the main chain of the polysaccharide.

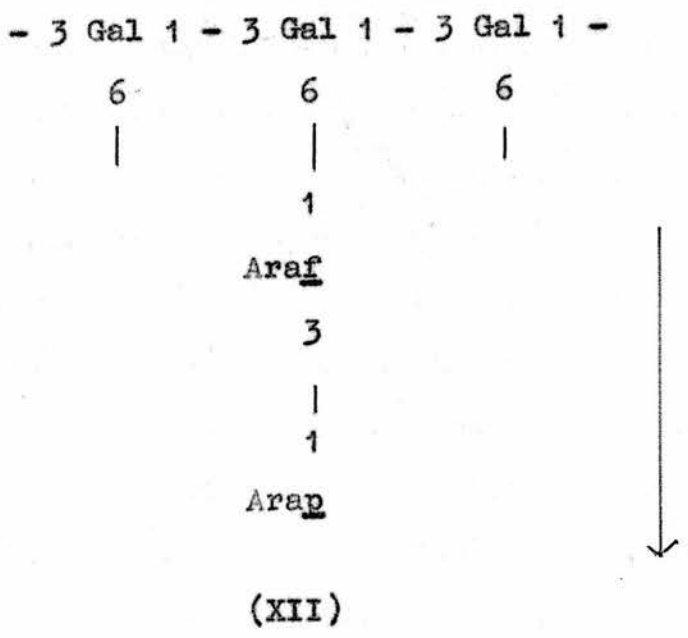
Araf 1 - 6 Gal 1 - (X)

Araf 1 - 6 Gal 1 - (XI)
3
|

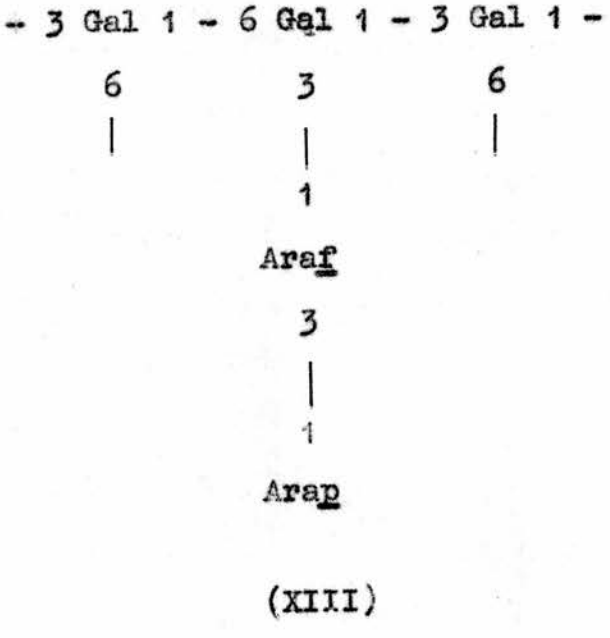
It is not possible on the present evidence to state whether all the non-reducing arabinofuranose end-groups are attached as in (X) or (XI), or whether both modes of attachment are of structural significance.

Only trace amounts of 2 - O - methylarabinose were detected among the cleavage products from the reduced acidic sugar fraction and it is not possible on this evidence to state how the non-terminal arabinofuranose residues are attached to galactose units. The isolation, however, of a degraded polysaccharide from the first Smith degradation of Japanese larch arabinogalactan A (33) which contained 10% arabinose shows that non-terminal arabinofuranose residues are attached to periodate-resistant galactose residues (XII, XIII).

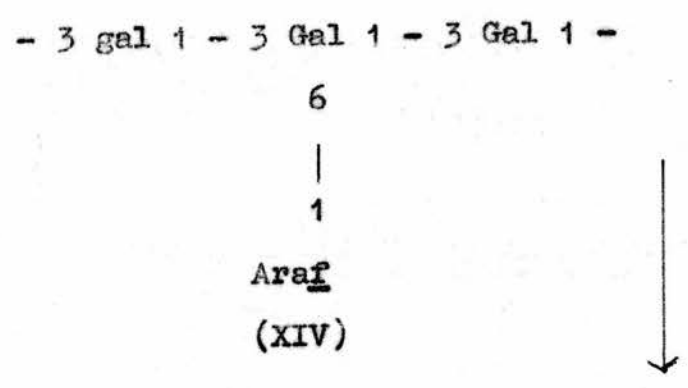
- 3 Gal 1 /



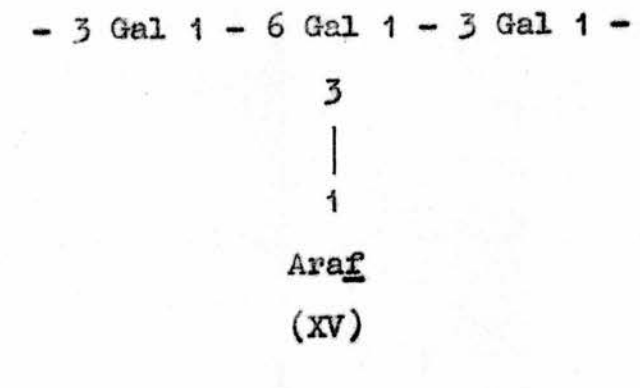
(XII)



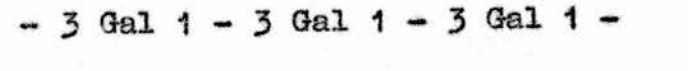
(XIII)



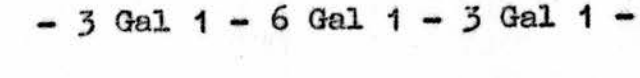
(XIV)



(XV)



(XVI)

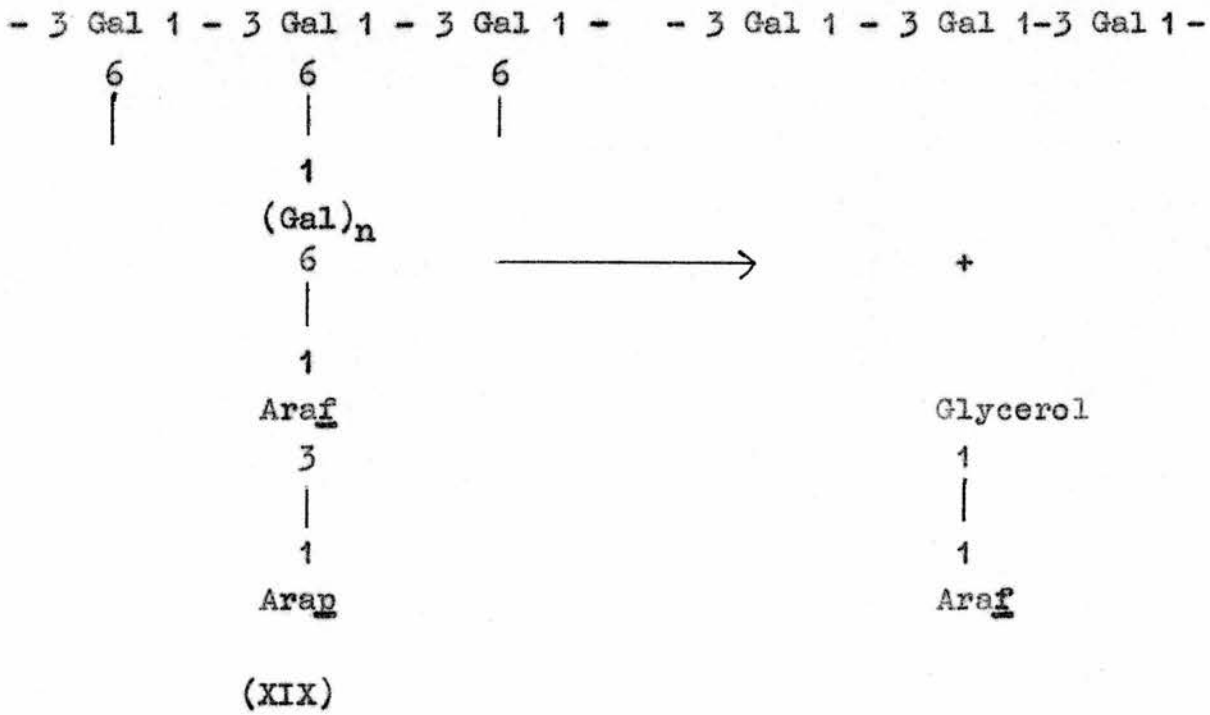


(XVII)

Evidence in favour of structural (XII) as the more important mode of attachment of non-terminal arabinofuranose residues to galactose units is provided by a comparison of the amounts of periodate consumed by the first and second Smith degraded polysaccharides (0.26 and 0.08 moles per sugar unit respectively). Both structures (XII) and (XIII) would./

would, on treatment with periodate followed by a Smith degradation, give degraded polysaccharides (XIV, XV) which would consume similar amounts of periodate. A second periodate oxidation and Smith degradation of structure (XV) would have resulted in the isolation of a further degraded polysaccharide (XVII) in which the relative proportion of (1 - 6)- linkages would have increased and the minimum amount of periodate consumed would have been 0.20 moles per sugar unit. Smith degradation of structure (XIV), however would be accompanied by a decrease in the relative proportion of (1 - 6)- linkages (XVI), which is consistent with the observed results.

Since no monosaccharide derivatives were detected among the low molecular weight products from the first Smith degradation of the polysaccharide very few - if any - of the non-terminal arabinofuranose residues can be linked to periodate-oxidisable galactose units (XIX).



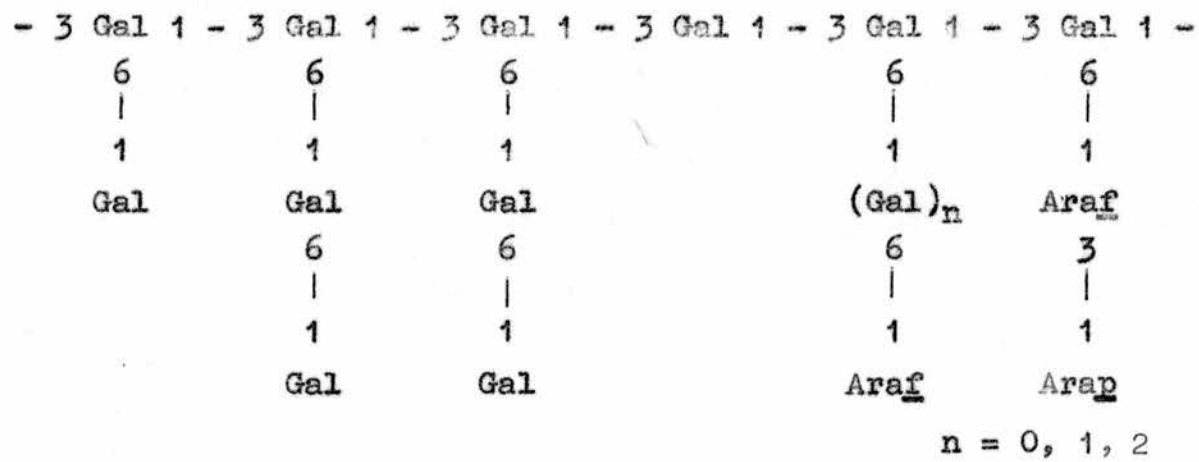
(XIX)

It /

It is clear from the results of the above investigations, together with those of Aspinall and Wood, that Japanese larch arabinogalactan A possesses a (1 - 3)- linked β - D - galactopyranose main chain to which are attached, through positions C6, D - galactopyranose - containing side-chains. The majority of these side-chains contain an average of two (1-6)- linked β - D - galactopyranose residues although a significant proportion of terminal non-reducing D - galactopyranose residues are linked directly to the galactan backbone.

Arabinose residues, which constitute approximately 14 molar % of the polysaccharide are present in the periphery of the molecule. Half of the residues are present as non-reducing L - arabinofuranose end-groups which are attached to position C6 of galactose residues. It is not possible on the present evidence to indicate whether they are attached directly to the galactan backbone or through one or more galactose residues. The remaining arabinose residues are accommodated as non-reducing terminal 3 - O - β - L - arabinopyranosyl - L - arabinofuranose units which are linked directly to the galactan backbone.

Proposed structure for Japanese larch arabinogalactan A



EXPERIMENTAL

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 : pyridine : water (8:2:1).

(C) Ethyl acetate : acetic acid : formic acid : water (18:3:1:4).

(D) Butan-1-ol : ethanol : water (4:1:5, upper layer).

(E) Butan-1-ol : acetic acid : water (4:1:5, upper layer).

(F) Butan-2-one, half saturated with water.

(G) Benzene : ethanol : water (169:47:15, upper layer).

The chromatograms were air-dried, and unless otherwise stated, sprayed with a saturated methylated spirits solution of aniline oxalate and developed at 130° for 3-4 minutes.

The value R_G refers to the distance travelled by a methylated sugar relative to the distance travelled by 2,3,4,6 - tetra - O - methyl - D - glucose. R_{Gal} and R_F similarly, refer to the distance travelled by sugars relative to D - galactose and the solvent front respectively.

Thick paper chromatographic separations were carried out on Whatman 3MM and Whatman 31 (extra thick) filter sheets which had previously been exhaustively extracted with water in a /

a Soxhlet extractor. The sugars were located by cutting thin side-strips and spraying with the above spray. The appropriate sections of the filter sheets were then cut out and the sugars eluted with water.

Evaporations were carried out at 40° under reduced pressure.

Optical rotations were observed at 18° ± 2°.

Cellulose columns were used to separate methylated sugar mixtures. The columns were packed dry and washed with water, butan - 1 - ol and finally with the eluting solvent.

The solvents were purified as follows:-

Light petroleum (b.p.100-120°) was shaken several times with concentrated sulphuric acid (10% v./v.), washed free of acid and distilled.

Butan - 1 - ol was refluxed for 2 hours with sodium hydroxide (1% w./v.) and distilled.

The sugar mixture, dissolved in the minimum amount of butan-1-ol, half saturated with water, was applied to the top of the column, allowed to soak into the absorbent, and eluted with the given solvents. The eluates were collected on an automatic fraction collector. Aliquot proportions were withdrawn from every fifth tube and examined chromatographically. Similar fractions were combined and evaporated to dryness. The residues were purified by dissolution in water, filtration through a charcoal pad, evaporation, dissolution in A.R. acetone, filtration /

filtration and evaporation to dryness. The filtrates were finally dried in vacuo over phosphorus pentoxide and weighed.

A charcoal-celite column was used to fractionate the partial hydrolysis products from Japanese larch arabinogalactan. Activated charcoal was washed several times with hot water and mixed with celite (grade 545) which had previously been boiled with concentrated hydrochloric acid : water (1:1) and washed free of acid. The column was packed as an aqueous slurry and washed well with water. The oligosaccharide mixture, in a small volume of water, was applied to the top of the column and eluted with the given solvents.

Neutral sugars were separated from aldobiouronic acids on a D.E.A.E. Sephadex column (37). D.E.A.E. Sephadex was stirred for 30 minutes with (a) 0.5 N hydrochloric acid and (b) 0.5 N sodium hydroxide. It was then washed with water till free of alkali. A perforated disc was placed in the bottom of a column, then a layer of glass wool, followed by a layer (1 cm.) of silver sand and finally a second layer of glass wool. The D.E.A.E. Sephadex was packed as an aqueous slurry and converted to the formate form by washing with 10 bed-volumes of 15% formic acid. The D.E.A.E. Sephadex was then washed with water till free of acid. The oligosaccharide mixture, dissolved in the minimum amount of water, was absorbed onto the top of the column and allowed to stand for a minimum period of 1 hour. Neutral sugars were eluted with water and acidic material with

3% /

3% formic acid. Formic acid was removed from the acidic solutions by continuous extractions for 12 hours with ether. The aqueous solutions were finally concentrated to a syrup and dried *in vacuo* over phosphorus pentoxide.

Gas-liquid partition chromatography was carried out on a "Pye Argon Chromatograph" according to the procedure of Bishop and Cooper (94,95). Separations were made on the following columns (120 x 0.5 cm.) at gas flow rates of 80 - 100 ml./min.; (a) 15% by weight of butan - 1, 4 - diol succinate polyester on acid-washed celite (80 - 100 mesh) at 175°, (b) 10% by weight of polyphenyl ether (m - bis - (m - phenoxyphenoxy) - benzene) on acid-washed celite at 200° and (c) 3% by weight of neo-pentyl glycol adipate on acid-washed celite at 150°. The methyl O - methyl glycosides were identified by comparing their retention times (T), relative to methyl 2,3,4,6 - tetra - O - methyl - β - D - glucopyranoside with those of authentic samples run under the same conditions.

Small Scale hydrolyses were carried out by heating samples (5 - 10 mg.) in sealed tubes at 100° for 4 - 6 hours with N acid (1-2 ml.).

Sulphuric acid solutions were neutralised with barium carbonate and filtered. Barium ions were removed with Amberlite resin IR - 120 (H) and the solutions were evaporated to dryness.

Hydrochloric acid solutions were neutralised with silver carbonate, filtered and the filtrates were evaporated to dryness. /

dryness. Colloidal silver salts were removed by extracting the residues with methanol or acetone. The extracts were then filtered and evaporated to dryness.

Methanolyses of methylated polysaccharides was achieved by refluxing with methanolic hydrogen chloride (4%) for 18 hours. Methyl glycosides and methylester methylglycosides of sugars were formed by heating samples (1 - 10 mg.) with methanolic hydrogen chloride (4%) at 100° in sealed tubes for 4 - 6 hours. Acid was neutralised with silver carbonate, which was filtered off and washed with chloroform. The filtrate and washings were combined and concentrated. When samples were prepared for gas-liquid chromatography, care was taken to ensure that they were not evaporated to dryness as the methyl glycosides of many methylated sugars are extremely volatile.

Borohydride reductions were carried out by adding potassium borohydride to an aqueous solution of the sugar and allowing the solution to stand overnight. Excess borohydride was destroyed and cations were removed by the addition of Amberlite resin IR - 120 (H). The solution was filtered and the filtrate was evaporated to dryness. Boric acid was removed by the repeated addition and evaporation of methanol.

Estimations of sugars were carried out by (a) the phenol-sulphuric acid method (96) and (b) a modification of the p - aminobenzoic acid method (97).

p - Aminobenzoic acid (3 ml; 2% w./v. in glacial acetic acid, /

acid, purified by redistilling over p - aminobenzoic acid) and sulphosalicylic acid (3 ml; 1.5% w./v. in glacial acetic acid, purified as above) were added to the sugar solutions (1 ml.), containing 20-120 μ g. sugar. The solutions were heated on a boiling-water bath for 70 minutes and allowed to stand at room temperature for 5 hours. Absorbance was measured at 370 mμ for hexoses and 540 mμ for pentoses on a "Unicam" S.P. 500 spectrometer.

Uronic acid anhydride determinations were carried out by the decarboxylation method (98) and the carbazole method (99).

Methoxyl contents were estimated by the Ziesel semi-micro procedure (100).

Estimation of triphenylmethoxyl content (101).

Tritylated polysaccharide (50 - 100 mg.) was treated with concentrated sulphuric acid (5 ml.) and gently agitated until dissolved. Water (50 ml.) was added slowly with swirling and the precipitated triphenylcarbinol was filtered into a preweighed porosity 4 sintered-glass crucible and washed till free of acid. The crucible was dried at 100° for 1 hour and reweighed. Due to the presence of some foreign material in most samples, the weighed precipitate was treated with hot methanol and reweighed in order to estimate the methanol-soluble material (triphenylcarbinol).

Methylation with silver oxide and methyl iodide.

The partially methylated polysaccharide was dissolved in /

in methyl iodide (purified by refluxing over dry silver oxide until neutral and redistilling in a dry system) and silver oxide was added in tenths to the refluxing mixture over a period of 4 hours. The mixture was refluxed for a further 4 hours, cooled and centrifuged. The residue was extracted continuously with boiling chloroform to ensure complete removal of the methylated polysaccharide. The chloroform extract and methyl iodide centrifugate were combined and concentrated to a syrup which was taken up in chloroform and washed with potassium cyanide solution (1% w./v. in water) to remove colloidal silver salts. The aqueous solution was washed 4 times with chloroform and the chloroform extract and washings were combined, washed with a little water, dried over anhydrous sodium sulphate and concentrated. The methylated polysaccharide was precipitated by pouring into light petroleum (60-80°, 20:1).

Methylation with silver oxide, methyl iodide and dimethylformamide (102).

The partially methylated material was dissolved in dimethylformamide and methyl iodide was added. The solution was stirred at room temperature and silver oxide was added in fifths over a period of 1 hour. Stirring was continued for a further 12 hours when the mixture was centrifuged. The residue was extracted continuously for 18 hours with boiling chloroform and the chloroform extract and centrifugate combined. The chloroform solution was treated with potassium cyanide as above, dried over anhydrous sodium sulphate and concentrated to a syrup.

Demethylations /

Demethylations were carried out by the procedure described by Bonner, Bourne and McNally (103).

Aniline derivatives of methylated sugars were prepared by refluxing the sugar (10 - 100 mg.) with an equimolecular amount of freshly distilled aniline in dry ethanol (10 ml.). The reaction was performed in an atmosphere of CO_2 and allowed to proceed for 30 minutes. On removal of the solvent the aniline derivative crystallised and was recrystallised from the given solvent.

Aldonolactones were prepared by dissolving the sugar (10 - 100 mg.) in water (5 ml.) and adding 10 to 20 drops of bromine to effect oxidation. The reaction was allowed to proceed in the dark for 3 days when excess bromine was removed by aeration and the solution was neutralised with silver carbonate. Silver ions were removed by treatment with hydrogen sulphide and the filtrate was evaporated to dryness. The residue was dissolved in acetone, filtered, evaporated to dryness and dried in vacuo over phosphorus pentoxide.

Aldonomides were prepared by treating the lactone (5 - 100 mg.) with methanolic ammonia (5 ml.) for 24 hours at 0^{o}. Evaporation of the solvent yielded a crystalline amide which was recrystallised from the given solvent.

Phenylhydrazine derivatives of aldonic acids were prepared by refluxing the aldonolactone (10 - 100 mg.) with an equimolecular amount of freshly distilled phenylhydrazine in dry /

dry ether (30 ml.). After 4 hours the solution was concentrated to a dark syrup which was heated on a boiling-water bath for 3 hours. On cooling, the phenylhydrazine derivative crystallised and was recrystallised from the given solvent.

The p - nitrobenzoyl derivative of 2,3,4 - tri - O - methyl - L - arabinose was prepared by dissolving the sugar (200 mg.) in dry pyridine (20 ml.) containing p - nitrobenzoyl chloride (220 mg.) and heating the solution at 60° for 40 minutes. The reaction mixture was allowed to stand overnight at room temperature and then treated with a saturated solution of sodium bicarbonate until effervescence stopped. The solution was extracted with chloroform and the extract was dried over anhydrous sodium sulphate, filtered and concentrated to a syrup which solidified on standing. 2,3,4 - Tri - O - methyl - L - arabinose - p - nitrobenzoate, after recrystallisation from methanol - ether, had m.p. = 128-129°.

Phenylosazones were prepared by heating the sugar (20 mg.) for 30 minutes on a boiling-water bath with redistilled phenylhydrazine (0.02 ml.), glacial acetic acid (0.02 ml.), water (0.5 ml.) and 2 drops of saturated sodium bisulphite solution. On dilution with water (3 ml.) the phenylosazone precipitated and was recrystallised from the given solvent.

Formaldehyde estimations.

The /

The amount of formaldehyde liberated on periodate oxidation of 3 - O - β - L - arabinopyranosyl - L - arabinose phenylosazone was determined colorimetrically by the method of Hough, Powell and Woods (104).

Periodate oxidation of methylated sugars was carried out according to the method of Lemieux and Bauer (105).

Consumption of periodate was determined spectrophotometrically by the method of Aspinall and Ferrier (106).

Formic acid estimations (107).

The polysaccharides (100 mg.) in water (30 ml.) was shaken in the dark with potassium chloride solution (10 ml., 16% w./v.) and sodium metaperiodate solution (20 ml., 0.3 M). A blank was run at the same time omitting the polysaccharide. Aliquot proportions (5 ml.) were withdrawn at regular time intervals and treated with ethylene glycol (1 ml.) to destroy excess periodate. The amount of formic acid released during the oxidation was determined by adding potassium iodate solution (2 ml., 10% w./v.) and titrating the liberated iodine with standard sodium thiosulphate.

METHYLATION STUDIES ON ARABINOGALACTAN A.

Hydrolysis of methylated polysaccharide(33)

Methylated arabinogalactan A (3.8 g.) was suspended in hydrochloric acid (2 N ; 140 ml.) at room temperature for 3 days when most of the polysaccharide had dissolved. Methanol (140 ml.) was added and the solution was heated very slowly to 100°, care being taken that no material precipitated out. The methanol was evaporated off and the solution was diluted with water to give a normal solution with respect to hydrochloric acid. Hydrolysis was continued for a further 6 hours (constant rotation) when the solution was cooled, neutralised with silver carbonate, filtered and concentrated to a syrup. The aqueous distillate was extracted with chloroform (3 x 50 ml.) and the chloroform extracts were combined with the hydrolysate and concentrated to a syrup. Colloidal silver salts were removed by extracting the syrup with methanol. The extracts were then concentrated to a syrup (3.5 g.).

Separation of methylated sugars.

The mixture of methylated sugars (3.5 g.) was separated by chromatography on a cellulose column (80 x 3 cm.). The column was eluted with light petroleum (b.p.100-120°) : butan-1-ol (70:30), saturated with water, followed by light petroleum : butan-1-ol (50:50), saturated with water and later with butan-1-ol, half saturated with water. The column was finally washed with water.

Methylated sugars from arabinogalactan A.

Fraction	Weight (in mg.)	T values Column (a)	T values Column (b)	R_G	Sugars present	
1	21			1.02	unknown sugar	tr.
			0.47	0.94	2,3,5-trimethylarabinose	tr.
		1.80	1.60	0.90	2,3,4,6-tetramethylgalactose	
			0.70	0.84	2,5-dimethylarabinose	
			1.09			
2	65			1.02	unknown sugar	tr.
		0.55	0.47	0.94	2,3,5-trimethylarabinose	
		0.72	0.60			
		1.82	1.60	0.90	2,3,4,6-tetramethylgalactose	
		1.06		0.78	2,3,4-trimethylarabinose	tr.
3	29	0.55	0.47	0.94	2,3,5-trimethylarabinose	
		0.71	0.60			
		1.80	1.61	0.90	2,3,4,6-tetramethylgalactose	
		1.06	0.82	0.78	2,3,4-trimethylarabinose	
				0.49	2,4-dimethylgalactose	tr.
4	11	1.80	1.61	0.90	2,3,4,6-tetramethylgalactose	
			0.70	0.84	2,5-dimethylarabinose	
			1.03			
		7.4	2.56	0.69	2,3,4-trimethylgalactose	
			2.87			
				0.49	2,4-dimethylgalactose	tr.
5	846	1.80	1.61	0.90	2,3,4,6-tetramethylgalactose	

Fraction	Weight (in mg.)	$\bar{T}$ values Column (a)	Column (b)	R_G	Sugars present	
6	121		1.60	0.90	2,3,4,6-tetramethylgalactose	
		1.85	0.70	0.84	2,5-dimethylarabinose	
		3.36	1.08			
		7.4	2.68	0.69	2,3,4-trimethylgalactose	
			2.92			
			4.38	0.49	2,4-dimethylgalactose	tr.
7	5	1.89	0.69	0.84	2,5-dimethylarabinose	
		3.39	1.04			
		1.04	0.79	0.78	2,3,4-trimethylarabinose	
8	60	1.88	0.69	0.84	2,5-dimethylarabinose	tr.
		3.38				
		1.05	0.79	0.78	2,3,4-trimethylarabinose	
9	30	1.88	0.71	0.84	2,5-dimethylarabinose	tr.
		1.05	0.81	0.78	2,3,4-trimethylarabinose	
10	33	4.18	2.07	0.69	2,4,6-trimethylgalactose	
		4.75	2.36			
		3.24	1.63		2,3,6-trimethylgalactose	tr.
11	100	4.10	2.10	0.69	2,4,6-trimethylgalactose	
		4.65	2.38			
		7.37	2.89		2,3,4-trimethylgalactose	
			2.65			
12	346	7.59	2.62	0.69	2,3,4-trimethylgalactose	
			2.89			

Fraction	Weight (in mg.)	T values Column (a)	T values Column (b)	R_G	Sugar present	
13	76			0.83	dimethylarabinose	tr.
			2.64	0.69	2,3,4-trimethylgalactose	tr.
			2.89			
				0.63	unknown sugar	tr.
			3.72	0.49	2,4-dimethylgalactose	
			4.40			
14	42		2.86	0.69	2,3,4-trimethylgalactose	tr.
			2.49,	0.54	2,6-dimethylgalactose	
			3.19			
			3.70			
15	14		2.47	0.54	2,6-dimethylgalactose	
			3.21			
			3.69	0.49	2,4-dimethylgalactose	
			4.36			
			1.11		2 - methylarabinose	
			1.47			
16	920		3.76	0.49	2,4-dimethylgalactose	
			4.46			
17	24			0.49	2,4-dimethylgalactose	tr.
				0.31	2 - methylgalactose	
18	17			0.49	2,4-dimethylgalactose	tr.
				0.31	2 - methylgalactose	
				0.26	3 - methylgalactose	
				0.19	arabinose	tr.
19	6			0.49	2,4-dimethylgalactose	
				0.26	3 - methylgalactose	
				0.19	arabinose	

Fraction	Weight (in mg.)	$\frac{T}{2}$ values Column (a)	Column (b)	R_G	Sugar present
20	4			0.49	2,4 - dimethylgalactose
21	7			0.49	2,4 - dimethylgalactose galactose methylated uronic acids
22	12			0.49 0.19	2,4 - dimethylgalactose arabinose galactose

Examination of Fractions

Fractions (1 - 4) (126 mg.).

Unknown sugar, 2,3,5 - tri - O - methyl - L - arabinose, 2,3,4,6 - tetra - O - methyl - D - galactose and unhydrolysed glycosides.

Paper chromatography in solvents D, F and G of the first four fractions indicated the presence of 2,3,5 - tri - O - methyl arabinose, 2,3,4,6 - tetra - O - methyl galactose and an unknown sugar (R_G = 1.02).

Gas-liquid chromatography of the derived methyl glycosides and hydrolysis followed by paper chromatography in the above solvents, showed, in addition to the sugars already detected, 2,5 - di - O - methyl arabinose, 2,3,4 - tri - O - methyl galactose, 2,3,4 - tri - O - methyl arabinose and traces of 2,4 - di - O - methyl galactose.

The four fractions were combined, hydrolysed with N hydrochloric acid for 4 hours, neutralised and concentrated to a syrup (35 mg.).

In view of the disappointing yield and in order to fully characterise 2,3,5 - tri - O - methyl arabinose, a second batch of methylated polysaccharide (2.5 g.) was hydrolysed. Evaporation of the solvent gave a syrup (2.3 g.).

The syrup was fractionated on a cellulose column (80 x 3 cm.). 2,3,5 - Tri - O - methylarabinose and 2,3,4,6 - tetra/

tetra - O - methylgalactose were eluted from the column with light petroleum (b.p. 100 - 120°): butan - 1 - ol (80:20), saturated with water. The remaining methylated sugars were eluted with water.

Subfraction a (131 mg.).

Unknown sugar, 2,3,5 - tri - O - methyl - L - arabinose and unhydrolysed glycoside.

Paper chromatography in solvents D, F and G showed that this fraction, $[\alpha]_D$, - 21° (c , 0.7 in water), contained 2,3,5 - tri-O - methylarabinose and an unknown sugar (R_G= 1.02). Gas-liquid chromatography of the methyl glycosides and hydrolysis followed by paper chromatography in solvents D, F and G, showed that a small amount of 2,3,4 - tri - O - methylgalactose was also present.

The derived 2,3,5 - tri - O - methyl - L - arabonamide, after recrystallisation from ethyl acetate, had m.p. and mixed m.p. = 132 - 133°.

Subfraction b (245 mg.).

Unknown sugar, 2,3,5 - tri - O - methyl - L - arabinose and 2,3,4,6 - tetra - O - methyl - D - galactose.

Paper chromatography in solvents D, F and G showed that this fraction contained 2,3,4,6 - tetra - O - methylgalactose with/

with trace amounts of 2,3,5 - tri - O - methylarabinose and an unknown sugar (R_G = 1.02).

Subfraction c (327 mg.).

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

This fraction was chromatographically identical to 2,3,4,6 - tetra - O - methyl - D - galactose in solvents D, F and G.

Subfraction d (water washings) (1.2 g.).

2,3,4,6 - Tetra - O - methyl - D - galactose, 2,5 - di - O - methyl - L - arabinose, 2,3,4 - tri - O - methyl - L - arabinose, tri - O - methyl - D - galactose and di - O - methyl - D - galactose.

Paper chromatography in solvent D showed that the above sugars were present in this fraction. Only a trace amount of 2,3,4,6 - tetra - O - methylgalactose was observed.

Fraction 5 (846 mg.).

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

Paper chromatography in solvents D, F and G, and gas-liquid chromatography of the methyl glycoside showed that this fraction, $[\alpha]_D$, + 97° (c , 0.9 in water), contained only 2,3,4,6 - tetra - O - methylgalactose. Demethylation gave galactose only.

The syrup crystallised after being seeded with 2,3,4,6 - tetra/

tetra - O - methyl - D - galactose and, after recrystallisation from ether- light petroleum (b.p. 60 - 80°) had m.p. and mixed m.p. = 67-68°, $[\alpha]_D$, + 144° ⟶ + 118° (c , 1.1 in water).

The derived 2,3,4,6 - tetra - O - methyl - N - phenyl - D - galactosylamine, after recrystallisation from ethanol, had m.p. and mixed m.p. = 198 - 199°.

Fraction 6 (121 mg.).

2,3,4,6 - Tetra - O - methyl - D - galactose, 2,5 - di - O - methyl - L - arabinose and unhydrolysed glycosides.

Paper chromatography in solvents D, F and G showed that this fraction, $[\alpha]_D$, + 68° (c , 0.8 in water), contained 2,3,4,6 - tetra - O - methylgalactose and 2,5 - di - O - methylarabinose. Gas-liquid chromatography of the methyl glycosides, and hydrolysis followed by paper chromatography in solvents D, F and G, showed that 2,3,4 - tri - O - methylgalactose and a trace of 2,4 - di - O - methylgalactose were also present.

The fraction was hydrolysed with N hydrochloric acid for 4 hours, neutralised and concentrated to a syrup (95 mg.).

The syrup was fractionated on a cellulose column (60 x 2 cm.). 2,3,4,6 - Tetra - O - methylgalactose and 2,5 - di - O - methylarabinose were eluted with light petroleum (b.p. 100 - 120°) : butan - 1 - ol (80:20), saturated with water. The remaining methylated sugars were eluted with water.

Subfraction/

Subfraction a (9 mg.).

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

This fraction was chromatographically identical to 2,3,4,6 - tetra - O - methyl - D - galactose in solvents D, F and G.

Subfraction b (2 mg.).

2,3,4,6 - Tetra - O - methyl - D - galactose and 2,5 - di - O - methyl - L - arabinose.

Paper chromatography in solvent D showed that this fraction contained 2,3,4,6 - tetra - O - methylgalactose and 2,5 - di - O - methylarabinose.

Subfraction c (43 mg.).

2,5 - Di - O - methyl - L - arabinose.

Paper chromatography in solvents D, F and G and gas-liquid chromatography of the methyl glycoside showed that this fraction, $[\alpha]_D$, - 10° (c, 0.8 in water), contained only 2,5 - di - O - methylarabinose.

The derived 2,5 - di - O - methyl - L - arabonamide, after recrystallisation from ethyl acetate, had m.p. and mixed m.p. = 127 - 128°.

Subfraction d (water washings) (35 mg.).

2,/

Subfraction d (water washings) (35 mg.).

2,3,4 - Tri - O - methyl - D - galactose, 2,4 - di - O - methyl - D - galactose and 2 - O - methyl - D - galactose.

Paper chromatography in solvent D showed that this fraction contained the above sugars.

Fraction 7 (5 mg.).

2,5 - Di - O - methyl - L - arabinose and 2,3,4 - tri - O - methyl - L - arabinose.

Paper chromatography in solvents D, F and G and gas-liquid chromatography showed that this fraction contained 2,5 - di - O - methylarabinose and 2,3,4 - tri - O - methylarabinose.

Fraction 8 (60 mg.).

2,5 - Di - O - methyl - L - arabinose and 2,3,4 - tri - O - methyl - L - arabinose.

Paper chromatography in solvents D, F and G, and gas-liquid chromatography of the methylglycosides showed that the main component of this fraction, $[\alpha]_D$, + 134° (c , 1.2 in water), was 2,3,4 - tri - O - methylarabinose with a trace of 2,5 - di - O - methylarabinose.

The derived 2,3,4 - tri - O - methyl - L - arabonophenylhydrazide, after recrystallisation from ethanol-water, had m.p. and mixed/

mixed m.p. = 158°.

Fraction 9 (30 mg.).

2,5 - Di - O - methyl - L - arabinose and 2,3,4 - tri - O - methyl - L - arabinose.

Paper chromatography in solvents D, F and G, and gas-liquid chromatography of the methyl glycosides showed that this fraction was similar to fraction 8.

Fraction 10 (33 mg.).

2,4,6 - Tri - O - methyl - D - galactose and 2,3,6 - tri - O - methyl - D - galactose.

Paper chromatography in solvents D and G showed that this fraction, $[\alpha]_D$, + 78° (c, 0.8 in water), contained tri - O - methylgalactose. Gas-liquid chromatography of the methyl glycosides showed that the main component was 2, 4, 6 - tri - O - methylgalactose with a trace of 2, 3, 6 - tri - O - methylgalactose.

The main component crystallised after several weeks in vacuo over phosphorus pentoxide and the derived 2,4,6 - tri - O - methyl - N - phenyl - D - galactosylamine, after recrystallisation from ethyl acetate, had m.p. and mixed m.p. = 173 - 174°.

Fraction 11 (100 mg.).

2,4,6 - Tri - O - methyl - D - galactose and 2,3,4 - tri - O - methyl - D/

D - galactose.

Paper chromatography in solvents D and F showed that this fraction, $[\alpha]_D$, + 96° (c , 0.9 in water), contained tri - O - methylgalactose. Gas-liquid chromatography of the methyl glycosides indicated the presence of 2,3,4 - tri - O - methylgalactose and a smaller amount of 2,4,6 - tri - O - methylgalactose.

The syrup was heated with ethanolic aniline and fractional crystallisation of the product from ethyl acetate afforded (a) 2,3,4 - tri - O - methyl - N - phenyl - D - galactosylamine (plates), m.p. and mixed m.p.=169°, (b) a mixture of needles and plate, m.p. = 156° and (c) a mixture of needles and plates, m.p. = 142° from which 2,4,6 - tri - O - methyl - N - phenyl - D - galactosylamine, m.p. and mixed m.p. = 172°, was separated.

Fraction 12 (346 mg.).

2,3,4 - Tri - O - methyl - D - galactose.

Paper chromatography in solvents D and F, and gas-liquid chromatography of the methyl glycoside showed that this fraction, $[\alpha]_D$, + 113° (c , 1.2 in water), contained only 2,3,4 - tri - O - methylgalactose. Demethylation gave only galactose. 2,3,4 - Tri - O - methyl - D - galactose monohydrate crystallised on standing and, after recrystallisation from ethyl acetate containing 1% water, had m.p. and mixed m.p. = 68°, $[\alpha]_D$, + 145° → +115° (c , 1.1 in water).

The derived 2,3,4 - tri - O - methyl - N - phenyl - D - galactosylamine,/

galactosylamine, after recrystallisation from ethyl acetate, had m.p. and mixed m.p. = 167 - 168°.

Fraction 13 (76 mg.).

Di - O - methyl - L - arabinose, 2,3,4 - tri - O - methyl - D - galactose, unknown sugar and unhydrolysed glycoside.

Paper chromatography in solvents D and F showed that this fraction contained tri - O - methylgalactose and an unknown sugar (R_G = 0.63). Gas-liquid chromatography of the methyl glycosides indicated that the main component was 2,4 - di - O - methylgalactose with a trace of 2,3,4 - tri - O - methylgalactose. The dimethylgalactose must have arisen from a product of incomplete hydrolysis.

A portion of the fraction (10 mg.) was dissolved in water (1 ml.) and methyl sulphate (1 ml.) and aqueous sodium hydroxide (30% w./v., 1 ml.) were added dropwise over a period of 2 hours. The reaction mixture was kept in an ice-bath and the solution was stirred vigorously in an atmosphere of nitrogen. After 24 hours the solution was heated on a boiling-water bath for 30 minutes to destroy excess methyl sulphate. The solution was then extracted continuously for 18 hours with chloroform and the chloroform extract was concentrated to a syrup (8 mg.). Gas-liquid chromatography of the methyl glycosides from the above syrup showed that the main component was 2,3,4,6 - tetra - O - methylgalactose with trace amounts of 2,3,4 - tri - O - methylgalactose and 2,4,6 - tri - O - methylgalactose, indicating that 2,4 - di - O - methylgalactose was originally/

originally present as a glycoside.

The remainder of the fraction was hydrolysed with N hydrochloric acid and the neutralised hydrolysate was concentrated to a syrup.

Paper chromatography in solvent D showed that 2,4 - di - O - methylgalactose and trace amounts of tri - O - methylgalactose, di - O - methylarabinose (R_G = 0.83) and an unknown sugar (R_G = 0.63) were present. Demethylation gave galactose and a trace of arabinose. The syrup was dissolved in acetone containing 1% water and left at 0° for 2 days when 2,4 - di - O - methyl - D - galactose crystallised. The sugar was heated with ethanolic aniline and recrystallisation of the product from acetone afforded 2,4 - di - O - methyl - N - phenyl - D - galactosylamine, m.p. and mixed m.p. = 216°.

Fraction 14 (42 mg.).

2,3,4 - Tri - O - methyl - D - galactose and 2,6 - di - O - methyl - D - galactose.

Paper chromatography in solvents D and F and gas-liquid chromatography of the methyl glycosides showed that this fraction, $[\alpha]_D$, +68° (c , 0.7 in water) contained 2,6 - di - O - methylgalactose as the main component with a trace of 2,3,4 - tri - O - methylgalactose.

A portion of the fraction (1 mg.) was subjected to periodate oxidation, and paper chromatography of the oxidised products in solvent D showed 2 - O - methyl-malondialdehyde (R_F = 0.17), indicative/

indicative of a 2,6 - di - O - methylhexose.

2,6 - Di - O - methyl - D - galactose crystallised on standing and after recrystallisation from chloroform-light petroleum (b.p. 60-80°), had m.p. and mixed m.p. = 111 - 113°, $[\alpha]_D$, + 56 → + 83° (c, 1.1 in water).

The derived 2,6 - di - O - methyl - N - phenyl - D - galactosylamine, after recrystallisation from ethyl acetate, had m.p. and mixed m.p. = 115°.

Fraction 15 (14 mg.)

2,6 - Di - O - methyl - D - galactose, 2,4 - di - O - methyl - D - galactose and 2 - O - methyl - L - arabinose.

Paper chromatography in solvents D and F showed that this fraction contained 2,6 - di - O - methylgalactose and 2,4 - di - O - methylgalactose. Gas-liquid chromatography of the methyl glycosides indicated the presence of both the above sugars together with 2 - O - methyl - L - arabinose. A portion of the fraction (1 mg.) was oxidised with sodium periodate but no 2 - O - methyl - malondialdehyde was observed. Demethylation gave galactose and arabinose.

Fraction 16 (920 mg.).

2,4 - Di - O - methyl - D - galactose.

Paper chromatography in solvent D and F and gas-liquid chromatography of the methyl glycoside showed that this fraction, $[\alpha]_D$, + 85° (c, 1.1 in water) contained only 2,4 - di - O - methyl-galactose./

galactose. Demethylation gave galactose only. 2,4 - Di - O - methyl - D - galactose monohydrate crystallised immediately on drying and, after recrystallisation from acetone containing 1% water, had m.p. and mixed m.p. = 97-98°, $[\alpha]_D$, + 121 ⟶ + 85° (c , 1.3 in water).

The derived 2,4 - di - O - methyl - N - phenyl - D - galactosylamine, after recrystallisation from acetone, had m.p. and mixed m.p. = 217°.

Fraction 17 (24 mg.).

2,4 - Di - O - methyl - D - galactose and 2 - O - methyl - D galactose.

Paper chromatography in solvent D showed that the main component of this fraction was 2 - O - methylgalactose with a trace of 2,4 - di - O - methylgalactose. Demethylation gave galactose only.

The syrup crystallised on seeding and recrystallisation from ethanol gave 2 - O - methyl - D galactose, m.p. and mixed m.p. = 155-157°.

Fraction 18 (17 mg.).

2,4 - Di - O - methyl - D - galactose, 2 - O - methyl - D - galactose, 3 - O - methyl - D - galactose and arabinose.

Paper chromatography in solvent D showed that this fraction contained the above sugars. Only trace amounts of 2,4 - di - /

di - O - methylgalactose and arabinose were observed.

Fraction 19 (6 mg.).

2,4 - Di - O - methyl - D - galactose, 3 - O - methyl - D - galactose and arabinose.

Paper chromatography in solvents A and D showed that this fraction contained the above sugars.

Fraction 20 (4 mg.).

2,4 - Di - O - methyl - D - galactose.

Paper chromatography in solvents A and D showed that 2,4 - di - O - methylgalactose was the only sugar present in this fraction.

Fraction 21 (7 mg.).

2,4 - Di - O - methyl - D - galactose, galactose and methylated uronic acids.

Paper chromatography in solvent E showed that this fraction contained two methylated uronic acids (R_G = 0.80 and 0.35). Paper chromatography in solvents A and D showed that galactose and 2,4 - di - O - methylgalactose were also present.

Fraction 22 (water washings) (12 mg.).

2,4 - /

Fraction 22 (water washings) (12 mg.).

2,4 - Di - O - methyl - D - galactose, arabinose and galactose.

Paper chromatography in solvents A and D showed that this fraction contained the above sugars.

Preparation of crystalline derivatives of 2,3,4 - tri - O - methyl - L - arabinose.

L - Arabinose (20 g.) was refluxed with methanolic hydrogen chloride (200 ml., 2% w./v.) until the solution was non-reducing (3.5 hours). The solution was neutralised with silver carbonate in the usual way and concentrated when methyl - β - L - arabinopyranoside crystallised out.

Recrystallisation from methanol furnished methyl - β - L - arabinopyranoside (6.9 g.), m.p. and mixed m.p. = 170°, $[\alpha]_D$, + 245° (c, 2.1 in water).

Methyl sulphate (50 ml.) and aqueous sodium hydroxide (100 ml., 30% w./v.) were added dropwise to a vigorously stirred solution of methyl - β - L - arabinopyranoside (5 g.) in water (150 ml.). Stirring was continued and similar additions were made on 3 successive days. The solution was heated on a boiling-water bath for 30 minutes to destroy excess methyl sulphate, neutralised with dilute sulphuric acid and concentrated to small volume (100 ml.). Methylated spirits (4 volumes) was added and the precipitated sodium/

sodium sulphate was removed at the centrifuge and washed well with methylated spirits. The centrifugate and washings were combined, concentrated to small volume (50 ml.) and extracted continuously with boiling chloroform for 18 hours. The chloroform extract was evaporated to dryness and dried in vacuo over phosphorus pentoxide to give a pale yellow syrup (5.4 g.).

Hydrolysis of a small portion, followed by paper chromatography in solvent D showed that small amounts of mono - and di - O - methyl - L arabinoses were present as well as the main component, 2,3,4 - tri - O - methyl - L - arabinose.

The partially methylated product (5.4 g.) was methylated to completion with methyl iodide (100 ml.) and silver oxide (20 g.). Evaporation of the solvent gave a syrup which crystallised after 2 days at 0°. Recrystallisation from light petroleum (b.p. 60-80°) furnished methyl - 2,3,4 - tri - O - methyl - β - L - arabinopyranoside (5.1 g.), m.p. = 43-45°, $[\alpha]_D$, + 251° (c , 0.9 in water).

The glycoside (2g.) was hydrolysed with N sulphuric acid for 4 hours and neutralised in the usual way. Evaporation of the solvent gave a colourless syrup (1.9g.) which had $[\alpha]_D$, + 135° (c , 1.0 in water).

The derived 2,3,4 - tri - O - methyl - L - arabonophenylhydrazide, after recrystallisation from ethanol-ether, had m.p. = 157°.

The derived 2,3,4 - tri - O - methyl - L - arabinose - p - nitrobenzoate, after recrystallisation from methanol-ether, had m.p. = 128-129°.

PARTIAL HYDROLYSIS STUDIES ON ARABINOGALACTAN A.

Partial hydrolysis of arabinogalactan A.

The polysaccharide (10g.) in hydrochloric acid (0.01N, 360 ml.) was heated on a boiling-water bath for 2.5 hours. The solution was cooled, neutralised with Amberlite resin IR - 4B (OH), filtered and concentrated to 50 ml. The degraded polysaccharide was precipitated with ethanol (4 volumes) and removed at the centrifuge. The centrifugate and washings were combined and concentrated to a syrup (683 mg.).

Separation of monosaccharides and oligosaccharides.

The mixture of monosaccharides and oligosaccharides (683 mg.) was separated on a charcoal-celite column (30 x 2.5 cm.) using water and aqueous ethanol (up to 5% ethanol v./v.) as eluents.

Fractions (500 ml.) were collected and evaporated to dryness. The residues were purified by extraction with water, filtration and evaporation to dryness.

Products/

Products from partial hydrolysis of arabinogalactan A.

Fraction	Eluent	Wt. in mg.	R_{Gal}	Sugars present
1	water	394	1.40	arabinose
			1.00	galactose
3	water	74	0.82	(1-3) - arabinobiose
5	2% aqueous ethanol	16	1.84	unknown sugar
			1.38	arabinose
			0.84	(1-3) - arabinobiose
			0.37	(1-6) - galactobiose
6	2% aqueous ethanol	10	1.78	unknown sugar
			1.40	arabinose
			1.21	unknown sugar
			0.90	unknown sugar
			0.80	(1-3) - arabinobiose
			0.74	unknown sugar
			0.34	(1-6) - galactobiose

In addition, eleven other fraction were collected (combined wt. = 112 mg.). Paper chromatographic examinations in solvent A indicated the presence of sugars with the following $R_{Gal.}$ values; 2.73, 2.22, 1.68, 1.50, 1.38 (arabinose), 1.16, 1.00 (galactose). /

(galactose), 0.92, 0.82 (3 - O - β - L - arabinopyranosyl - L - arabinose), 0.75, 0.62, 0.54 (3 - O - β - D - galactopyranosyl - D - galactose), 0.43, 0.35 (6 - O - β - D - galactopyranosyl - D - galactose), 0.14 and 0.11.

Examination of Fractions.

Fraction 1.

Paper chromatography in solvent A showed that this fraction (394 mg.) contained galactose and arabinose.

Fraction 3.

This fraction (74 mg., $R_{Gal.}$ = 0.82 and 0.67 in solvents A and C respectively), $[\alpha]_D$, + 157° (c , 2.1 in water), was chromatographically identical to 3 - O - β - L - arabinopyranosyl - L - arabinose. Hydrolysis followed by paper chromatography gave only arabinose.

Methyl sulphate (0.5 ml.) and aqueous sodium hydroxide (0.5 ml. 30% w./v.) were added dropwise over a period of 2 hours to a portion of the fraction (10 mg.) in water (1 ml.). The solution was stirred vigorously at 0°, in an atmosphere of nitrogen, for 12 hours. Stirring was continued and aliquot proportions of methyl sulphate (1 ml.) and aqueous sodium hydroxide (2 ml.) were added dropwise over a period of 4 hours. Similar additions of the reagents were made on 3 successive days. The solution was heated on a boiling-water bath for 30 minutes to destroy excess methyl sulphate, neutralised with sulphuric acid and extracted continuously with chloroform for 18 hours. The chloroform extract was evaporated to dryness and dried in vacuo over phosphorus pentoxide. The residue/

residue (8 mg.) was methylated to completion with methyl iodide (5 ml.) and silver oxide (1 g.). Evaporation of the solvent gave a syrup (5 mg.).

Paper chromatography of the hydrolysis products in solvent D and gas-liquid chromatography of the methyl glycosides indicated the presence of approximately equimolecular proportions of 2,3,4 - tri - O - methylarabinose (R_G = 0.78, T = 1.03 column (a), 0.80 column (b)) and 2,4 - di - O - methylarabinose (R_G = 0.61, T = 2.22, 2.30 column (a), 1.08, 1.10 column (b)), and a trace of 2,5 - di - O - methylarabinose (T = 1.87, 3.41 column (a), 0.67 column (b)).

The derived 3 - O - β - L - arabinopyranosyl - L - arabinose phenylosazone, after recrystallisation from aqueous ethanol, had m.p. and mixed m.p. = 233° and gave an identical X-ray powder photograph to the one obtained from an authentic sample. Periodate oxidation of the phenylosazone gave 1.08 and 1.36 molecules of formaldehyde after 30 and 300 minutes respectively, but no mesoxalaldehyde -1,2 - bisphenylhydrazone. This result is indicative of a 3-O - substituted pentose phenylosazone.

Fractions (5 and 6).

Paper chromatography in solvent A showed that the main component of both fractions (combined wt. = 26 mg.) was 6 - O - β - D - galactopyranosyl - D - galactose. The two fractions were combined and fractionated by paper chromatography on Whatman No.1 filter/

filter paper to give 6 - O - β - D - galactopyranosyl - D - galactose (12 mg., $R_{Gal.}$ = 0.35).

The derived 6 - O - β - D - galactopyranosyl - D - galactose phenylosazone gave an identical X-ray powder photography to the one obtained from an authentic specimen.

OXIDATION STUDIES ON ARABINOGALACTAN A.

Scheme leading to the isolation of methylated aldobiouronic acids.

Polysaccharide

↓ Tritylation

Tritylated polysaccharide

↓ Methylation

Methylated tritylated polysaccharide

↓ Detritylation

Methylated polysaccharide

↓ Oxidation

Oxidised methylated polysaccharide

↓ Partial hydrolysis

Methylated neutral sugars + methylated aldobiouronic acids

Tritylation of polysaccharide.

The polysaccharide (8g.) and trityl chloride (55g.) in pyridine (240 ml.) and dimethylformamide (240 ml.) were stirred at 100° under anhydrous conditions until all the solid had dissolved (45 minutes). The reaction mixture was left at room temperature for 5 days when the solution was concentrated to small volume (100 ml.) and the tritylated polysaccharide was precipitated with methanol (4 volumes) and removed at the centrifuge. The residue was washed with methanol (4 x 200 ml.), dissolved in chloroform (70 ml.), reprecipitated with methanol and removed at the centrifuge. The tritylated polysaccharide was washed with methanol (4 x 100 ml.) followed by ether (4 x 100 ml.), air-dried and finally dried *in vacuo* over phosphorus pentoxide to give a cream product (11.1 g.), $[\alpha]_D$, + 31° (c , 0.7 in chloroform) [Found : Tr, 38.3%, carbohydrate (phenol-sulphuric acid), 59.3%].

Methylation of tritylated polysaccharide (108).

Barium oxide (35g.) and methyl iodide (140 ml.) were added to a solution of the tritylated polysaccharide (9.9g.) in dimethylsulphoxide (200 ml.). The suspension was stirred at room temperature for 2 days when inorganic salts were removed at the centrifuge. The residue was washed with chloroform (4 x 100 ml.) and the centrifugate and chloroform washings were combined. Chloroform was then added until the precipitation of inorganic salts was complete. Inorganic salts were removed at the centrifuge and washed /

washed with chloroform (5 x 100 ml.). The centrifugate and washings were combined and washed with aqueous sodium thiosulphate (15% w./v.). The resulting colourless solution was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness. The residue was dissolved in chloroform, washed with water to remove traces of dimethylsulphoxide, dried over anhydrous sodium sulphate and concentrated to small volume (60 ml.). The methylated tritylated polysaccharide was precipitated with light petroleum, removed at the centrifuge and dried *in vacuo* over phosphorus pentoxide to give a pale yellow product (9.4g.) [Found : O Me, 16.3%].

The partially methylated product was methylated twice with methyl iodide (40 ml.), dimethylformamide (120 ml.) and silver oxide (40g.) to give methylated tritylated arabinogalactan (9.2g.) [Found : O Me, 19.7%, not raised on further methylation with the above reagents].

The above product (8.9g.) was methylated twice with methyl iodide (150 ml.) and silver oxide (30g.) to give methylated tritylated arabinogalactan A (8.2g.), $[\alpha]_D$, - 43° (c, 0.5 in chloroform) [Found : Tr, 33.7%, O Me, 23.2%, not raised on further methylation. Calc. : Tr, 33.6%, O Me, 27.0%].

Examination of the cleavage products by gas-liquid chromatography on columns (a) and (b) and by paper chromatography insolvent D indicated the presence of mono - O - methylgalactoses, 2,4 - di - O - methylgalactose, 2,3,4 - tri - O - methylgalactose, 2,4,6 - tri - O - methylgalactose, 2 - O - methylarabinose, 2,3, - di - O - methylarabinose, /

methylarabinose, 2,3,4 - tri - O - methylarabinose and traces of 2,3,4,6 - tetra - O - methylgalactose, 2,5 - di - O - methylarabinose and 2,3,5 - tri - O - methylarabinose.

Detritylation of methylated tritylated polysaccharide.

All attempts to detritylate the methylated tritylated polysaccharide by (a) catalytic hydrogenolysis (109) with 10% platinum on charcoal and reduced platinum oxide (Adam's catalyst) and (b) reductive hydrogenolysis with lithium aluminium hydride, failed.

Detritylation with acid.

Methylated tritylated polysaccharide (8.1g.) was dissolved in methanol : chloroform (160 ml. 1:7) containing 1% (w./v.) hydrogen chloride and allowed to stand at room temperature for 3 hours. After neutralisation with silver carbonate and centrifugation the centrifugate and washings were combined and concentrated slightly (100 ml.). The solution was shaken with aqueous potassium cyanide (30 ml. 1% w./v.) to remove colloidal silver salts, when 3 layers separated. The bottom layer (chloroform) was separated, washed with water, dried over anhydrous sodium sulphate and concentrated (20 ml.). Precipitation with light petroleum gave methylated polysaccharide (fraction A, 2.4g.). The middle layer (chloroform-water emulsion) was washed with water and evaporated to dryness. The residue was extracted with chloroform and the extracts were concentrated (20 ml.) and poured in light petroleum to give methylated /

methylated polysaccharide (fraction B, 0.5g.).

The chloroform - insoluble material from the middle layer, the top layer, and all aqueous washings were combined, deionised with Amberlite resins IR-120 (H) and IR-4B (OH) and freeze-dried to give methylated polysaccharide (fraction C, 1.7g.).

The chloroform - soluble material (fractions A and B) was fractionated into 4 fractions by stepwise extraction with boiling light petroleum containing increasing proportions of chloroform. Details of the fractionation are given below (table 8). The trityl content of each fraction was found to be nil. The optical rotations of fractions 3 and 4 were observed in chloroform (c, ca.1) while that of fraction C was observed in water (c, 0.8).

Table 8

Fraction	% chloroform in extractant	Weight (g.)	O Me %	$[\alpha]_D$
1	50	0.03	-	-
2	60	0.27	-	-
3	80	1.79	34.1	-23°
4	100	0.52	31.8	-21°
c	-	1.67	29.1	-4°

Gas-liquid chromatography of the cleavage products from methanolyses of fractions 3 and C showed that both fractions contained the following methylated sugars :- 2,3,5 - tri - O - methylarabinose (trace, $\underline{\underline{T}}$ = 0.47 column (b)), 2,3,4 - tri - O - methylarabinose ($\underline{\underline{T}}$ = 0.82 column (b), 0.90 column (c)), 2,3 - di - O - methylarabinose ($\underline{\underline{T}}$ = 0.65, 0.82, 1.03 column (b), 1.25, 1.82 column (c)), 2,3,4,6 - tetra - O - methylgalactose (trace, $\underline{\underline{T}}$ = 1.58 column (b)) 2,4,6 - tri - O - methylgalactose (trace, $\underline{\underline{T}}$ = 2.31, column (b), 4.20 column (c)), 2,3,4 - tri - O - methylgalactose ($\underline{\underline{T}}$ = 2.53, 2.80 column (b), 6.85 column (c)) and 2,4 - di - O - methylgalactose ($\underline{\underline{T}}$ = 3.48, 4.11 column (b)). The five polysaccharide fractions were then combined to give detritylated methylated arabinogalactan A (4.1g.).

Oxidation of detritylated methylated polysaccharide (110)

Chromium trioxide (680 mg.) in glacial acetic acid (40 ml.) containing 2% of water (v./v.) was added to a solution of the detritylated methylated polysaccharide (4.1 g.) in glacial acetic acid (80 ml.). The solution was allowed to stand at room temperature for 60 hours when a further quantity of oxidant (680 mg.) was added. After 2 days at room temperature methanol (3 ml.) was added to destroy excess chromium trioxide and the solution was left at room temperature for 30 minutes. Water (100 ml.) was added and the solution was extracted with chloroform (5 x 100 ml.). The chloroform extracts were washed with water and concentrated to a syrup to give oxidised methylated polysaccharide (fraction D, 2.4 g.).

The aqueous layer and washings from the chloroform extraction /

extraction were combined, deionised with Amberlite resin IR - 120 (H) and methyl - di - N - octylamine in chloroform (5% v./v., 5 x 100 ml.), and freeze-dried to give oxidised methylated polysaccharide (Fraction E, 1.1 g.).

Both fractions were contaminated with inorganic material. A sample from fraction D was purified by extraction with boiling chloroform containing 20% light petroleum. The extract was filtered, concentrated and poured into light petroleum. The precipitated oxidised methylated polysaccharide had $[\alpha]_D$, -21°, (c, 1.0 in chloroform) [Found: OMe, 32.8%].

Samples from fractions D and E were hydrolysed with N sulphuric acid at 100° for 4 hours and neutralised in the usual manner. A paper chromatographic examination of the products in solvent E showed that three acidic sugars (R_G = 0.51, 0.74, 0.93) together with neutral monomers were present in both hydrolysates. The two polysaccharide fractions were then combined to give oxidised methylated arabinogalactan A (3.1 g.).

Partial hydrolysis of oxidised methylated polysaccharide.

Oxidised methylated polysaccharide (3.1 g.) was heated with N sulphuric acid (60 ml.) on a boiling-water bath for 4 hours. The solution was cooled, neutralised with barium hydroxide and barium carbonate, treated with Amberlite resin IR - 120 (H) to remove barium ions, and concentrated to a syrup (2.7 g.).

The syrup was absorbed on to a D.E.A.E. Sephadex column (grade A25, 6 x 3 cm.) in the formate form. Elution with water gave /

gave a neutral sugar fraction (1.9 g.) and elution with 3% formic acid gave an acid sugar fraction (580 mg.).

Examination of neutral sugar fraction

Gas-liquid chromatography of the derived methyl glycosides indicated the presence of the following sugars in the neutral sugar fraction : 2,3,5 - tri - O - methylarabinose (trace, $\underline{T}$ = 0.54 column (a), 0.45 column (b)), 2,3,4 - tri - O - methylarabinose ($\underline{T}$ = 0.80 column (b)), 2,3, - di -O - methylarabinose ($\underline{T}$ = 1.50, 1.81, 2.08 column (a), 0.65, 0.80 column (b)), 2 - O - methylarabinose ($\underline{T}$ = 5.70 column (a), 1.10, 1.31, 1.54 column (b)), 2,3,4,6 - tetra - O - methylgalactose ($\underline{T}$ = 1.72 column (a), 1.54 column (b)), 2,4,6 - tri - O - methylgalactose (trace, $\underline{T}$ = 3.94, 4.42 column (a), 1.97, 2.26 column (b), 2,3,4, - tri - O - methylgalactose ($\underline{T}$ = 7.0 column (a), 2.51, 2.75, column (b)) and 2,4 - di - O - methylgalactose ($\underline{T}$ = 3.55, 4.05 column (b)).

Examination of acidic sugar fraction

A portion of this fraction (20 mg.) was refluxed with methanolic hydrogen chloride (3%) overnight. After neutralisation with silver carbonate, the mixture of methyl glycosides was examined by gas-liquid chromatography on column (b). The result obtained are shown in table 9 .

A second portion of the fraction (50 mg.), after conversion /

conversion to the methyl ester methyl glycosides, was refluxed with lithium aluminium hydride (75 mg.) in tetrahydrofuran for 2 hours. Excess hydride was destroyed by water, the mixture was taken to dryness, and the residue was exhaustively extracted with acetone to give a syrup (45 mg.). The cleavage products from methanolysis of this reduced material were examined by gas-liquid chromatography on column (b). The results obtained are shown in table 10.

A further portion of the acid sugar fraction (100 mg.) was methylated with methyl iodide (0.5 ml.), dimethylformamide (1.5 ml.) and silver oxide (0.5 g.). Evaporation of the solvent gave a syrup (90 mg.). A portion of this syrup (40 mg.) was methanolysed and neutralised as above, and the resulting mixture of methyl glycosides examined by gas-liquid chromatography on column (b). The results obtained are shown in table 9.

The remainder of the syrup obtained from methylation of the acidic sugar fraction was reduced with lithium aluminium hydride (75 mg.) in tetrahydrofuran (15 ml.) after conversion to the methyl ester methyl glycosides. The cleavage products from methanolysis of this reduced remethylated materialwere examined by gas-liquid chromatography. The results obtained are shown in table 10.

Table 9 .

Methyl glycosides of	T for methyl glycosides from acidic sugars	T for methyl glycosides from remethylated acidic sugars
2,3,4 - Trimethyl-galactose	2.47, 2.71	2.36,* 2.73
2,3,4 - Trimethyl-galacuronic acid	3.65, (4.00)	3.68, 4.03
2,4 - Dimethyl-galactose	3.40, (4.00)	

The T values within parenthesis correspond to more than one methyl glycoside.

Table 10 .

Methyl glycosides of	T for methyl glycosides from reduced acidic sugars	T for methyl glycosides from reduced remethylated acidic sugars
2,3 - Dimethyl-arabinose	0.64, 0.82, 0.97	0.63, 0.81, 0.95
2,3,4 - Trimethyl-galactone	2.47, 2.72	2.46, 2.73
2,4 - Dimethyl-galactose	3.40, 4.02	
Unknown		2.16*

In addition to the $\underline{T}$ values quoted above two peaks with low retention times (0.88, 0.99) were observed in both unreduced samples. These could have been due to 2,3 - di - $\underline{O}$ - methylaraburonic acid, the retention times of which are not known.

The presence of an unrecognised peak ($\underline{T}$ = 2.16) on the chromatogram of the methanolysis products from the reduced remethylated acid sugar fraction, and the distortion of the first methyl 2,3,4 - tri - $\underline{O}$ - methylgalactoside peak ($\underline{T}$ = 2.36 as compared with 2.47 for an authentic sample) on the chromatogram of the methanolysis products from the methylated acidic sugar fraction, indicate that an unknown methyl glycoside(s) with similar retention times as methyl 2,4,6 - tri - $\underline{O}$ - methylgalactoside ($\underline{T}$ = 1.97 and 2.25) was present. However, analyses of several sugar mixtures containing varying proportions of authentic methyl 2,3,4 - tri - $\underline{O}$ - methylgalactoside and methyl 2,4,6 - tri - $\underline{O}$ - methylgalactoside showed that the second methyl 2, 4, 6 - tri - $\underline{O}$ - methylgalactoside peak ($\underline{T}$ = 2.25) and the first methyl 2,3,4 - tri - $\underline{O}$ - methylgalactoside peak ($\underline{T}$ = 2.46) could be recognised even when only traces of methyl 2,4,6 - tri - $\underline{O}$ - methylgalactoside were present.

Although 2 - $\underline{O}$ - methylarabinose was not detected among the cleavage products from methanolysis of the reduced acidic sugar fraction, hydrolysis of this material followed by paper chromatography in solvent F gave a faint trace of 2 - $\underline{O}$ - methylarabinose $\left\{R = \begin{matrix} 1.38 \\ 2,4 - \text{dimethylgalactose} \end{matrix}\right\}$.

S E C T I O N II.

ARAUCARIA BIDWILLI GUM.

DISCUSSION.

The sample of gum polysaccharide used in the present investigation was kindly provided by Professor A. J. Birch. The polysaccharide had been isolated as a white amorphous powder by precipitation with acidified ethanol from an aqueous extract of the resin.

Preliminary investigations showed that the polysaccharide, $[\alpha]_D$, + 11°, had uronic anhydride content, 10.1%. Hydrolysis furnished galactose (46.5 moles) arabinose (12.6 moles) rhamnose (3.5 moles) and a faint trace of xylose. In addition two aldobiouronic acids were present which were identified chromatographically as 6 - O - (glucopyranosyluronic acid) - galactose and 6 - O - (4 - O - methylglucopyranosyluronic acid) - galactose.

Partial hydrolysis studies.

A sample of the polysaccharide (polysaccharide A) was hydrolysed for 4 hours at 100° with N sulphuric acid and the products were adsorbed on to a D.E.A.E. Sephadex column in the formate form. The neutral sugars, which were eluted with water, were fractionated by chromatography on thick paper to give D - galactose, L - arabinose and L - rhamnose which were characterised by crystalline derivatives.

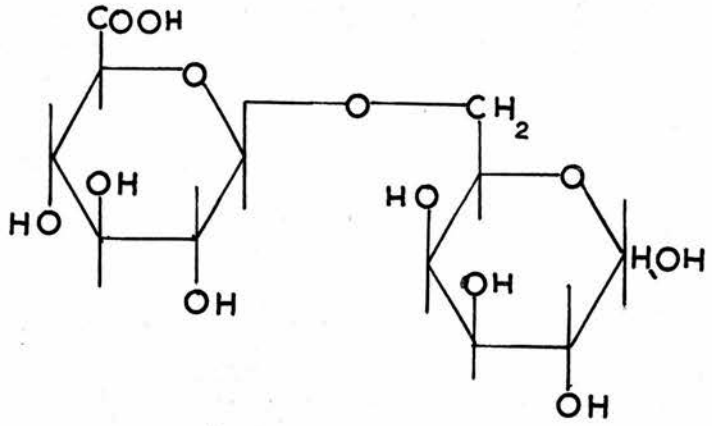
The acidic sugars were eluted with 3% formic acid and fractionation by chromatography gave two fractions.

Fraction /

Fraction 1, $[\alpha]_D$, 0° contained only one sugar which was chromatographically identical to 6 - O - β - D - (glucopyranosyluronic acid) - D - galactose. Hydrolysis of the fraction gave only galactose and glucuronic acid. Reduction of the derived methyl ester methyl glycoside with potassium borohydride followed by hydrolysis gave galactose and glucose. The latter sugar must have arisen from reduction of glucuronic acid.

Methylation of the fraction afforded a syrup which failed to crystallise. A gas-liquid chromatographic examination of the cleavage products from methanolysis showed approximately equal amounts of methyl 2,3,4 - tri - O - methylgalactoside and the methyl ester of methyl 2,3,4 - tri - O - methylglucuronoside with a trace of methyl 2,3,5 - tri - O - methylgalactoside.

On the above evidence, the sugar is assigned the following structure:-

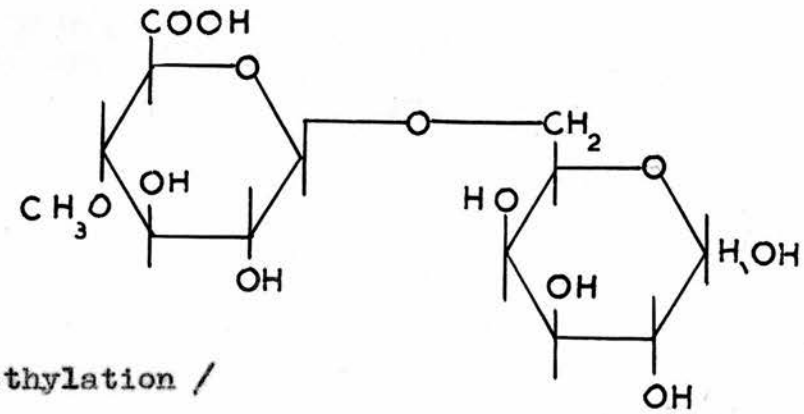


Fraction 2, $[\alpha]_D$, $+3^\circ$, was chromatographically identical to 6 - O - (4 - O - methyl - β - D - glucopyranosyluronic acid) - D - galactose and on hydrolysis gave galactose and an acidic sugar with the same chromatographic mobility as 4 - O - methylglucuronic acid. /

acid. Reduction of the derived methyl ester methyl glycoside with potassium borohydride, followed by hydrolysis gave galactose and 4 - O - methylglucose. The latter sugar must have arisen from reduction of 4 - O - methylglucuronic acid. Periodate oxidation of the hydrolysis products from the reduced sugar furnished 2 - O - methylerythrose. The latter sugar must have arisen from periodate oxidation of a 4 - O - methylhexose.

Methylation of the fraction afforded a syrup which failed to crystallise. A chromatographic examination of the cleavage products from methanolysis showed approximately equal amounts of methyl 2,3,4 - tri - O - methylgalactoside and the methyl ester of methyl 2,3,4 - tri - O - methylglucuronoside together with smaller amounts of methyl 2,3,5 - tri - O - methylgalactoside, methyl 2,3,4,6 - tetra - O - methylgalactoside and an unknown ($\underline{T}$ = 3.04 column (a)). The origin of the latter two minor components is unknown.

The above evidence shows that the major component of this fraction had the following structure:-



Methylation /

Methylation studies

Polysaccharide A was methylated with barium hydroxide and methyl sulphate to give a partially methylated product which had O Me, 31.9%. Methylation was completed by two treatments with methyl iodide and silver oxide. The fully methylated polysaccharide had $[\alpha]_D$, - 49°, O Me, 42.8%.

The cleavage products from the methylated polysaccharide were examined by gas-liquid chromatography of the methylglycosides and paper chromatography of the sugars. The following methyl ethers were detected.

Methyl ether	Relative amounts
2,3,4 - Trimethylrhamnose	+
2,3,5 - Trimethylarabinose	++
2,3,4 - Trimethylarabinose	trace
2,5 - Dimethylarabinose	trace
2,3,4,6 - Tetramethylgalactose	++++
2,4,6 - Trimethylgalactose	++
2,3,4 - Trimethylgalactose	+++
2,4 - Dimethylgalactose	++++
2 - Monomethylgalactose	trace
2,3,4 - Trimethylglucuronic acid	+++

A sample of the fully methylated polysaccharide was depolymerised with methanolic hydrogen chloride and hydrolysed with $\underline{\underline{N}}$ sulphuric acid. The products were adsorbed on to a D.E.A.E. /

D.E.A.E. Sephadex column in the formate form, the neutral sugars were removed with water and the acidic sugars were eluted with 3% formic acid. An examination of the cleavage products from the acidic sugar fraction by gas-liquid chromatography of the methyl glycosides and paper chromatography of the sugars showed 2,3,4 - tri - O - methylgalactose and 2,3,4 - tri - O - methylglucuronic acid. A portion of the acidic sugar fraction, after conversion to the methyl ester methyl glycoside, was reduced with potassium borohydride. An examination of the cleavage products by gas-liquid chromatography of the methyl glycosides and paper chromatography of the sugars indicated the presence of 2,3,4 - tri - O - methylgalactose and 2,3,4 - tri - O - methylglucose.

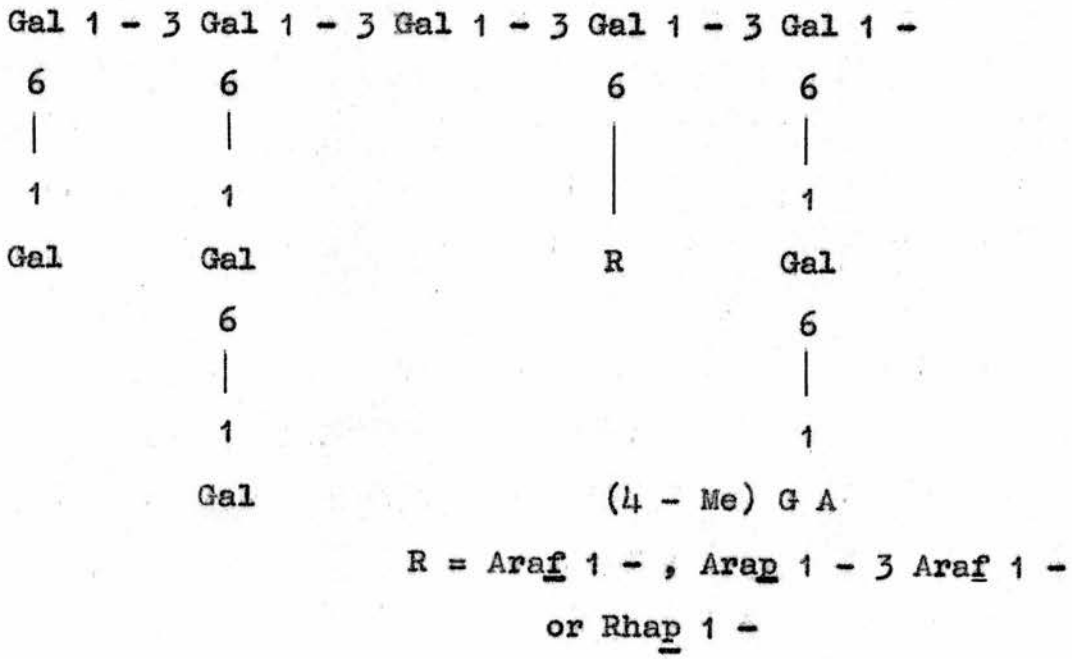
The above methylation results clearly show that the polysaccharide is highly branched. 2,3,4,6 - Tetra - O - methylgalactose, 2,4,6 - tri - O - methylgalactose, 2,3,4 - tri - O - methylgalactose and 2,4 - di - O - methylgalactose were present in largest amount indicating that the framework of the polysaccharide consists of (1 - 3)-and (1 - 6)- linked galactose units.

All the rhamnose residues and the majority of the arabinose residues are present as non-reducing end-groups; the former in the pyranose form and the latter in the furanose form. The detection of trace amounts of 2,5 - di - O - methylarabinose and 2,3,4 - tri - O - methylarabinose indicates that a small proportion of the arabinose residues are accommodated as 3 - O - β - L - arabinopyranosyl - L - arabinofuranose groups. The existence of this structural unit in the polysaccharide was subsequently supported by partial hydrolysis /

hydrolysis evidence when trace quantities of a disaccharide with the same chromatographic mobility as 3 - O - β - L - arabinopyranosyl - L - arabinose was observed.

The acidic residues are present as non-reducing end-group. The detection of 2,3,4 - tri - O - methylgalactose as the only neutral sugar among the hydrolysis products from the acidic sugar fraction shows that all the acidic residues are linked to periodate oxidisable galactose residues by a (1 - 6) - linkage.

From the methylation and partial hydrolysis evidence one possible partial structure for polysaccharide A is shown below.



R = Araf 1 - , Arap 1 - 3 Araf 1 - or Rhap 1 -

Periodate oxidation studies.

Polysaccharide A was oxidised with sodium metaperiodate.

1.43 /

1.43 moles of oxidant were consumed with the liberation of 0.64 moles of formic acid per sugar unit. From the methylation and partial hydrolysis evidence it can be seen that the residues which will consume 2 moles of periodate and liberate 1 mole of formic acid are terminal non-reducing galactose, (1 - 6) - linked galactose, glucuronic acid and terminal non-reducing arabinopyranose residues. It follows then that 64% of the residues in the polysaccharide are present in this form and of the remaining 36%, 15% are present as terminal non-reducing 4 - O - methylglucuronic acid and terminal non-reducing arabinofuranose residues.

In order to obtain information on the distribution of the (1 - 3) - linked galactose residues, the polysaccharide was oxidised with sodium metaperiodate until no more reagent was consumed. The oxidised residues were removed from the molecule, after reduction with potassium borohydride, by treatment with N sulphuric acid at room temperature. The resulting degraded polysaccharide was recovered by precipitation with ethanol. The supernatant liquid and ethanol washings were combined and concentrated to give syrup B. The degraded polysaccharide (polysaccharide B), $[\alpha]_D$, + 26°, on hydrolysis gave galactose and arabinose in the approximate ratio of 20 to 1, and a trace of a third reducing substance (R_{Gal} = 2.02), but no non-reducing substances.

The degraded polysaccharide was subjected to a second periodate oxidation and Smith degradation to give a further degraded polysaccharide (polysaccharide C), $[\alpha]_D$, + 30°, which on hydrolysis gave galactose and trace of arabinose and a third reducing substance (R_{Gal} = 2.02), but no non-reducing substances. Evaporation of the supernatant /

supernatant liquid and ethanol washings from the precipitation of polysaccharide C gave syrup C.

A chromatographic examination of the two low molecular weight fractions (syrups B and C) showed mainly glycerol with smaller amounts of two other non-reducing substances, but no reducing sugars. Hydrolysis of the two syrups gave galactose, arabinose (syrup B only) and the above three non-reducing subtances. The presence of the above reducing sugars in these fractions is due mainly - if not entirely - to contamination of the syrups with polymeric material.

The two degraded polysaccharides were examined by partial hydrolysis, periodate oxidation and methylation. Both polysaccharides proved extremely difficult to methylate and fully methylated derivatives were not obtained. The results of these experiments together with those from similar studies on the undegraded polysaccharide are shown below.

Partial hydrolysis studies.

Disaccharides	Polysaccharides		
	A	B	C
(1 - 3)- galactobiose	small	large	large
(1 - 6)- galactobiose	large	small	trace
(1 - 3)- arabinobiose	trace	none	none

Periodate /

Periodate oxidation studies.

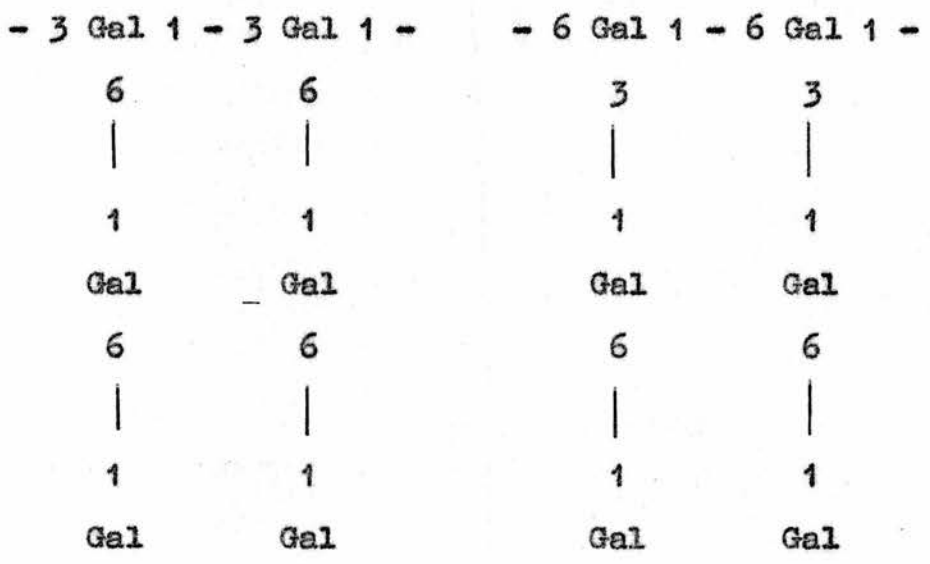
Polysaccharide	Reagent consumed Moles/sugar unit.	Formic acid liberated Moles/sugar unit.
Polysaccharide A	1.43	0.64
Polysaccharide B	0.27	0.09
Polysaccharide C	0.15	0.04

Methylation studies.

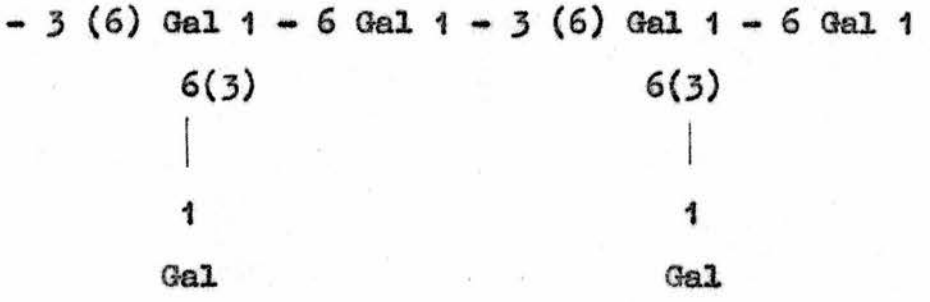
Methyl ether.	Methylated polysaccharides. A	B	C
2,3,4 - Trimethylrhamnose	trace	-	-
2,3,5 - Trimethylarabinose	++	+	-
2,3,4 - Trimethylarabinose	trace	-	-
2,5 - Dimethylarabinose	trace	-	-
2,3,4,6 - Tetramethylgalactose	++++	+	+
2,4,6 - Trimethylgalactose	++	++++	++++
2,3,4 - Trimethylgalactose	+++	trace	trace
2,6 - Dimethylgalactose	-	++	++
2,4 - Dimethylgalactose	++++	+	+
2 - Monomethylgalactose	trace	++	++
2,3,4 - Trimethylglucuronic acid	+++	-	-

From /

From the methylation studies on the undegraded polysaccharide, where 2,3,4,6 - tetra - O - methylgalactose, 2,3,4 - tri - O - methylgalactose and 2,4 - di - O - methylgalactose were found in largest amount, three possible partial structures for the galactan framework are shown below (1, 11, 111).



(1) (11)



(111)

Since polysaccharide residues were isolated from the products of both Smith degradations, the polysaccharide must possess /

possess blocks of periodate - resistant residues. Structure (111) can therefore be eliminated as only isolated periodate - resistant residues would be present. Structure (11) can also be eliminated as the polysaccharide residue obtained from a Smith degradation of such a structure (IV) would contain no periodate - resistant residues and would, consequently, fragment when subjected to a second Smith degradation.

- 6 Gal 1 - 6 Gal 1 -

(IV)

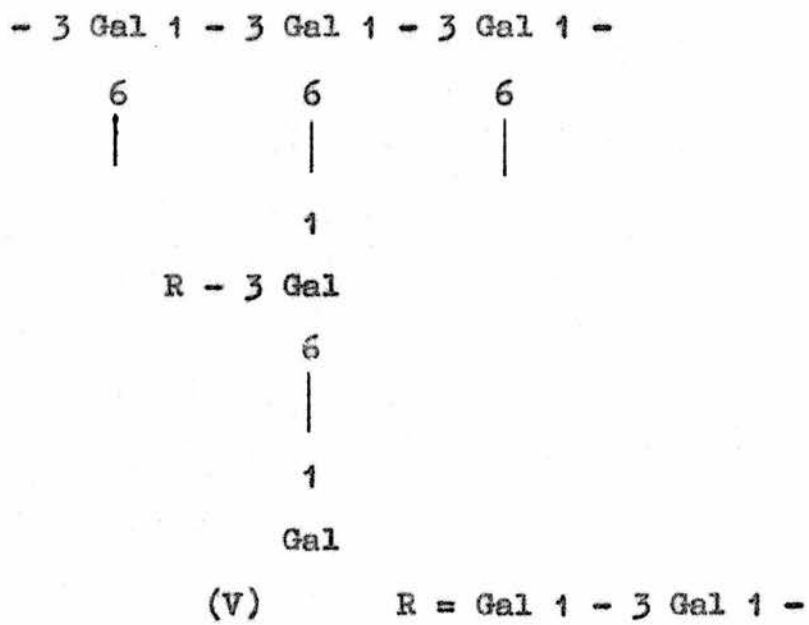
Evidence in favour of structure (1) representing a major part of the galactan framework was provided by periodate oxidation, partial hydrolysis and methylation studies on the two degraded polysaccharides.

The amount of periodate consumed by the polysaccharide decreased after each Smith degradation indicating that the polysaccharide was approximating to a (1 - 3)- linked galactan. Partial hydrolyses of the two degraded polysaccharides gave 3 - O - galactopyranosyl-galactose with only traces of the (1-6)-linked isomer, whereas the undegraded polysaccharide furnished on partial hydrolysis, large amounts of 6 - O - galactopyranosyl - galactose with smaller amounts of the (1 - 3)- linked isomer. It is evident therefore, that the (1 - 3)- linkages between galactose residues predominate in the interior chains of the molecule.

Both Smith degraded polysaccharides proved extremely difficult /

difficult to methylate and, like Smith degraded mountain larch arabinogalactan (85), fully methylated derivatives were not obtained. In the absence of any evidence indicating the presence of a significant number of (1 - 4)- linkages in the undegraded polysaccharide, the appreciable quantities of 2 - O - methylgalactose and 2,6 - di - O - methylgalactose found in the methylation studies on the two degraded polysaccharides are attributed to incomplete methylation. The large amounts of 2,4,6 - tri - O - methylgalactose and the virtual absence of 2,3,4 - tri - O - methylgalactose among the cleavage products from both methylated degraded polysaccharides provide further evidence in favour of a (1 - 3)- linked galactan backbone.

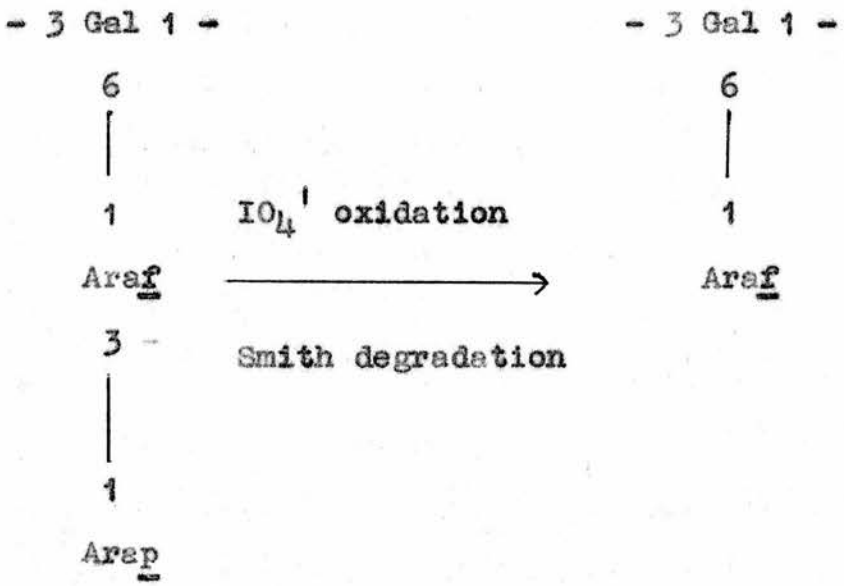
The trace amounts of 6 - O - galactopyranosyl-galactose which were found among the partial hydrolysis products even after the second Smith degradation suggests that branching may occur in some of the galactose containing side-chains (V).



(V) R = Gal 1 - 3 Gal 1 -

The /

The small amount of terminal non-reducing arabinofuranose found in the methylated degraded polysaccharide arises from the 3 - O - arabinopyranosyl-arabinofuranose groupings in the undegraded polysaccharide (VI).



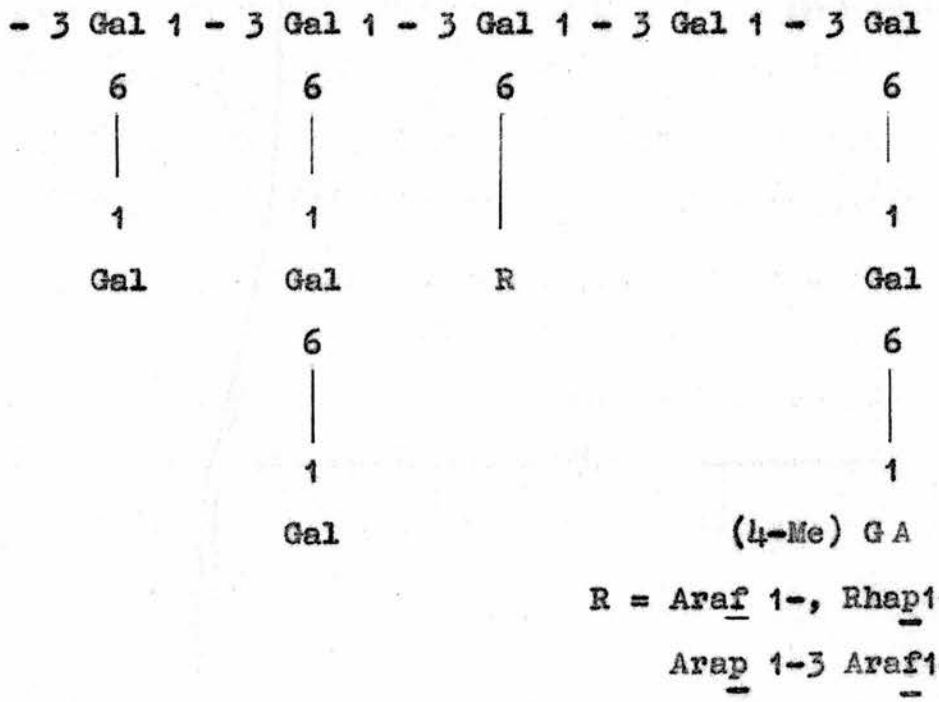
(VI)

The results of the investigations on the polysaccharide from Araucaria bidwilli gum clearly show that it contains a galactan framework consisting of a main chain of (1-3)- linked D - galactopyranose residues to which are attached, through positions C 6, D - galactopyranose-containing side-chains. Since appreciable quantities of 2,4,6 - tri - O - methylgalactose were found among the cleavage products from the methylated polysaccharide, not all the main chain galactose residues carry side-chains. Some of the galactose-containing side-chains are terminated by glucuronic acid (or the 4 - O - methyl ether). Arabinose residues are /

are present mainly as non-reducing end-groups in the furanose form. Rhamnopyranose residues, which are present in small proportion only, appear to be accounted for entirely in the form of non-reducing end groups.

It is not yet possible on the present evidence to indicate how the aldobiouronic acid, arabinose and rhamnose residues are incorporated in the general molecular structure of the polysaccharide and the structure shown below indicates only one possible site of attachment of these units.

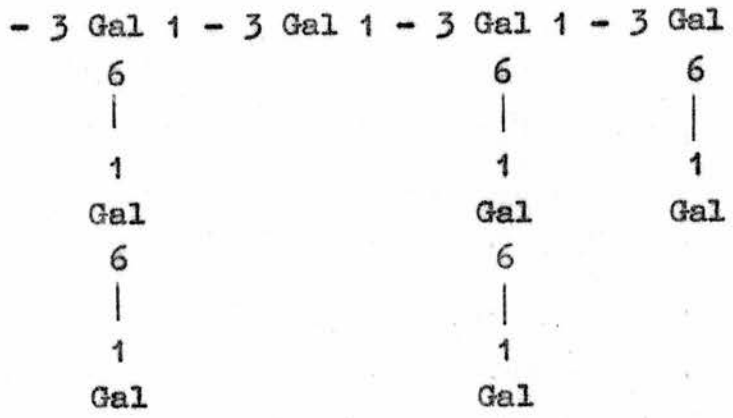
Proposed structure for *Araucaria bidwillii* gum polysaccharide.



Comparison of the structural features of Japanese larch arabinogalactan A and *Araucaria bidwilli* gum with those of other coniferous wood arabinogalactans and galactan-based gums.

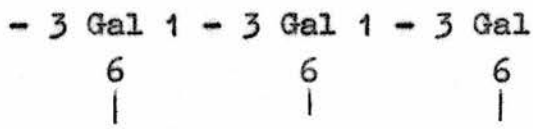
Methylation and partial hydrolyses studies on the two polysaccharides which have been the subjects of the present investigations have shown that both polysaccharides possess highly branched frameworks consisting of (1-3)- and (1-6)- linked D - galactopyranose residues. Periodate oxidation followed by Smith degradations led to the isolation of degraded polysaccharides in which (1-3) - linked D - galactose residues were shown, by methylation, partial hydrolyses and periodate oxidation studies, to be predominant. These results show that both polysaccharides possess similar galactan frameworks consisting of (1-3)- linked D - galactopyranose main chains to which are attached, through positions C6, galactose-containing side-chains (VII). Similar studies on other coniferous wood arabinogalactans (22, 32, 33, 63-70, 72-76, 82-86) and the polysaccharides from *Acacia senegal* gum (36, 87-89) *Khaya senegalensis* gum (B fraction) (36), *Acacia pycnantha* gum (90-92) and *Asafoetida* gum (93) have shown that these polysaccharides possess a similar type of galactan framework (VII).

- 3 Gal 1 /



(VII)

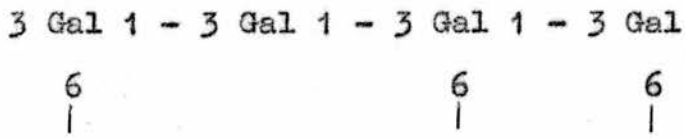
In Japanese larch arabinogalactan A, as in the other coniferous wood arabinogalactans and Acacia senegal gum (gum arabic) virtually all the residues in the main chain are substituted at position C6 (VIII) as only trace amounts of 2,4,6 - tri - O - methyl-galactose were found among the hydrolysis products from the methylated polysaccharide.



(VIII)

In Araucaria bidwilli gum, however, relatively large amounts of 2,4,6 - tri - O - methylgalactose were detected on examination of the cleavage products from the methylated polysaccharide, suggesting that an appreciable proportion of the main chain galactose residues do not carry side-chains. In this respect the polysaccharide is similar to Khaya senegalensis gum (B fraction), Acacia pycnantha gum and Asafoetida gum. In the latter gum approximately /

approximately half of the main chain galactose residues are not further substituted (IX).



(IX)

A characteristic feature of the above gums, which for convenience will be termed galactan-based gums, is the presence of glucuronic acid residues, often present in part as the 4 - O - methyl ether. The acidic residues in Araucaria bidwilli gum, as are the majority and frequently all the acidic residues in other galactan-based gums, are linked to position C6 of galactose residues as the only aldobiouronic acids isolated as products of partial hydrolysis from the gum were 6 - O - (β - D - glucopyranosyluronic acid)- D - galactose and 6 - O - (4 - O - methyl - β - D - glucopyranosyluronic acid) - D - galactose (X).

(4 - Me) GA 1 - 6 Gal

(X)

The detection of 2,3,4 - tri - O - methylglucuronic acid and the fully etherified aldobiouronic acid 6 - O - (2,3,4 - tri - O - methylglucuronic /

methylglucuronic acid) - 2,3,4 - tri - O - methylgalactose as the only acidic sugars among the cleavage products from the methylated polysaccharide indicates that all the acidic residues in Araucaria bidwilli gum are present as non-reducing end-groups and that aldobiouronic acid units (XI) must be attached as side-chains to the galactan backbone. The precise mode of attachment is not known.

(4 - Me) GA 1 - 6 Gal 1 -

(XI)

Similar studies on other galactan-based gums have shown that all the acidic residues in Acacia pycnantha gum, Asafoetida gum and degraded Acacia senegal gum from which the acid-labile residues had been removed, and four out of five of the acidic-residues in Khaya senegalensis gum (B fraction) are also present as non-reducing-end groups. In Acacia pycnantha gum and degraded Acacia senegal gum, like Araucaria bidwilli gum, all the acidic residues are linked to side-chain galactose residues (XI).

Small amounts of 2,3,4 - tri - O - methylglucuronic acid were detected among the cleavage products from methylated Japanese larch arabinogalactan A but it is impossible on the available evidence to state how these terminal non-reducing residues are incorporated in the general molecular structure of the molecule. In the arabinogalactans from tamarack larch (82-84) and maritime pine (86), where glucuronic acid accounts for approximately 3% of the residues, and mountain larch (85) /

(85) which contains 7% glucuronic acid, it has been shown that, like Araucaria bidwillii gum and other galactan-based gums, the acidic residues are present as non-reducing end-groups and are attached to position C6 of galactose residues.

Both Japanese larch arabinogalactan A and Araucaria bidwillii gum contain L-rhamnose and/or L - arabinose residues which are easily cleaved by acid. In Araucaria bidwillii gum, as in all the other rhamnose-containing galactan-based gums, the only rhamnose derivative found among the cleavage products from the methylated polysaccharide was the 2,3,4 - tri- O - methyl ether, therefore, the small proportion of these sugar residues are present as end-groups in the pyranose form (XII). Both Japanese larch arabinogalactan A and Araucaria bidwillii gum, like the other coniferous wood arabinogalactans and galactan-based gums, contain non-reducing arabinofuranose end-groups (XIII). In addition non-reducing arabinopyranose and non-terminal arabinofuranose groups are also present, the latter two arabinose residues accounting for only a very small proportion of the arabinose content of Araucaria bidwillii gum. Methylation and partial hydrolyses studies on both polysaccharides has shown that at least some, and possibly all, the arabinopyranose and non-terminal arabinofuranose residues are accommodated as 3 - O - β - L - arabinopyranosyl - L - arabinofuranose end-groups (XIV).

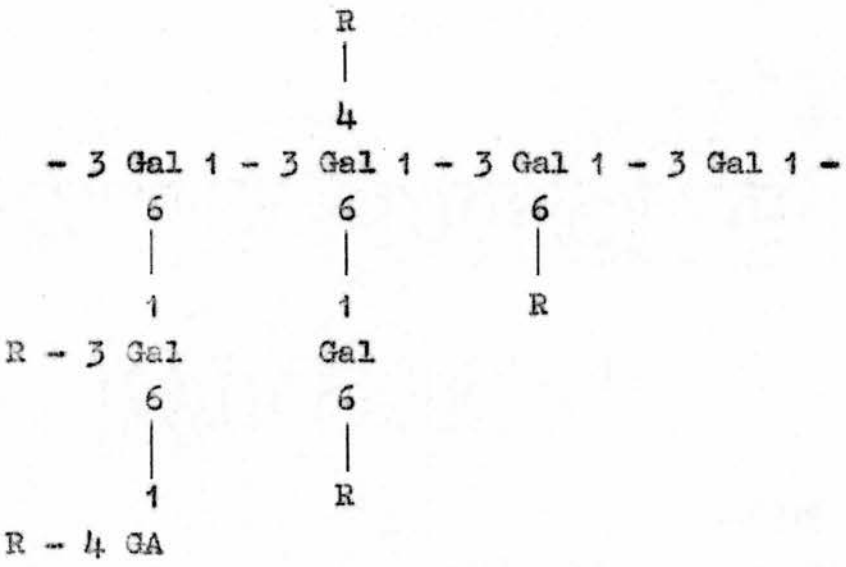
Rhap 1 - (XII)

Araf 1 - (XIII)

Arap 1 - 3 Araf 1 - (XIV)

The /

The structure shown below (XV) is a general structure embodying all the known sites of attachment of acid-labile units to the galactan framework from polysaccharides of both groups although not all the sites indicated are necessarily encountered in every polysaccharide. The nature of the acid-labile units known to be present in individual polysaccharides is shown in table II.

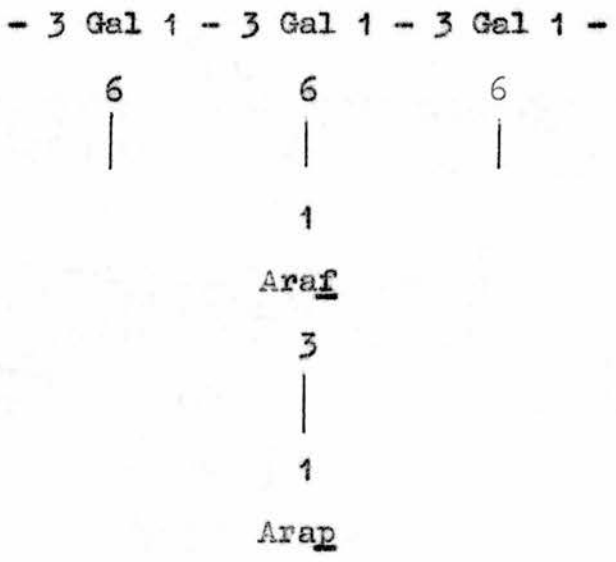


R = acid labile unit

(XV)

A comparison of the proportion of methyl ethers formed on hydrolyses of the methylated polysaccharides and of the methylated degraded polysaccharides from which the acid-labile units had been removed by mild acid hydrolysis, has in many galactan-based gums indicated the positions of linkage of acid-labile units to the galactan framework. Where such a comparison has been made, in the majority of cases it has been shown that some acid-labile units are attached /

attached to position C6 of the galactose residues in the main chains (XV). In Araucaria bidwilli gum there is as yet little information concerning the mode of attachment of acid-labile unit. However at least some of the non-terminal arabinofuranose residues must be attached to periodate-resistant galactose residues, probably those in the main chain (XVI), as periodate oxidation followed by a Smith degradation resulted in the isolation of a degraded polysaccharide which contained 5% arabinose.



(XVI)

In Acacia senegal gum rhamnopyranose residues have been shown to be attached to position C4 of glucuronic acid units (XVII) since 4 - O - α - L - rhamnopyranosyl - D - glucose was isolated as a product of acetolysis from the carboxyl reduced gum (88). It is clear that such a mode of attachment in Araucaria bidwilli gum is impossible /

impossible as all the acidic units are present as non-reducing end-groups and consequently the rhamnose residues must be attached, as in Acacia pycnantha gum, to galactose and, possibly, arabinofuranose units.

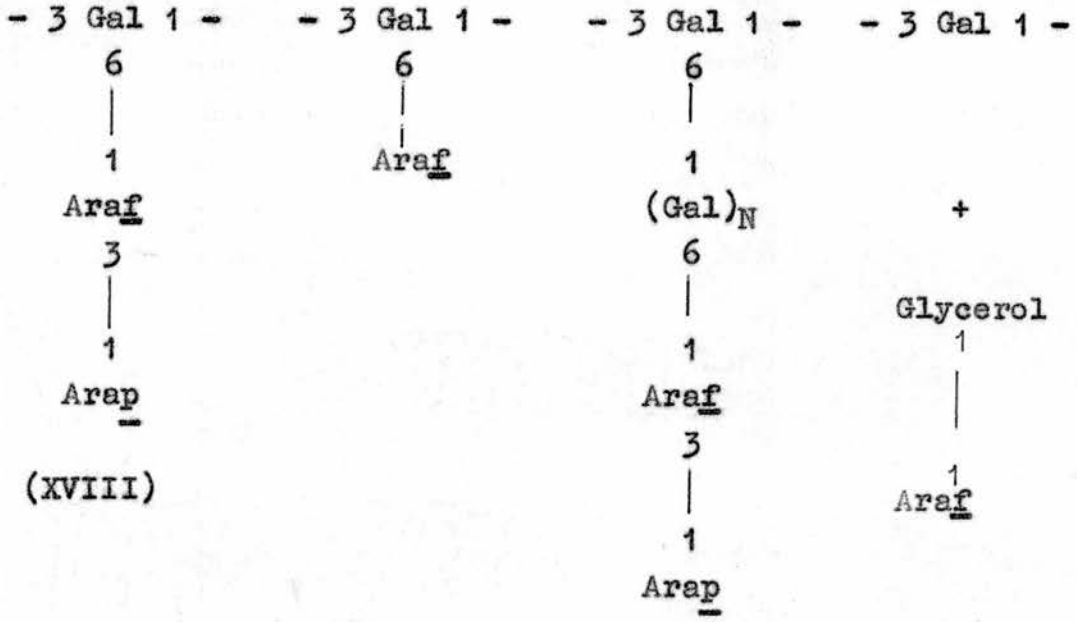
Rhap 1 - 4 G A 1 -

(XVII)

The primary hydroxyl groups of arabinofuranose and terminal galactose residues in Japanese larch arabinogalactan A were oxidised to carboxylic acid groups thereby stabilising the furanose linkages to acid attack. Partial hydrolysis studies on the oxidised polysaccharide has shown that arabinose residues, as in European larch arabinogalactan (76) and like many of the acid-labile units in galactan-based gums, are linked to position C6 of galactose residues.

The isolation of a degraded polysaccharide which contained 10% arabinose from periodate oxidised and Smith degraded Japanese larch arabinogalactan A (33) showed that non-terminal arabinofuranose units were attached to periodate-resistant galactose residues, very probably those in the main chain (XVIII). The absence of any monosaccharide derivatives among the low molecular weight degradation products suggested that no non-terminal arabinofuranose residues were attached to (1-6)- linked galactose residues in the side chains (XIX). Similar degradation studies on other coniferous wood arabinogalactans led to the same conclusions concerning the mode of attachment of the majority of non-terminal arabinofuranose to galactose residues, although /

although in European larch arabinogalactan (76), Western larch arabinogalactan B (but not A) (70), and mountain larch arabinogalactan (85) small proportions of the non-terminal arabinofuranose residues are probably linked as in (XIX).



It is becoming increasingly apparent that the coniferous wood arabinogalactans and the galactan-based gums form a spectrum of polysaccharides which have a main chain of (1-3)- linked D - galactopyranose residues to which are attached, through positions C6, side-chains of (1-6)- linked D - galactose residues. Acid-labile units, and acidic residues when present, are situated in the periphery of the molecule and there is no evidence to indicate their presence in the interior chains. The nature of peripheral units known to be present in polysaccharides from both groups is shown in table II .

The /

The relative proportion of the various sugar residues in these polysaccharides vary considerably with the galactan based gums containing in general a smaller proportion of galactose residues (table 2).

Table II

Peripheral units	Polysaccharides in groups						
	1	2	3	4	5	6	7
Araf 1 -	+	+	+	+	+	+	+
Araf 1 - 3 Araf 1 -	-	-	-	-	+	-	-
Arap 1 - 3 Araf 1 -	+	+	+	-	-	+	+
Galp 1 - 3 Araf 1 -	-	-	-	-	-	+	-
Xylp 1 - 3 Araf 1 -	-	-	+	-	-	-	-
Rhap 1 -	-	-	-	-	+	+	+
Rhap 1 - ? Gal 1 -	-	-	-	-	+	-	+
Rhap 1 - 4 G A 1 -	-	-	-	-	-	+	-
G A 1 - 6 Gal 1 -	-	+	+	+	+	+	+
4 - Me - GA 1 - 6 Gal 1 -	-	-	-	+	-	-	+

1 Japanese, European and Western larch arabinogalactans.

2 Tamarack and mountain larch arabinogalactans.

3 Maritime pine arabinogalactan.

4 Asafoetida gum and Khaya senegalensis gum (B fraction).

5 Acacia pycnantha gum.

6 Acacia senegal gum.

7 Araucaria bidwilli gum.

E X P E R I M E N T A L

Summary of work done

Araucaria bidwilli gum (Polysaccharide A).

	Hydrolysis
IO_4^-	Relative proportion of constituents
oxidation	Partial hydrolysis
Smith	Characterisation of neutral and acidic sugars
degradation	Methylation
	Periodate oxidation

↓

Degraded Araucaria bidwilli gum (Polysaccharide B).

	Hydrolysis
IO_4^-	Relative proportion of constituents
oxidation	Partial hydrolysis
Smith	Methylation
degradation	Periodate oxidation

↓

Degraded Araucaria bidwilli gum (Polysaccharide C).

Hydrolysis

Partial hydrolysis

Methylation

Periodate oxidation

PRELIMINARY INVESTIGATIONS

Hydrolysis of Polysaccharide A.

Polysaccharide A (5 mg.) $[\alpha]_D$, + 11° (c 0.5 in water), was hydrolysed with N sulphuric acid (2 ml.) for 4 hours at 100°. Paper chromatography in solvents A and C gave galactose, arabinose, rhamnose and two acidic oligosaccharides. A trace of xylose was observed on chromatograms which had been heavily spotted.

The acidic sugars were separated from the neutral sugars on a D.E.A.E. Sephadex column (grade A.25, 4 x 1 cm.). After conversion to the methyl ester methyl glycosides, the acidic sugars were reduced with potassium borohydride and hydrolysed with N sulphuric acid. Paper chromatography in solvent A gave galactose, glucose (R_{Gal} = 1.18) and 4 - O - methylglucose (R_{Gal} = 1.90).

Sugar proportions in polysaccharide A.

Polysaccharide A (16 mg.) was hydrolysed with N sulphuric acid (3 ml.) for 4 hours at 100°. The solution was neutralised by extraction with L.A.2 liquid resin in chloroform (5% v./v., 5 x 20 ml.), stirred with Amberlite resin IR - 120 (H) to remove traces of the liquid resin, filtered and evaporated to dryness. The residue was fractionated by paper chromatography on Whatman No.1 filter paper in solvent B.

A colorimetrical estimation (97) of the neutral sugars gave galactose (46.5 moles), arabinose (12.6 moles) and rhamnose (3.5 moles).

PARTIAL HYDROLYSIS STUDIES ON POLYSACCHARIDE A.

Partial hydrolysis of Polysaccharide A.

Polysaccharide A (10 mg.) was heated with sulphuric acid (0.5 N, 3 ml.) on a boiling-water bath for 1 hour. The solution was neutralised with Amberlite resin IR - 4B (OH), filtered and evaporated to dryness. Paper chromatography in solvent A gave arabinose (R_{Gal} = 1.35), galactose, and 6 - O - galactopyranosyl - galactose (R_{Gal} = 0.39) as the main components with smaller amounts of 3 - O - arabinopyranosyl - arabinose (R_{Gal} = 0.82), 3 - O - galactopyranosyl - galactose (R_{Gal} = 0.53) and higher galactose-containing oligosaccharides.

Isolation of neutral and acidic sugars.

Polysaccharide A (2g.) was hydrolysed with N sulphuric acid (50 ml.) for 4 hours at 100°. The solution was 95% neutralised with barium hydroxide, centrifuged and neutralised to completion with Amberlite L.A.2 liquid resin in chloroform (5% v./v., 5 x 20 ml.) The solution was stirred with Amberlite resin IR - 120 (H) to remove traces of liquid resin, filtered and concentrated to a syrup (1.9g.).

The syrup was fractionated on a D.E.A.E. Sephadex column (grade A.25, 6 x 3 cm.) to give neutral sugars (1.4g.) and acidic sugars (420 mg.).

Separation /

Separation of neutral sugars.

The neutral sugars were separated into 3 fractions by paper chromatography on 3 MM filter sheets in solvent B.

Characterisation of neutral sugars.

Fraction 1.

This fraction (1.1g., R_{Gal} = 1.00 in solvent A) was chromatographically identical to D - galactose. The sugar, after recrystallisation from absolute alcohol, had $[\alpha]_D$, + 79° (c, 0.8 in water), m.p. = 163°, undepressed on admixture with D - galactose.

Fraction 2.

This fraction (161 mg., R_{Gal} = 1.36 in solvent A) was chromatographically identical to L - arabinose. The sugar, after recrystallisation from ethanol containing 20% water, had $[\alpha]_D$, + 104° (c, 0.9 in water), m.p. = 159°, undepressed on admixture with L - arabinose.

Fraction 3.

This fraction (50 mg., R_{Gal} = 1.97 in solvent A) was chromatographically identical to L - rhamnose. The sugar, after recrystallisation from ethanol containing 30% water, had $[\alpha]_D$, + 6.7° (c, 0.7 in water), m.p. = 93°, undepressed on admixture with L - rhamnose /

rhamnose monohydrate.

Separation of acidic sugars.

Paper chromatography in solvent C showed that 2 aldobiouronic acids with identical R_{Gal} values to 6 - O (β - D - glucopyranosyluronic acid) - D - galactose (R_{Gal} = 0.20) and 6 - O - (4 - O - methyl - β - D - glucopyranosyluronic acid) - D - galactose (R_{Gal} = 0.61), and D - glucuronic acid (R_{Gal} = 1.21) were present.

The mixture was separated by paper chromatography on Whatman 31 (extra thick) filter sheets in solvent C to give aldobiouronic acid A (216 mg., R_{Gal} = 0.20) and aldobiouronic acid B (70 mg., R_{Gal} = 0.61).

Characterisation of aldobiouronic acid A.

Aldobiouronic acid A (R_{Gal} = 0.20 in solvent C) had $[\alpha]_D$, 0° (c, 1.5 in water). Hydrolysis with 2N sulphuric acid for 6 hours and subsequent paper chromatographic examination of the neutralised hydrolysate gave approximately equal proportions of galactose and glucuronic acid. Aldobiouronic acid A (2 mg.), after conversion to the methyl ester methyl glycoside, was reduced with potassium borohydride and hydrolysed with N sulphuric acid. A chromatographic examination of the neutralised hydrolysate in solvent A gave approximately equal amounts of galactose and glucose.

Methyl sulphate (1 ml.) and aqueous sodium hydroxide (1 ml. 30% w./v.) were added dropwise over a period of 2 hours to a solution /

solution of aldobiouronic acid A (100 mg.) in water (5 ml.). The solution was stirred vigorously at 0° in an atmosphere of nitrogen for 12 hours. Stirring was continued at room temperature and methyl sulphate (3 ml.) and aqueous sodium hydroxide (6 ml.) were added dropwise over a period of 5 hours. Similar additions of the reagents were made on 4 successive days. The reaction mixture was heated on a boiling-water bath for 30 minutes to destroy excess methyl sulphate, cooled, acidified with sulphuric acid and poured into methylated spirits (4 volumes). The precipitated sodium sulphate was removed at the centrifuge and washed with methylated spirits (4 x 50 ml.). The centrifugate and washings were combined, made slightly alkaline with sodium hydroxide, and concentrated to small volume (50 ml.). The aqueous solution was acidified with sulphuric acid and extracted with chloroform (4 x 100 ml.). The chloroform extracts were washed with water and evaporated to dryness. The silver salt of the partially methylated aldobiouronic acid was prepared by dissolving the residue in water (10 ml.) and stirring with silver carbonate (50 mg.). Excess silver carbonate was removed at the centrifuge and washed with water (4 x 20 ml.). The centrifugate and washings were combined and freeze-dried to give a light brown product (120 mg.).

The partially methylated sugar was methylated twice with methyl iodide (10 ml.) and silver oxide (1.5 g.). Evaporation of the solvent gave a light brown syrup (70 mg.).

Examination of the cleavage products by gas-liquid chromatography of the methyl glycosides, and paper chromatography of the sugars /

sugars in solvent E indicated the presence of 2,3,4 - tri - O - methylglucuronic acid (R_G = 0.87, $\underline{T}$ = 2.50, 3.21 column (a), 1.78, 2.20 column (b)), 2,3,4 - tri - O - methylgalactose (R_G = 0.69, $\underline{T}$ = 7.28 column (a), 2.61, 2.88 column (b)) and a small amount of 2,3,5 - tri - O - methylgalactose ($\underline{T}$ = 4.43 column (a)).

Characterisation of aldobiouronic acid B.

Aldobiouronic acid B (R_{Gal} = 0.61 in solvent C) had $[\alpha]_D$, + 3° (c, 0.9 in water). Hydrolysis with 2N sulphuric acid for 6 hours and subsequent paper chromatographic examination of the products showed approximately equal proportions of galactose and 4 - O - methylglucuronic acid. Aldobiouronic acid B, after conversion to the methyl ester methylglycoside was reduced with potassium borohydride and hydrolysed with N sulphuric acid. A chromatographic examination of the products in solvent A showed approximately equal amounts of galactose and 4 - O - methylglucose (R_{Gal} = 1.90). A portion of the hydrolysate (3 mg.) was subjected to periodate oxidation (105). Paper chromatography of the oxidised products gave 2 - O - methylerythrose (R_F= 0.55 in solvent D). The formation of this sugar is indicative of 4 - O - methylglucose.

Aldobiouronic acid B (50 mg.) was converted to the fully methylated derivative by the same methylation procedure as was used for aldobiouronic acid A. Gas-liquid chromatography of the cleavage products from methanolysis indicated the presence of 2,3,4 - tri - O - methylgalactose ($\underline{T}$ = 7.25 column (a), 2.49, 2.74 column (b)), 2,3,4 - tri - O - methylglucuronic acid ($\underline{T}$ = 2.47, 3.17 column (a), 1.68, /

1.68, 2.07 column (b)) and smaller amounts of 2,3,5 - tri - O - methylgalactose ($\underline{T}$ = 4.39 column (a), 1.85 column (b)), 2,3,4,6 - tetra - O - methylgalactose ($\underline{T}$ = 1.78 column (a), 1.52 column (b)) and an unknown substance ($\underline{T}$ = 3.04 column (a)).

METHYLATION STUDIES ON POLYSACCHARIDE A.

Methylation of polysaccharide A (III).

A suspension of polysaccharide A (400 mg.) in ether (100 ml.) containing diazomethane (1% w./v.) was vigorously stirred for 18 hours. The resulting methyl ester of the polysaccharide was removed at the centrifuge, dissolved in warm dimethylsulphoxide (10 ml.), cooled and diluted with dimethylformamide (10 ml.). The solution was stirred at 0° for 30 minutes when barium hydroxide octahydrate (10 g.) was added. Stirring was continued at 0° and aliquot proportions of methyl sulphate (2 ml.) were added after 30, 90, 120 and 180 minutes. After 30 minutes the ice-bath was removed and stirring was continued for 2 days. The reaction mixture was then dialysed against running tap-water for 5 days, treated with Amberlite resin IR - 120 (H) to remove cations and freeze-dried to give partially methylated polysaccharide A (355 mg.) [Found: OMe, 31.2%].

The partially methylated material was methylated to completion with methyl iodide (2 ml.), dimethylformamide (6 ml.) and silver oxide (2g.) to give methylated polysaccharide A (210 mg.), $[\alpha]_D$ - 49° (c, 0.6 in chloroform) [Found: O Me, 42.8% not raised on further methylation].

Examination of methylated polysaccharide A.

The methylated polysaccharide was methanolysed and the resulting /

resulting mixture of methyl glycosides was analysed by gas-liquid chromatography. The results obtained are shown in table 12 . Trace amounts of 2 - O - methylgalactose (R_{Gal} = 1.70) were observed on a paper chromatographic examination of the hydrolysis products in solvent A.

Table 12

Methyl Ether	T Values Column (a)	T Values Column (b)	Relative amounts
2,3,4 - Trimethylrhamnose	0.47	0.47	+
2,3,5 - Trimethylarabinose	0.56	0.47	++
	0.73	0.60	
2,3,4 - Trimethylarabinose	1.06	0.83	tr
2,5 - Dimethylarabinose		0.70	tr
2,3,4,6 - Tetramethylgalactose	1.80	1.52	++++
		1.60	
2,4,6 - Trimethylgalactose	4.16	2.07	++
	4.74	2.37	
2,3,4 - Trimethylgalactose	7.48	2.60	+++
		2.88	
2,4 - Dimethylgalactose		3.57	++++
		4.24	
2,3,4 - Trimethylglucuronic	2.50	1.77	+++
acid	3.24	2.21	
2 - Monomethylgalactose	-	-	tr

Methylated /

Methylated polysaccharide A (50 mg.) was partially methanolysed by refluxing with methanolic hydrogen chloride (5 ml., 2% W./v.) for 2 hours. The solution was neutralised in the usual way and concentrated to a syrup. The partially methanolised product was then hydrolysed with N sulphuric acid at 100° for 4 hours, neutralised with barium hydroxide and barium carbonate, treated with Amberlite resin IR - 120 (H) to remove barium ions and concentrated to small volume (5 ml.).

The acidic sugars were separated from the neutral sugars on a D.E.A.E Sephadex column (grade A 25, 4 x 1 cm.). Gas-liquid chromatography of the cleavage products from methanolysis showed the methyl glycosides of 2,3,4 - tri - O - methylglucuronic acid ($\underline{T}$ = 2.50, 3.22 column (a), 1.77, 2.22 column (b)) and 2,3,4 - tri - O - methylgalactose ($\underline{T}$ = 7.5 column (a), 2.66, 2.94 column (b)). A portion of the acidic fraction, after conversion to the methyl ester methylglycoside, was reduced with potassium borohydride. Paper chromatography of the hydrolysis products in solvent D gave 2,3,4 - tri - O - methylgalactose (R_G = 0.72) and 2,3,4 - tri - O - methylglucose (R_G = 0.88). No di - O - methylgalactose or di - O - methylglucose was observed.

PERIODATE OXIDATION STUDIES ON POLYSACCHARIDE A.

Periodate consumption of polysaccharide A.

The amount of reagent consumed when polysaccharide A (100 mg.) was oxidised with sodium metaperiodate (10 ml., 0.15 M) was determined spectrophotometrically by the method of Aspinall and Ferrier (106) and found to be 1.43 moles per sugar unit.

Formic acid release from polysaccharide A.

The amount of formic acid released by polysaccharide A on oxidation with potassium metaperiodate was determined by the method of Halsall, Hirst and Jones (107) and found to be 0.64 moles per sugar unit.

Smith degradation of polysaccharide A.

Polysaccharide A (8.0 g.) was dissolved in sodium metaperiodate solution (800 ml. 0.15 M) and allowed to stand at room temperature in the dark for 90 hours. Ethylene glycol (7 g.) was added to destroy excess periodate, and the solution was left for 30 minutes. Sodium ions were removed with Amberlite resin IR - 120(H) and the resulting acid solution was neutralised with barium hydroxide and barium carbonate. Barium salts were removed at the centrifuge and washed with water (4 x 100 ml.). The centrifugate and washings were combined, treated with potassium borohydride (11 g.) and allowed /

allowed to stand at room temperature for 2 days. The solution was then stirred for 15 minutes with Amberlite resin IR - 120 (H) to destroy excess borohydride and remove cations, filtered and evaporated to dryness. Boric acid was removed by the repeated addition and evaporation of methanol.

The residue was dissolved in N sulphuric acid (200 ml.) and left at room temperature for 3 hours. The solution was neutralised in the usual way, concentrated to small volume (50 ml.) and poured into ethanol (4 volumes). The precipitate was removed at the centrifuge, washed with ethanol (4 x 75 ml.), dissolved in water and reprecipitated to give polysaccharide B (2.07 g.), $[\alpha]_D$, + 26° (c, 0.7 in water).

The degraded polysaccharide on hydrolysis gave galactose, arabinose and a trace of reducing substance (R_{Gal} = 2.02 in solvent A) but no non-reducing substance.

The supernatant liquid and ethanol washings from the precipitation of polysaccharide B were combined and concentrated to give syrup B (2.8 g.). Paper chromatography of the syrup in solvent C indicated the presence of glycerol (R_{Gal} = 2.38) and two other non-reducing substances (R_{Gal} = 2.08 and 1.91) but no reducing sugars. Hydrolysis of the syrup followed by paper chromatography gave galactose, arabinose and the above three non-reducing substances.

Sugar proportions in polysaccharide B.

The polysaccharide was hydrolysed with N sulphuric acid at 100° /

100° for 4 hours and the products were fractionated on Whatman No.1 filter paper in solvent B. A colorimetrical estimation (97) of the sugar fractions showed that the ratio of galactose to arabinose was approximately 20 to 1.

Partial hydrolysis of polysaccharide B.

A sample of the polysaccharide was heated with sulphuric acid (0.5 N) at 100° for 1 hour. Paper chromatography of the products in solvent A indicated the presence of (1-3) - galactobiose (R_{Gal} = 0.53) (1-3) - galactotriose (R_{Gal} = 0.23) and a smaller amount of (1-6) - galactobiose (R_{Gal}= 0.35).

Methylation of polysaccharide B.

Methyl sulphate (1 ml.) and aqueous sodium hydroxide (1 ml., 30% w./v.) were added dropwise over a period of 2 hours to a solution of polysaccharide B (150 mg.) in water (5 ml.). The reaction mixture was stirred for 12 hours at 0° in an atmosphere of nitrogen. Stirring was continued and methyl sulphate (4 ml.) and aqueous sodium hydroxide (8 ml.) were added dropwise. Similar additions of the reagents were made on 6 successive days. After the third addition acetone was added to keep the partially methylated polysaccharide in solution. The solution was heated on a boiling-water bath for 30 minutes, cooled, neutralised with sulphuric acid and poured into methylated spirits (4 volumes). The precipitated sodium sulphate was removed at the centrifuge and washed with methylated spirits (4 x 50ml.). The /

The centrifugate and washings were combined, concentrated to small volume (50 ml.) and extracted continuously with chloroform for 18 hours. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in chloroform and poured into light petroleum (20 volumes). The resulting precipitate was removed at the centrifuge to give partially methylated polysaccharide B (120 mg.) [Found: O Me, 32.1%].

The partially methylated polysaccharide was methylated 6 times with methyl iodide (10 ml.) and silver oxide (2g.), and twice with methyl iodide (0.25 ml.), dimethylformamide (0.75 ml.) and silver oxide (250 mg.) to give methylated polysaccharide B (51 mg.), $[\alpha]_D$, - 16°, (c, 0·7 in chloroform) [Found: O Me, 37.4%].

The cleavage products from the methylated polysaccharide were examined by gas-liquid chromatography of the methyl glycosides and paper chromatography of the sugars in solvents D and F. The results obtained are shown below.

Methyl /

Methyl ether	$\underline{T}$ values Column (a)	Column (b)	R_G values solvent D	Relative amounts
2,3,5 - Trimethylarabinose	0.53	0.47		+
	0.70	0.72		
2,3,4,6 - Tetramethylgalactose	1.72	1.54	0.90	+
2,4,6 - Trimethylgalactose	3.92	1.98		++++
	4.46	(2.26)	0.78	
2,3,4 - Trimethylgalactose	7.0			trace
2,6 - Dimethylgalactose	9.5	(2.26)		++
		2.90		
		(3.46)	0.59	
2,4 - Dimethylgalactose		(3.46)		+
		4.02		
2 - Monomethylgalactose			0.34	++

The $\underline{T}$ values in parentheses are common to more than one sugar.

Periodate consumption of polysaccharide B.

The amount of reagent consumed when polysaccharide B (100 mg.) was oxidised with sodium metaperiodate (10 ml., 0.15 $\underline{M}$) was determined spectrophometrically by the method of Aspinall and Ferrier (106) and found to be 0.27 moles per sugar unit.

Formic /

Formic acid release of polysaccharide B.

The amount of formic acid released by polysaccharide B on oxidation with potassium metaperiodate was determined by the method of Halsall, Hirst and Jones (107) and found to be 0.09 moles per sugar unit.

Smith degradation of polysaccharide B.

Polysaccharide B (1.3 g.) was oxidised with sodium metaperiodate (130 ml., 0.15 M) for 48 hours, reduced with potassium borohydride (2 g.) and hydrolysed with cold sulphuric acid (1 N, 50 ml.) by the same procedures as were used for the degradation of polysaccharide A. Precipitation with ethanol gave polysaccharide C (771 mg.), $[\alpha]_D$, + 30° (c, 0.6 in water).

The degraded polysaccharide on hydrolysis gave galactose, traces of arabinose and a reducing substance (R_{Gal} = 2.02) but no non-reducing substances.

The supernatant liquid and ethanol washings from the precipitation of polysaccharide C were combined and concentrated to give syrup C. Paper chromatography of the syrup in solvent C indicated the presence of glycerol (R_{Gal} = 2.38) and two other non-reducing substances (R_{Gal} = 2.08 and 1.91) but no reducing substances. Hydrolysis of the syrup followed by paper chromatography gave galactose and the above three non-reducing substances.

Partial /

Partial hydrolysis of polysaccharide C.

Polysaccharide C was partially hydrolysed under the same conditions as were employed for the partial hydrolysis of polysaccharides A and B. The products were examined chromatographically in solvent C when (1 - 3) - galactobiose and (1 - 3) - galactotriose with only a trace of (1 - 6) - galactobiose were observed.

Methylation of polysaccharide C.

Polysaccharide C (150 mg.) was methylated 7 times with methyl sulphate (4 ml.) and aqueous sodium hydroxide (8 ml., 30% w./v.) by the same procedure as was used for the methylation of polysaccharide B, to give partially methylated polysaccharide C (125 mg.) [Found: O Me, 31.6%].

The partially methylated product was methylated 6 times with methyl iodide (10 ml.) and silver oxide (2 g.), and twice with methyl iodide (0.25 ml.), dimethylformamide (0.75 ml.) and silver oxide (250 mg.) to give methylated polysaccharide C (56 mg.), $[\alpha]_D$ -12 ° (c, 0.8 in chloroform) [Found : O Me , 36.6%].

The cleavage products from the methylated polysaccharide were examined by gas-liquid chromatography of the methyl glycosides and paper chromatography of the sugars in solvents D and F. The results obtained are shown below.

Methyl /

Methyl ether	$\underline{T}$ values column (a)	R_G values solvent D	R_G values solvent F	Relative amounts
2,3,4,6 - Tetramethylgalactose	1.73	0.90		+
2,4,6 - Trimethylgalactose	3.92			++++
	4.42	0.78		
2,3,4 - Trimethylgalactose	7.1			trace
2,6 - Dimethylgalactose	9.5	0.59	1.00*	++
2,4 - Dimethylgalactose			0.64*	+
2- Monomethylgalactose		0.34		++

*Quoted as $R_{2,6}$ - Dimethylgalactose values.

Periodate oxidation of polysaccharide C.

The amount of reagent consumed when polysaccharide C (100 mg.) was oxidised with sodium metaperiodate (10 ml. 0.15 $\underline{M}$) was determined spectrophotometrically by the method of Aspinall and Ferrier (106) and found to be 0.15 moles per sugar unit.

Formic acid release from polysaccharide C.

The amount of formic acid released by polysaccharide C on oxidation with potassium metaperiodate was determined by the method of Halsall, Hirst and Jones (107) and found to be 0.04 moles per sugar unit.

Bibliography

1) E.L. Hirst, J., 1955, 2974.

2) J.W.T. Merewether, Holzforschung, 1955, II, 65.

3) A.G. Norman, "The Biochemistry of Cellulose, the Polyuronides, Lignin", Oxford, 1937.

4) W. Overbeck, H.F. Müller, Svensk. Papperstidn., 1942, 45, 357.

5) C. Schuerch, J. Amer. Chem. Soc., 1952, 74, 5061.

6) E. Hägglund, "Chemistry of Wood", 1951, 416.

7) J.W.T. Merewether, Australian Pulp and Paper Ind. Tech. Assoc. Proc., 1951.

8) L.E. Wise, "Wood Chemistry", 2nd Ed., New York, 1952, 369.

9) A.G. Norman, "Cellulose and Cellulose derivatives" 2nd Ed. Part 1, New York, 1954, 466.

10) F.E. Brauns, "The Chemistry of Lignin", New York, 1952, 14.

11) A. Adams, Ind. Eng. Chem., 1921, 13, 786.

12) K. Freudenberg, "Tannin, Cellulose, Lignin," Berlin, 1933, 139.

13) B.O. Lindgren, Acta. Chem. Scand., 1958, 12, 447.

14) R. Nelson and C. Schuerch, J. Polymer Sci., 1956, 22, 435.

15) E.F. Kurth, Ind. Eng. Chem. Annal. Ed., 1939, II, 203.

16) C.F. Cross and J. Bevan, J., 1889, 55, 199.

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

18) E. Schmidt, Y.C. Tang and W. Jandebeur, Cellulosechemie, 1931, 12, No.7, 201.

19) A.G. Norman, Biochem. J., 1937, 31, 1579.

20) R.L. Mitchell and G.J. Ritter, J. Amer. Chem. Soc., 1940, 62, 1958.

21) H.O. Mosimann and T. Svedberg, Kolloid-Zeitschr., 1942, 100, 99.

22) W.G. Campbell, E.L. Hirst and J.K.N.Jones, J., 1948, 774.

23) /

23) J.F. Carson and W.D. Mackay, J. Amer. Chem. Soc., 1948, 70, 293.

24) I.A. Preece and K.G. Mackenzie, J. Inst. Brew., 1952, 58, 353.

25) G.A. Adams, Canad. J. Chem., 1957, 35, 556.

26) G.A. Adams, Tappi, 1957, 40, 721.

27) G.O. Aspinall and T.M. Wood, J. 1963, 1686.

28) S. Angell and F.W. Norris, Biochem. J. 1936, 30, 2159.

29) A.S. Jones, Biochim. Biophys. Acta., 1953, 10, 607.

30) B.C. Bera, A.S. Foster and M. Stacey, J., 1955, 3788

31) S.A. Barker, M. Stacey and G. Zweifel, Chem. and Ind., 1957, 330.

32) H.O. Bouveng and B. Lindberg, Acta. Chem. Scand., 1958, 12, 1977.

33) T.M. Wood, Ph.D. Thesis, Edinburgh, 1961.

34) W. Heri, H. Neukom and H. Deuel, Helv. Chim. Acta., 1961, 44, 1939.

35) N.R. Ringertz and P. Reichard, Acta. Chem. Scand., 1960, 14, 303.

36) R. Young, Ph.D. Thesis, Edinburgh, 1963.

37) M. Schmidt, Biochim. Biophys. Acta., 1962, 63, 346.

38) I.A. Preece and R. Hobkirk, Chem. and Ind., 1955, 257.

39) D.R. Briggs, E.F. Garner and F. Smith, Nature, 1956, 178, 154.

40) B.A. Lewis and F. Smith, J. Amer. Chem. Soc., 1957, 79, 3979

41) T. Svedberg and K.O. Patterson, "The Ultracentrifuge", Oxford, 1940.

42) C.T. Bishop, Canad. J. Chem., 1953, 31, 134.

43) R.L. Whistler and L. Hough, J. Amer. Chem. Soc., 1953, 75, 4918.

44) G.O. Aspinall and R.J. Ferrier, J., 1957, 4188.

45) R.L. Whistler and D.I. McGilvary, J. Amer. Chem. Soc., 1955, 77, 2212.

46) R.L. Whistler and W.M. Corbett, J. Amer. Chem. Soc., 1955, 77, 3822.

47) R.L. Whistler and G.E. Lauterback, J. Amer. Chem. Soc., 1958, 80, 1987.

48) R.L. Whistler and W.M. Corbett, J. Amer. Chem. Soc., 1955, 77, 6328.

49)/

49) R.L. Whistler and J.N. Be Miller, J. Amer. Chem. Soc., 1956, 78, 1163.

50) R. Montgomery, F. Smith and H.C. Srivastava, J. Amer. Chem. Soc., 1957, 79, 698.

51) F. Smith and H.C. Srivastava, J. Amer. Chem. Soc., 1957, 79, 892.

52) J.K.N. Jones, E. Merler and L.E. Wise, Canad. J. Chem., 1957, 35, 634.

53) J.K.N. Jones, G.B. Purves and T.E. Timell, Canad. J. Chem., 1961, 37, 1059.

54) A.R.N. Gorrod and J.K.N. Jones, J., 1954, 2522.

55) A. Roudier, Bull. Soc. Chim. France, 1961, 976.

56) J.K.N. Jones and L.E. Wise, J., 1952, 2750.

57) T.E. Timell, J. Amer. Chem. Soc., 1959, 81, 4989.

58) J.K. Gillham and T.E. Timell, Canad. J. Chem., 1958, 36, 410, 1467.

59) A. Roudier and H. Gillet, Assoc. techn. ind. papertière Bull., 1963, 17, 145.

60) B. Lindberg and H. Mier, Svensk Papperstidn., 1957, 60, 785.

61) I. Groon and B. Lindberg, Acta. Chem. Scand., 1958, 12, 453.

62) E. Merler and L.E. Wise, Tappi, 1958, 41, 80.

63) E.V. White, J. Amer. Chem. Soc., 1941, 63, 2871.

64) E.V. White, J. Amer. Chem. Soc., 1942, 64, 302.

65) E.V. White, J. Amer. Chem. Soc., 1942, 64, 1507.

66) E.V. White, J. Amer. Chem. Soc., 1942, 64, 2838.

67) H.O. Bouveng and B. Lindberg, Acta. Chem. Scand., 1956, 10, 1515.

68) H.O. Bouveng, Acta. Chem. Scand., 1959, 13, 1869.

69) H.O. Bouveng, Acta. Chem. Scand., 1959, 13, 1877.

70) H.O. Bouveng, Acta. Chem. Scand., 1961, 15, 78.

71) V.C. Barry and P.W.D. Mitchell, J., 1954, 4020.

72) G.O. Aspinall, E.L. Hirst and E. Ramstad, J., 1958, 593.

73) M. Heidelberger, J. Amer. Chem. Soc., 1955, 77, 4308.

74)/

74) J.K.N. Jones, J. 1953, 1672.

75) G.O. Aspinall and T.M. Wood, unpublished results.

76) G.O. Aspinall and A. Nicolson, J., 1960, 2503.

77) M. Abdel-Akker, J.K. Hamilton, R. Montgomery and F. Smith, J. Amer. Chem. Soc. 1955, 77, 4308.

78) A.B. Anderson, W.Z. Hassid and W.H. Wadman, J., Amer. Chem. Soc., 1954, 76, 4097.

79) C.T. Bishop, Canad. J. Chem. 1957, 35, 1010.

80) G.A. Adams, Canad. J. Chem., 1958, 36, 755.

81) R.A. Laidlaw and G.A. Smith, Chem. and Ind., 1962, 462.

82) G.A. Adams, Canad. J. Chem., 1960, 38, 280.

83) G.A. Adams and S. Haq, Canad. J. Chem., 1961, 39, 1563.

84) G.A. Adams, C.T. Bishop and B. Urbas, Canad. J. Chem., 1963, 41, 1522.

85) J.K.N. Jones, J. Polymer Sci. Part C. No.2, 1963, 63.

86) L. Eberhard and A.J. Roudier, Bull. Soc. Chim. France, and unpublished results.

87) F. Smith and P. Montgomery, "Plant Gums and Mucilages", New York, 1959, 241 and references there cited.

88) G.O. Aspinall, A.J. Charlson, E.L. Hirst and R. Young, J., 1963, 1696.

89) P. Andrews and J.K.N. Jones, J., 1955, 583.

90) E.L. Hirst and A.S. Perlin, J., 1954, 2622.

91) A.S. Perlin, Analyt. Chem., 1955, 27, 396.

92) G.O. Aspinall, E.L. Hirst and A. Nicolson, J., 1959, 1697.

93) J.K.N. Jones and G.H.S. Thomas, Canad. J. Chem., 1961, 39, 192.

94) C.T. Bishop and F.P. Cooper, Canad. J. Chem. 1960, 38, 388.

95) G.O. Aspinall, J., 1962, 1676.

96) M. Dubois, K.A. Gillies, J.K. Hamilton, P.A. Rebers and F. Smith, Analyt. Chem., 1956, 28, 350.

97) J. Ek and E. Hultman, Nature, 1958, 181, 780.

98)/

98) D.M.W. Anderson, Talanta, 1959, 2, 73.

99) E.A. McComb and R. McCready, Analyt. Chem., 1952, 24, 1630.

100) R. Belcher and A.L. Godbert, "Semi-micro Quantitative Organic Analysis", London, 1954.

101) W.M. Hearon, G.D. Hiattand and C.R. Fordyce, J. Amer. Chem. Soc., 1943, 65, 2449.

102) R. Kuhn, I. Löw and H. Trischman, Angew. Chem., 1955, 67, 32.

103) T.C. Bonner, E.J. Bourne and S. McNally, J., 1960, 2929.

104) L. Hough, D.B. Powell and B.M. Woods, J., 1956, 4799.

105) H.F. Bauer and R.U. Lemieux, Canad. J. Chem., 1953, 31, 814.

106) G.O. Aspinall and R.J. Ferrier, Chem. and Ind., 1957, 1216.

107) T.G. Halsall, E.L. Hirst and J.K.N. Jones, J., 1947, 1427.

108) S.N. Harshe, P.P. Singh and H.C. Srivastava, Tetrahedron Letters, 1963, 27, 1869.

109) F. Micheel, Chem. Ber., 1932, 65, 262.

110) Y. Hirasaka, Yakugaku Zasshi, 1964, 83, 976.

111) R. Kuhn and H. Trischmann, Chem. Ber., 1963, 96, 284.

ABSTRACT OF THESIS

Name of Candidate RONALD M. FAIRWEATHER

Address 35 Fountainhall Road, Edinburgh, 9.

Degree Doctor of Philosophy *Date* 30th November 1964

Title of Thesis The chemistry of wood-cell wall polysaccharides, with special reference to the galactan components.

Methylated Japanese larch arabinogalactan A was hydrolysed to give the following methylated sugars:- 2,3,5-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-arabinose, 2,5-di-O-methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose, 2,6-di-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose and 2-O-methyl-D-galactose.

Partial hydrolysis of arabinogalactan A with 0.01N hydrochloric acid afforded 3-O-β-L-arabinopyranosyl-L-arabinose.

The primary hydroxyl groups of terminal non-reducing galactose and arabinofuranose residues in the polysaccharide were oxidised to carboxylic acid groups via procedures involving tritylation, methylation, detritylation and oxidation. Hydrolysis of the resulting oxidised methylated polysaccharide afforded neutral and acidic sugar fractions. A chromatographic examination of the cleavage products from the acidic sugar fraction showed 2,3,4-tri-O-methylgalactose, 2,4-di-O-methylgalactose and 2,3,4-tri-O-methylgalacturonic acid. Reduction of the acidic sugar fraction with lithium aluminium hydride followed by a chromatographic examination of the cleavage products showed 2,3-di-O-methylarabinose, 2,3,4-tri-O-methylgalactose and 2,4-di-O-methylgalactose. A portion of the acidic sugar fraction was remethylated and a chromatographic examination of the cleavage products showed 2,3,4-tri-O-methylgalactose and 2,3,4-tri-O-methylgalacturonic acid. Reduction of the remethylated acidic sugar fraction with lithium aluminium hydride, followed by a chromatographic examination of the cleavage products, showed 2,3-di-O-methylarabinose and 2,3,4-tri-O-methylgalactose.

The structural significance of these results is discussed.

Araucaria bidwilli gum had $[\alpha]_D$, +11°. The polysaccharide was partially hydrolysed with N sulphuric acid when the following sugars were identified:- D-galactose, L-arabinose, L-rhamnose, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

A chromatographic examination of the cleavage products from the fully methylated polysaccharide showed:- 2,3,4-tri-O-methylrhamnose, 2,3,5-tri-O-methylarabinose, 2,3,4-tri-O-methylarabinose, 2,5-di-O-methylarabinose, 2,3,4,6-tetra-O-methylgalactose, 2,4,6-tri-O-methylgalactose, 2,3,4-tri-O-methylgalactose, 2,4-di-O-methylgalactose, 2-O-methylgalactose and 2,3,4-tri-O-methylglucuronic acid.

The polysaccharide was oxidised with sodium metaperiodate and the resulting polyaldehyde was reduced with potassium borohydride. The polyalcohol was hydrolysed with N sulphuric acid at room temperature to give a degraded polymer and low molecular weight material. The degraded polysaccharide was subjected to a second periodate oxidation and Smith degradation to give a further degraded polysaccharide. Both Smith degraded polysaccharides were shown by methylation, partial hydrolysis and periodate oxidation studies to consist substantially of (1-3)-linked galactopyranose residues.

The /

Use other side if necessary.

The structural significance of these results is discussed.

The structural features of Japanese larch arabinogalactan A and Araucaria bidwilli gum are compared with those of other coniferous wood arabinogalactans and galactan-based gums.